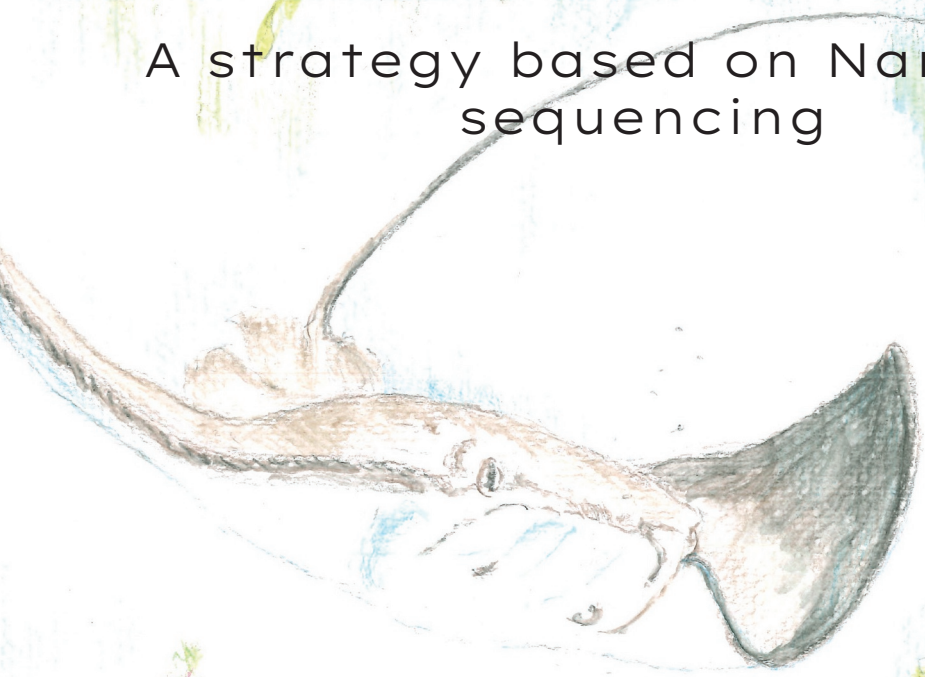
Improving North Sea biodiversity monitoring using novel molecular approaches

A strategy based on Nanopore sequencing



Karlijn Doorenspleet

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Karlijn Doorenspleet

Thesis

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CONTENTS

Chapter 1	General Introduction	7
Chapter 2	Advancing molecular macrobenthos biodiversity monitoring: a comparison between Nanopore and Illumina based metabarcoding and metagenomics	23
Chapter 3	The long and the short of it: Nanopore based eDNA metabarcoding of marine vertebrates works; sensitivity and specificity depend on amplicon lengths	79
Chapter 4	The potential of eRNA to assess the local biodiversity in North Sea bivalve reefs	121
Chapter 5	Integrating current and future molecular tools for standardised monitoring of North Sea biodiversity and ecosystem dynamics	163
Chapter 6	Shellfish reefs as Essential Fish Habitats: complementary insights from eDNA and BRUVs in the North Sea	189
Chapter 7	General Discussion	259
References		275
Summary		325
Acknowledgments		328
About the author		331
List of publications		332
Authorship statement		333
Training and education statement		335

Chapter 1

General introduction

Karlijn Doorenspleet

1. GENERAL INTRODUCTION

1.1 The many faces of the North Sea

1.1.1 The North Sea

The North Sea is a temperate shallow sea (40–200m) on the European continental shelf that borders Great Britain, Norway, Denmark, Germany, the Netherlands, Belgium, and France (Holligan et al., 1989). It connects with the Atlantic Ocean through the English Channel in the south and with the Norwegian Sea in the north. Strong tidal and wind forces characterise water dynamics and circulation in the North Sea, as well as inflow from the Atlantic Ocean, which mixes with freshwater inputs from rivers and colder outflows of the Baltic Sea (Eisma et al., 1997; Otto et al., 1990; van der Molen & Pätsch, 2022).

Due to its dynamic nature, the North Sea harbours a vast arsenal of zones, habitats, and ecosystems. Most iconic are the coastal and intertidal zones, such as the Wadden Sea, which stretches along the Dutch, German, and Danish coasts, characterised by coastline, tidal flats, salt marshes, and shallow dunes that create a highly productive system (UNESCO World Heritage Centre [UNESCO], n.d). Additionally, the North Sea includes offshore sandbanks, such as the Dogger Bank, where sediment movement and water column mixing shape animal community compositions on the seafloor (Beermann et al., 2023; Sonnewald & Türkay, 2012; Wieking & Kröncke, 2003). Habitats, such as the Borkum reef ground, form mosaics of sandy and stony patches (Coolen et al., 2015; Kühne & Rachor, 1996), while farther North, the shelf includes deeper muddy and transitional zones such as the Skagerrak. Throughout the North Sea, benthic organisms (organisms that live on or within the seabed), such as polychaetes, bivalves, echinoderms, and crustaceans, play critical roles in structuring the North Sea ecosystem by remineralising, water filtering, stabilising sediments, grazing and providing food for higher trophic organisms (Clare et al., 2015; Weinert et al., 2022). Bivalve species as *M. modiolus* and *O. edulis,* as well as polychaetes as *Lanice sp.* and *Sabellaria sp.,* are reef builders, creating three-dimensional structures for other taxonomic groups to hunt and hide. These reefs also stabilise currents and sediment, contributing to coastal protection (Coolen et al., 2015; Jenkins et al., 2018; van der Reijden et al., 2019). The North Sea also hosts more than 200 species of marine vertebrates, including benthic fish such as plaice, turbot, cod, and haddock, and pelagic species such as herring and mackerel that rely on planktonic or benthic prey (Daan et al., 1990)

(Figure 1). The North Sea is also home to many shark and ray species, for example, blue skates, and marine mammals such as the harbour porpoise, all of which are important for food-web balance and are indicators for ecosystem health (Daan, Heessen, et al., 2005; Reijnders & Lankester, 1990; Sguotti et al., 2016).

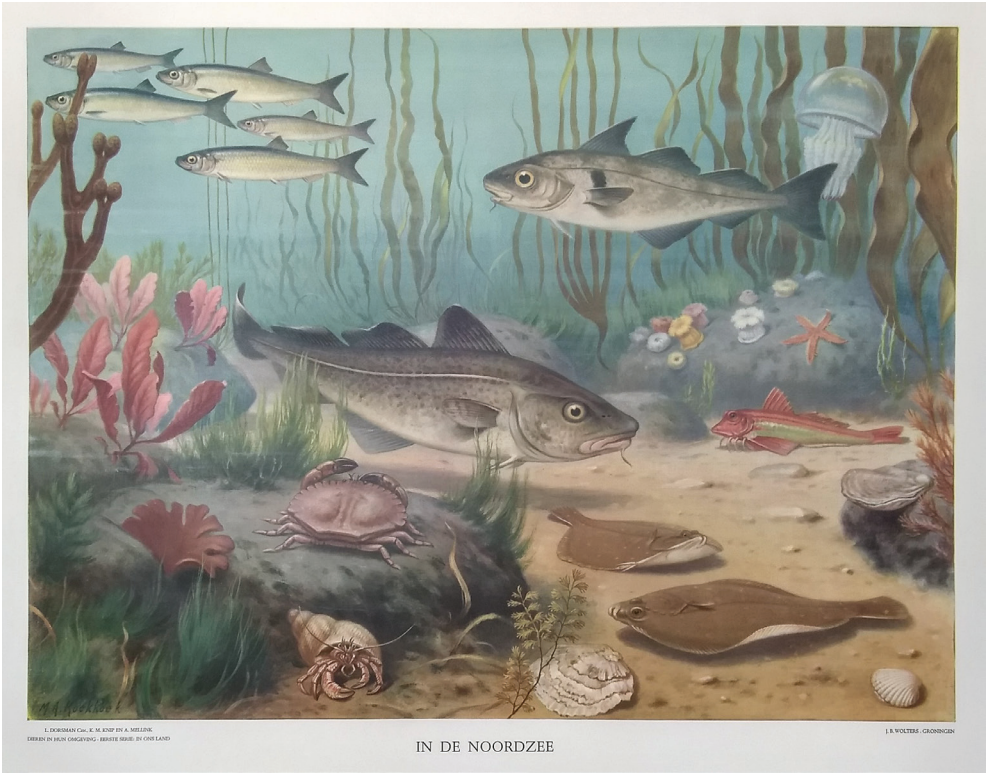


Figure 1: Adapted from "In de Noordzee" by M.A. Koekoek.

1.1.2 The history of the North Sea: transforming to an industrialised area

For more than a thousand years, the North Sea has been used for fishing, and this began with opportunistic gathering in local coastal areas throughout the medieval period (Hoffmann, 2005; Holm et al., 2022). Around the 1500s, fisheries shifted to organised, seasonal, and long-distance fisheries that, together with the salt-preservation technique, led to a 'fish revolution' with long-lasting social and economic impacts on European countries (Holm et al., 2022). Mainly, herring was fished in mass amounts between the 16th and 19th centuries (Holm et al., 2022). Also, biogenic reefs, such as flat oyster and mussel beds, were abundant and harvesting them became of considerable economic importance, especially

in England (Olsen, 1883; Thurstan et al., 2024). Among the largest in the North Sea was the flat oyster reef around the German Bight area covering roughly 1.5 million ha (Olsen, 1883; Thurstan et al., 2024a; Thurstan et al., 2024b). This reef was harvested for centuries, with evidence of harvested shells dating back to the Mesolithic period (Lewis et al., 2016; Milner, 2002; Tauber, 1981). The flat oyster population remained abundant until the 20th century, when increased engine power led to greater overfishing and the subsequent decimation of oyster reefs. This decline led to reduced water quality, and the introduction of Bonamia disease ultimately led to the functional extinction of flat oyster from the North Sea (Thurstan et al., 2024b; zu Ermgassen et al., 2025).

The industrial revolution brought significant changes to the fisheries sector, transport and urbanisation of coastal areas introducing steam and diesel engines, metal hulls, and mechanical trawls, alongside capitalised, centralised fleets and onshore processing (Engelhard, 2008). These technical advancements significantly improved catch efficiency and distance coverage but also led to large-scale habitat destruction and increased bycatch (Couce et al., 2020; Thurstan et al., 2010). In the 20th century, further improvements in equipment and technological developments, such as on-board refrigeration and factory fleets (Heidbrink, 2011), in combination with a rapidly growing human population and thus market demand, large-scale destruction of habitats, resulted in a record removal of fish and oyster stocks (Jackson et al., 2001). Consequently, both the size and the number of catches declined, ultimately resulting in the collapse of several species, such as herring (Dickey-Collas et al., 2010), cod (Engelhard et al., 2014), and plaice (Rijnsdorp, 1996) between the 60s and the 80s.

1.1.3 A way forward: a nature-inclusive North Sea

Fortunately, awareness of the vulnerability of marine ecosystems is increasing, and European citizens and their societies have increasingly recognised the vital services they provide (Cruz & Manuel, 2021; Luisetti & Schratzberger, 2023). These ecosystem services include food supply, as the North Sea ecosystem is highly productive, providing seafood, including fish, shellfish, and other edible species, thereby contributing to human well-being (Groot et al., 2011). It also delivers coastal protection, carbon sequestration, nutrient cycling, cultural values and traditions, tourism, and recreation (Maes et al., 2012).

For example, the North Sea was also an important migration route for several whale species (e.g. Humpack whale, Gray whale, Sperm whale) and strandings (Haelters & Kerckhof, 2010; Smeenk, 1997; Swartz et al., 2006) were seen as a sign (from God) or a warning of disaster in medieval times, and later served as a public spectacle (Smeenk, 1997) before they were considered valuable as natural history museum specimens (Simon-Ekeland & Delsett, 2024) or as an ecological indication (Pierce et al., 2007).

Thus, the conventional justification for conservation and ecosystem management is based on the significance of ecosystem services for human use and dependence. However, it is also increasingly argued that marine ecosystems have a value independent of human use. In other words, the oceans and associated ecosystems have an intrinsic value and are described under ethical frameworks such as deep ecology and biocentrism (Johnson, 1993). Thus, natural processes and non-human life are seen as morally significant on their own, and this perspective expands the ethical grounds for nature conservation beyond resource use or ecosystem services (Batavia & Nelson, 2017).

North Sea ecosystems, and ecosystems in general, can thus be viewed as entities deserving moral consideration or legal protection alongside humanity, not only for the services they provide but also as part of life in general (Burgers et al., 2020). This way of thinking has sparked legal and policy debates and explored the possibility of treating natural entities (e.g., rivers, forests, coral reefs) as legal entities or granting them "legal personhood" (Burgers et al., 2020). Although these proposals remain controversial and established legal frameworks are rare, such frameworks have been proposed for the North Sea (Burgers et al., 2020) and highlight that conservation should not depend solely on anthropocentric views (Batavia & Nelson, 2017). This reframing, combining ecosystem services, human wellbeing and intrinsic value, provides a more robust argumentation and policy grounds for stronger protective measures and environmental regulation in response to growing anthropogenic pressure. Within this context, conservation of the North Sea is based on ecosystem-based management and holds that the integrity of ecosystems should be protected as a whole (Arkema et al., 2006). This results in three main conservation approaches that are used alongside each other (Figure 2):

1. Nature-inclusive industrial design
2. The designation of Marine Protected Areas (MPA) and rewilding
3. Active restoration of degraded or vulnerable habitats.

Nature-inclusive industrial design, in which industrial development intentionally incorporates ecological considerations to enhance biodiversity and ecosystem functioning (Hernandez-Santin et al., 2022; O'Leary et al., 2023). For example, offshore wind farms (OWFs) in the North Sea are not only designed and built for energy production, but also sometimes designated as ecology enhancing habitats (Hermans et al., 2020), such as reef-like scour protection (Kingma et al., 2024) and placing artificial reefs in OWF areas to coordinate human use with ecosystem functioning.

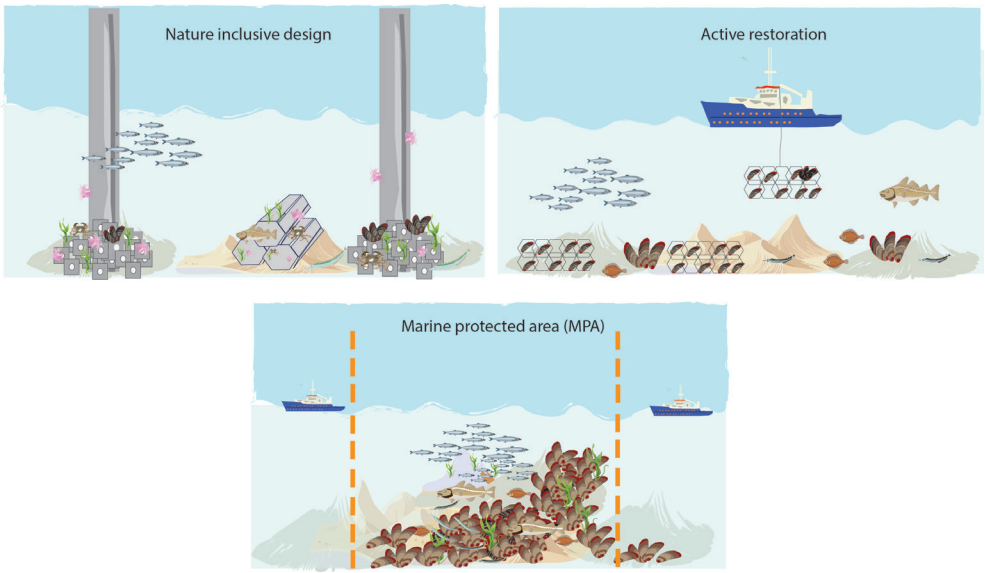


Figure 2: Representation of three approaches for ecosystem protection: nature-inclusive design, such as porous scour protection and artificial reef structures that function as habitat. Active restoration, where certain species such as raster with mussels are reintroduced in certain areas to promote the regrowth of mussel beds. The designation of marine protected areas, where fishing and/or entry is prohibited.

The designation of MPAs, as recently occurred for some parts of the Dogger Bank (Marine Management Organisation, 2022), aims to maintain vulnerable habitats and reefs by (partly) closing the area to certain types of fishing activities (Lindeboom, 1995). Active restoration aims to restore ecosystems that are degrading or have been lost and requires human intervention to restore characteristic habitats (Duarte et al., 2020).

Examples of these are the active restoration of native oysters (*O. edulis*) reefs by seeding juveniles, adults, and empty mussel shells in e.g., Borkum and the Dogger bank (Didderen et al., 2019.; P. zu Ermgassen et al., 2021), placing oyster and mussel settlement modules in OWFs (Kamermans et al., 2018), and reseeding seagrass meadows in the Dutch Wadden Sea (Govers et al., 2022). Together, these approaches align with the concept of rewilding, which has recently gained increased attention (Clover, 2022). Rewilding is a combination of passive and active protection and restoration approaches, including allowing natural colonisation, using geoengineering, and actively restoring biogenic habitats (Quintana et al., 2025). These approaches have been increasingly used in several North Sea ecosystems with considerable beneficial effects in some cases (Clover, 2022).

The diversity of initiatives to restore and maintain a functioning North Sea ecosystem occurs against the background of a rapidly changing climate, which further complicates local mitigation (Sguotti et al., 2022; Thorpe et al., 2022) adding an extra layer of uncertainty on how North Sea ecosystems will respond and adapt.

1.2. Changing how we monitor change in the North Sea

1.2.1 Monitoring for evidence-based decision making

To guide effective ecosystem management in the North Sea, European environmental directives enforce measures that require "good ecological status" as formulated by the water framework directive (WFD, 2000/60/EC) for coastal and transitional water or "good environmental status" that needs to be maintained throughout the whole North Sea (MSFD, 2008/56/EC). In addition, the Natura 2000 network obligates countries to protect designated habitats and species to ensure their "favourable conservation status" (Natura 2000 network, Habitat Directive). To do so, projects in industry and conservation need to rely on robust, transparent monitoring methods that detect ecological change driven by environmental or human drivers. This monitoring evaluates whether management decisions were effective and comply with directive frameworks (Borja et al., 2010). However, the heterogeneous offshore character of the North Sea, as well as the scale and location of industrial and conservation projects, makes monitoring challenging both logistically and financially.

1.2.2 Conventional invasive monitoring has negative effects

Conventional monitoring methods often rely on labour-intensive sampling approaches and campaigns. For benthic organisms, surveys typically use techniques such as scraping, grab sampling, box coring, or trawling. (A. D. Rijnsdorp et al., 2016, 2018). After collection and sorting, these samples often require individual processing and detailed taxonomic identification, both of which are time-consuming and require taxonomic expertise (Danovaro et al., 2016; Pawlowski et al., 2018). Monitoring of the mobile community (e.g., fish and other vertebrates) relies on netting, trapping, and trawl surveys, including the international bottom trawl survey, which is carried out to monitor the abundance and spatial distribution of fish for recruitment indices (ICES, 2019). Although such large-scale European-wide research offers valuable insight into biomass and distribution shifts, these methods are invasive, disturb vulnerable and valuable habitats, and require large research vessels that are often costly (Daan, Gislason, et al., 2005; Eggleton et al., 2018; Teletchea, 2009). In addition, these methods do not fully cover the ecological spectrum, thereby missing cryptic or small/fast species and population dynamics, nor can they sample the full complexity of environmental networks and trophic profiles (Fediajevaite et al., 2021; Shea et al., 2023). Therefore, robust, non-invasive, and cost-effective monitoring approaches are needed that provide accurate data over short time frames across large spatial and temporal scales.

1.2.3 Alternative non-invasive monitoring methods

Recently, there has been significant development in the availability of non-invasive monitoring methods, each with its own strengths and limitations (McGeady et al., 2023). Acoustic methods, including hydrophones (Hubert et al., 2020) and active sonar systems, can monitor species presence, biomass, and behaviour (Holmes et al., 2006). Multibeam echosounders can provide highly detailed maps of the seabed, reef structures, fish schools, and biomass (Lopez et al., 2016; Melvin & Cochrane, 2015). Additionally, passive acoustic methods can be used in low visibility and at night to detect nocturnal species (Verfuss et al., 2018). However, they require complex data processing and may be disturbed by background noise (Bordoux et al., 2025). Remote sensing using satellite or aerial imagery can detect ecological features such as algal blooms, kelp forests, and shallow reefs and can provide repeated snapshots, but has only a limited ability to penetrate below the surface, especially in turbid North Sea waters (Kachelriess et al., 2014; Ma et al., 2023). Diving surveys are beneficial for highly detailed observations but challenging in North Sea

conditions, limited by depth and safety, and require high labour costs (Shields & Lee, 1998). Video-based tools such as DROP cams, remote or autonomous underwater vehicles (ROVs, AUVs), and baited remote underwater video systems (BRUVs) allow the observation of benthic and pelagic species in complex habitats without disturbance (de Mendonça & Metaxas, 2021; Schramm et al., 2020). Nevertheless, video tools are sometimes limited by field-of-view constraints, limited battery power, and large datasets that require time-intensive analysis (de Mendonça & Metaxas, 2021). Finally, molecular tools for monitoring are increasingly used and contribute a new toolbox for routine biomonitoring (Danovaro et al., 2016).

1.3 The future of North Sea biodiversity monitoring with molecular methods.

1.3.1 The principles of metabarcoding for biodiversity monitoring

Molecular methods have great potential for monitoring, as they can detect species based on genetic material rather than physical observation (Danovaro et al., 2016). All marine organisms shed nucleic acids, such as DNA and RNA, into the water column by releasing skin cells, mucus, faeces, gametes, or by decomposing or releasing free-form DNA (Deiner et al., 2017). Collectively, these traces of nucleic acids are called environmental (e)NA and eDNA is relatively stable and can persist in the water column for days to weeks (Ficetola et al., 2008; Taberlet et al., 2012). eRNA, on the other hand, is less stable and degrades more rapidly, over hours to days (Jo, 2023). Traditionally, nucleic acids can be extracted directly from captured animals taken through swaps, blood, or tissue from individuals (van der Loos & Nijland, 2021).

Among molecular methods, metabarcoding is among the most rapidly evolving and widely applied tools in molecular marine ecology (Deiner et al., 2017). First, nucleic acid traces are collected from a water sample by filtration. Then, DNA is extracted from the filter, and the extract is used to amplify (copy) a target region of a gene, which is often located on mitochondrial DNA. To do so, a specific set of molecular markers (primers) is selected to amplify a target DNA region. The choice of primer is essential, as universal primers (e.g., COI or 18S) to maximise the number of targeted species or be group-specific (e.g., MiFish (12S)) to improve fish-specific resolution but fail to detect other taxonomic groups (Miya, 2022). With the help of a polymerase chain reaction (PCR), temperatures rhythmically change to enable denaturation (opening) of the double-

stranded DNA, binding (annealing) of the primer to the target region, and to initiate enzymatic amplification of the DNA region by linking nucleotides, thereby elongating the DNA strand (Lynch 1990). By repeating this temperature rhythm, the target
DNA can be amplified exponentially, thereby improving detection. Then, the amplified DNA is sequenced using high-throughput sequencing, which can read and translate millions of DNA sequences; these sequences are then processed using bioinformatics pipelines and compared to sequences in a reference database to assign final taxonomy (Figure 3). Molecular methods such as metabarcoding for marine biodiversity assessment hold potential for routine monitoring, as they enable highly sensitive species detection and can detect rare, cryptic, and low-abundant species that are often missed in visual or acoustic monitoring surveys (Shea et al., 2023). Moreover, if the laboratory infrastructure is in place, samples can be collected and processed rapidly, and the technique is cost-effective, making it a powerful non-invasive monitoring tool.

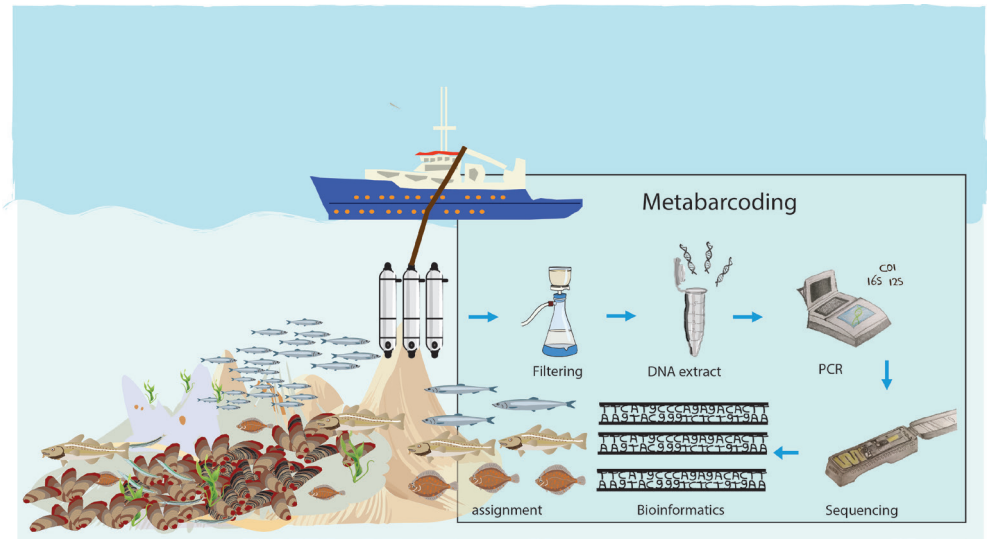


Figure 3: Sampling of water for eDNA or eRNA-based metabarcoding and the different processing steps that follow for metabarcoding for assessing the biodiversity of North Sea systems.

1.3.2 Limitations of metabarcoding

Despite its potential, the metabarcoding method still has several limitations that currently hamper its use as a stand-alone routine biomonitoring method (Beng & Corlett, 2020). A central challenge is the reduced ability to obtain quantitative information, as read counts can indicate

relative abundances of DNA molecules but are not directly proportional to biomass, as different species shed at different rates and dilution and degradation is strongly influenced by environmental factors (Shelton et al., 2023). The metabarcoding method also cannot obtain individual traits, such as body size, age class, condition, or sex, which are essential for population dynamics or stock assessments (Miya, 2022).

Biases that can occur during laboratory processes, such as primer biases, can lead to overrepresentation, underrepresentation, false-positive, and false-negative detections (van der Loos & Nijland, 2021). In addition, the choice of primer may hamper identification, at the species level, of certain closely related taxonomic groups (Miya, 2022). A current limitation still is that, reference databases such as Bold, GenBank, and NCBI are incomplete for many species, especially invertebrates and accurate species identification relies on the completeness and accuracy of these databases (van der Loos & Nijland, 2021; Weigand et al., 2019).

Interpreting eDNA-based metabarcoding in an ecological context is especially challenging. The degradation rate of eDNA depends on temperature, UV exposure, microbial activity, and hydrodynamics (Collins et al., 2018; Lamb et al., 2022). Lack of information about current and transport of eDNA further increase uncertainties about the spatial and temporal origin of the DNA (Harrison et al., 2019). It is thus challenging to determine whether the metabarcoding result reflects organisms currently present, those that recently passed through, or those originating from upstream locations (Beng & Corlett, 2020; Jo, 2023).

1.3.3 Technological developments bring new opportunities

Rapid technological developments are opening new opportunities to improve molecular biodiversity assessments by strengthening what can be detected from eDNA samples (Hu et al., 2021; Jahnke et al., 2022; Zhao et al., 2021). Different sequencing platforms are available, of which Illumina is the most favoured, as it produces highly accurate reads, albeit short ones. Platforms such as Oxford Nanopore are becoming increasingly popular because they provide long-read sequencing, generating reads spanning thousands of base pairs, enabling the use of markers targeting longer fragments to improve taxonomic resolution (Bleidorn, 2016; J. S. Johnson et al., 2019). In addition, Nanopore devices are portable, enabling sequencing to be performed remotely offshore, such as on research vessels, field stations, or even wind farms (Chen et al., 2023; Maggini et al., 2024). However, Nanopore sequencing typically has a higher error

rate than conventional sequencing platforms, such as Illumina, which requires different bioinformatics processing and more extensive error correction to avoid identification biases (Baloğlu et al., 2021; Egeter et al., 2022).

Other developments, such as the ability to capture different nucleic acids like eRNA, may improve ecological interpretability, as eRNA degrades much faster in the water column, thereby reducing spatial-temporal uncertainties (Jo, 2023). PCR-free approaches, such as metagenomics, can sequence all genetic material simultaneously, thereby potentially improving DNA abundance estimates and species identification (Bernatchez et al., 2024; Monchamp et al., 2022; Theissinger et al., 2023). In addition, metagenomics can provide information on the presence of functional genes, trophic interactions, and population profiles, but it remains costly and is limited by incomplete reference genomes (Serivichyaswat et al., 2025).

To summarise, while new sequencing platforms and tools hold great promise for improving molecular biodiversity assessment in North Sea biodiversity monitoring, their integration into the monitoring toolbox remains limited. It is therefore important to pay more attention to comparing the novel with conventional molecular biodiversity methods and more traditional non-invasive methods. This will unveil the extent to which current molecular metabarcoding methods provide meaningful contributions to routine monitoring frameworks. Thus, it needs to be evaluated whether molecular advances can complement and improve existing monitoring methods to study the biodiversity of a changing North Sea.

1.4 Aims of this thesis

The main aim of this thesis is to evaluate and verify molecular monitoring techniques to improve North Sea biodiversity, applying these methods to assess biodiversity in natural reefs, and providing more reliable monitoring tools for evidence-based ecosystem management and environmentally conscious industrial development. As previously mentioned, with activities such as offshore wind, fisheries, and restoration efforts likely to expand, and with the need to follow development against the background of climate change, there is a growing need for scalable, cost-effective, non-invasive monitoring tools that deliver high-resolution ecological data. This thesis focuses on molecular methods for biodiversity monitoring.

Three research objectives are formulated to address the overarching aim:

Objective 1: to validate more flexible, cost-effective, and simpler sequencing platforms and methodological workflows by comparing them with conventional approaches, supporting future harmonised and standardised monitoring frameworks.

Objective 2: to develop molecular monitoring approaches that enhance species-level resolution and provide improved spatial and temporal resolution, thereby advancing understanding of ecological patterns in North Sea ecosystems.

Objective 3: to apply these optimised tools to assess the biodiversity of natural reef systems in the North Sea and to complement detection patterns with conventional surveys to gain a comprehensive understanding of how reef density influences species and trait composition.

1.5 Outline of this thesis

The overall aim was achieved by developing the workflow and bioinformatics pipeline (Chapter 3) for Nanopore metabarcoding and comparing conventional Illumina MiSeq and Novaseq platforms (Chapter 2). Long-read molecular markers (Chapter 3) and eRNA metabarcoding (Chapter 4) were developed and applied to samples from natural and artificial reef systems (Chapters 3, 4, 6) to detect diversity and functional trait patterns in marine invertebrates, demonstrating the potential of metabarcoding for evidence-based monitoring of developments in the North Sea.

Objective 1, concerning the development and validation of a new sequencing method, is addressed in Chapters 2 and 3. In Chapter 2, the comparability of Nanopore metabarcoding of morphologically identified benthos samples was tested by comparing Nanopore metabarcoding, amplified with a COI primer pair, with Illumina MiSeq COI metabarcoding and a Novaseq shotgun approach. The results were compared with morphological findings to validate them. Additionally, in Chapter 3, a new bioinformatics workflow, DECONA, is introduced and assessed across several settings to enable the most accurate species-level assignment of error-prone nanopore reads. This chapter also details the software implemented and the pipeline's user-friendliness.

Objective 2, which investigates innovative metabarcoding methods, is covered in Chapters 3, 4, and 5. Chapter 3 outlines the development of

a primer targeting a 2kb segment within the 12S and 16S mitochondrial DNA regions and assesses its potential for eDNA-based assessments of fish and vertebrate diversity. The primer was initially evaluated in silico to determine its value for identifying species within closely related genera, compared to a commonly used 12S ~150 bp primer. It was then further tested by comparing 2kb and 12S results from a bulk sample and an aquarium eDNA sample with known species composition, as well as in situ eDNA samples from various locations in the North Sea. In Chapter 4, the eRNA fraction was metabarcoded using the widely used 12S primer and compared with eDNA and 2kb results from samples collected on a natural *M. modiolus* reef in Scottish Noss Head. The spatial and temporal resolution was examined by comparing diversity and community composition patterns between nucleic acid fractions and fragment lengths. Chapter 5 reviews and discusses the potential of new molecular tools to enhance marine monitoring in the North Sea, with particular focus on database availability, standardisation, and harmonisation.

Objective 3, is to apply the novel metabarcoding techniques to better understand the species composition natural and artificial reef systems, is addressed in Chapters 3, 4, and 6. In Chapter 3, results from 2kb and 12S metabarcoding were used to describe vertebrate diversity in a wind park, a sandy area, and the natural Stony Reef called the Borkum Reef grounds. Chapter 4 utilised 12S, 2kb, and eRNA metabarcoding to describe the diversity of an *M. modiolus* reef across different spatial and temporal scales. For Chapter 6, intensive sampling was carried out across the *M. modiolus* reef at Noss Head, Scotland, and the shellfish reef in the Voordelta, both within an MPA, at multiple locations with varying reef densities for eDNA analysis. 12S metabarcoding revealed community composition and functional traits of marine vertebrates across reef sites with different reef densities. The eDNA metabarcoding data were further complemented by BRUV findings from both locations.

Chapter 7 discusses all chapters in relation to their objectives and how they collectively achieve the primary goal. It also provides broader perspectives on how novel technology supports our understanding of the North Sea and outlines future steps for standardised monitoring.

Chapter 2

Advancing molecular macrobenthos biodiversity monitoring: a comparison between Oxford Nanopore and Illumina based metabarcoding and metagenomics

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ABSTRACT

DNA-based methods and developments of sequencing technologies are integral to macrobenthos biodiversity studies, and their implementation as standardized monitoring methods is approaching. Evaluating the efficacy and reliability of these technological developments is crucial for macrobenthos biodiversity assessments.

In this study, we compared three DNA-based techniques for assessing the diversity of bulk macrobenthos samples from the Belgian North Sea. Specifically, we compared amplicon sequencing using Illumina MiSeq and portable real-time sequencing of Oxford Nanopore versus shotgun sequencing using Illumina NovaSeq sequencing. The 313 bp mitochondrial COI metabarcoding fragment served as the target region for the metabarcoding analysis.

Our results indicate that Oxford Nanopore and MiSeq metabarcoding had similar performances in terms of alpha and beta diversity, revealing highly similar location-specific community compositions. The NovaSeq metagenomics method also resulted in similar alpha diversity, but slightly different community compositions compared to the metabarcoding approach. Despite these differences, location-specific community compositions were maintained across all platforms.

Notably, read counts from the NovaSeq metagenomic analysis showed the weakest correlation to size corrected morphological abundance and there were mismatches between morphological identification and all DNA based findings which are likely caused by a combination of factors such as primer efficiency and an incomplete reference database.

Our findings underscore the critical importance of database completeness prior to implementing DNA-based techniques as standardized monitoring method, especially for metagenomics. Nevertheless, our findings emphasize that Oxford Nanopore metabarcoding proves to be a viable alternative to the conventional Illumina MiSeq metabarcoding platform for macrobenthos biodiversity monitoring.

INTRODUCTION

The European Union established the Marine Strategy Framework Directive (MSFD, 2008/56/EC), alongside the European Water Framework Directive (WFD, 2000/60/EC) and the European network for protected areas for conserving the most valuable species and habitats (Natura 2000 network, Habitat Directive). These directives form the basis of ecosystem management and use a variety of monitoring-based assessments (e.g., Borja et al., 2010) that aim to inform (non-) governmental decisions on marine environmental health. However, taxonomy-based monitoring practices rely on great taxonomic expertise, are time-consuming and limited in taxonomic resolution (Danovaro et al., 2016; Pawlowski et al., 2018), particularly when identifying understudied taxonomic groups and species at different life stages (Ammon et al., 2018; Gleason et al., 2023). Consequently, DNA-based technological developments have gained particular interest for their potential in studying marine biodiversity (Bucklin et al., 2011; Cordier et al., 2019; Elbrecht et al., 2017; Lanzén et al., 2017; Leray & Knowlton, 2016). A reduction in labour time and the growing reference sequence libraries demonstrate DNA based methods to be a promising alternative for monitoring (Baird & Hajibabaei, 2012; DeSalle & Goldstein, 2019; Gostel & Kress, 2022; Leray & Knowlton, 2015). Different DNA-based monitoring studies have demonstrated correspondence with morphological findings (Derycke et al., 2021; Mauffrey et al., 2021). As a result, DNA-based methods are repeatedly proposed as a suitable routine biodiversity assessment strategy to inform policy (Aylagas et al., 2020; Goodwin et al., 2017; Hering et al., 2018; Hinz et al., 2022; Pawlowski et al., 2018; Thalinger et al., 2019).

Generally, DNA-based biodiversity assessments rely on DNA metabarcoding, which enables the identification of species from environmental samples by amplifying a short DNA fragment using universal primer pairs. This amplified DNA is then sequenced with next-generation sequencing platforms to identify the taxa found in samples (Taberlet et al., 2012). As biases can be introduced at each stage (L. M. van der Loos & Nijland, 2021), decisions need to be made on the sampling method and the appropriate use of preservation techniques (Gaither et al., 2011; Ransome et al., 2017), DNA extraction methods, using sufficient replicates (Van den Bulcke et al., 2021), and using appropriate primer pair(s) (Creer et al., 2016; Devloo-Delva et al., 2018; Leray & Knowlton, 2016). Bioinformatics pipelines are also crucial to take into consideration as the

pipeline choice, and the processing settings greatly influence the output (Antich et al., 2021).

Current developments in DNA-based methods have resulted in the availability of several different third-generation sequencing platforms, such as Illumina, Ion Torrent, Oxford Nanopore sequencing and Pacific Biosciences (Hu et al., 2021). These platforms provide exciting opportunities to study the environment in convenient ways that include obtaining abundance data (Klunder et al., 2022), epigenetic modifications (Zhao et al., 2023) and population genetics (Jahnke et al., 2022). For metabarcoding of gene fragments shorter than 500 bp, Illumina technologies are currently the standard platform because of its high accuracy (M. Meyer & Kircher, 2010). In comparison to Illumina MiSeq, the Oxford Nanopore sequencing platform measures an electrical current that is produced when the nucleotides of a sequence pass through a transmembrane nanopore, allowing for real-time sequencing and base calling (Bleidorn, 2016; Y. Wang et al., 2021). The advantages of Oxford Nanopore sequencing include lower costs, the sequencing of long fragments and its suitability for real-time in-field experiments (Krehenwinkel et al., 2019). However, the error rates of raw Oxford Nanopore sequences are currently higher (87-99% accuracy) compared to Illumina platforms, therefore different bioinformatics processing pipelines have been used to circumvent this problem (Baloğlu et al., 2021; Doorenspleet et al., 2025; Egeter et al., 2020). Short read Oxford Nanopore sequencing has previously been shown to be consistent with Illumina MiSeq in low-diversity samples (Egeter et al., 2020; van der Reis et al., 2023). However, comparisons of the sequencing platforms with high diversity samples were only performed using bacterial communities, and show a lack of several taxa (Heikema et al., 2020; Stevens et al., 2023). Thus, it remains unclear to what extent short read Oxford Nanopore sequencing is directly comparable to Illumina data.

Recently, metagenomics sequencing have gained interest as alternatives to metabarcoding for community analysis (Bernatchez et al., 2024; Theissinger et al., 2023). Shotgun sequencing can bypass some methodological disadvantages that are inherent to metabarcoding such as PCR (Zhou et al., 2013) and primer amplification bias (Leray & Knowlton, 2015), because DNA is directly processed for sequencing. Moreover, shotgun sequencing has been suggested to cover the full spectrum of biota in a sample and provide a correlation with morphological biodiversity studies (Monchamp et al., 2022). This method is seen as a viable contestant to metabarcoding methods to monitoring genetic diversity (Bista et al.,

2018; Lopez et al., 2022; Monchamp et al., 2022). However, metagenomics can be limited by i) the sequencing depth, which has become more cost-efficient with the advent of current sequencing platforms such as NovaSeq, and ii) the availability and quality state of reference sequences within widely used databases (Weigand et al., 2019), given that the shotgun sequencing process is non-selective. Thus, it is inconclusive whether shotgun sequencing is currently more useful for both diversity detection and relative abundance data for macrobenthos studies.

In this study, we compared different DNA-based methods: paired end Illumina MiSeq metabarcoding, Oxford Nanopore MinION metabarcoding of the 313 bp COI mitochondrial fragment and Illumina NovaSeq Metagenomics shotgun sequencing. We used bulk macrobenthos community samples collected from different soft-bottom habitats along the Belgium North Sea. By using different DNA-based methods and sequencing platforms for metabarcoding, we assessed the suitability of these methods for monitoring benthic community composition and diversity. For the metabarcoding methods, we used a curated COI North Sea invertebrate reference database and compared it to a metagenomics method using the complete NCBI-nt database. We hypothesized that the metabarcoding data from both sequencing platforms and the shotgun metagenomics method are generally robust and resemble the morphologically identified community both in alpha and beta diversity.

MATERIALS AND METHODS

Sample collection

Sampling was conducted at four locations in the Belgian North Sea that contained different macrobenthos communities with high, medium, and low diversity (see Breine et al., 2018) (Figure 1). The bulk samples have been previously used to optimize the metabarcoding protocols and to test the method reproducibility (Derycke et al., 2021; Van den Bulcke et al., 2021, 2023). Samples were collected from a coastal muddy fine-sand habitat with a high taxa diversity of sessile tube-forming organisms and high bioturbation (location 120 - *Abra alba* community). Samples were also collected from a medium sand habitat with a medium taxa diversity of mobile organisms (location 330 - *Nepthys cirrosa* community), a coarse sand habitat with high taxa diversity for sessile interstitial species (location 840 - *Hesionura elongata* community) and lastly, a muddy habitat with low taxa diversity (location ZVL - *Macoma balthica* community)

(Breine et al., 2018). A Van Veen grab (with a sampling surface area of 0.1m^2) was used to collect three biological replicates per location (A, B, C). On average, each sample consisted of 9.3 Liters of material. All sediment samples were sieved using a 1 mm sieve, and the remaining material (for example, shells and rocks) was fixed using absolute ethanol and stored at -20 °C prior to further processing.

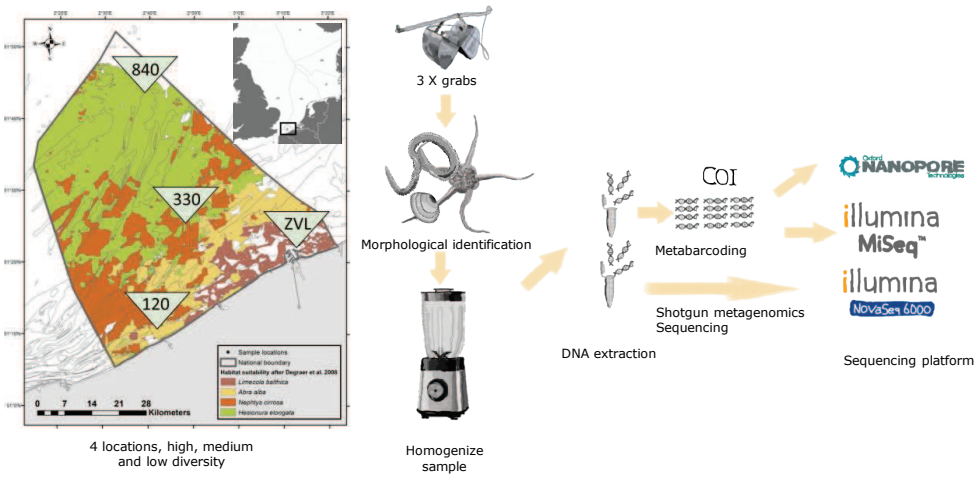


Figure 1: Experimental set-up. Graphical presentation of methods used in this study. Samples were taken in the Belgian part of the North Sea with known macrobenthos communities (see Breine et al., 2018 for details). The samples were taken in triplicates using a van Veen grab on 4 different locations with high medium and low biodiversity. The animals found in the samples were either morphologically identified or homogenized for DNA extraction. For both amplicon based and metagenomics, different subsamples were used for DNA extraction. Depending on DNA based method, either a COI 313 bp fragment was amplified (Nanopore or Illumina MiSeq) or DNA was directly sequenced with Illumina Novaseq shotgun sequencing for metagenomics.

Morphological identification

The morphological identification followed the protocols described by Derycke et al. (2021) and van den Bulcke et al. (2021; 2023). Organisms from one replicate per location (120 - B, 840 - C, 330 - C, ZVL - A) were identified to species level and juveniles to genus level, except for specimens belonging to Nemertea, Anthozoa and Oligochaeta, which were identified up to phylum, subphylum, and order level, respectively. The complete list of species identified in each location is available (Table S1). Species were recorded per individual hence no biomass information was available for this dataset. To correct for the lack of biomass data, the

count data were multiplied by the average size (from each size class). This was obtained to correlate morphology abundance data and the read count of each DNA based method for the identified species.

DNA extraction

For molecular comparison, all specimens isolated from each field replicate were retained to obtain a bulk sample. Bulk samples were homogenized with a blender or, if the sample was < 100 ml, with a mortar and pestle. Subsamples of 6 ml were taken and stored in Eppendorf tubes at -20 °C before processing in different institutes (Table S2). Samples used for Illumina MiSeq and Oxford Nanopore sequencing were extracted at Naturalis Biodiversity Centre (Leiden, The Netherlands) and used by Wageningen University and research (Wageningen, The Netherlands) for amplification and sequencing with Oxford Nanopore. Samples used for Illumina NovaSeq were processed at Nord University (Bodø, Norway). For every location, three subsamples of 2ml were used for DNA extraction. For location 840C, ZVLA and ZVLC, DNA extract from ILVO was used (Table S2, Oostende, Belgium) due to low recovery of sample material. All institutes (Nord, Naturalis and ILVO) used the same extraction protocol according to Van den Bulcke et al. (2023). In short, the Eppendorf tubes were centrifuged for 3 min at 10,000 RPM, and the supernatant was removed. Samples were incubated at 50 °C for 1 hr to evaporate the remaining ethanol. Three subsamples from each biological replicate (3X3) were incubated overnight at 56 °C in the power-beads tube of the DNeasy PowerSoil Kit (QIAGEN), supplemented with 10 µL of proteinase K (20 mg/ml).

The DNA extracted from each subsample was pooled and cleaned using the Wizard DNA clean-up system (Promega, USA) and eluted in 50 µL TE buffer. After processing, samples were stored at -20 °C before amplification or shotgun sequencing (Figure 1).

PCR amplification

For amplification, a 313 bp COI fragment was amplified using the forward mlCOIlintF primer (Leray et al., 2013) with the reverse jgHCO2198 primer (Geller et al., 2013) in combination with Oxford nanopore and Illumina MiSeq extension sequence (printed red in table 1). However, a modified version of the primers was used by replacing deoxy-inosines (I) with degenerated bases (N) (Table 1). Amplification was performed on each sample in triplicate. Each reaction contained 8.5 µL nuclease-free water, 12.5 µL 2x KAPA HiFi HotStart ReadyMix (Roche, USA), 0.75 µL (10 µM)

forward and 0.75 µL (10 µM) reverse primer and 2.5 µL of DNA template. For Oxford Nanopore sequencing, DNA template was diluted 10x prior to amplification. PCR conditions were 3 min at 95 °C, 35 cycles of 30 s at 98 °C, 30 s at 57 °C, 30 s at 72 °C and a final extension for 1 min at 72 °C. PCR replicates were pooled, and a clean-up was performed using a 2:1 mixture with AMPURE beads (Beckman Coulter Inc., USA) and >70% ethanol. Amplification was confirmed using gel electrophoresis (1% gel, ethidium bromide).

Table 1: Primers used for metabarcoding. Primers used for the Illumina MiSeq and Nanopore metabarcoding method. Sequences indicated in bold are the platform specific adapters to the primers.

	Oxford Nanopore	
Forward	miCOIintF: 5′- **TTTCTGTTGGTGCTGATATTGC**GGWACWGGWT-GAA CWGTWTAYCCYCC-3′	Leray et al. (2013)
reverse	jgHCO2198:5′-**ACTTGCCTGTCGCTCTATCTTC**TANACYTCNGGRTGN CCRA ARAAYCA-3′	Geller et al. (2013)
	Illumina Miseq	
Forward	miCOIintF: 5′-**TCGTCGGCAGCGTCAGATGTGTATAAGAGA-CAG**GGWACWGG WTGAACWGTWTAYCCYCC-3′	Leray et al. (2013)
reverse	jgHCO2198:5′-**GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG**TA NA CYTCNGGRTGNCCRAARAAYCA-3′	Geller et al. (2013)

Illumina MiSeq metabarcoding

Index PCR

For the index PCR, 5 µL nuclease-free water, 12.5 µL 2X KAPA HiFi Hot-Start ReadyMix (Roche, USA) and 2.5 µL of each index primer (Nextera XT primer 1 and 2) without modifications was used with 2.5 µL of initial pooled PCR product. The PCR program was 3 min at 95 °C, 8 cycles of 30 s at 95 °C, 30 s at 55 °C, 30 s at 72 °C and a final extension for 3 min at 72 °C. Amplification was confirmed using gel-electrophoresis (1% gel, ethidium bromide). The purified Index PCR products were equimolarly pooled and sequenced using the Illumina MiSeq 2X300bp platform (v2), with an addition of 20% of PhiX (sequenced by Baseclear BV.).

Bioinformatics of Illumina MiSeq reads.

After Illumina MiSeq sequencing, the quality of the demultiplexed reads was checked using *MultiQC* (Ewels et al., 2016), and primers were removed using *Trimmomatic* (Bolger et al., 2014). Amplicon sequence variants (ASV) were generated using the *DADA2* pipeline in the *Dada2* v1.17.0

package (Callahan et al., 2016) in R Studio v4.0.2 (R Core Team, 2020). Standard settings were used and an error rate of 3 mismatches was allowed. Reads with a quality score lower than 30 were removed. Unique paired-end reads were determined, merged, and filtered for chimeras for each sample with the removeBimeraDenovo function using the *Dada2* v1.17.0 package (Callahan et al., 2016). Taxonomy was assigned using the *assignTaxonomy* function in the *Dada2* package (Q. Wang et al., 2007) using the *Ribosomal Database Project* (RDP) (Q. Wang et al., 2007) with a minimum bootstrap confidence parameter of 80.

Reference database for metabarcoding methods

A morphologically curated database that contains 1992 COI sequences of 565 North Sea invertebrate species was used for taxonomic identification (dx.doi.org/10.5883/DS-GEANS1). This database was assembled from public and an in-house marine macrobenthos COI sequences that were obtained during multiple diversity monitoring campaigns throughout the North Sea (GEANS, 2021). During these campaigns, missing species were actively collected, vouchered and barcodes when possible.

Oxford Nanopore metabarcoding

Oxford Nanopore sequencing

The PCR barcoding kit 96 (EXP-PCB096) was used for the barcoding PCR, and the sequence library was prepared with the SQK-LSK114 kit (Oxford Nanopore Technologies, UK). Several adaptations deviated from the manufacturer's instructions: barcoding PCR was achieved in a total volume of 10 µL using 0.3 µL 10 µM PCR barcode primer pair and 10-50 ng amplicon. The following PCR program was used: initial denaturation at 95 °C for 3 min, 15 cycles of 95 °C for 10 s, 62 °C for 15 s, 65 °C for 90 s, followed by a final extension at 65 °C for 180 s.

The concentration of the barcoded PCR products was measured using the Qubit HS kit (Thermo Fisher Scientific, USA) on the non-purified products, after which barcoded PCR products were pooled in equimolar ratios. The pooled amplicon sequence library was cleaned twice using AMPURE beads (Beckman Coulter Inc., USA). The first clean-up step used 70% ethanol and the second used Short Fragment Buffer (SFB) to enrich for the target size fragments. After end prep and adapter ligation, the library was again washed with SFB during the final clean-up of the protocol. A total of 5 µL library containing 98.5ng DNA was loaded onto an R10.4.1

flow cell (Oxford Nanopore Technologies, UK) mounted on a Minion Mk1C device.

Oxford Nanopore Sequence read processing

Oxford Nanopore Sequence read processing was performed according to the post-processing protocol as described by Doorenspleet et al., (2023). Basecalling of the fast5 pass files was performed using *Guppy* (Version 6.5.7, Oxford Nanopore Technologies, UK) in super high accuracy (SUP) mode. The *Decona* pipeline was used (https://github.com/Saskia-Oosterbroek/decona) for trimming, clustering, and taxonomic assignment of the reads. Raw base-called reads were trimmed to between 250-400 bases for each sequence. A cluster similarity of 85% was set as the clustering threshold of the sequences. Medaka consensus sequences were generated from each cluster larger than 5 reads (Decona -f -q 10 -T 18 -l 300 -m 320 -c 0.85 -g "GGWACWGGWTGAACWGTWTAYCCYCC;max_error_rate=0.1;min_overlap=20... TANACYTCNGGRTGNCCRAARAAYCA;max_error_rate=0.1;min_overlap=19" -n 5 -r 0.99 -R 500 -k 6 -M).

Taxonomic assignment

The consensus sequences were classified using *BLASTn* (NCBI, version 2.11.0), prior to further processing, the classifications were manually checked on inconsistent classifications. For taxonomic identification, the Top 5 hits were considered for each consensus sequence. Of these, the best match was used for taxonomic assignment based on hits with the highest e-value. The best match was assigned to species level when there was a minimal alignment length of 250 nucleotides with <4 bp mismatches and >98% identity. The best match was assigned to genus level with >97% identity, Family level with >95% identity, >93% for Order level and >90% for Phylum level identification. The North Sea invertebrate species reference database was used for taxonomic identification (dx.doi.org/10.5883/DS-GEANS1). This database was the same as used for the Illumina MiSeq metabarcoding method, but not for the metagenomics method.

Read abundance correction

After taxonomic assignments, a tag correction was performed on the Oxford Nanopore data to correct the tag jumping that had occurred. Tag jumping had occurred when both forward and reverse barcode tags were on sequences that did not belong to that barcode. After troubleshooting, most of the contamination could be alleviated by removing 1% of the total read count of each species from each barcode. This correction is intended

to correct proportionally for the total read abundance of each taxon. Although no positive control sample was used for this study, PCR negative controls did not show any positive band nor higher DNA concentrations after barcode PCR. However, the raw data contained a low amount of reads of the most commonly occurring species in these. As the same species also occurred in the ZVL samples with low read abundance where we did not expect them based on the Illumina results, we decided to remove these reads by a minimum 1% read abundance correction. Therefore, we decided to remove these reads by a 1% read abundance correction and this appeared to reduce these observations.

First, the total read count was calculated for all species in all barcodes using the mutate() function in the *dplyr* v1.1.0 package. Second, 1% of the total read count was calculated and rounded to a whole number of reads. Finally, 1% of the total read count of each species was subtracted from each barcode.

NovaSeq shotgun sequencing

Preparations for sequencing

After DNA extraction and clean-up, libraries were directly prepared using NEBNext® Ultra™ II DNA Library Prep Kit for Illumina (New England Biolabs, USA). Samples were indexed using NEBNext® Multiplex Oligos for Illumina (New England Biolabs, USA). The final quality check was conducted using the Agilent Tapestation system using hds1000 screen tape (Agilent Technologies, USA). Samples were pooled in equimolar concentrations and sent to the Norwegian Sequencing Center in Oslo to be sequenced using the NovaSeq S4 quarter flow cell (2x150).

Sequence read processing

The NovaSeq reads were trimmed using cutadapt (--minimum-length=100, q=30), and merged with *PEAR v 1.7.2* (Zhang et al., 2014) using the default parameters. Reads were then assembled using idba_ud (Peng et al., 2012) using default parameters. Reads were mapped back to the contigs using BBMap (http://sourceforge.net/projects/bbmap/) with the parameter -minid set to 90. BLASTn was used to align the contigs with settings set at >97 % identity, e-value set at -10, and alignment length equal and over 100 bp. Taxonomic assignments to more than one phylum per query were removed and then the match with the highest bit-score was kept for the data analysis. Single alignments were discarded for the data analysis.

Reference databases for metagenomics method

To compare the performance of different databases, contigs were locally aligned to four sets of databases. One, the curated COI database for North Sea specific marine invertebrates (geans v4, dx.doi.org/10.5883/DS-GEANS1). Two, the metagenomics data were also aligned a manually curated NCBI-nt database (original complete NCBI-nt downloaded on 22.05.2022) that mainly contained marine eukaryotes. Three, the nuclear entries of the curated NCBI-nt database only, and four, the mitochondrial entries of the curated NCBI-nt database only. The complete NCBI-nt database including both nuclear and mitochondrial entries gave the most optimal result, and therefore this database was used for the comparison with the metabarcoding methods.

Data analysis

Data analysis was carried out in RStudio v4.2.2 (R Studio v4.2.2). A rarefaction curve (*Vegan* v2.6.4) was plotted to understand the effect of differential sequencing depths between samples. Each sample showed a flattening curve (Figure S1), which indicated that for each DNA-based method, an appropriate sequencing depth was achieved. Therefore, the data were not rarefied but transformed using a $\log_{10}$ transformation. All reads from the metabarcoding method (Illumina MiSeq and Oxford Nanopore) were compared on ASV /consensus sequence level to verify the initial assignment between sequences (>97% percentage identity). Phylum level comparisons were made to determine the differences between general taxonomic composition between the different DNA based methods (Miseq, Nanopore, NovaSeq). For visualisation of the database comparison with the Novaseq results, a Pie chart was used (ggplot2, v3.4.0). Species level richness, and Shannon diversity index (H, log 10 transformed) of the read counts were calculated using the diversity() function (*Vegan* v2.6.4) and visualized using boxplot (ggplot2, v3.4.0). Normality of the data was tested using Shapiro-Wilk for normal distribution and Q-Q plots. Based on these results a 2-way ANOVA using the aov() function (stats, v3.6.2) was carried out to determine the differences between sampling locations and the DNA-based method used and whether an interaction effect could be observed. For a pairwise comparison, a post/hoc analysis was performed using the Tuckey test (HSD) using the TukeyHSD() function (stats, v3.6.2). For beta diversity, non-metric multidimensional scaling ('bray') was performed on each dataset separately (Oxford Nanopore metabarcoding, Illumina MiSeq metabarcoding and Shotgun metagenomics sequencing) in combination with *betadisper* to check for homogeneity of variance. A PERMANOVA was used along with a pairwise post-hoc

analysis using a Bonferroni correction (both available in adonis2(), *Vegan* v2.6.4) to analyse which locations were significantly different from each other, within each dataset. A Spearman rank correlation was used (stats v3.6.2), to compare the size class corrected morphological abundance findings with each DNA-based method.

RESULTS

Read processing comparison between DNA-based methods

From the Illumina MiSeq metabarcoding samples, a total of 10,206,087 sequences were obtained with 8,692,409 used after processing. Of these, 8,330,994 reads were used as ASVs for taxonomy assignment (39 species). An overview is available of the sequencing output of each DNA-based method after sequencing and processing (Table S3). From the Oxford Nanopore metabarcoding dataset, 2,426,017 reads were basecalled. This is a lower sequencing depth than commonly used for Oxford Nanopore sequencing (van der Reis et al., 2023). However as indicated, the lower sequencing depth was not reflected in the rarefaction curves indicating that it was not informative to sequence any further. Of the basecalled reads, 1,841,385 remained after clustering and consensus building. A total of 1,191,853 of the remaining reads were used as consensus sequences for taxonomy (48 species). From the 3,060,417,120 shotgun reads obtained from the Illumina NovaSeq metagenomics run, 2,425,520,473 reads passed the quality values for direct taxonomic assignment of the reads. For the species assignment using the COI curated invertebrate database, 42,262 reads (39 species) could be assigned to species level whereas the complete NCBI database with marine eukaryotes was used, a total of 8,512,483 (36 species) reads could be assigned to species level, which represented 0.35% of the total filtered reads. For direct comparison of each species for each dataset see further.

Initial comparison and Novaseq metagenomics reference database choice.

Direct comparison to the processed metabarcoding sequences between Oxford Nanopore (consensus sequences) and Illumina MiSeq (ASVs), indicated that most of the sequences were similar between methods (Figure S2). In addition, phylum level assignment showed that both the number of phyla as well as the proportion of phyla is very similar between metabarcoding methods (Figure S3).

Of the reads that were used for taxonomic classification of the NovaSeq metagenomics dataset, 67.85% (5.775.720 reads) could be assigned to the nuclear fraction of the DNA whereas 7.08% (602.684 reads) was assigned to mitochondrial DNA (Figure 2a). Between the taxonomic assignment of the nuclear (Figure 2b) and mitochondrial fraction (Figure 2c), the nuclear fraction represents more Annelida whereas the mitochondrial fraction represents more Mollusca. In addition, the nuclear fraction represents more phyla that are present with <1%. Within the comparison of alignments with the curated COI marine invertebrate database, the amount of Annelida was 14.32 % lower with a total of 5.08% (figure 2e). The proportions between phyla obtained with the complete marine NCBI-nt data (Figure 2d), was used for the diversity comparison between methods, as this covered the most diversity. However, the proportions between phyla obtained through NovaSeq were very dissimilar to the proportions observed in the metabarcoding methods (Figure S3).

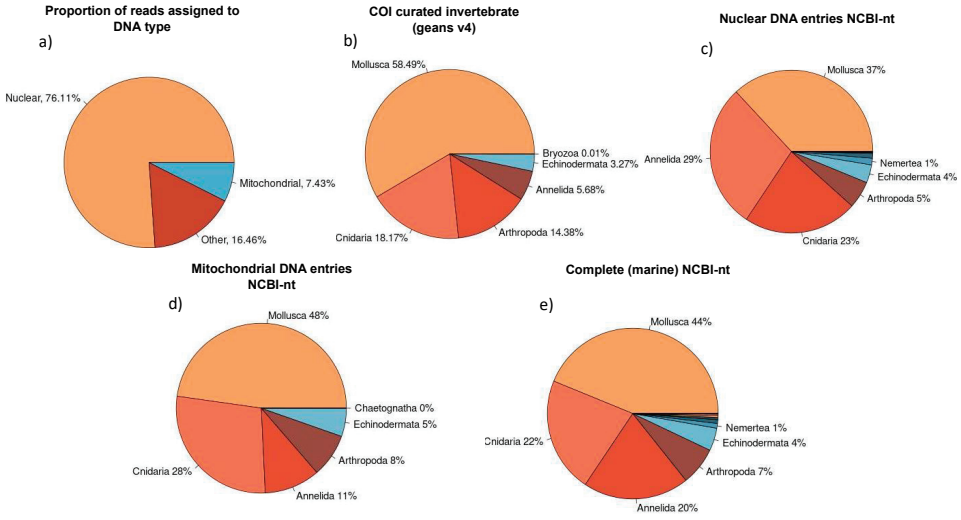


Figure 2: Proportion phyla found with each database using NovaSeq metagenomics. Pie charts showing (A) the proportions of types of DNA within the Novaseq metagenomics method. (B) Pie chart showing the phyla found using Illumina Shotgun metagenomics in combination with the COI curated marine invertebrate database, (C) the marine Nuclear DNA NCBI-nt database (D) the marine mitochondrial NCBI-nt database and (E) the complete marine NCBI-nt database.

Alpha diversity obtained with the three DNA-based methods

A two-way ANOVA showed a significant interaction effect between sequencing techniques (Illumina MiSeq, Nanopore, NovaSeq), and location (120, 330, 840 and ZVL) for species richness ($F = 4.34$, $p < 0.01$). A

main effect was also observed for location (F = 98.79 p < 0.01) and no significant difference was found between methods (p = 0.23). Post-hoc analysis using a Tuckey's HSD test showed a significant difference between location 120 and all other locations (Table S4), where 120 had the highest richness (Figure 3a). A significant difference was also observed between ZVL and all other locations (Table S4), where ZVL had the lowest richness. No significant difference was found between location 330 and 840 (p = 0.97). As for the interaction effect, the NovaSeq showed no significant different effect between ZVL and location 330 and 840. A two-way ANOVA showed a significant interaction of Shannon indices between sequencing techniques and locations (F = 7.67, p < 0.01, Table S4). The main effects were only significant for the factor location (F = 104.49, p < 0.01, Table S4) and for the factor method (F = 7.31, p = <0.05). Post-hoc analysis using a Tuckey's HSD test showed significant differences in Shannon indices between location 120, and all other locations (Table S5), where 120 had the highest Shannon index (Figure 3b). With the exception of the NovaSeq analysis, a significant difference was also found between ZVL and all other locations (Table S4), where ZVL had the lowest Shannon index (Figure 3b). Overall, these results highlight that all sequencing techniques similarly observe alpha diversity between locations, except for the richness and Shannon of the Novaseq shotgun metagenomics in location ZVL as more species were observed than the metabarcoding approaches. Shannon indices were also higher for NovaSeq for the low diversity location ZVL.

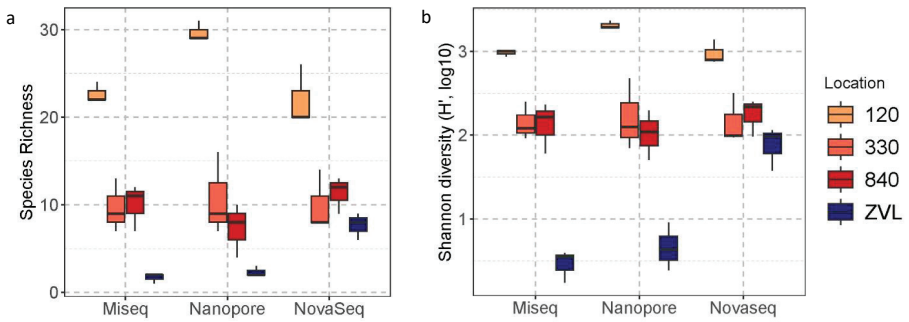


Figure 3: Boxplots of alpha diversities between locations of each DNA based method. The color represents the different location 120, 330, 840 and ZVL. The facets represent each DNA-based method, Amplicon, Illumina MiSeq and Nanopore. Panel (A) represents the number of species (species richness) and panel (B) represents the Shannon diversity for each location and DNA-based method.

Beta diversity obtained from the three DNA-based methods

The PERMANOVA demonstrated significant differences in macrobenthic community compositions between locations ($F = 16.05$, $p < 0.01$) and between techniques used ($F = 3.25$, $p < 0.01$) (Table S5, Figure 4). In addition, a significant interaction effect was observed between location and the DNA-based approach used ($F = 2.48$, $p < 0.01$), indicating that benthic community composition and location depend on the DNA-based method used and the other way around (Table S5). Post-hoc pairwise PERMANOVA tests showed a significant difference in community composition between all locations ($p < 0.01$). Surprisingly, post-hoc analysis showed no significant difference in community composition between either sequencing technique. The NMDS plot showed a clear clustering for each location, except for locations 330 and 840 (Figure 4). The plot also illustrates that location was responsible for the biggest contrasts in community composition, indicating that the location explained most of the variation between community compositions. Similarly, the NMDS plot also indicated that NovaSeq has a different community composition in some locations, particularly within location ZVL. An interaction effect was also observed, indicating that the effect of sequencing platform choice on species composition depends on the community present. Sequencing platforms choice did not affect the species composition for Illumina MiSeq and Oxford Nanopore metabarcoding, as these approaches clustered together within each location.

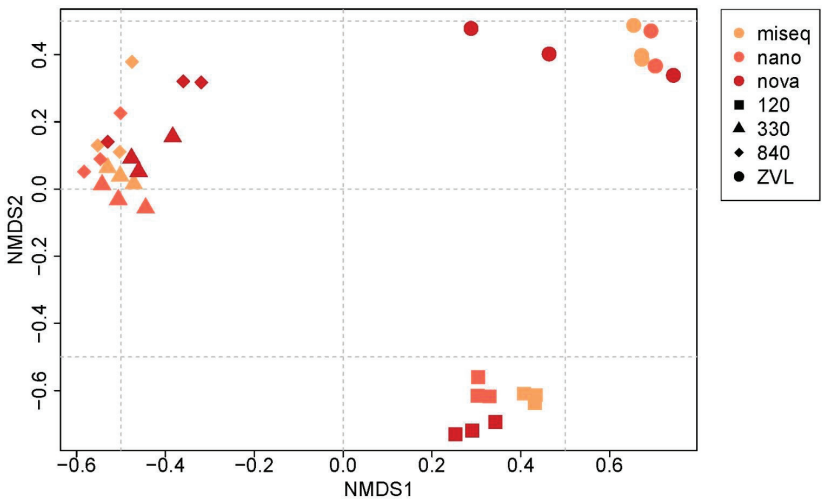
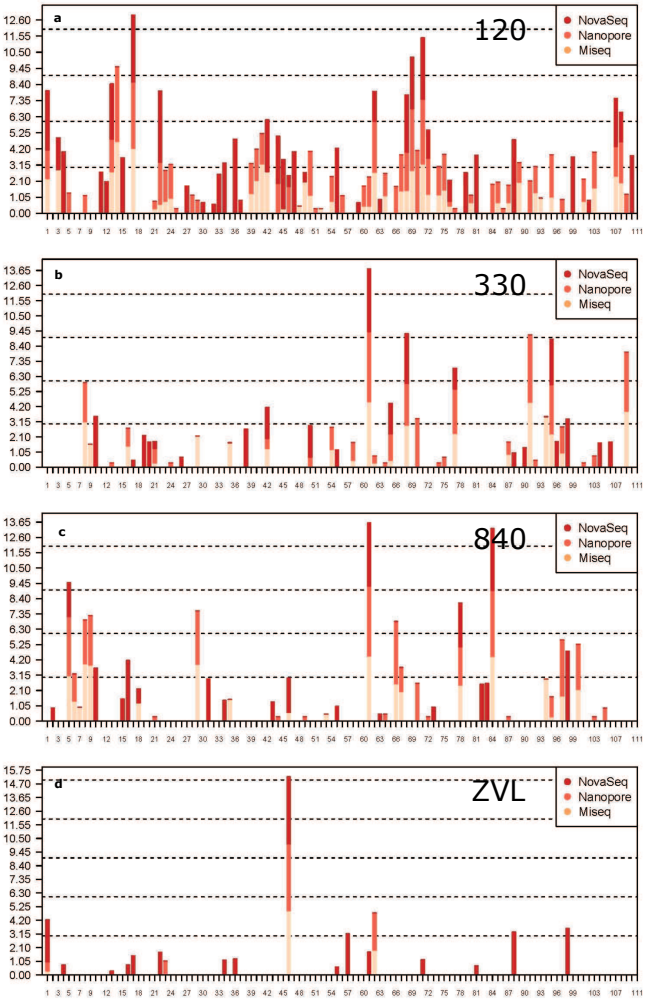


Figure 4: NMDS ordination plot (Bray–Curtis dissimilarity) of similarities of communities between samples and DNA based method. Colors indicate the different DNA based methods whereas the shapes indicate the different locations on species level (log10 transformation).

Comparison of DNA-based methods for assessing location-specific species composition

All three DNA-based methods (Figure 5) shared 25 species and an additional 29 species were shared between just the metabarcoding methods (Figure 5, S4). At location 120, 23 species were shared between the two metabarcoding methods whereas only 12 species were shared between all three DNA-based methods (Figure 5, S4). The Oxford Nanopore metabarcoding data contained 7 unique species for all locations (Figure 5b, Figure S4). Interestingly, NovaSeq contained 38 unique species for all locations, and most of these unique species were found in all locations (Figure 5b, Figure S4). Several species such as, *Crepidula fornicata*, *Eumida mackiei* and *Magelona mirabilis*, occurred at location 330 using the Oxford Nanopore dataset but with less reads than in location 120 (Figure 5b). However, these species were only present in location 120 using the MiSeq dataset. Nevertheless, all DNA-based methods detected unique species with each method.



1) Abra alba
2) Acraspedanthus ferax
3) Acrocnida brachiata
4) Actinia tenebrosa
5) Anapagurus laevis
6) Aonides paucibranchiata
7) Asbjornsenia pygmaea
8) Bathyporeia elegans
9) Bathyporeia guilliamsoniana
10) Bathyporeia sp.
11) Cirriformia sp.
12) Cirriformia tentaculata
13) Crepidula fornicata
14) Cylista troglodytes
15) Diadumene lineata
16) Diogenes pugilator
17) Echinocardium cordatum
18) Echinocyamus pusillus
19) Ectopleura dumortierii
20) Ectopleura larynx
21) Electra pilosa
22) Ensis leei
23) Eucheilota maculata
24) Eumida mackiei
25) Eunereis longissima
26) Eutima gegenbauri
27) Exaiptasia pallida
28) Fabulina fabula
29) Gastrosaccus spinifer
30) Glycera alba
31) Glycera lapidum
32) Glycera sp.
33) Glycera tridactyla
34) Haloclava producta
35) Hesionura elongata
36) Hormathia digitata
37) Hubrechtella juliae
38) Jesogammarus spinopalpus
39) Lagis koreni
40) Lanice conchilega
41) Liocarcinus holsatus
42) Liocarcinus marmoreus
43) Liponema brevicornis
44) Loimia ramzega
45) Lutraria lutraria
46) Macoma balthica
47) Macoma calcarea
48) Magallana gigas
49) Magelona johnstoni
50) Magelona mirabilis
51) Malmgrenia lunulata
52) Malmgrenia sp.
53) Megaluropus agilis
54) Membranipora membranacea
55) Metridium senile
56) Microprotopus maculatus
57) Moerella iridescens
58) Monocorophium acherusicum
59) Mytilus sp.
60) Nephtys assimilis
61) Nephtys cirrosa
62) Nephtys hombergii
63) Nephtys sp.
64) Notomastus latericeus
65) Nototropis swammerdamei
66) Ophelia borealis
67) Ophiothrix fragilis
68) Ophiura albida
69) Ophiura ophiura
70) Other
71) Owenia fusiformis
72) Pagurus bernhardus
73) Paracalanus parvus
74) Pariambus typicus
75) Pholoe baltica
76) Phyllodoce mucosa
77) Pinnotheres pisum
78) Pisidia longicornis
79) Pocillopora damicornis
80) Poecilochaetus serpens
81) Polybius henslowii
82) Polygordius appendiculatus
83) Pomacea canaliculata
84) Processa modica
85) Psammechinus miliaris
86) Pseudocuma longicorne
87) Sabellaria spinulosa
88) Sagartia ornata
89) Sagartiogeton sp.
90) Sagitta setosa
91) Scolelepis bonnieri
92) Scoloplos armiger
93) Spio decorata
94) Spio goniocephala
95) Spiophanes bombyx
96) Spiophanes cf.
97) Spisula elliptica
98) Spisula solida
99) Sthenelais boa
100) Streptodonta pterochaeta
101) Streptosyllis websteri
102) Strongylocentrotus purpuratus
103) Tellimya ferruginosa
104) Temora longicornis
105) Thia scutellata
106) Ectopleura sp.
107) Tritia reticulata
108) Tubificoides diazi
109) Urothoe brevicornis
110) Venerupis corrugata

Figure 5: Relative read abundance of each species found between locations and DNA-based method. Bar plot showing the relative read abundance (log_{10}) of all species found. Colors represent the different DNA-based methods. (A) The relative read abundance of location 120, (B) the relative read abundance of location 330, (C) the relative read abundance of location 840 and (D) the relative read abundance of location ZVL.

Comparison of species presence and abundance between DNA-based methods and morphology

For the morphological identification, one biological replicate was available (120B, 330C, 840C and ZVLA) for each location. This resulted in the identification of 56 species. A total of 39, 13, 10 and three species were identified at locations 120, 330, 840 and ZVL, respectively (Table S1). Of the 56 species identified, 22 species were identified using morphology only (Figure 6a, table S7). Of these 22 species, 13 did not have a reference sequence available in the chosen reference database (Table S7, coloured in red). In addition, 9 of the species that did not have a reference sequence available belonged to the phylum Annelida (Table S7), which are known to have low primer efficiency and are therefore harder to include in COI reference databases (Carr et al., 2011). Of the 32 species that were identified using both DNA-based methods and morphology, 12 species were identified using all three DNA-based methods (Figure 6a), 11 species were identified using the metabarcoding-based methods only (Figure 6a). Lastly, eight species that were identified morphologically were identified only with the NovaSeq metagenomics method whereas an additional three (Nanopore) and two (MiSeq) were unique for the metabarcoding method. (Figure 6a).

Three species were identified with all three DNA-based methods (Figure 6a), that were not identified with morphology and an additional eight were identified using only the metabarcoding methods. These eight species included *Scolelepois bonnieri* and *Cylista troglodytes* which represented a substantial amount of the identified reads (Table S6). This indicated that the DNA-based methods can identify species that are missed using morphological identification. Oxford Nanopore identified an additional nine species that were unique (Figure 6a) but found as singletons (Table S6).

A Spearman rank correlation between morphological size class corrected counts and relative read abundance between each DNA-based method across all locations showed the highest positive correlation with both

the metabarcoding methods (Sp = 0.237 (MiSeq), Sp = 0.27 (Nanopore) (Figure 6b, c, d). However, the metagenomics method only showed a weak correlation (Sp =0.097) with the size class corrected morphological counts (Figure 6d).

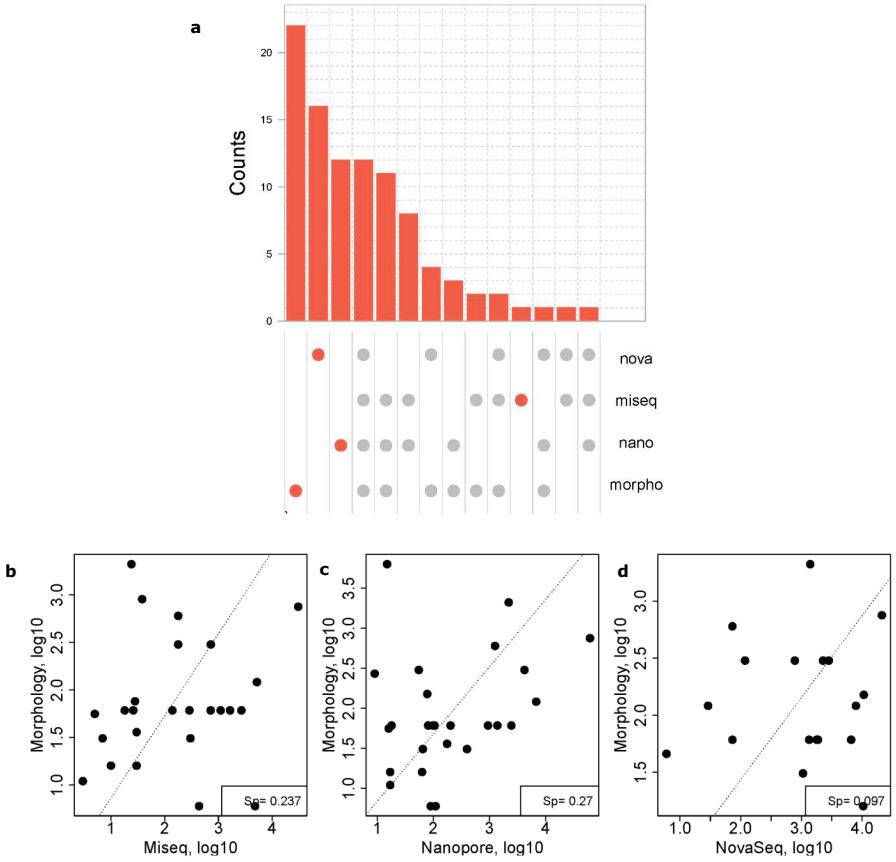


Figure 6: Comparison of species overlap and relative abundance between morphology and DNA-based methods. (A) UpSet plot showing the overlapping and unique species found using each DNA-based method in all the morphologically identified samples. Where the orange dots refer to unique species per method, and the gray dots shared among the respective methods. A Spearman correlation of the overlapping species abundance between morphology and (B) Illumina MiSeq metabarcoding (C) Oxford Nanopore metabarcoding and (D) NovaSeq shotgun metagenomics.

DISCUSSION

We compared three different DNA-based methods: two amplicon-based metabarcoding approaches (MiSeq, Nanopore) and a shotgun metagenomics approach (NovaSeq). We assessed the robustness between techniques in terms of alpha and beta diversity to understand the suitability for macrobenthos monitoring. We have demonstrated that the two metabarcoding methods showed a similar diversity richness and Shannon as well as location-specific species compositions. The NovaSeq metagenomics method also showed similar alpha diversities, but the community composition was slightly different, mostly due to taxonomic assignments that were uniquely found with this method. Interestingly, 22/56 species found in the morphological dataset were not identified with molecular techniques and shows that more than half of the species were identified. Nevertheless, all methods showed that most species were shared within each location.

Macrobenthos communities are highly similar using metabarcoding despite using different sequencing platforms, platform specific considerations remain

No difference was found between alpha and beta diversity when comparing a standardized lab protocol with Illumina MiSeq sequencing (Van den Bulcke et al., 2023) to Oxford Nanopore metabarcoding. Furthermore, both methods identified similar species composition and community structure at each location and generally, shared similar ASVs/consensus sequences and proportions of phyla. Therefore, this study clearly demonstrated that Oxford Nanopore and Illumina MiSeq metabarcoding are equally suited for monitoring macrobenthos biodiversity. This is in line with recent comparisons (Chang et al., 2023; van der Reis et al., 2023; Voorhuijzen-Harink et al., 2019) showing that the Oxford Nanopore datasets are overall comparable with Illumina MiSeq results. This study demonstrates that, even for high-diversity samples, both sequencing platforms perform comparably in terms of macrobenthos diversity assessments, however platform specific considerations remain, including bioinformatics pipelines and sequencing error rates, that may impact species detection and read count estimations. Oxford Nanopore sequencing identified some species that were uniquely present in this dataset. These exclusive taxa were found mostly in very low relative read abundance and did not influence the alpha and beta diversity. These detections could be explained as an effect of the stochastic nature of PCR that is observed in each dataset (L. M. van der Loos & Nijland, 2021). Indeed,

previously reported assessments of reproducibility of laboratories showed minor variations between detected species (Van den Bulcke et al., 2021).

Another potential explanation for these differences is that the Oxford Nanopore chemistry and its protocols might be more prone to 'barcode leakage' (Framst et al., 2024). The Oxford Nanopore dataset contained some species with only several reads in one of the replicates, while in another location these species were abundant as is the case for example *Crepidula fornicata* and *Eumidia mackei*. Even though a simple correction of barcode leakage was used for this study (as presented in the materials and methods), it is possible that tag jumping, or barcode leakage was still a problem for this dataset. Although this could not be confirmed as no positive control was considered, several independent projects within the same lab that used the same barcoding protocol had similar issues. After routine testing, this problem was found to be related to the temperature at which barcoded samples were pooled for the Oxford Nanopore sequencing library. Barcode leakage problems have been reported often in metabarcoding studies (Beentjes et al., 2019; L. M. van der Loos & Nijland, 2021). Therefore, it is important to consider protocols that minimize the possibility of barcode leakage. This includes using negative and positive controls at each stage, minimizing the handling and amplification of tagged products, or correcting for a crossover of tags between samples (Beentjes et al., 2019). Similar to other platforms, Oxford Nanopore uses two cycles of PCR, one for the amplification of the region of interest and a second PCR for the barcode attachment using Oxford Nanopore-specific protocols and kits for barcoding. The protocol used for this study could be optimized in such a way that individual samples are amplified with already tagged initial barcodes to circumvent the second PCR step (Srivathsan et al., 2021) or by using amplicon-free barcode kits (Toxqui Rodríguez et al., 2023; van der Reis et al., 2023).

The bioinformatics pipeline used in this study could also be responsible for the small differences observed between the metabarcoding results. The 10.4.1 flow cells were used, which greatly improved the quality of the metabarcoding data (Zhang et al., 2023). Nevertheless, clustering and consensus building of the data with bioinformatics processing might also result in overlooking some elements of the biodiversity (Brandt et al., 2021). Therefore, the reads that were not included in a cluster for consensus building were also considered when they met the threshold for taxonomy assignment. This inclusion may have led to the detection of

additional species within the individual samples, although this is unlikely since the singletons did not contain any additional species (Table S8).

Despite these minor differences between metabarcoding methods, this did not lead to significant differences in commonly used biodiversity indices. Therefore, this study suggests that Oxford Nanopore and Illumina MiSeq metabarcoding are highly similar for macrobenthos monitoring. However, platform specific challenges need to be considered, as Illumina generally provides high-accuracy short read data effective for fine scale taxa identification. In contrast, Nanopore offers sequencing real-time and long- read reads albeit typically with a higher error rate resulting in more intensive error correction during downstream analysis.

NovaSeq Shotgun sequencing identified the most method specific species.

The primer and PCR-free shotgun metagenomics method yielded similar alpha diversities compared to the metabarcoding methods but resulted in a slightly different community composition between methods within location. These different community compositions between methods were mostly due to additional species that were only found using the metagenomics method. Nevertheless, most species that were detected by NovaSeq metagenomics were also detected by the two metabarcoding methods suggesting that either method can describe the general community equally well.

This study, therefore, reflects current environmental metagenomics studies that show equal or higher levels of biodiversity compared to amplicon-based approaches (Bista et al., 2018; Garlapati et al., 2019; Monchamp et al., 2022; Paula et al., 2022). However, these studies, were not targeting a certain taxonomic group and therefore, reflected a wider spectrum of biota. In contrast, this study aimed to test whether metagenomics is suitable for specifically species/level macrobenthos biodiversity monitoring and therefore the data presented are not directly comparable to other metagenomics studies.

The highest number of uniquely found species was found with the metagenomics method, which was especially apparent in the low diversity samples (ZVL). The most straightforward explanation would be contamination between the high and low diversity samples, as these species were not found in the morphological analysis. Although molecular methods are always prone to cross contaminations in the lab (L. M.

van der Loos & Nijland, 2021), it is in this case less likely since part of the extractions used in this study for ZVL previously were also used in other studies (Van den Bulcke et al., 2023) and did not experience similar problems.

Alternative explanations for these findings are 1) that misidentification could have occurred due to the use of a database (NCBI) with mislabelled references (Sinniger et al., 2016) or 2) false positive detection of species that are genetically similar but do not occur in the North Sea as is the case for *Actinia tenebrosa* (Ayre, 1985). Furthermore, 3) the species is present but were not picked up with the metabarcoding method because these species did not have a curated COI sequence. As most of the additionally found species with the metagenomics approach belonged to cnidarians, it is possible that during morphological identifications only part of the animal was present in the initial sample and was therefore also never recorded for the morphological analysis (Lobo et al., 2017).

Although the complete NCBI reference database with relevant species was used, still only 0.35% of the metagenomics reads were assigned to a species. Reference databases that contain environmental sequences are mainly focused on genetic regions that are popular for metabarcoding (Weigand et al., 2019) and references of full (mito)genomes are still in their infancy (Blasiak et al., 2020; Leray et al., 2022), it is not surprising that such a low percentage of reads are assigned. Thus, the present findings align with previous research that suggest that shotgun sequencing metagenomics is hardly feasible for environmental studies (Ficetola & Taberlet, 2023; Quince et al., 2017). These results also indicate the necessity to improve reference databases, particularly for full (mito)genomes of macrobenthos, as the limited availability of references largely contributed to the low number of reads that are assigned.

Similarly, there was a weak correlation between species that were identified with the metagenomics approach and the morphologically identified species when correcting for size class. Unfortunately, no biomass data was obtained from the morphologically identified samples, which would be a more appropriate representation of the actual abundance. Nevertheless, a better correlation was found with the metabarcoding data, which is in contrast with findings that suggest a better correlation between found metagenomics reads and biomass (Bista et al., 2018). Since in the study by Bista et. al., the complete mitogenomes of all species in the mock community was available, the lack of correlations in this study

further suggests that the metagenomics method is presently not feasible for reliable DNA-based monitoring of macrobenthos biodiversity. In the future, focussing on reference databases and especially databases that contain complete (mito)genomes will greatly improve detection rates and improve DNA-based monitoring.

Mismatches between morphology and DNA based methods not only linked to reference sequence availability

As typical for metabarcoding studies, several species were found with metabarcoding that were not reported during the morphological analysis. This is not surprising as these species are either missed during morphological sorting or can only be identified to a higher taxonomic level (Lobo et al., 2017; Robinson et al., 2022). For this study this was apparent for the Anthozoa *Cylista* that was found with high read count (>13000 reads), whose tissue becomes hard or impossible to recognize when not intact.

Most taxa were detected using both morphology and a DNA-based technique, but there were 22 species that were not identified using any DNA-based method. This was especially apparent in the high diversity samples. Of these, 13 species could not be identified due to incompleteness of the curated database, which especially in relation to marine invertebrates, has often been reported as a limiting factor (Aylagas et al., 2016; Günther et al., 2018; Steyaert et al., 2020; Willassen et al., 2022). Several efforts have been made to improve (Lavrador et al., 2023) and increase the coverage of these databases (Leray et al., 2018; Radulovici et al., 2021). Approximately 22-43% of all European marine species have reference sequences available in the widely used reference database BOLD (Weigand et al., 2019). Multiple studies in the North Sea have emphasized the importance of enhancing these databases (Christodoulou et al., 2021), indicating a need for sustained long-term efforts. To improve the reference database used here, much effort has been put in vouchering as many species as possible by collaborating internationally with molecular scientists (https://northsearegion.eu/geans/about/). However, after morphological sorting of samples of this study, samples were preserved in formaldehyde which is known to hamper DNA extraction from these specimens therefore a one-on-one comparison was not possible. In addition, of the 13 unidentified species due to incompleteness of the reference database, 7 could be found in the COI NCBI database (manual search, 2025), suggesting that the curated database may be less complete in terms of species presence, but was on the other hand referenced with

a voucher species and could therefore be confirmed as the correct identification.

However, as 9 species did have the potential to be picked up with the used reference database, more factors should explain mismatches between methods. One explanation is the procedure in the field as small differences may occur in species composition between the grabs. 12 out of the 22 species that were not identified with any DNA based method, had an abundance of only 1 specimen in the morphologically assigned grab sample. Although others with only 1 morphological assignment were identified (see table S6), 4 species that were not found in the comparison, (*Aonide paucibranciata, Nototrips Swammerdamei, Phyllodoce mucosa* and *Streptodonta pterochaeta*) did occur in other replicas of at least one of the DNA based methods. This suggesting that small differences between grabs explain the mismatch to a certain extend.

During lab procedures biases can also occur, for example primer efficiency have also been often reported to influence metabarcoding results (L. M. van der Loos & Nijland, 2021). Of the 13 species that were missing in the reference database, 9 species belonged to Annelida. Annelida, and especially certain Polychaeta, are known to be difficult to sequence using the conventional markers because this group has high variation within the COI region and therefore has lower primer binding efficiency (Carr et al., 2011). This have caused the COI reference databases to be biased towards a lower representation of Annelida. Contrariwise, as the primer efficiency is lower for Annelida, it is likely that the chosen COI primer is not representing the taxonomic group well. This is emphasized by the fact that an additional 5 annelids were not detected with a DNA based method while they were represented in the reference database. Using multiple markers that are more specific to certain taxonomic groups might therefore greatly improve the capability to detect species from metabarcoding methods. However, consequently this may increase laboratory time and costs (Cordier et al., 2019; Gielings et al., 2021; A. Meyer et al., 2021). Metagenomic methods can also improve the detection of annelids as this technique doesn't rely on the amplification of specific regions. Nevertheless, the metagenomics method in this study did not result in the retrieval of more annelids.

Other factors such as the taxonomic assignment of the species using the algorithms of RDP or selecting the best scoring match per query can also be of influence as the size and training set of especially RDP influences

the sensitivity of detecting certain species (Ritari et al., 2015). Finally, the taxonomic assignment of the species can be wrong. In this case however, this is less likely as this survey is conducted by experts and samples were quality controlled (see Derycke et al., 2021) suggesting that this had little influence on the results.

In conclusion, although DNA-based methods missed 22 species compared to morphological analysis, the DNA-based methods detected additional species that otherwise remained undetected. This is in line with previous reports, also confirming that DNA-based methods and morphology should remain complementary (Cahill et al., 2018; Kelly et al., 2017; A. Meyer et al., 2021). However, improvements to the databases, the use of multiple replicates and the choice of multi-marker approach for specific taxonomic groups might further improve detection sensitivity and at some point, may surpass morphological identification.

CONCLUSIONS

In recent years, there has been an increased interest in implementing DNA-based tools into routine biodiversity monitoring practices. To achieve this, standardized protocols are necessary to ensure reproducibility and data robustness across studies and regions, particularly as sequencing technology is evolving rapidly (Van den Bulcke et al., 2023).

In this study, we showed that similar alpha and beta diversity patterns were found regardless of the metabarcoding platform used. Thus, Illumina MiSeq and Oxford Nanopore sequencing results are, at this stage, highly similar and can both be used to monitor macrobenthos biodiversity, although platform specific challenges remain. In addition, NovaSeq metagenomics has the potential for environmental monitoring and showed similar communities. However, metagenomics at this stage does not surpass metabarcoding methods, as it did not improve representation of abundance data, nor did it identify more species that are not efficiently amplified by the COI primer. Incomplete reference databases still hamper detection of metagenomics sequences but mismatches between morphological and DNA based findings are also influenced by fieldwork choices, primer biases and bioinformatics choices. These findings demonstrate that portable, real-time Oxford Nanopore sequencing is ready to be integrated for standard monitoring practices and emphasize the importance

of improving sequence reference databases to implement and enhance metagenomics methods for robust and harmonized monitoring practices.

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AUTHOR CONTRIBUTIONS

Karlijn Doorenspleet: Laboratory work, analysis, writing and reviewing with emphasis on writing the draft. Amalia Mailli: Laboratory work, analysis, writing and reviewing with emphasis on visualization of the results. Annelies de Backer Concept: Writing and reviewing, project administration, funding acquisition. Sofie Derycke concept, methodology writing and reviewing, project administration, funding acquisition. Berry van den Hoorn: writing and reviewing. Kevin Beentjes: laboratory work, writing and reviewing. Henning Reis: Writing, reviewing and supervision. Albertinka Murk: Supervision, writing and reviewing. Reindert Nijland: Concept, supervision, project administration writing and reviewing.

DATA AVAILABILITY

The raw data as well as the processed data and the used scripts including descriptive metadata are available in the Marine data Archieve under https://doi.org/10.14284/682 (MiSeq), https://doi.org/10.14284/683 (Nanopore) and https://doi.org/10.14284/684 (NovaSeq). Custom R scripts used for the Data Analysis can be accessed at: https://github.com/amalia03/GEANS_Platform_Comparison_Scripts/blob/main/tax_geans.R.

The data generated from the metabarcoding approach with Illumina MiSeq, as well as the morphological identification have been previously used for the publication by van der Bulke et al. 2023. The data has been used to compare datasets generated from the same samples with the same protocol in different labs. This was to show whether the methods were robust between institutes. In our approach however, we use the dataset of one lab only and the morphology data to compare this with other DNA based methods and sequencing platforms and therefore serves another purpose.

SUPPLEMENTARY MATERIAL

Supplementary figures

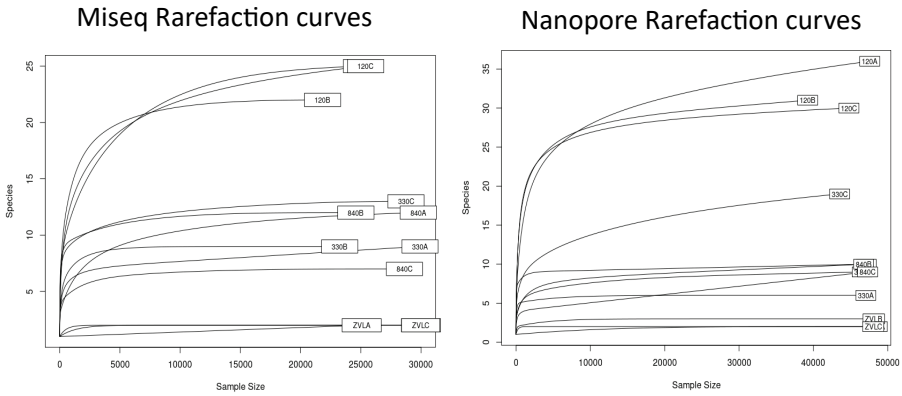
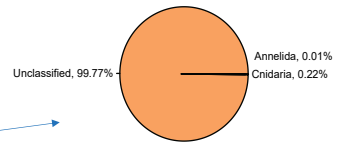


Figure S1: Rarefaction curves of the metabarcoding methods (Illumina MiSeq, Oxford Nanopore) of each location

a

Total percentage Nanopore consensus sequences matching MiSeq ASVs (>97% identity)	98,86%
From the matching ASVs, the Percentage of differently assigned taxonomies for the nanopore dataset	1.1%



b

Total percentage MiSeq ASV matching Nanopore consensus sequences (>97% identity)	98.79%
From the matching ASVs, the Percentage of differently assigned taxonomy assignments for the MiSeq dataset	1.21%

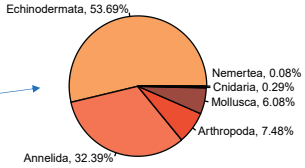


Figure S2: ASV level comparison between metabarcoding methods. Comparison of the ASVs (MiSeq) and consensus sequences (Nanopore) that match on sequence level (97% identity). Table in (A) represents the percentage of matching Nanopore consensus sequences with MiSEq ASVs, and from those matching sequences the percentage of differently assigned taxonomies. The pie chart represents what these differently assigned taxonomies are for the nanopore dataset. Table in (B) represents the percentage of matching Illumina MiSeq ASVs with Nanopore consensus sequences, and from those matching sequences the percentage of differently assigned taxonomies. The pie chart represents what these differently assigned taxonomies are for the MiSeq dataset

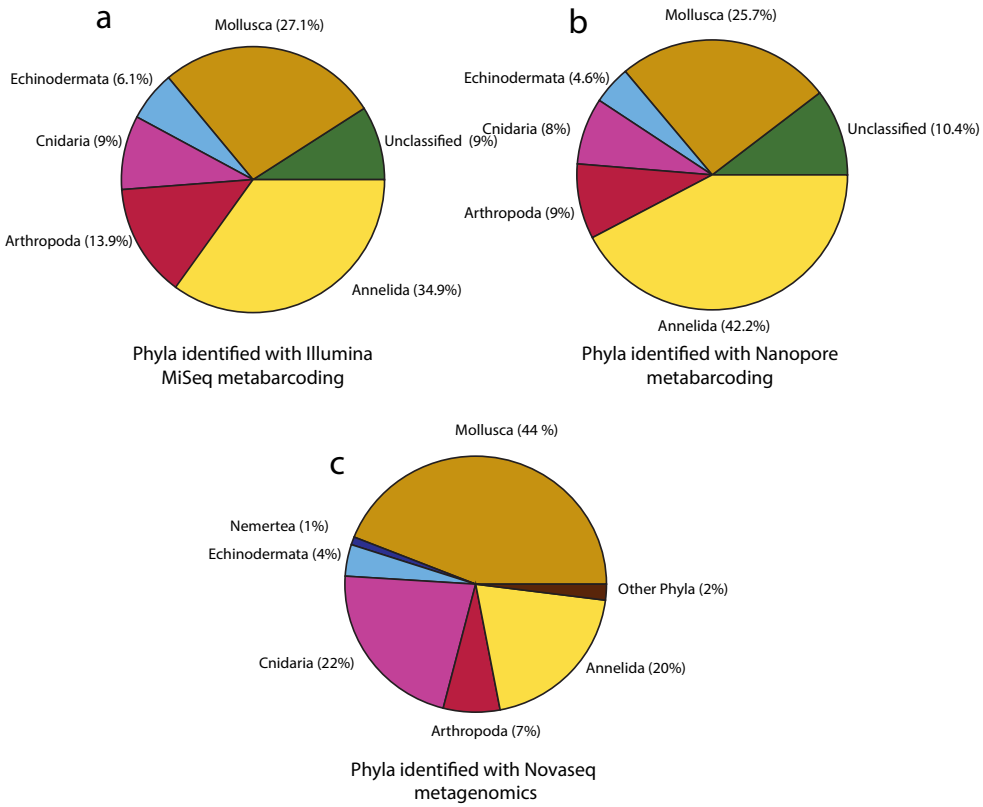


Figure S3: Pie charts of the found phyla for each DNA based method. (A) Illumina MiSeq metabarcoding (B) Nanopore MinION metabarcoding (C) Illumina Novaseq shotgun metagenomics (using the complete NCBI-nt (marine) database.

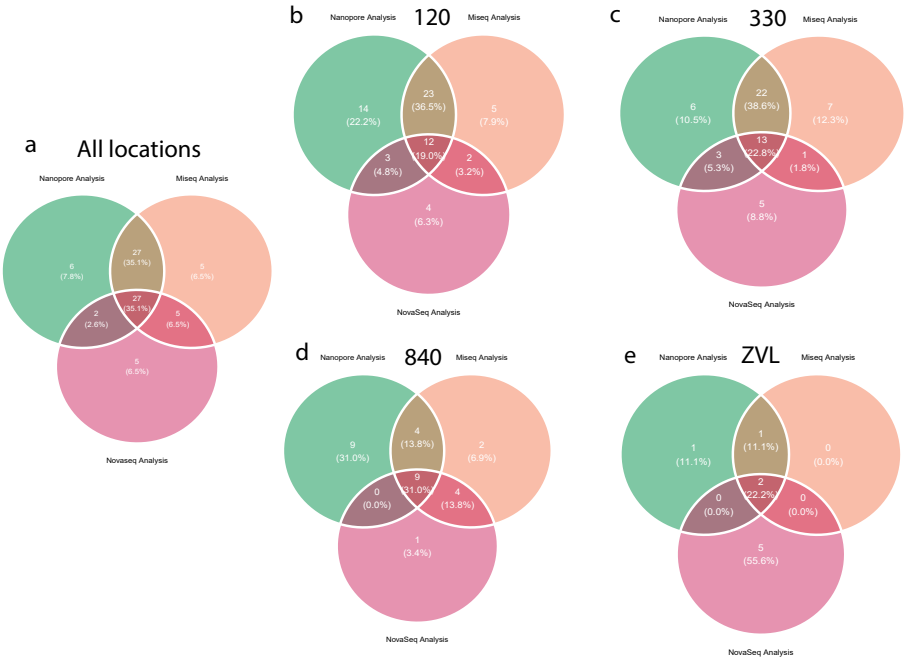


Figure S4: Venn diagram of species presence between DNA-based methods and locations. Venn diagram of the overlapping and unique species between DNA based method with all species from all replicates Diagram (A) represent the overlap between methods in all locations, (B) location 120, (C) location 330, (D) location 840 and (E) location ZVL.

Supplementary Tables

Table S1: Morphological observations of each species for each location. Columns size class indicate the size class of each species. Size class corrected data were calculated by multiply the average size class with the number of individuals.

	120	330	840	ZVL	Totaal	sizeclass	sizeclass_ average	sizeclass_ correction
Abludomelita obtusata	7	1	0	0	8	sr<10	5	40
Abra alba	3	0	0	1	4	sr11-20	15	60
Acrocnida brachiata	2	0	0	0	2	sr101-200	150	300
Aonides oxycephala	1	0	0	0	1	sr21-100	60	60
Aonides paucibranchiata	0	0	1	0	1	sr21-100	60	60
Apolochus neapolitanus	1	0	0	0	1	sr<10	5	5
Bathyporeia elegans	0	1	0	0	1	sr<10	5	5
Bathyporeia guilliamsoniana	0	0	1	0	1	sr<10	5	5
Capitella minima	2	0	0	0	2	sr21-100	60	120
Caulleriella alata	6	0	0	0	6		15	90
Crepidula fornicata	1	0	0	0	1	sr21-100	60	60
Diogenes pugilator	0	1	0	0	1	sr11-20	15	15
Echinocardium cordatum	2	0	0	0	2	sr21-100	60	120
Ensis leei	1	0	0	0	1	sr101-200	150	150
Eteone longa	5	0	0	0	5	sr101-200	150	750
Eumida sanguinea	11	0	0	0	11	sr21-100	60	660
Fabulina fabula	18	0	0	0	18	sr11-20	15	270
Gastrosaccus spinifer	1	1	2	0	4	sr11-20	15	60
Glycera lapidum	0	2	0	0	2	sr101-200	150	300
Glycera tridactyla	3	0	0	0	3	sr11-20	15	45
Harmothoe glabra	3	0	0	0	3	sr21-100	60	180
Hesionura elongata	0	4	1	0	5	sr11-20	15	75
Kurtiella bidentata	5	0	0	0	5	sr<10	5	25
Lagis koreni	1	0	0	0	1	sr21-100	60	60
Limecola balthica	0	0	0	5	5	sr11-20	15	75
Liocarcinus holsatus	1	0	0	0	1	sr21-100	60	60
Liocarcinus marmoreus	0	0	1	0	1	sr21-100	60	60
Magelona johnstoni	15	0	0	0	15	sr21-100	60	900

Table S1: Morphological observations of each species for each location. Columns size class indicate the size class of each species. Size class corrected data were calculated by multiply the average size class with the number of individuals. (*continued*)

	120	330	840	ZVL	Totaal	sizeclass	sizeclass_ average	sizeclass_ correction
Mediomastus fragilis	20	1	0	0	21	sr21-100	60	1260
Monocorophium acherusicum	0	2	0	0	2	sr<10	5	10
Mytilus edulis	3	0	0	0	3	sr21-100	60	180
Nephtys cirrosa	0	4	1	0	5	sr101-200	150	750
Nephtys hombergii	4	0	0	0	4	sr101-200	150	600
Notomastus latericeus	17	0	1	0	18	sr201-500	350	6300
Nototropis swammerdami	0	2	0	0	2	sr<10	5	10
Ophiura albida	1	0	0	0	1	sr21-100	60	60
Ophiura ophiura	5	0	0	0	5	sr21-100	60	300
Owenia fusiformis	6	0	0	0	6	sr201-500	350	2100
Pagurus bernhardus	1	0	0	0	1	sr21-100	60	60
Pariambus typicus	11	0	0	0	11	sr<10	5	55
Petricolaria pholadiformis	0	0	0	1	1	sr21-100	60	60
Pholoe baltica	2	0	0	0	2	sr11-20	15	30
Phyllodoce mucosa	1	0	0	0	1	sr101-200	150	150
Pisidia longicornis	0	0	2	0	2	sr11-20	15	30
Protodriloides chaetifer	0	1	0	0	1	sr11-20	15	15
Pseudopolydora pulchra	1	0	0	0	1		15	15
Sigalion mathildae	1	0	0	0	1	sr101-200	150	150
Spiophanes bombyx	5	0	0	0	5	sr21-100	60	300
Spirobranchus lamarckii	1	0	0	0	1	sr11-20	15	15
Spisula elliptica	0	1	0	0	1	sr11-20	15	15
Sthenelais boa	2	0	0	0	2	sr101-200	150	300
Streptodonta pterochaeta	0	0	1	0	1	sr11-20	15	15
Tanaissus lilljeborgi	0	0	1	0	1	sr<10	5	5
Tellimya ferruginosa	7	0	0	0	7	sr<10	5	35
Tryphosa nana	1	0	0	0	1	sr<10	5	5
Urothoe brevicornis	0	12	0	0	12	sr<10	5	60
Venerupis corrugata	2	0	0	0	2	sr21-100	60	120
Total species number	**39**	**13**	**10**	**3**				

Table S2: Volumes of initial material for extraction. Start volume of BULK material for each extraction. Samples labeled DNA were used directly as DNA extracts.

Sample material received from ILVO		
	Metagenomics (Nord university)	Amplicons (Naturalis)
120A	6ml	6ml
120B	6ml	6ml
120C	6ml	6ml
330A	6ml	6ml
330B	6ml	6ml
330C	6ml	6ml
840A	6ml	6ml
840B	6ml	6ml
840C	6ml	DNA82 +DNA83+DNA84
ZVLA	2ml + DNA32 +DNA57	DNA32 +DNA86**+DNA87***
ZVLB	6ml	6ml
ZVLC	6ml	DNA93+DNA95+DNA96
Where the "DNA" sample refers to the respective DNA extracts directly obtained from ILVO see publication		
Van den Bulcke, L., De Backer, A., Wittoeck, J., Beentjes, K., Maes, S., Christodoulou, M., ... & Derycke, S. (2023). DNA metabarcoding on repeat: Sequencing data of marine macrobenthos are reproducible and robust across labs and protocols. Ecological Indicators, 150, 110207.		
**	8 ul	
***	5 ul	

Table S3: read count before and after porocessing for each DNA based method

DNA Method	Sample	Output Reads	Filtered reads	Assembled contigs/reads for taxonomy
novaseq	120A	325601204	280039801	4849167
novaseq	120B	227831344	206654794	2615230
novaseq	120C	294266452	249606555	3869151
novaseq	330A	259484921	189759557	3157847
novaseq	330B	370625502	290446200	5196941
novaseq	330C	241406566	235315213	3598688
novaseq	840A	407105954	297489186	5457584
novaseq	840B	407293585	290899142	4937478
novaseq	840C	138941208	92321117	2224152
novaseq	ZVLA	326501645	240071589	4747334
novaseq	ZVLB	15323971	11938131	144745
novaseq	ZVLC	46034768	40979188	544434
ILLumina	120A	797248	706309	677582
ILLumina	120B	782399	677298	652431
ILLumina	120C	766004	670603	642446
ILLumina	330A	726463	651547	640904
ILLumina	330B	691436	590555	576895
ILLumina	330C	920456	822291	813114
ILLumina	840A	886151	796760	789261
ILLumina	840B	1419428	1223034	1190692
ILLumina	840C	531724	471231	468239
ILLumina	ZVLA	948577	826367	708023
ILLumina	ZVLB	1208840	807457	744530
ILLumina	ZVLC	527361	448957	426877
Nanopore	120A	104493	65369	64212
Nanopore	120B	213119	164664	81156
Nanopore	120C	205291	153795	106256
Nanopore	330A	159996	124071	120255
Nanopore	330B	242368	190046	102981
Nanopore	330C	281977	217043	111843
Nanopore	840A	85183	65486	65160
Nanopore	840B	143013	111683	56858
Nanopore	840C	351204	266695	1155
Nanopore	ZVLA	89920	69987	69939

Table S3: read count before and after porocessing for each DNA based method (*continued*)

DNA Method	Sample	Output Reads	Filtered reads	Assembled contigs/reads for taxonomy
Nanopore	ZVLB	122271	93042	92822
Nanopore	ZVLC	427182	319504	319216

Table S4: 2 way anova between location and DNA based method. Yellow is non-significant observations. This is for NovaSeq alignment length >= 100 and max counts >=5

	Species richness				
	Two way analysis of Variance				
	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Location	3	2147,194	715,731	104,741	6,35E-14
Method	2	21,5	10,75	1,573	0,22803
Location *Method	6	178,1	29,6759	4,3428	0,0042
Residuals	24	164	6,833	NA	NA

		Tukey HSD			
		diff	lwr	upr	p adj
Location	330-120	-14,667	-18,066	-11,267	8,69E-11
	840-120	-15,222	-18,622	-11,823	4,02E-11
	ZVL-120	-20,889	-24,288	-17,490	6,67E-14
	840-330	-0,556	-3,955	2,844	0,9688
	ZVL-330	-6,222	-9,622	-2,823	0,0002
	ZVL-840	-5,667	-9,066	-2,267	0,0006
Method	Nanopore-Miseq	1,5	-1,165073281	4,165073281	0,3540
	Novaseq-Miseq	1,75	-0,915073281	4,415073281	0,2489
	Novaseq-Nanopore	0,25	-2,415073281	2,915073281	0,9702
Location *Method	330:Miseq-120:Miseq	-13	-20,69576401	-5,304235985	0,000142241
	840:Miseq-120:Miseq	-12,66666667	-20,36243068	-4,970902652	0,000206591
	ZVL:Miseq-120:Miseq	-21	-28,69576401	-13,30423599	3,94E-08
	120:Nanopore-120:Miseq	7	-0,695764015	14,69576401	0,09861977
	330:Nanopore-120:Miseq	-12	-19,69576401	-4,304235985	0,00043753
	840:Nanopore-120:Miseq	-15,33333333	-23,02909735	-7,637569319	1,10E-05
	ZVL:Nanopore-120:Miseq	-20,33333333	-28,02909735	-12,63756932	7,31E-08
	120:NovaSeq-120:Miseq	-0,666666667	-8,362430681	7,029097348	0,999999962
	330:NovaSeq-120:Miseq	-12,66666667	-20,36243068	-4,970902652	0,000206591
	840:NovaSeq-120:Miseq	-11,33333333	-19,02909735	-3,637569319	0,000929611
	ZVL:NovaSeq-120:Miseq	-15	-22,69576401	-7,304235985	1,58E-05
	840:Miseq-330:Miseq	0,333333333	-7,362430681	8,029097348	1
	ZVL:Miseq-330:Miseq	-8	-15,69576401	-0,304235985	0,036644699
	120:Nanopore-330:Miseq	20	12,30423599	27,69576401	1,00E-07
	330:Nanopore-330:Miseq	1	-6,695764015	8,695764015	0,999997286
	840:Nanopore-330:Miseq	-2,333333333	-10,02909735	5,362430681	0,99212168
	ZVL:Nanopore-330:Miseq	-7,333333333	-15,02909735	0,362430681	0,071656116

		Shannon Diversity			
		Two way analysis of Variance			
	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Location	3	19,80109948	6,600366492	104,4904668	6,52E-14
Method	2	0,923845532	0,461922766	7,312703852	0,003311753
Location *Method	6	2,906149381	0,48435823	7,667879903	0,000112189
Residuals	24	1,516011945	0,063167164	NA	NA

		Tukey HSD			
		diff	lwr	upr	p adj
Location	330-120	-0,918261598	-1,245097344	-0,591425852	3,17E-07
	840-120	-0,964419846	-1,291255591	-0,6375841	1,34E-07
	ZVL-120	-2,091941413	-2,418777159	-1,765105667	3,97E-14
	840-330	-0,046158248	-0,372993994	0,280677498	0,979
	ZVL-330	-1,173679816	-1,500515561	-0,84684407	3,44E-09
	ZVL-840	-1,127521568	-1,454357314	-0,800685822	7,46E-09
Method	Nanopore-Miseq	0,120096285	-0,136138769	0,376331339	0,4817
	Novaseq-Miseq	0,383565303	0,127330249	0,639800357	0,0028
	Novaseq-Nanopore	0,263469018	0,007233964	0,519704072	0,0430
Location *Method	330:Miseq-120:Miseq	-0,834021778	-1,573935582	-0,094107975	0,01798464
	840:Miseq-120:Miseq	-0,861942014	-1,601855817	-0,12202821	0,013136852
	ZVL:Miseq-120:Miseq	-2,523638262	-3,263552066	-1,783724459	4,50E-10
	120:Nanopore-120:Miseq	0,329287588	-0,410626215	1,069201392	0,891121373
	330:Nanopore-120:Miseq	-0,775242905	-1,515156708	-0,035329101	0,034322905
	840:Nanopore-120:Miseq	-0,968909476	-1,70882328	-0,228995673	0,00383375
	ZVL:Nanopore-120:Miseq	-2,324352123	-3,064265926	-1,584438319	2,43E-09
	120:Novaseq-120:Miseq	-0,009360398	-0,749274202	0,730553405	1
	330:Novaseq-120:Miseq	-0,82559292	-1,565506724	-0,085679117	0,019757431
	840:Novaseq-120:Miseq	-0,742480857	-1,482394661	-0,002567054	0,048669335
	ZVL:Novaseq-120:Miseq	-1,107906665	-1,847820469	-0,367992862	0,00074991
	840:Miseq-330:Miseq	-0,027920235	-0,767834039	0,711993568	1
	ZVL:Miseq-330:Miseq	-1,689616484	-2,429530287	-0,94970268	1,07E-06
	120:Nanopore-330:Miseq	1,163309367	0,423395563	1,90322317	0,000391086
	330:Nanopore-330:Miseq	0,058778874	-0,68113493	0,798692677	0,999999985
	840:Nanopore-330:Miseq	-0,134887698	-0,874801501	0,605026106	0,999917709
	ZVL:Nanopore-330:Miseq	-1,490330344	-2,230244148	-0,750416541	9,24E-06

Table S4: 2 way anova between location and DNA based method. Yellow is non-significant observations. This is for NovaSeq alignment length >= 100 and max counts >=5 (*continued*)

	Species richness			
	Two way analysis of Variance			
120:NovaSeq-330:Miseq	12,33333333	4,637569319	20,02909735	0,000300478
330:NovaSeq-330:Miseq	0,333333333	-7,362430681	8,029097348	1
840:NovaSeq-330:Miseq	1,666666667	-6,029097348	9,362430681	0,999574857
ZVL:NovaSeq-330:Miseq	-2	-9,695764015	5,695764015	0,997812402
ZVL:Miseq-840:Miseq	-8,333333333	-16,02909735	-0,637569319	0,025869397
120:Nanopore-840:Miseq	19,66666667	11,97090265	27,36243068	1,37E-07
330:Nanopore-840:Miseq	0,666666667	-7,029097348	8,362430681	0,999999962
840:Nanopore-840:Miseq	-2,666666667	-10,36243068	5,029097348	0,978260688
ZVL:Nanopore-840:Miseq	-7,666666667	-15,36243068	0,029097348	0,051488093
120:NovaSeq-840:Miseq	12	4,304235985	19,69576401	0,00043753
330:NovaSeq-840:Miseq	3,55E-15	-7,695764015	7,695764015	1
840:NovaSeq-840:Miseq	1,333333333	-6,362430681	9,029097348	0,999950054
ZVL:NovaSeq-840:Miseq	-2,333333333	-10,02909735	5,362430681	0,99212168
120:Nanopore-ZVL:Miseq	28	20,30423599	35,69576401	1,16E-10
330:Nanopore-ZVL:Miseq	9	1,304235985	16,69576401	0,01264512
840:Nanopore-ZVL:Miseq	5,666666667	-2,029097348	13,36243068	0,304987868
ZVL:Nanopore-ZVL:Miseq	0,666666667	-7,029097348	8,362430681	0,999999962
120:NovaSeq-ZVL:Miseq	20,33333333	12,63756932	28,02909735	7,31E-08
330:NovaSeq-ZVL:Miseq	8,333333333	0,637569319	16,02909735	0,025869397
840:NovaSeq-ZVL:Miseq	9,666666667	1,970902652	17,36243068	0,006066277
ZVL:NovaSeq-ZVL:Miseq	6	-1,695764015	13,69576401	0,236138163
330:Nanopore-120:Nanopore	-19	-26,69576401	-11,30423599	2,61E-07
840:Nanopore-120:Nanopore	-22,33333333	-30,02909735	-14,63756932	1,18E-08
ZVL:Nanopore-120:Nanopore	-27,33333333	-35,02909735	-19,63756932	1,93E-10
120:NovaSeq-120:Nanopore	-7,666666667	-15,36243068	0,029097348	0,051488093
330:NovaSeq-120:Nanopore	-19,66666667	-27,36243068	-11,97090265	1,37E-07
840:NovaSeq-120:Nanopore	-18,33333333	-26,02909735	-10,63756932	5,02E-07
ZVL:NovaSeq-120:Nanopore	-22	-29,69576401	-14,30423599	1,59E-08
840:Nanopore-330:Nanopore	-3,333333333	-11,02909735	4,362430681	0,906661921
ZVL:Nanopore-330:Nanopore	-8,333333333	-16,02909735	-0,637569319	0,025869397
120:NovaSeq-330:Nanopore	11,33333333	3,637569319	19,02909735	0,000929611
330:NovaSeq-330:Nanopore	-0,666666667	-8,362430681	7,029097348	0,999999962
840:NovaSeq-330:Nanopore	0,666666667	-7,029097348	8,362430681	0,999999962
ZVL:NovaSeq-330:Nanopore	-3	-10,69576401	4,695764015	0,951153982
ZVL:Nanopore-840:Nanopore	-5	-12,69576401	2,695764015	0,476148818

		Shannon Diversity			
		Two way analysis of Variance			
	120:Novaseq-330:Miseq	0,82466138	0,084747576	1,564575184	0,019963284
	330:Novaseq-330:Miseq	0,008428858	-0,731484945	0,748342662	1
	840:Novaseq-330:Miseq	0,091540921	-0,648372882	0,831454725	0,999998369
	ZVL:Novaseq-330:Miseq	-0,273884887	-1,01379869	0,466028917	0,965413301
	ZVL:Miseq-840:Miseq	-1,661696249	-2,401610052	-0,921782445	1,44E-06
	120:Nanopore-840:Miseq	1,191229602	0,451315798	1,931143405	0,000281961
	330:Nanopore-840:Miseq	0,086699109	-0,653214694	0,826612913	0,999999075
	840:Nanopore-840:Miseq	-0,106967462	-0,846881266	0,632946341	0,999991884
	ZVL:Nanopore-840:Miseq	-1,462410109	-2,202323912	-0,722496305	1,26E-05
	120:Novaseq-840:Miseq	0,852581615	0,112667812	1,592495419	0,014602006
	330:Novaseq-840:Miseq	0,036349093	-0,70356471	0,776262897	1
	840:Novaseq-840:Miseq	0,119461156	-0,620452647	0,85937496	0,999975225
	ZVL:Novaseq-840:Miseq	-0,245964652	-0,985878455	0,493949152	0,983976498
	120:Nanopore-ZVL:Miseq	2,852925851	2,113012047	3,592839654	3,34E-11
	330:Nanopore-ZVL:Miseq	1,748395358	1,008481554	2,488309161	5,81E-07
	840:Nanopore-ZVL:Miseq	1,554728786	0,814814983	2,29464259	4,55E-06
	ZVL:Nanopore-ZVL:Miseq	0,19928614	-0,540627664	0,939199943	0,997028242
	120:Novaseq-ZVL:Miseq	2,514277864	1,77436406	3,254191667	4,86E-10
	330:Novaseq-ZVL:Miseq	1,698045342	0,958131538	2,437959145	9,82E-07
	840:Novaseq-ZVL:Miseq	1,781157405	1,041243601	2,521071208	4,15E-07
	ZVL:Novaseq-ZVL:Miseq	1,415731597	0,675817793	2,1556454	2,13E-05
	330:Nanopore-120:Nanopore	-1,104530493	-1,844444296	-0,364616689	0,000780296
	840:Nanopore-120:Nanopore	-1,298197064	-2,038110868	-0,558283261	8,13E-05
	ZVL:Nanopore-120:Nanopore	-2,653639711	-3,393553514	-1,913725907	1,57E-10
	120:Novaseq-120:Nanopore	-0,338647987	-1,07856179	0,401265817	0,873028949
	330:Novaseq-120:Nanopore	-1,154880509	-1,894794312	-0,414966705	0,000431747
	840:Novaseq-120:Nanopore	-1,071768446	-1,811682249	-0,331854642	0,00114732
	ZVL:Novaseq-120:Nanopore	-1,437194254	-2,177108057	-0,69728045	1,67E-05
	840:Nanopore-330:Nanopore	-0,193666571	-0,933580375	0,546247232	0,997673578
	ZVL:Nanopore-330:Nanopore	-1,549109218	-2,289023022	-0,809195414	4,84E-06
	120:Novaseq-330:Nanopore	0,765882506	0,025968703	1,50579631	0,037958546
	330:Novaseq-330:Nanopore	-0,050350016	-0,790263819	0,689563788	0,999999997
	840:Novaseq-330:Nanopore	0,032762047	-0,707151756	0,772675851	1
	ZVL:Novaseq-330:Nanopore	-0,332663761	-1,072577564	0,407250043	0,884780177
	ZVL:Nanopore-840:Nanopore	-1,355442647	-2,09535645	-0,615528843	4,22E-05

Table S4: 2 way anova between location and DNA based method. Yellow is non-significant observations. This is for NovaSeq alignment length >= 100 and max counts >=5 (*continued*)

	Species richness			
	Two way analysis of Variance			
120:NovaSeq-840:Nanopore	14,66666667	6,970902652	22,36243068	2,27E-05
330:NovaSeq-840:Nanopore	2,666666667	-5,029097348	10,36243068	0,978260688
840:NovaSeq-840:Nanopore	4	-3,695764015	11,69576401	0,763067013
ZVL:NovaSeq-840:Nanopore	0,333333333	-7,362430681	8,029097348	1
120:NovaSeq-ZVL:Nanopore	19,66666667	11,97090265	27,36243068	1,37E-07
330:NovaSeq-ZVL:Nanopore	7,666666667	-0,029097348	15,36243068	0,051488093
840:NovaSeq-ZVL:Nanopore	9	1,304235985	16,69576401	0,01264512
ZVL:Novaseq-ZVL:Nanopore	5,333333333	-2,362430681	13,02909735	0,385601932
330:NovaSeq-120:NovaSeq	-12	-19,69576401	-4,304235985	0,00043753
840:NovaSeq-120:NovaSeq	-10,66666667	-18,36243068	-2,970902652	0,00197538
ZVL:NovaSeq-120:NovaSeq	-14,33333333	-22,02909735	-6,637569319	3,26E-05
840:NovaSeq-330:NovaSeq	1,333333333	-6,362430681	9,029097348	0,999950054
ZVL:NovaSeq-330:NovaSeq	-2,333333333	-10,02909735	5,362430681	0,99212168
ZVL:NovaSeq-840:NovaSeq	-3,666666667	-11,36243068	4,029097348	0,843313669

	Shannon Diversity			
	Two way analysis of Variance			
120:Novaseq-840:Nanopore	0,959549078	0,219635274	1,699462881	0,004275535
330:Novaseq-840:Nanopore	0,143316556	-0,596597248	0,883230359	0,999852026
840:Novaseq-840:Nanopore	0,226428619	-0,513485185	0,966342422	0,991520873
ZVL:Novaseq-840:Nanopore	-0,138997189	-0,878910993	0,600916614	0,999889843
120:Novaseq-ZVL:Nanopore	2,314991724	1,575077921	3,054905528	2,64E-09
330:Novaseq-ZVL:Nanopore	1,498759202	0,758845399	2,238673006	8,42E-06
840:Novaseq-ZVL:Nanopore	1,581871265	0,841957462	2,321785069	3,39E-06
ZVL:Novaseq-ZVL:Nanopore	1,216445457	0,476531654	1,956359261	0,000209991
330:Novaseq-120:Novaseq	-0,816232522	-1,556146325	-0,076318718	0,021921077
840:Novaseq-120:Novaseq	-0,733120459	-1,473034262	0,006793345	0,053683864
ZVL:Novaseq-120:Novaseq	-1,098546267	-1,83846007	-0,358632463	0,000837217
840:Novaseq-330:Novaseq	0,083112063	-0,656801741	0,823025866	0,999999406
ZVL:Novaseq-330:Novaseq	-0,282313745	-1,022227549	0,457600058	0,957577672
ZVL:Novaseq-840:Novaseq	-0,365425808	-1,105339612	0,374487996	0,812843902

Table S5: PERMANOVA OF THE NMDS

PERMANOVA Analysis					
	Df	SumOfSqs	R2	F	Pr(>F)
Location	3	7,33989424	0,514929006	16,04521822	0,001
variable	2	0,989843323	0,069442286	3,245738618	0,001
Location:variable	6	2,26484467	0,158889785	2,475507532	0,001
Residual	24	3,659604571	0,256738923	NA	NA
Total	35	14,2541868	1	NA	NA

Table S5: PERMANOVA OF THE NMDS

	Post hoc pairwise Adonis						
	pairs	Df	SumsOfSqs	F.Model	R2	p.value	p.adjusted
Location	120 vs 330	1	2,858058801	14,40651218	0,473796932	0,002	0,012
	120 vs 840	1	2,319421305	8,540460822	0,348015503	0,001	0,006
	120 vs ZVL	1	2,957113007	15,59176152	0,493538846	0,001	0,006
	330 vs 840	1	0,782965561	3,229305809	0,167936682	0,005	0,03
	330 vs ZVL	1	3,169297746	19,74214163	0,552349152	0,001	0,006
	840 vs ZVL	1	2,593611061	11,09668746	0,409521919	0,001	0,006
Method	Miseq vs Nanopore	1	0,157179348	0,394518832	0,017616759	0,9	1
	Miseq vs NovaSeq	1	0,621043548	1,533942162	0,065179992	0,142	0,426
	Nanopore vs NovaSeq	1	0,706372338	1,754639546	0,07386513	0,079	0,237

Table S6: Presence absence of each species in each DNA based method, to which Phylum the belong and the read count.
Only replicates that were identified morphologically were used to determine presence/ absesnce of gener and species (replicates 120B, 330C, 840C, ZVLA)
Abundance is based on Counts for the morphological analysis and OTU alignments for Naturalis and Nanopore
Table of all species and their total abundance for the Miseq, Nanopore and Novaseq methods.

	Morphology	Miseq	Nanopore	NovaSeq	Abundance	Phylum
Abludomelita obtusata	TRUE	FALSE	FALSE	FALSE	8, 0, 0, 0	Arthropoda
Abra alba	TRUE	TRUE	TRUE	TRUE	4, 25, 17, 6596	Mollusca
Acraspedanthus ferax	FALSE	FALSE	FALSE	TRUE	0, 0, 0, 7	Cnidaria
Acrocnida brachiata	TRUE	TRUE	FALSE	TRUE	2, 719, 0, 117	Echinodermata
Actinia tenebrosa	FALSE	FALSE	FALSE	TRUE	0, 0, 0, 2182	Cnidaria
Aonides oxycephala	TRUE	FALSE	FALSE	FALSE	1, 0, 0, 0	Annelida
Aonides paucibranchiata	TRUE	FALSE	FALSE	FALSE	1, 0, 0, 0	Annelida
Apolochus neapolitanus	TRUE	FALSE	FALSE	FALSE	1, 0, 0, 0	Arthropoda
Bathyporeia elegans	TRUE	TRUE	TRUE	FALSE	1, 434, 109, 0	Arthropoda
Bathyporeia guilliamsoniana	TRUE	TRUE	TRUE	FALSE	1, 4781, 89, 0	Arthropoda
Bathyporeia sp.	FALSE	FALSE	FALSE	TRUE	0, 0, 0, 53	Arthropoda
Capitella minima	TRUE	FALSE	FALSE	FALSE	2, 0, 0, 0	Annelida
Caulleriella alata	TRUE	FALSE	FALSE	FALSE	6, 0, 0, 0	Annelida
Crepidula fornicata	TRUE	TRUE	TRUE	TRUE	1, 287, 104, 1787	Mollusca
Cylista troglodytes	FALSE	TRUE	TRUE	FALSE	0, 13271, 22912, 0	Cnidaria
Diogenes pugilator	TRUE	TRUE	TRUE	TRUE	1, 29, 16, 10420	Arthropoda
Echinocardium cordatum	TRUE	TRUE	TRUE	TRUE	2, 5184, 6834, 7895	Echinodermata
Echinocyamus pusillus	FALSE	TRUE	FALSE	TRUE	0, 14, 0, 4	Echinodermata
Electra pilosa	FALSE	FALSE	TRUE	FALSE	0, 0, 1, 0	Bryozoa
Ensis leei	TRUE	FALSE	TRUE	TRUE	1, 0, 77, 10654	Mollusca
Eteone longa	TRUE	FALSE	FALSE	FALSE	5, 0, 0, 0	Annelida
Eucheilota maculata	FALSE	FALSE	TRUE	FALSE	0, 0, 21, 0	Cnidaria
Eumida mackiei	FALSE	TRUE	TRUE	FALSE	0, 3, 23, 0	Annelida
Eumida sanguinea	TRUE	FALSE	FALSE	FALSE	11, 0, 0, 0	Annelida
Eunereis longissima	FALSE	FALSE	TRUE	FALSE	0, 0, 1, 0	Annelida
Fabulina fabula	TRUE	FALSE	TRUE	FALSE	18, 0, 8, 0	Mollusca
Gastrosaccus spinifer	TRUE	TRUE	TRUE	FALSE	4, 2670, 2466, 0	Arthropoda
Glycera alba	FALSE	FALSE	FALSE	TRUE	0, 0, 0, 4	Annelida
Glycera lapidum	TRUE	FALSE	FALSE	TRUE	2, 0, 0, 779	Annelida
Glycera tridactyla	TRUE	FALSE	FALSE	TRUE	3, 0, 0, 5	Annelida
Haloclava producta	FALSE	FALSE	FALSE	TRUE	0, 0, 0, 23	Cnidaria

	Species	Miseq	Nanopore	NovaSeq	Abundance (read count)	Phylum	In GEANS
1	Abra alba	TRUE	TRUE	TRUE	181, 78, 8884	Mollusca	TRUE
2	Acraspedanthus ferax	FALSE	FALSE	TRUE	0, 0, 7	Cnidaria	FALSE
3	Acrocnida brachiata	TRUE	FALSE	TRUE	719, 0, 117	Echinodermata	TRUE
4	Actinia tenebrosa	FALSE	FALSE	TRUE	0, 0, 10342	Cnidaria	FALSE
5	Anapagurus laevis	TRUE	TRUE	TRUE	1333, 11169, 205	Arthropoda	TRUE
6	Aonides paucibranchiata	TRUE	TRUE	FALSE	23, 70, 0	Annelida	TRUE
7	Asbjornsenia pygmaea	TRUE	FALSE	FALSE	8, 0, 0	Mollusca	TRUE
8	Bathyporeia elegans	TRUE	TRUE	FALSE	10279, 1510, 0	Arthropoda	TRUE
9	Bathyporeia guilliamsoniana	TRUE	TRUE	FALSE	7157, 2367, 0	Arthropoda	TRUE
10	Bathyporeia sp.	FALSE	FALSE	TRUE	0, 0, 7650	Arthropoda	FALSE
11	Cirriformia sp.	FALSE	FALSE	TRUE	0, 0, 481	Annelida	FALSE
12	Cirriformia tentaculata	FALSE	FALSE	TRUE	0, 0, 117	Annelida	FALSE
13	Crepidula fornicata	TRUE	TRUE	TRUE	523, 127, 4104	Mollusca	TRUE
14	Cylista troglodytes	TRUE	TRUE	FALSE	47865, 75594, 0	Cnidaria	TRUE
15	Diadumene lineata	FALSE	FALSE	TRUE	0, 0, 4242	Cnidaria	TRUE
16	Diogenes pugilator	TRUE	TRUE	TRUE	29, 16, 14217	Arthropoda	TRUE
17	Echinocardium cordatum	TRUE	TRUE	TRUE	16959, 21977, 21880	Echinodermata	TRUE
18	Echinocyamus pusillus	TRUE	FALSE	TRUE	17, 0, 8	Echinodermata	TRUE
19	Ectopleura dumortierii	FALSE	FALSE	TRUE	0, 0, 158	Cnidaria	FALSE
20	Ectopleura larynx	FALSE	FALSE	TRUE	0, 0, 54	Cnidaria	TRUE
21	Electra pilosa	TRUE	TRUE	TRUE	2, 12, 2	Bryozoa	TRUE
22	Ensis leei	TRUE	TRUE	TRUE	3, 548, 41939	Mollusca	TRUE
23	Eucheilota maculata	TRUE	TRUE	FALSE	5, 116, 0	Cnidaria	TRUE
24	Eumida mackiei	TRUE	TRUE	FALSE	9, 153, 0	Annelida	TRUE
25	Eunereis longissima	FALSE	TRUE	FALSE	0, 1, 0	Annelida	TRUE
26	Eutima gegenbauri	FALSE	FALSE	TRUE	0, 0, 4	Cnidaria	FALSE
27	Exaiptasia pallida	FALSE	FALSE	TRUE	0, 0, 60	Cnidaria	FALSE
28	Fabulina fabula	FALSE	TRUE	FALSE	0, 14, 0	Mollusca	TRUE
29	Gastrosaccus spinifer	TRUE	TRUE	FALSE	8075, 4512, 0	Arthropoda	TRUE
30	Glycera alba	FALSE	FALSE	TRUE	0, 0, 4	Annelida	FALSE
31	Glycera lapidum	FALSE	FALSE	TRUE	0, 0, 779	Annelida	FALSE

Table S6: Presence absence of each species in each DNA based method, to which Phylum the belong and the read count. (*continued*)

	Morphology	Miseq	Nanopore	NovaSeq	Abundance	Phylum
Harmothoe glabra	TRUE	FALSE	FALSE	FALSE	3, 0, 0, 0	Annelida
Hesionura elongata	TRUE	TRUE	FALSE	FALSE	5, 27, 0, 0	Annelida
Hormathia digitata	FALSE	FALSE	FALSE	TRUE	0, 0, 0, 56151	Cnidaria
Jesogammarus spinopalpus	FALSE	FALSE	FALSE	TRUE	0, 0, 0, 53	Arthropoda
Kurtiella bidentata	TRUE	FALSE	FALSE	FALSE	5, 0, 0, 0	Mollusca
Lagis koreni	TRUE	TRUE	TRUE	FALSE	1, 17, 80, 0	Annelida
Liocarcinus holsatus	TRUE	TRUE	TRUE	FALSE	1, 1642, 98, 0	Arthropoda
Liocarcinus marmoreus	TRUE	TRUE	FALSE	TRUE	1, 138, 0, 1339	Arthropoda
Loimia ramzega	FALSE	FALSE	TRUE	FALSE	0, 0, 1, 0	Annelida
Macoma balthica	TRUE	TRUE	TRUE	TRUE	5, 25066, 48475, 124943	Mollusca
Magelona johnstoni	TRUE	TRUE	FALSE	FALSE	15, 37, 0, 0	Annelida
Magelona mirabilis	FALSE	TRUE	TRUE	FALSE	0, 14, 383, 0	Annelida
Malmgrenia lunulata	FALSE	FALSE	TRUE	FALSE	0, 0, 1, 0	Annelida
Mediomastus fragilis	TRUE	FALSE	FALSE	FALSE	21, 0, 0, 0	Annelida
Membranipora membranacea	FALSE	TRUE	TRUE	FALSE	0, 5, 40, 0	Bryozoa
Metridium senile	FALSE	FALSE	FALSE	TRUE	0, 0, 0, 3	Cnidaria
Microprotopus maculatus	FALSE	FALSE	TRUE	FALSE	0, 0, 7, 0	Arthropoda
Moerella iridescens	FALSE	FALSE	FALSE	TRUE	0, 0, 0, 1253	Mollusca
Monocorophium acherusicum	TRUE	TRUE	TRUE	FALSE	2, 2, 16, 0	Arthropoda
Mytilus edulis	TRUE	FALSE	FALSE	FALSE	3, 0, 0, 0	Mollusca
Mytilus sp.	FALSE	FALSE	FALSE	TRUE	0, 0, 0, 4	Mollusca
Nephtys assimilis	FALSE	TRUE	TRUE	FALSE	0, 2, 18, 0	Annelida
Nephtys cirrosa	TRUE	TRUE	TRUE	TRUE	5, 30361, 62484, 21142	Annelida
Nephtys hombergii	TRUE	TRUE	TRUE	TRUE	4, 177, 1261, 72	Annelida
Notomastus latericeus	TRUE	FALSE	TRUE	FALSE	18, 0, 14, 0	Annelida
Nototropis swammerdamei	FALSE	TRUE	TRUE	FALSE	0, 2, 66, 133	Arthropoda
Nototropis swammerdami	TRUE	FALSE	FALSE	FALSE	2, 0, 0, 0	Arthropoda
Ophiothrix fragilis	FALSE	TRUE	TRUE	FALSE	0, 9, 87, 0	Echinodermata
Ophiura albida	TRUE	TRUE	TRUE	TRUE	1, 713, 942, 1870	Echinodermata
Ophiura ophiura	TRUE	TRUE	TRUE	TRUE	5, 177, 4224, 2269	Echinodermata
Other	FALSE	FALSE	TRUE	FALSE	0, 0, 10183, 0	N/A
Owenia fusiformis	TRUE	TRUE	TRUE	TRUE	6, 23, 2213, 1396	Annelida
Pagurus bernhardus	TRUE	TRUE	TRUE	TRUE	1, 17, 202, 72	Arthropoda
Pariambus typicus	TRUE	TRUE	TRUE	FALSE	11, 4, 15, 0	Arthropoda
Petricolaria pholadiformis	TRUE	FALSE	FALSE	FALSE	1, 0, 0, 0	Mollusca

	Species	Miseq	Nanopore	NovaSeq	Abundance (read count)	Phylum	In GEANS
32	Glycera sp.	FALSE	FALSE	TRUE	0, 0, 3	Annelida	FALSE
33	Glycera tridactyla	FALSE	FALSE	TRUE	0, 0, 350	Annelida	FALSE
34	Haloclava producta	FALSE	FALSE	TRUE	0, 0, 1903	Cnidaria	FALSE
35	Hesionura elongata	TRUE	FALSE	FALSE	78, 0, 0	Annelida	TRUE
36	Hormathia digitata	FALSE	FALSE	TRUE	0, 0, 69855	Cnidaria	TRUE
37	Hubrechtella juliae	FALSE	FALSE	TRUE	0, 0, 6	Nemertea	FALSE
38	Jesogammarus spinopalpus	FALSE	FALSE	TRUE	0, 0, 433	Arthropoda	FALSE
39	Lagis koreni	TRUE	TRUE	FALSE	19, 88, 0	Annelida	TRUE
40	Lanice conchilega	TRUE	TRUE	FALSE	136, 109, 0	Annelida	TRUE
41	Liocarcinus holsatus	TRUE	TRUE	FALSE	1642, 98, 0	Arthropoda	TRUE
42	Liocarcinus marmoreus	TRUE	TRUE	TRUE	550, 4, 2454	Arthropoda	TRUE
43	Liponema brevicornis	FALSE	FALSE	TRUE	0, 0, 20	Cnidaria	FALSE
44	Loimia ramzega	FALSE	TRUE	TRUE	0, 89, 1213	Annelida	TRUE
45	Lutraria lutraria	TRUE	FALSE	TRUE	1, 0, 1632	Mollusca	TRUE
46	Macoma balthica	TRUE	TRUE	TRUE	84939, 143941, 143331	Mollusca	TRUE
47	Macoma calcarea	FALSE	FALSE	TRUE	0, 0, 10491	Mollusca	FALSE
48	Magallana gigas	TRUE	FALSE	FALSE	2, 0, 0	Mollusca	TRUE
49	Magelona johnstoni	TRUE	TRUE	TRUE	113, 1, 3	Annelida	TRUE
50	Magelona mirabilis	TRUE	TRUE	TRUE	14, 751, 153	Annelida	TRUE
51	Malmgrenia lunulata	FALSE	TRUE	FALSE	0, 1, 0	Annelida	TRUE
52	Malmgrenia sp.	TRUE	FALSE	FALSE	1, 0, 0	Annelida	FALSE
53	Megaluropus agilis	TRUE	FALSE	FALSE	2, 0, 0	Arthropoda	TRUE
54	Membranipora membranacea	TRUE	TRUE	FALSE	21, 73, 0	Bryozoa	TRUE
55	Metridium senile	FALSE	FALSE	TRUE	0, 0, 17148	Cnidaria	TRUE
56	Microprotopus maculatus	FALSE	TRUE	FALSE	0, 13, 0	Arthropoda	TRUE
57	Moerella iridescens	FALSE	FALSE	TRUE	0, 0, 1443	Mollusca	FALSE
58	Monocorophium acherusicum	TRUE	TRUE	FALSE	2, 16, 0	Arthropoda	TRUE
59	Mytilus sp.	FALSE	FALSE	TRUE	0, 0, 4	Mollusca	FALSE
60	Nephtys assimilis	TRUE	TRUE	FALSE	2, 19, 0	Annelida	TRUE
61	Nephtys cirrosa	TRUE	TRUE	TRUE	65397, 130743, 44036	Annelida	TRUE
62	Nephtys hombergii	TRUE	TRUE	TRUE	562, 2783, 89	Annelida	TRUE
63	Nephtys sp.	FALSE	FALSE	TRUE	0, 0, 9	Annelida	FALSE
64	Notomastus latericeus	TRUE	TRUE	FALSE	13, 31, 0	Annelida	TRUE
65	Nototropis swammerdamei	TRUE	TRUE	FALSE	2, 66, 133	Arthropoda	TRUE
66	Ophelia borealis	TRUE	TRUE	FALSE	360, 18904, 0	Annelida	TRUE

Table S6: Presence absence of each species in each DNA based method, to which Phylum the belong and the read count. (*continued*)

	Morphology	Miseq	Nanopore	NovaSeq	Abundance	Phylum
Pholoe baltica	TRUE	TRUE	TRUE	FALSE	2, 6, 64, 0	Annelida
Phyllodoce mucosa	TRUE	FALSE	FALSE	FALSE	1, 0, 0, 0	Annelida
Pinnotheres pisum	FALSE	TRUE	TRUE	TRUE	0, 218, 1179, 28	Arthropoda
Pisidia longicornis	TRUE	TRUE	TRUE	TRUE	2, 300, 398, 1060	Arthropoda
Pocillopora damicornis	FALSE	FALSE	FALSE	TRUE	0, 0, 0, 20	Cnidaria
Poecilochaetus serpens	FALSE	FALSE	TRUE	FALSE	0, 0, 1, 0	Annelida
Polybius henslowii	FALSE	FALSE	FALSE	TRUE	0, 0, 0, 6212	Arthropoda
Pomacea canaliculata	FALSE	FALSE	FALSE	TRUE	0, 0, 0, 116	Mollusca
Protodriloides chaetifer	TRUE	FALSE	FALSE	FALSE	1, 0, 0, 0	Annelida
Psammechinus miliaris	FALSE	FALSE	TRUE	FALSE	0, 0, 3, 0	Echinodermata
Pseudopolydora pulchra	TRUE	FALSE	FALSE	FALSE	1, 0, 0, 0	Annelida
Sabellaria spinulosa	FALSE	FALSE	TRUE	FALSE	0, 0, 4, 0	Annelida
Sagartia ornata	FALSE	FALSE	FALSE	TRUE	0, 0, 0, 8	Cnidaria
Scolelepis bonnieri	FALSE	TRUE	TRUE	FALSE	0, 13631, 21870, 0	Annelida
Scoloplos armiger	FALSE	FALSE	TRUE	FALSE	0, 0, 2, 0	Annelida
Sigalion mathildae	TRUE	FALSE	FALSE	FALSE	1, 0, 0, 0	Annelida
Spio goniocephala	FALSE	TRUE	FALSE	FALSE	0, 3096, 0, 0	Annelida
Spiophanes bombyx	TRUE	FALSE	TRUE	FALSE	5, 0, 54, 0	Annelida
Spirobranchus lamarckii	TRUE	FALSE	FALSE	FALSE	1, 0, 0, 0	Annelida
Spisula elliptica	TRUE	TRUE	TRUE	FALSE	1, 9, 62, 0	Mollusca
Spisula solida	FALSE	FALSE	FALSE	TRUE	0, 0, 0, 2228	Mollusca
Sthenelais boa	TRUE	FALSE	FALSE	TRUE	2, 0, 0, 2819	Annelida
Streptodonta pterochaeta	TRUE	FALSE	FALSE	FALSE	1, 0, 0, 0	Annelida
Tanaissus lilljeborgi	TRUE	FALSE	FALSE	FALSE	1, 0, 0, 0	Arthropoda
Tellimya ferruginosa	TRUE	TRUE	TRUE	FALSE	7, 29, 175, 0	Mollusca
Tryphosa nana	TRUE	FALSE	FALSE	FALSE	1, 0, 0, 0	Arthropoda
Tubificoides diazi	FALSE	FALSE	TRUE	FALSE	0, 0, 6, 0	Annelida
Urothoe brevicornis	TRUE	TRUE	TRUE	FALSE	12, 1097, 1393, 0	Arthropoda
Venerupis corrugata	TRUE	FALSE	FALSE	TRUE	2, 0, 0, 28	Mollusca

	Species	Miseq	Nanopore	NovaSeq	Abundance (read count)	Phylum	In GEANS
67	Ophiothrix fragilis	TRUE	TRUE	FALSE	135, 268, 0	Echinodermata	TRUE
68	Ophiura albida	TRUE	TRUE	TRUE	847, 1091, 8065	Echinodermata	TRUE
69	Ophiura ophiura	TRUE	TRUE	TRUE	637, 10476, 2269	Echinodermata	TRUE
70	Other	FALSE	TRUE	FALSE	0, 15497, 0	N/A	FALSE
71	Owenia fusiformis	TRUE	TRUE	TRUE	1687, 16319, 10000	Annelida	TRUE
72	Pagurus bernhardus	TRUE	TRUE	TRUE	17, 209, 72	Arthropoda	TRUE
73	Paracalanus parvus	FALSE	FALSE	TRUE	0, 0, 8	Arthropoda	FALSE
74	Pariambus typicus	TRUE	TRUE	FALSE	15, 70, 0	Arthropoda	TRUE
75	Pholoe baltica	TRUE	TRUE	FALSE	32, 211, 0	Annelida	TRUE
76	Phyllodoce mucosa	TRUE	TRUE	TRUE	2, 1, 23	Annelida	TRUE
77	Pinnotheres pisum	TRUE	TRUE	TRUE	218, 1180, 28	Arthropoda	TRUE
78	Pisidia longicornis	TRUE	TRUE	TRUE	300, 398, 1060	Arthropoda	TRUE
79	Pocillopora damicornis	FALSE	FALSE	TRUE	0, 0, 461	Cnidaria	FALSE
80	Poecilochaetus serpens	TRUE	TRUE	FALSE	4, 2, 0	Annelida	TRUE
81	Polybius henslowii	FALSE	FALSE	TRUE	0, 0, 6216	Arthropoda	FALSE
82	Polygordius appendiculatus	FALSE	FALSE	TRUE	0, 0, 330	Annelida	FALSE
83	Pomacea canaliculata	FALSE	FALSE	TRUE	0, 0, 378	Mollusca	FALSE
84	Processa modica	TRUE	TRUE	TRUE	26533, 34619, 17332	Arthropoda	TRUE
85	Psammechinus miliaris	TRUE	TRUE	FALSE	4, 21, 0	Echinodermata	TRUE
86	Pseudocuma longicorne	FALSE	TRUE	FALSE	0, 1, 0	Arthropoda	TRUE
87	Sabellaria spinulosa	TRUE	TRUE	FALSE	11, 20, 0	Annelida	TRUE
88	Sagartia ornata	FALSE	FALSE	TRUE	0, 0, 64088	Cnidaria	FALSE
89	Sagartiogeton sp.	TRUE	TRUE	FALSE	108, 18, 0	Cnidaria	FALSE
90	Sagitta setosa	FALSE	FALSE	TRUE	0, 0, 21	Chaetognatha	FALSE
91	Scolelepis bonnieri	TRUE	TRUE	FALSE	31923, 48897, 0	Annelida	TRUE
92	Scoloplos armiger	TRUE	TRUE	FALSE	22, 49, 0	Annelida	TRUE
93	Spio decorata	TRUE	FALSE	FALSE	9, 0, 0	Annelida	TRUE
94	Spio goniocephala	TRUE	FALSE	FALSE	3958, 0, 0	Annelida	TRUE
95	Spiophanes bombyx	TRUE	TRUE	TRUE	220, 3146, 1383	Annelida	TRUE
96	Spiophanes cf.	FALSE	FALSE	TRUE	0, 0, 59	Annelida	FALSE
97	Spisula elliptica	TRUE	TRUE	FALSE	61, 6978, 0	Mollusca	TRUE
98	Spisula solida	FALSE	FALSE	TRUE	0, 0, 68047	Mollusca	TRUE
99	Sthenelais boa	FALSE	FALSE	TRUE	0, 0, 4610	Annelida	FALSE
100	Streptodonta pterochaeta	TRUE	TRUE	FALSE	147, 1255, 0	Annelida	TRUE
101	Streptosyllis websteri	TRUE	TRUE	FALSE	5, 29, 0	Annelida	TRUE

Table S6: Presence absence of each species in each DNA based method, to which Phylum the belong and the read count. (*continued*)

	Morphology	Miseq	Nanopore	NovaSeq	Abundance	Phylum

	Species	Miseq	Nanopore	NovaSeq	Abundance (read count)	Phylum	In GEANS
102	Strongylocentrotus purpuratus	FALSE	FALSE	TRUE	0, 0, 6	Echinodermata	FALSE
103	Tellimya ferruginosa	TRUE	TRUE	FALSE	44, 221, 0	Mollusca	TRUE
104	Temora longicornis	FALSE	FALSE	TRUE	0, 0, 47	Arthropoda	FALSE
105	Thia scutellata	FALSE	TRUE	FALSE	0, 7, 0	Arthropoda	TRUE
106	TPA_asm: Ectopleura	FALSE	FALSE	TRUE	0, 0, 54	Cnidaria	FALSE
107	Tritia reticulata	TRUE	TRUE	TRUE	268, 82, 1400	Mollusca	TRUE
108	Tubificoides diazi	TRUE	TRUE	TRUE	103, 450, 81	Annelida	TRUE
109	Urothoe brevicornis	TRUE	TRUE	FALSE	7771, 12471, 0	Arthropoda	TRUE
110	Venerupis corrugata	FALSE	FALSE	TRUE	0, 0, 5743	Mollusca	FALSE

Table S7: List of species that were not found in any DNA based method. Curated database? Represents whether or not the species was present in the GEANS macrobenthos database.

	Morphology	Miseq	Nanopore	NovaSeq	Abundance	Phylum	Curated database?
Abludomelita obtusata	TRUE	FALSE	FALSE	FALSE	8, 0, 0, 0	Arthropoda	YES
Aonides oxycephala	TRUE	FALSE	FALSE	FALSE	1, 0, 0, 0	Annelida	NO
Aonides paucibranchiata	TRUE	FALSE	FALSE	FALSE	1, 0, 0, 0	Annelida	YES
Apolochus neapolitanus	TRUE	FALSE	FALSE	FALSE	1, 0, 0, 0	Arthropoda	YES
Capitella minima	TRUE	FALSE	FALSE	FALSE	2, 0, 0, 0	Annelida	NO
Caulleriella alata	TRUE	FALSE	FALSE	FALSE	6, 0, 0, 0	Annelida	NO
Eteone longa	TRUE	FALSE	FALSE	FALSE	5, 0, 0, 0	Annelida	YES
Eumida sanguinea	TRUE	FALSE	FALSE	FALSE	11, 0, 0, 0	Annelida	NO
Harmothoe glabra	TRUE	FALSE	FALSE	FALSE	3, 0, 0, 0	Annelida	NO
Kurtiella bidentata	TRUE	FALSE	FALSE	FALSE	5, 0, 0, 0	Mollusca	NO
Mediomastus fragilis	TRUE	FALSE	FALSE	FALSE	21, 0, 0, 0	Annelida	NO
Mytilus edulis	TRUE	FALSE	FALSE	FALSE	3, 0, 0, 0	Mollusca	YES
Nototropis swammerdami	TRUE	FALSE	FALSE	FALSE	2, 0, 0, 0	Arthropoda	YES
Petricolaria pholadiformis	TRUE	FALSE	FALSE	FALSE	1, 0, 0, 0	Mollusca	NO
Phyllodoce mucosa	TRUE	FALSE	FALSE	FALSE	1, 0, 0, 0	Annelida	YES
Protodriloides chaetifer	TRUE	FALSE	FALSE	FALSE	1, 0, 0, 0	Annelida	NO
Pseudopolydora pulchra	TRUE	FALSE	FALSE	FALSE	1, 0, 0, 0	Annelida	NO
Sigalion mathildae	TRUE	FALSE	FALSE	FALSE	1, 0, 0, 0	Annelida	YES
Spirobranchus lamarckii	TRUE	FALSE	FALSE	FALSE	1, 0, 0, 0	Annelida	NO
Streptodonta pterochaeta	TRUE	FALSE	FALSE	FALSE	1, 0, 0, 0	Annelida	YES
Tanaissus lilljeborgi	TRUE	FALSE	FALSE	FALSE	1, 0, 0, 0	Arthropoda	NO
Tryphosa nana	TRUE	FALSE	FALSE	FALSE	1, 0, 0, 0	Arthropoda	NO
						14 annelida	
					9 annelida	13 no db	

Table S8: Singletons used for species identification of Nanopore method. The species that were found in the fraction of reads that remained Singleton after processing the Nanopore reads with Decona.

See online publication

Chapter 3

The Long and the Short of It: Nanopore-Based eDNA Metabarcoding of Marine Vertebrates Works; Sensitivity and Species-Level Assignment Depend on Amplicon Lengths

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ABSTRACT

To monitor the effect of nature restoration projects in North Sea ecosystems, accurate and intensive biodiversity assessments are vital. DNA-based techniques and especially environmental (e)DNA metabarcoding is becoming a powerful monitoring tool. However, current approaches rely on genetic target regions under 500bp, offering limited taxonomic resolution.

We developed a method for long-read eDNA metabarcoding, using Nanopore sequencing of a longer amplicon and present DECONA, a read processing pipeline to enable improved identification of marine vertebrate species. We designed a universal primer pair targeting a 2kb region of fish mitochondrial DNA and compared it to the commonly used MiFish primer pair targeting a ~170bp region.

In silico testing showed that 2kb fragments improved accurate identification of closely related species. Analyzing eDNA from a North Sea aquarium showed that sequences from both primer pairs could be assigned to most species, and additional species level assignments could be made through the 2kb primer pair. Interestingly, this difference was opposite in eDNA from the North Sea, where not the 2kb but the MiFish primer pair detected more species.

This study demonstrates the feasibility of using long-read metabarcoding for eDNA vertebrate biodiversity assessments. However, our findings suggests that longer fragments are less abundant in environmental settings, but not in aquarium settings, suggesting that longer fragments may provide a more recent snapshot of the community. Thus, long-read metabarcoding can expand the molecular toolbox for biodiversity assessments by improving species-level identification and may be especially useful when the temporal origin of the eDNA signal is better understood.

Keywords
eDNA, North Sea, metabarcoding, nanopore, long read, fish

INTRODUCTION

North Sea fish populations are sensitive to disturbances such as fisheries, nutrient run-off and increasing sea water temperatures (Andersen et al., 2020; Capuzzo et al., 2018; Hofstede et al., 2010; Krehenwinkel et al., 2019; O'Brien et al., 2019). Combined management strategies such as reduced fishing (Couce et al., 2020), designation of marine protected areas (MPA), and placing artificial hard substrates such as offshore wind parks are suggested to facilitate rehabilitation of the North Sea ecosystem (Claudet, 2018; Degraer et al., 2020; Didderen et al., 2019; Kamermans et al., 2018). To understand how North Sea fish population dynamics are affected by these strategies, development and validation of methods that map fish population diversity and density is crucial. Conventional marine fish biomonitoring practices largely rely on destructive methods that involve netting and trapping (Daan et al., 2005; Reiss et al., 2010). These methods are costly, time-consuming and require expert taxonomic visual identification skills (Mateos-Rivera et al., 2020; Teletchea, 2009). In addition, conventional methods have limited sampling efficiencies and may be disruptive to the environment (Eggleton et al., 2018). Thus, it is crucial to develop precise and non-invasive biomonitoring solutions that are also time and cost efficient (Goodwin et al., 2017).

Environmental DNA (eDNA) based fish species identification has gained substantial attention in the last decade, as it can detect the presence of fish species based on a small amount of DNA present in seawater. It has been shown to be highly sensitive for non-indigenous species detection (Ficetola et al., 2008) and identification of spawning and migration patterns (Thalinger et al., 2019). Short amplicon eDNA metabarcoding has become an increasingly popular tool to perform fish community assessment for identification of ecological relevant fish species from an array of ecosystems (Deiner et al., 2017; Miya et al., 2015; Ruppert et al., 2019; Taberlet et al., 2012; Thomsen et al., 2012a). Also in the North Sea, species identified through metabarcoding of eDNA samples showed to be comparable to species caught in fyke nets in the same area (Bleijswijk et al., 2020).

The standardization of eDNA metabarcoding as marine monitoring strategy is still under development. Species-specific differences occur e.g., in degree of skin cell shedding, degradation rates vary depending on temperature and season, and unknown dilution factors depending on currents all make quantification of the results challenging (Beng & Corlett,

2020; Lacoursière-Roussel et al., 2016; Sassoubre et al., 2016; Seymour et al., 2018). The sample preparation, metabarcoding technique and workflow will determine the quality of the results and thus the species detection quality and possible biases (Beng & Corlett, 2020; van der Loos & Nijland, 2021). Important steps in the protocol include decisions about methods of sampling and DNA extraction (Bessey et al., 2020; Hunter et al., 2005), primer and PCR settings (Doi et al., 2019; Sard et al., 2019; Zhang et al., 2020), sequencing technology (Egeter et al., 2020; Singer et al., 2019; Truelove et al., 2019), post-sequencing data handling (Santos et al., 2020) and reference databases used (Hestetun et al., 2020; McGee et al., 2019).

Especially choice of primer pair and targeted DNA region are crucial for successful fish detection with eDNA (Beng & Corlett, 2020). Several universal fish primer pairs are described and most target regions of the mitochondrial genome as there is a high copy number of this genome per cell (Schon, 2000). The most used primer pairs target different short regions from 100 to 500 nucleotides of the 12S rRNA (Miya et al., 2015; Riaz et al., 2011; Taberlet et al., 2012), 16S rRNA (DiBattista et al., 2017; Evans et al., 2016), cytochrome B (Thomsen et al., 2012b) and COI gene (Balasingham et al., 2018). Although primer pairs targeting short 12S regions are most used and considered as a standard (Shu et al., 2020), a longer target amplicon size facilitates distinguishing between closely related species and hence improves species level identification (Zhang et al., 2020). The use of multiple primer pairs is also suggested to increase taxonomic resolution (Evans et al., 2016; Miya et al., 2015; Zhang et al., 2020) and improved species level detectability has been demonstrated in lakes (Sard et al., 2019). Thus, using longer fragments and multiple markers can enhance the taxonomic resolution in metabarcoding studies.

Long read sequence analysis has been shown to be useful for species identification before in barcoding studies using Sanger sequencing (Hebert et al., 2003). Sanger sequencing accurately provides a DNA sequence from one individual but lacks the possibility of sequencing mixed communities like metabarcoding methods can (Kappel et al., 2017). On the other hand, commonly used Illumina platforms do not allow the use of long reads due to its ability to sequence with high accuracy but with a maximum read length of 500bp (Tan et al., 2019). Fortunately, third generation sequencing as available from Oxford Nanopore Technologies (ONT) and Pacific Biosciences enables the generation of ultra-long sequences and from mixed communities (Bleidorn, 2016). This can be used

for eDNA studies that are based on primer pairs targeting longer regions, covering several mitochondrial marker genes. This long amplicon approach was demonstrated to be successful in microbial metabarcoding studies and improved taxonomic assignment to species level (Johnson et al., 2019; Shin et al., 2016). Historically, the main limitation of nanopore sequencing was the large error rate of 5 to 10% (Jain et al., 2015). This error rate can be overcome with bioinformatics tools to generate reliable consensus sequences and thus increase sequence accuracy (Baloğlu et al., 2021; Carradec et al., 2018; Egeter et al., 2022; Sahlin et al., 2021). To our knowledge, a bioinformatics pipeline that require little command-line experience and generate a species list directly from raw sequence data from multiplexed metabarcoding experiments is not yet available for Nanopore short and long read metabarcoding. However, once installed, such a pipeline would greatly facilitate the development of DNA monitoring, as it also becomes feasible for non-experts in bioinformatics.

This study assesses the utility of long and short read eDNA metabarcoding for fish and vertebrates using Oxford Nanopore sequencing. We present a new bioinformatics pipeline DECONA to analyze the obtained data, and we discuss the optimal settings of DECONA depending on amplicon length and sequencing chemistry. A new primer pair was developed, specific for fish and other vertebrates targeting a 2kb fragment of both the 12S and 16S region of the mitochondrial rRNA genes. The primer pair was compared to the commonly used universal short read MiFish primer pair targeting a ~170 bp region of the 12S mitochondrial rRNA (Miya et al., 2015). We compared primer pairs *in silico* on several genetically similar species (<3 nucleotide differences between species), to identify the discrimination power of each primer pair to species level. We also compared the sensitivity and taxonomic resolution resulting from both primer pairs using samples from a North Sea Ray Reef aquarium with a known species composition and field samples from distinct locations and habitat types in the North Sea. The DECONA pipeline was developed especially for Oxford Nanopore sequence data to increase the accuracy with which sequences can be assigned to species level and to reduce the bioinformatics skills required for analysis of the sequences.

MATERIALS AND METHODS

Sample collection – Ray reef aquarium

Two 1L water samples were collected from the aquarium, just under the water surface using a 1L plastic container pre-sterilized with bleach **(figure 1A**). One negative field control filter was taken by filtering demineralized tap water (**table S1**). The aquarium has a volume of 200m^3 artificial seawater and represents a North Sea reef that contains bony fish, sharks, and rays with a total of 18 species (**table S2**, for species list and abundance). The water temperature was 13°C, the salinity at 32.0‰ and the pH at 8.2 at the day of sampling.

Sample collection – Gemini Wind Park/Borkum Reef Grounds

From Gemini Wind Park/Borkum Reef Grounds, samples were collected from inside Gemini Wind Park (54.0109N, 6.0781E), halfway between Gemini Wind Park and the Borkum Reef Grounds on sandy substrate (53.8645N, 6.2145E) (Sandy bottom) and at Borkum Reef Grounds (53.7016N, 6.3467E using the WGS84 reference system). All samples were taken in July 2020 at slack tide during neap tides. Data on environmental parameters at the North Sea sampling locations were retrieved from the Copernicus Marine Service's Data Portal. Salinity varied in July 2020 between 31.8‰ and 34.4‰, temperature 15.7-18.6°C, and pH 7.9-8.1. Three 1L replicates were collected at each location by sampling seawater using 2.5L Niskin bottles at 0.5-1m above the seafloor (**figure 1B**). One negative field control filter was taken by filtering demineralized tap water (**table S1**).

Sample collection – Shipwrecks

For the shipwrecks, samples were collected around three different shipwrecks in the North Sea while SCUBA diving in July 2019: wreck 1 (55.1821N, 03.4446E, wreck 2 (55.2609N, 03.5117E) and wreck 3 (55.0774N, 02.5087E) **(figure 1C).** On the sampling days and locations, salinity ranged between 34.2‰ and 34.27‰, temperature between 11-12°C and pH was 8.06. Wreck 1 was sampled at 36 metres depth, Wreck 2 at 32 meters depth and wreck 3 at 30 meters depth. At each sample location, three replicates of Several litres of water were collected at North Sea wreck sites near the bottom by scuba diving. The pump lever of a hand-operated pump (ProPlus air & siphon pump 2-in-1 red, EAN 8717568798967) was operated 15-20x to completely flush out the pump-housing and tubing, before a punch balloon (Punch balloons,

EAN 8021886316360) was attached to the outflow tube using a connector made of a 15ml tube with the tip cut off. A 1mm mesh was secured over the inflow tube with a rubber band. The inflow tube was held at the intended sampling site, and water was pumped in the balloon. The filled balloon was then clamped using 2 plastic sealing clips (BEVARA sealing clip, IKEA), and the balloon was stored in a mesh bag clipped to the diver's wing. One negative field control filter was taken by filtering tap water (**table S1**).

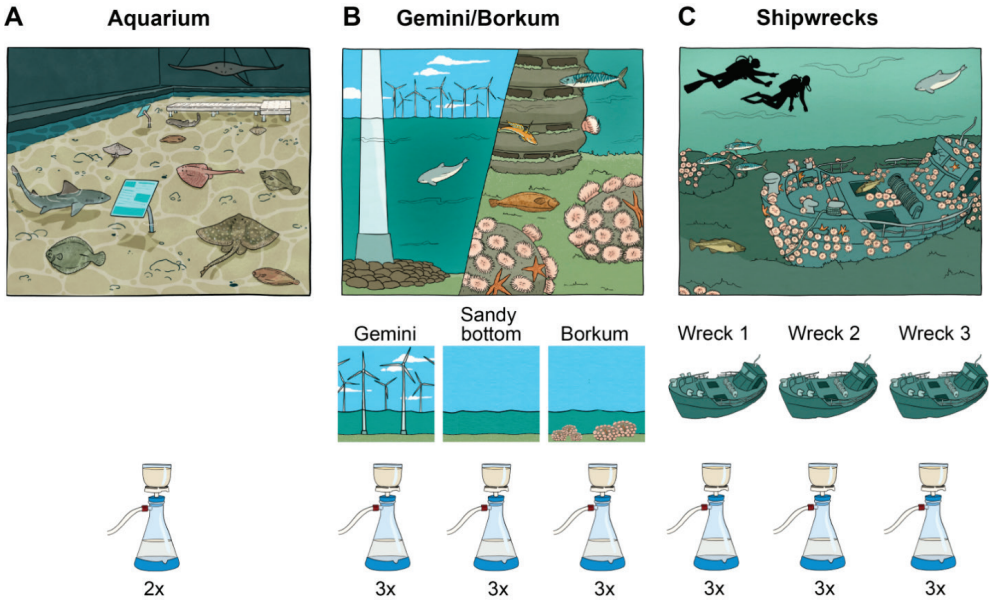


Figure 1: Sampling design of **A)** North Sea "Ray Reef" aquarium, Dolfinarium, Harderwijk, the Netherlands. 2x 1 liter water just under the water surface using a 1L plastic containers. **B)** Borkum/Gemini where samples were taken in Gemini Wind Park, halfway between Gemini and Borkum on a sandy bottom and on the Borkum Reef Grounds. Seawater was collected using 2.5L Niskin bottles. **C)** North Sea shipwrecks with three different shipwreck locations where samples were taken near shipwrecks while diving, using an underwater pump with a balloon attached.

Filtering sample water

All samples were immediately filtered using Thermo Scientific Nalgene Rapid-Flow sterile disposable Filter Units CN (Cellulose nitrate) with a pore size of 0.8µm. Filters were then individually placed in 2mL screw-cap Eppendorf tubes. The tubes were prefilled with 400µL Zymo DNA/RNA shield (Zymo, USA) preservative. Samples were immediately stored at -20°C for a maximum of one month before further processing.

Primer design

Primer design is based on the adjacent ribosomal genes 12S and 16S of the mitochondrial genome of bony fish present in the North Sea according to a curated database of Dutch species (Naturalis Biodiversity Center, Nederlands soortenregister) consulted in 2019. The primer pair was designed *in silico* in Geneious prime 2019.0.4 (Kearse et al., 2012) and based on the NCBI available mitochondrial genomes of the target species (**table S3**). A consensus sequence for each species was constructed when multiple genomes were available from the same species using default settings of the MAFFT alignment tool (v7.450, Katoh & Standley, 2013) incorporated in Geneious. Consensus sequences of all species were aligned and forward and reverse primers was designed manually by locating regions with low genetic variation between target species. This resulted in a long read universal fish primer pair (**table 1**) targeting a 2kb fragment from ~450bp downstream the start of the 12S rRNA gene in forward direction and ~300bp upstream the end of the 16S rRNA gene (**figure 2A**). The 5' ends of the primers were extended with an ONT tag to allow for direct PCR based sample barcoding in downstream library preparation. To validate the 2kb primer pair *in silico*, the primer pair was aligned against a curated North Sea database (see below) using Geneious prime 2023.0.4 (Kearse et al., 2012) in the "test with saved primers" mode (Primer3.2.3.7) allowing for 2 mismatches in the binding region. The primer pair was further validated with cutadapt v1.15 (Martin, 2011) and showed that all mitochondrial sequences present in the database aligned with the primer pair in the target region.

Table 1: Primer sequences and characteristics of the newly designed forward and reverse primer for the 2kb target region, including the ONT-specific primer extension enabling PCR barcoding (underlined).

	Sequence	T_m†	G/C content
Fish_12S_fw1-ONT	TTTCTGTTGGTGCTGATATTGCGGATTAGATACCCYACTATGY	56.3°C - 60.4°C	38.1-47.6%
Fish_16S_rv1-ONT	ACTTGCCTGTCGCTCTATCTTCGATTGCGCTGTTATCCCTRG	61.2°C - 64.1°C	50-55%

† Calculations by ThermoFisher Scientific Tm calculator for Phire DNA polymerase, for the sequence specific part only

In silico comparisons of species groups with little interspecific genetic differences.

In silico comparative alignments were made from different taxonomic groups relevant for this study (e.g sharks, rays, wrasses, gurnards, flatfishes, gobies, sand eels, mullets etc., data not shown) of (partial) mitochondrial references from the NCBI database. Genetically closely related species were aligned using Muscle 5.1 (Edgar, 2004) multiple alignment tool in Geneious prime (**table S4**, for accession numbers). Target regions of the different primer pairs were identified using "saved primers mode" (Primer3.2.3.7) allowing for 2 mismatches in the binding region.

DNA extraction

Two different DNA extraction kits were used for different datasets due to the availability of kits in our lab at the time of processing. DNA from the aquarium samples was extracted using the DNeasy Blood & Tissue kit (Qiagen, USA). 20µL Proteinase K was added to the samples in DNA/RNA shield, together with 400µL lysis buffer and followed by 400µL 70% ethanol. Further DNA extraction was performed using this kit following the protocol for tissue samples. DNA concentrations were measured using a Qubit 2.0 Fluorometer (Invitrogen, USA). DNA from filters from the two North Sea datasets were extracted using the Quick-DNA miniprep kit (Zymo, USA) according to the manufacturer's instructions. Details of both protocols are also given at protocols.io (dx.doi.org/10.17504/protocols.io.6yfhftn).

Mock community

To further test the test the primer design, DNA extracts of 12 North Sea fish species from different taxonomic groups were pooled in equimolar concentrations. This mock community contained: *Arnoglossus laterna, Chelon labrosus, Chelon ramada, Gadus morhua, Gasterosteus aculeatus, Petromyzon marinus, Neogobius melanostomus, Phycis blennoides, Salmo trutta, Scophthalmus rhomus, Solea solea* and *Trisopterus luscus* (**table S14**). Of *Phycis blennoides, Scophthalmus rhomus and Petromyzon marinus*, no reference sequence is available, therefore these species were also separately amplified and sequenced, and the consensus sequence was used to correctly identify the species in the mock community.

Amplification

For PCR amplification of the samples with the 2kb primer pair (aquarium, North Sea and shipwreck samples), 10µL 2x Phire Tissue Direct PCR Master Mix (ThermoFisher Scientific, USA) was used. To the master mix

0.4µL of each primer (10mM), 0.5µL eDNA template and nuclease free water (NFW) was added to a total of 20µL. eDNA template was replaced with 0.5µL NFW in case of PCR controls. Samples that were amplified with the MiFish primer pair (aquarium and North Sea) consisted of 5µL 2x Phire Tissue Direct PCR Master Mix in combination with 1µL template and 0.2µL of each primer, and NFW added to a total of 10µL. To reduce the effect of stochastic heterogeneity in PCR amplification, each sample was amplified using 3 PCR replicates. For the amplification with the 2kb primer pair of the aquarium samples PCR settings were 98°C 180s, 98° 8s sec, 57°C for 10s, 72°C 30s, and 72°C 3min with 36 cycles. For amplification with the MiFish primer pair, PCR settings were 98°C 180s, 98° 10 sec, 59.6°C for 8, 72°C 10s, and 72°C 3min with 35 cycles. PCR replicates were pooled prior to purification with SPRI magnetic beads (2:1 ratio).

Nanopore sequencing

All samples were barcoded using the PCR barcoding kit 96 (EXP-PCB096), and sequencing libraries were created using the SQK-LSK114 kit (Oxford Nanopore Technologies Ltd., UK). The following adaptations were made from the manufacturer's instructions: barcoding PCR was performed in a total volume of 15µL containing 0.3µL PCR barcode primer pair and 10-50ng amplicon. The applied barcode PCR program was as follows: initial denaturation at 95°C for 180s, 15 cycles of 95°C for 15s, 62°C for 15s (10s for MiFish), 65°C for 90s, followed by a final extension at 65°C for 180s. A negative control was taken along in which NFW was added instead of first-round PCR amplicon template. After the barcoding PCR, sample concentration was estimated using the Qubit HS kit on the non-purified barcoded PCR products, and samples were pooled in equimolar ratios. The pooled amplicon sequence library was cleaned using SPRI magnetic beads, washed once with freshly prepared 70% ethanol and once with a 2:1 mixture of Long Fragment Buffer (LFB) and Short Fragment Buffer (SFB) (LFB and SFB are supplied with the Ligation sequencing kits from ONT) to enrich for the 2kb target size fragments, and only SFB for MiFish samples. During final clean-up, the library was again washed in a 2:1 mixture of LFB and SFB (2kb) or SFB only (MiFish). A maximum of 100ng DNA was loaded on a primed flow cell to prevent overloading of the flow cell. For the 2kb samples, sequencing was performed until a sequencing depth of 4082320 reads. MiFish samples were sequenced with a sequencing depth of 2.059.490 reads per barcode for aquarium and Gemini wind park/ Borkum reef ground samples and 15.046.781 reads from Wreck samples. Sequencing was performed with a R10.4.1 flow cell on an Oxford

Nanopore MinION Mk1C device with a sequencing speed of 450 bases per second. For all PCRs, negative controls were considered (**table S1**).

Sequence read processing with DECONA

To process sequencing data and generate consensus sequences from mixed samples we designed a bioinformatics pipeline called DECONA (https://github.com/Saskia-Oosterbroek/DECONA). This pipeline clusters Nanopore reads, aligns them, creates a consensus sequence, and has the option to apply ONT specific polishing. The DECONA pipeline takes fastq files as input. The DECONA pipeline starts with filtering the fastq files on desired length and quality score with Nanofilt v2.8.0 (De Coster et al., 2018). Then, cutadapt v4.8 (Martin, 2011) is optionally used to trim primer sequences from the reads. CD-hit v4.8.1, a program that clusters reads based on short words rather than sequence alignment is used to cluster the reads based on a set percentage of similarity (W. Li et al., 2002). The clustered reads are subsequently aligned using Minimap2 v2.21 (H. Li, 2018). Based on these alignments, Racon v1.4.20 is used to build the initial draft consensus sequence of each cluster (Vaser et al., 2017) which is then optionally polished by Medaka v1.4.3 (Oxford Nanopore Technologies Ltd., UK)

In this research, base-calling of the raw fast5 files was performed using Guppy (Version 6.5.7, Oxford Nanopore Technologies Ltd., UK) in super high accuracy (SUP) mode for the MiFish samples of Aquarium and Gemini/Borkum reef ground. For all 2kb and MiFish samples of the Wreck dataset, basecalling of pod5 files was performed using Dorado (Version 0.8.1, Oxford Nanopore Technologies Ltd., UK) After this, DECONA1.5 was used to filter the fragments of the 2kb primer pair dataset at 1800-2350 bases and cluster these reads at 95% similarity. For the fragments of the MiFish primer pair dataset filtering was set between 160 and 240 bases and clustering at 97% similarity. All data were filtered at the default quality score of Q10. Large clusters were set to be randomly subsampled to a maximum cluster size of 500 reads. Medaka polished consensus sequences were created from each cluster larger than 5 reads. Initial polished consensus sequences were re-clustered at 99%. The commands used to run DECONA were as follows:

2kb R10: decona -f -T 32 -l 1800 -m 2350 -g "GGATTAGATACCCYACTATGY;max_error_rate=0.1;min_overlap=17...CYAGGGATAACAGCGCAATC;max_error_rate=0.1;min_overlap=17" -n 10

-r -o 0.99 -R 500 -k 6 -M -c 0.95 -b /home/reindert/Blast_database/eDNA_NZ_23/North_sea_232

MiFish R10: decona -f -T 32 -l 160 -m 240 -g "GTYGGTAAAWCTCGTGCCAGC;max_error_rate=0.1;min_overlap=20...CAAACTYGGATTAGATACCCCACTAT;max_error_rate=0.1;min_overlap=20" -n 10 -r -o 0.99 -R 500 -k 6 -M -c 0.97 -b /home/reindert/Blast_database/eDNA_NZ_23/North_sea_232

DECONA settings

Different combinations of raw read Q-score (-q) and cluster similarity (-c) settings were tested to determine their optimal use in various laboratory choices. Samples from the ray reef aquarium, including both 2kb and MiFish samples, were utilized to assess optimal settings for different amplicon target lengths. Additionally, various cluster similarity settings were tested on the wreck samples to determine optimal settings for different sequencing chemistries. Optimal settings were determined by running all combinations of cluster similarities (0.80, 0.85, 0.90, 0.95, 0.97, 0.99 (2kb only), and 1) and Q-scores (8, 10, 12, 15, 17, and 20) in looped DECONA runs. Total reads, consensus sequences (clusters), and identified species were recorded for each setting. Based on the highest number of species found, the optimal settings were chosen for the consensus building with DECONA.

Curated North Sea fish reference database building

For taxonomic identification, an in-house reference database was compiled based on whole mitochondrial genome sequences available in the NCBI database for North Sea fish species (last search October 2024, Table S5). When the whole mitochondrial genome was not available, available sequences of (fragments of) the 12S and/or the 16S rRNA genes from these species were added to the database. To validate correct species identification, closely related species that do not occur in the North Sea were also added to the database. Although our primer design was based on mitogenomes of bony fish, the resulting primer pair turned out to be universal not only to bony fish, but also to elasmobranchs and other marine vertebrates. Therefore, these taxonomic groups were also added to the database. In addition, frequently occurring contaminants as of human, chicken, cow, and pig were added to the database to prevent a large portion of unidentified reads resulting from contamination. The complete database consisted of 536 sequences of which 113 were complete

mitogenomes and 30 were complete 12S and 16S regions **(table S5)**. The database contained 222 unique species.

Taxonomic assignment of consensus sequences

The BLASTn (NCBI, version 2.11.0) function that was built within the DECONA environment was used against our North Sea fish reference database for taxonomic assignment of the consensus sequences derived from DECONA. To automate further assessment of the BLASTn output for accurate species-level identification, a script was developed in R studio (2022.12.0) and integrated into DECONA. This script can be found at github.com/karlijn-doorenspleet/decona-postprocessing/. This script retrieved the taxonomic lineage from NCBI using *taxize* (v0.9.96, Chamberlain & Szöcs, 2013). The top five hits were considered based on the highest e-value for each consensus sequence. Within each top five hit, sequence was labelled as *unclassified* on species level if they had the same e-value, percentage identity and alignment length but did not share the same taxon (species, genus, family, order, class, phylum). After that, the top hit (based on e-value) was kept for further quality threshold control. As such, species that shared the same sequence and had a high similarity hit with the reference database, were excluded from species level taxonomic assignment to avoid misidentifications (for examples of such cases, see **table S6**). Of all the taxonomically assigned sequences, top hits were considered for species level assignment, based on specific thresholds per amplicon length. For the 2kb fragment the thresholds were a minimal alignment length of 1100 nucleotides with <30 mismatches and >98% identity for species level assignments. Hits with percentage identities >97% sequences were assigned on genus level and with >95% sequences were assigned at family level but not further considered for this study. The following thresholds were considered based for the MiFish fragment: <4 mismatches, >98% identity, and a minimal alignment length of 100 nucleotides. Assignments that did not meet these thresholds were renamed to *unclassified* and this was applied to both 2kb and MiFish fragments. The finding of *Cheliyoditchus lucerna* in the ray reef aquarium (2kb) and *Molva molva* in the Gemini/Borkum dataset (2kb) are indicated with a star, as for these findings a species level assignment of *Cheliyoditchus kumu* and *Molva dypterygia* was found respectively. The assumingly correct species was reported with a star, as incorrect species identification happened due to the lack of 12-16S fragments of the North Sea species in the database.

Analysis of taxonomic assignments

Rarefaction curves were plotted (vegan package, v 2.6-4, **table S7**) and showed flatting curves, indicating that enough sequencing depth was reached for all samples, and no samples were further rarefied or removed. Sequence abundance was log10 transformed for all datasets. Reads classified as belonging to the genera *Homo*, *Ovis*, *Gallus*, *Bos* and other non-marine animals were set to *unclassified*, along with all consensus sequence that did not have a hit with a database on species level (see **table S8** for the read percentage of non-target hits per barcode). For alpha diversity, both Shannon indices and observed values were calculated and were tested using Shapiro-Wilk for normal distribution of the data, two-way ANOVA to test for significant differences between alpha diversities, primer pair and location, and post hoc Tuckey HSD test for pairwise comparison. For beta diversity, non-metric multidimensional scaling ('bray') was performed in combination with *betadisper* to check for homogeneity of variance and PERMANOVA to analyse the effect of treatments between samples (adonis, vegan). Post-hoc analysis was performed using the *pairwise.adonis* package in combination with *devtools* when applicable. Sequencing of control samples and PCR controls resulted no reads at all or, non-target species (e.g. *Homo sapiens*) in all control samples. Only the Wreck MiFish control samples, that contained *Pomatoschistus microps* which did not occur in any other samples. Control samples were therefore excluded from further analysis (**table S1**).

In silico comparisons of species groups with little interspecific genetic differences.

In silico comparative alignments were made from different taxonomic groups relevant for this study (e.g sharks, rays, wrasses, gurnards, flatfishes, gobies, sand eels, mullets etc., data not shown) of (partial) mitochondrial references from the NCBI database. Genetically closely related species were aligned using Muscle 5.1 (Edgar, 2004) multiple alignment tool in Geneious prime (**table S3**, for accession numbers). Target regions of the different primer pairs were identified using "saved primers mode" (Primer3.2.3.7) allowing for 2 mismatches in the binding region.

RESULTS

In silico comparison of primer pair performance on closely related taxa

In silico alignments show *Pleuronectes platessa* and *Platichthys flesus* target regions differ 1 nucleotide when using the MiFish primer pair (99.4% similarity) whereas the target region of the 2kb primer pair has 12 nucleotide differences (99.3% similarity) **(figure 2B)**. *Clupea harengus* and *Sprattus sprattus* diverged by 4 nucleotides (98.3% similarity) in the MiFish target region and their 2kb target region showed a pattern of 29 nucleotide differences (98.6% similarity) (**figure 2C**). Sand eel species *Ammodytes marinus*, *Ammodytes personatus*, *Ammodytes tobianus* and *Hyperoplus lanceolatus* also show 1 nucleotide mismatch (99.6% similarity) between all species for the MiFish target region. From the 2kb target region, *Ammodytes marinus* differed from *Ammodytes tobianus* and *Hyperoplus lanceolatus* with 29 and 30 nucleotide differences respectively, (98,3% similarity) whereas between *Ammodytes tobianus* and *Hyperoplus lanceolatus* the genetic diversity remains low with 3 nucleotide differences (99,8% similarity) (**figure 2D**). *Raja brachyura* and *Raja microocellata* showed 4 nucleotide differences for the MiFish fragment (98,3% similarity), while *Raja montagui* differed 2 nucleotides with from both *Raja microocellata and Raja brachyura* (99,1% similarity). Nucleotide differences greatly increase when comparing the 2kb region: 41 nucleotide differences between *Raja brachyura* and *Raja microocellata* (97,7% similarity), 47 between *Raja brachyura* and *Raja montagui* (98% similarity), and finally 53 between *Raja microocellata and Raja montagui* (97,4% similarity) (**Figure 2E**).

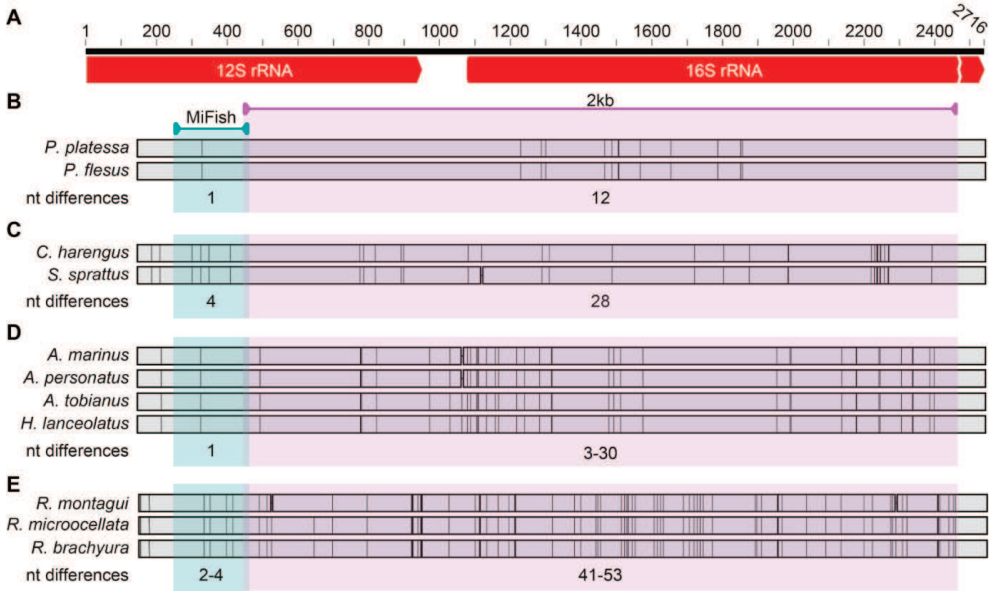


Figure 2. A) The position of the new 2kb primer pair (violet) and the MiFish primer pair (blue). **B)** Genetic diversity between *Platichtys flesus* and *Pleuronectes platessa* for the target regions of the different primer pairs. The MiFish target region has 1 mismatch whereas the 2 kb region has 12 mismatches **C)** *Sprattus sprattus* and *Clupea harengus*, where the MiFish target regions contains 4 mismatches and the 2kb target regions contains 28 mismatches **D)** four different *Ammodytes* species (*Ammodytes marinus*, *Ammodytes personatus*, *Ammodytes tobianus* and *Hyperoplus lanceolatus*) with 1 mismatch in the Mifish target region and 3-30 mismatches in the 2kb target region. **E)** *Raja montagui*, *Raja microocellata and Raja brachyura.* Between 2-4 (MiFish) and 41-53 mismatches (2kb). The (range of the) number of pairwise differences is indicated below each alignment.

Optimal DECONA settings are different per primer pair

Testing of different Q-score and cluster similarity settings in DECONA shows the optimal settings differ per experimental setup. For the 2kb amplicon, the number of unique clusters and species is highest with a cluster similarity of 0.95 (clusters and species) and 0.97 (species). Q-score is of lesser influence, especially when considering unique species (**figure 3A and C**). The MiFish amplicon shows the highest number of clusters and species when setting a clustering similarity of 0.97. Also here, Q-score influences this number to a lesser extent: anything between Q8 and Q17 results in a similar number of clusters or species (**figure 3B and D**). The total read count drops for both primer pairs when settings are too stringent (i.e., Q20 or clustering similarity of 1) (**figure 3E and F**).

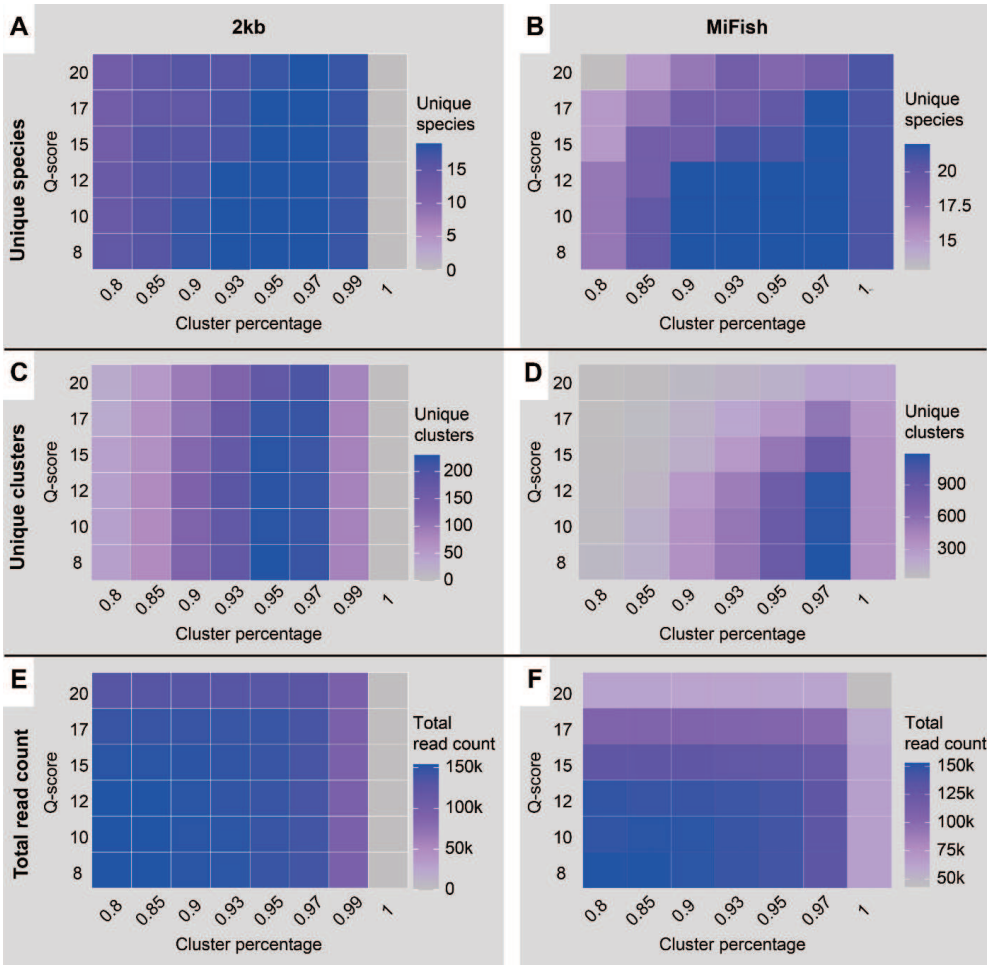


Figure 3: comparison of DECONA settings for the R10 SUP basecalled reads. The number of unique species that are found when using reads from a certain minimal q-score (8,10,12,15,17,20) in combination with different minimal % identity threshold values for clusters (cluster percentage, 0.8,0.85,0.9,0.93,0.95,0.97,0.99 (2kb only) and 1) of **A)** 2kb reads and **B)** Mifish reads. Color gradients indicate the number of species found. Where **C)** is the number of unique clusters found with each minimal q-score in combination with each cluster percentage for 2kb and **D)** MiFish. Color gradients indicate the number of unique clusters found. **E)** The reads that remain with each minimal q-score in combination with each cluster percentage for 2kb and **F)** MiFish. Color gradients indicate the total reads found.

Most diversity obtained with both primer pairs in aquarium samples, but species composition varies

Sequencing of aquarium samples yielded 529.064 (2kb) and 220.963 (MiFish) reads, of which 453.714 (2kb) and 152.395 (MiFish) reads were used for consensus building (**table S9**). A barcode distribution of 226.856±160.905 (2kb) and 76.198±8.010 (MiFish) reads per barcode was achieved. Shapiro-Wilk showed normally distributed data (Shannon: p=0.325, Observed p=0.406) and no significant difference in Shannon index (t-test: p= 0.5476) or richness (t-test: p=0.350) although on average more species were found with the 2kb primer pair (**table S10**).

Analysis of the mock community showed that all species could be detected using the 2kb Primer pair **(table S14**). From the aquarium samples, Eight out of 18 species present in the aquarium could be detected with both primer pairs and an additional seven species could be obtained with 2kb sequence assignments (**figure 4A**). One additional species could be detected with MiFish sequence assignments. Four species were detected with both primer pairs but were not reported as aquarium inhabitants. Sequences from both primer pairs were sometimes incorrectly assigned to a species that belonged to the same genus as the species present in the aquarium (e.g. *Mustelus manazo*, **figure 4B**). A total of 2 species that resided in the aquarium could not be detected in the eDNA samples by either of the primer pairs. These had only one or two individuals in the aquarium of a total of 301 individual fish individuals. *Scopthalmus rhombus* was not present in the reference database of either primer pair. Read count per species and sample can be found in **table S11**.

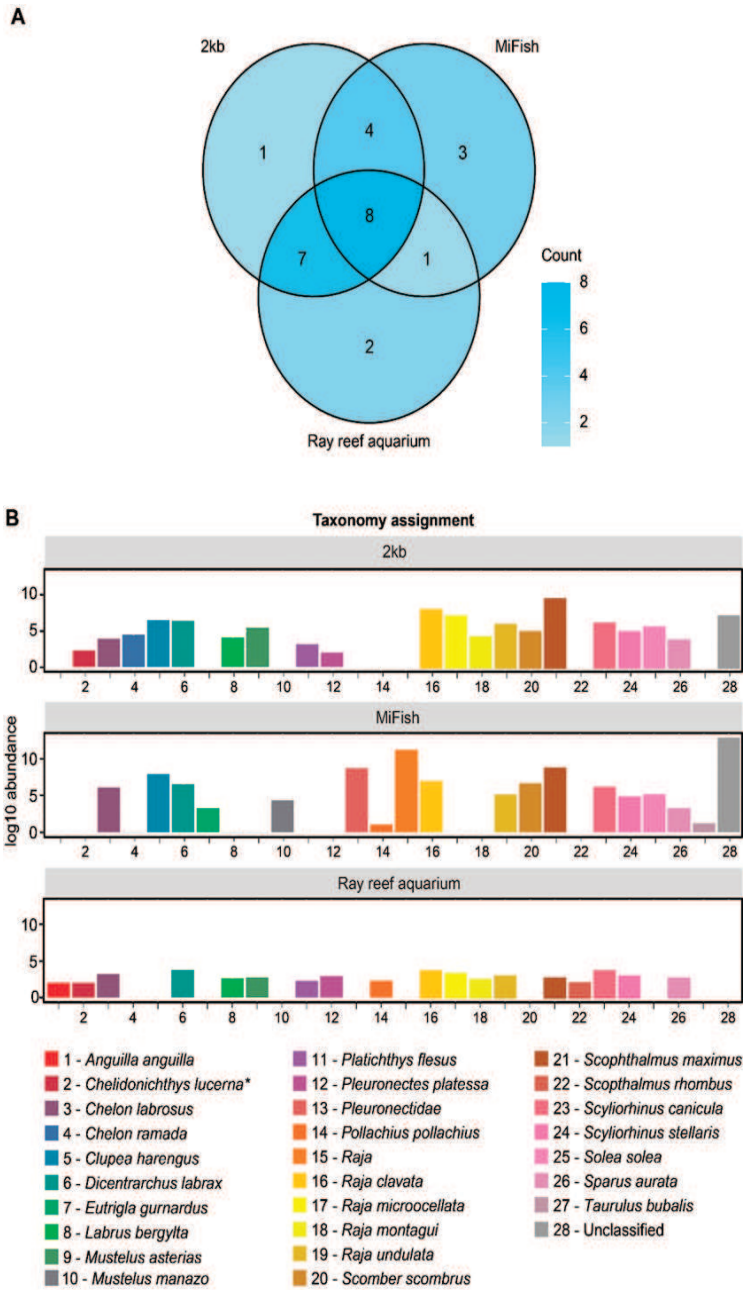


Figure 4. MiFish and 2kb primer pair in comparison to the species composition of the North Sea Ray Reef aquarium. Taxonomy assignment on species level unless specified otherwise. **A)** Venn diagram showing the species overlap in morphological species counts and the primer used **B)** Bar plot of the species-specific differences observed between the actual species present in the Ray aquarium and the results from the analysis using the two different primer pairs.

No significant difference in alpha, beta diversity in North Sea field samples from different habitats

A total of 1.627.781 (2kb) and 2.059.490 (MiFish) reads were obtained from the North Sea samples collected at Borkum, Gemini and sandy bottom. 959.538 (2kb) and 981.304 (MiFish) reads were used for consensus building **(table S9),** with a barcode distribution of 106.615± (2kb) and 108.864±88.918 (MiFish) reads per barcode. Samples were normally distributed (Shapiro-Wilk, Shannon index: p=0.401, Observed: p=0.662) and no significant difference in richness was found between primer pairs (Observed, 2-way ANOVA, p= 0151) nor locations (Observed, 2-way ANOVA: p= 0.981) (**figure 5A**). Shannon index was also not significantly different between primer pairs (Shannon, 2-way ANOVA: p=0.069), and between locations (Shannon, 2-way ANOVA: p= 0.7305). The NMDS ordination plot of the beta diversity (Bray Curtis index) shows clustering of sample replicates within location except for the Borkum reef ground processed with the 2kb primer pair (indicated in colours). Additionally, clustering of primer pairs can be observed within locations (indicated in shapes) (**figure 5B**). The effects of location and choice of primer pair were verified with statistical analysis. Homogeneity of variances between samples was found (betadisper: p=0.424) and PERMANOVA showed a significant effect of choice of primer pair (adonis: p=0.001) and location (adonis, p=0.019) and a significant interaction effect (adonis: p=0.031). More details of the statistical results are given in **table S10**. Both primer choices showed that unique species were observed with either method. *Clupea harengus,* and *Trisopterus luscus* were only observed using the MiFish primer pair, whereas *Ammodytes marinus*, *Limanda limanda*, *Raja microocellata* and *Ctenolabrus rupestris*are unique for the 2kb primer pair (**figure 5C** and **table S12**).

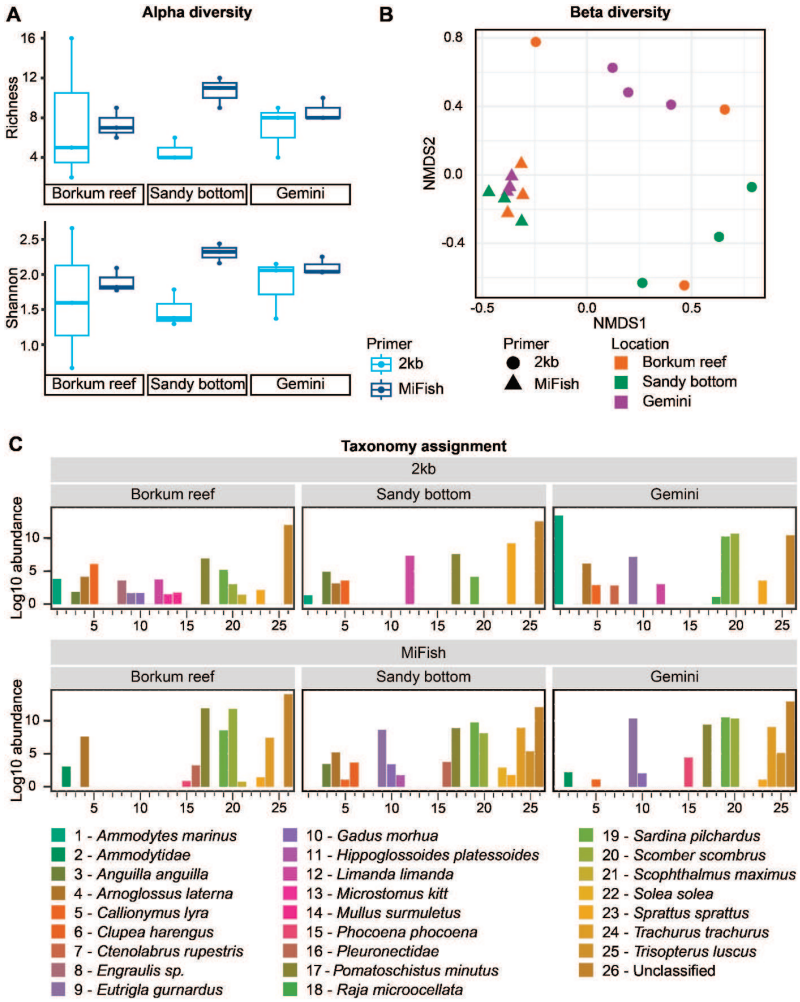


Figure 5. MiFish and 2kb primer pairs comparison of different eDNA samples taken in the Borkum reef, Gemini wind park and Sandy bottom. Taxonomy assignment on species level. **A)** Alpha diversity comparison of richness and evenness, **B)** NMDS ordination plot (Bray) showing the similarity between samples. **C)** species comparison barplot of the species-specific differences between the different location and primers.

eDNA samples taken at different shipwrecks significantly differ in alpha and beta diversity

Sequencing shipwreck samples resulted in 1.677.936 for 2kb and 1.301.036 reads for MiFish, of which 1.217.497 (2kb) and 1.126.749 (MiFish) reads were used for clustering and consensus building (**table S9**). A barcode distribution of 186.437±129.453 (2kb) and 144.559±104.799 (MiFish) reads per barcode was achieved. Shapiro-Wilk showed normally distributed data (Shannon: p=0.605, Observed: p=0.235) and there was a significant difference in richness between primer pairs (Observed, 2-way ANOVA: p=0.029) and between locations (Observed, 2-way ANOVA: p=0.026) and no significant interaction effect was found (p = 0.435). There was only a significant difference between Wreck 1 and Wreck 3 (Tuckey HSD, p = 0.026). A significant difference in Shannon index was observed between primer pairs (Shannon index, 2-way ANOVA: p=0.010) and between locations (Observed, 2-way ANOVA: p=0.014) and no significant interaction effect was found (p = 0.8705, **figure 6A**). There was only a significant difference in Shannon index between wreck 1 and 3 (p=0.011). The NMDS ordination plot shows clustering between wrecks (indicated in colors) but also between primer pair (indicated in shapes) (**figure 6B**). PERMANOVA showed a significant difference in beta diversity between wrecks (PERMANOVA, p=0.001) and Primer (PERMANOVA, p= 0.001) and an interaction effect was also observed (p= 0.007). Samples were homogeneous (betadisper, p=0.575). Nevertheless, overall, the species compositions were consistent between primer choice in each location, albeit MiFish detected more species. In addition, both primer pairs picked up unique species where the 2kb primer pair for example identified, *Melanogrammus aeglefinus*, *Merlangius merlangus* and *Limanda limanda*. MiFish on the other hand had unique findings of *Clupea harengus*, *Anguilla anguilla*, and *Trachurus trachurus* **(figure 6C** and **table S13**).

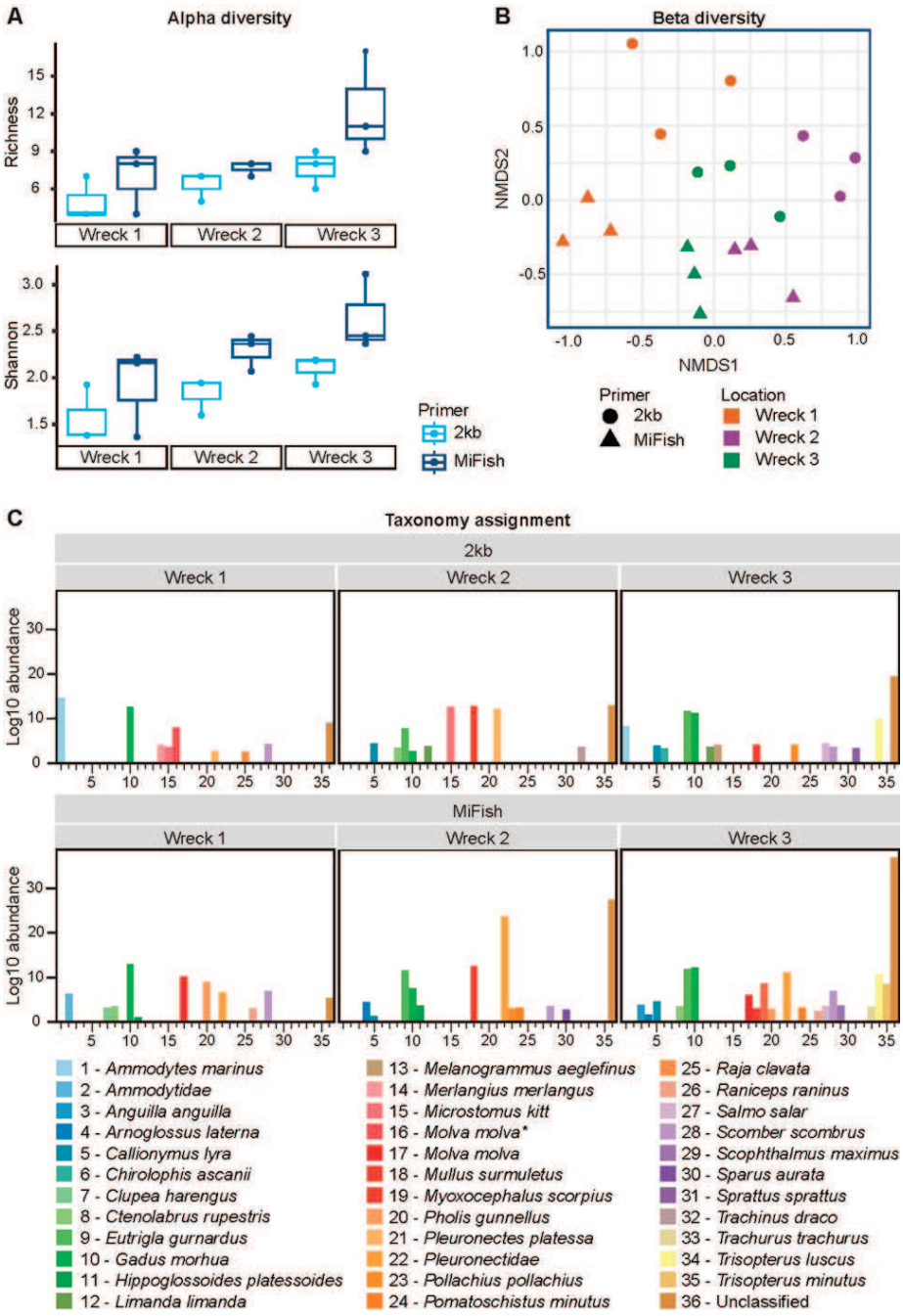


Figure 6. MiFish and 2kb primer pairs comparison of different eDNA samples taken from different shipwrecks in the North Sea. Taxonomy assignment on species level. **A)** Alpha diversity comparison of richness and evenness, **B)** NMDS ordination plot (Bray) showing the similarity between samples. **C)** species comparison bar plot of the species-specific differences between the different location and primers.

DISCUSSION

With the rise of Oxford nanopore sequencing, it now becomes increasingly feasible to use long read sequencing in metabarcoding studies. We introduced and tested the settings of the bioinformatics pipeline DECONA that enables processing of raw read Nanopore data to species assignment with just one line of code. In addition, we explored the utility of a longer amplicon fragment by comparing it to a commonly used short amplicon. Utility was tested by using an *in silico* approach, and subsequently, we tested the primer pair on samples from an aquarium with a known species composition as well as on field samples. We showed that the DECONA pipeline improves the accuracy of Nanopore reads to enable species level assignment and that the most optimal settings for DECONA depend on methodological choices. We also showed that the designed primer pair is not only bony fish specific, but also targets elasmobranchs. *In sillico* results show that longer target fragments can help increase correct species level assignments for genetically closely related species. This is also reflected in the results from the Aquarium samples: *Platichthys flesus* and *Pleuronectus platessa* are both detected with the 2kb primer pair but cannot be identified to species level with MiFish primer pair and are listed as *Pleuronectidae.* The same is true for several *Raja* species. Aquarium samples also showed that most of the species were shared between both amplicon fragment lengths but that more species could be correctly identified with the 2kb primer pair, mainly due to improved species level assignment. In the field samples, the alpha diversity was generally higher for the short fragment size, possibly due to lower abundance of longer fragments in the environment. Nevertheless, we have demonstrated that it is possible to use long and short amplicons for species level eDNA metabarcoding using Nanopore sequencing.

Although ONT based long read metabarcoding is shown to be successful in bacterial studies (Krehenwinkel et al., 2019; Matsuo et al., 2021), there is a limited number of reports that validated its use for marine biodiversity studies (Semmouri et al., 2021). Thus, these results help build a foundation to further study the added value of long read sequencing in marine vertebrate biodiversity assessments. Nevertheless, it remains challenging to adequately demonstrate possible strengths of long read amplicons sequencing as methodological choices are often different between studies (Ruppert et al., 2019; Wang et al., 2021). Also in this study, different methodological choices were made, including a limited number and different approaches of sampling, as offshore marine conditions

often restricted effective sampling efforts. Nevertheless, studies that address longer fragments, especially with diluted, environmental samples will improve the understanding of how long amplicons may enhance eDNA based marine biodiversity assessments.

DECONA enhances read accuracy and species assignment, but settings should be tailored to the experiment at hand

As it was possible to adequately assign reads to species level, the developed sequence read processing pipeline DECONA enables a consensus sequence accuracy as species level assignments were accepted from 98%, which is comparable to Illumina read accuracy (Caporaso et al., 2011). There is limited automated bioinformatics processing reported in Nanopore based studies, especially for metabarcoding (Santos et al., 2020). This study introduces the DECONA pipeline (https://github.com/saskia-oosterbroek/DECONA), thereby contributing to the availability of bioinformatics software to process Oxford Nanopore sequence output. Once installed, one line of code suffices to correctly run the pipeline and enables data processing for scientists with limited experience with the command line. The bioinformatics tools integrated in DECONA are well established programs in genomics and transcriptomic studies. For example, tools such as CD-Hit (Huang et al., 2010) have previously been used in the analysis of Nanopore sequence data for clustering and consensus building of fish amplicon-based sequences (Voorhuijzen-Harink et al., 2019). Reference based polishing was successfully applied when identifying benthic organisms on autonomous reef monitoring structures (Jin et al., 2020) using *minibarcoder.py* (Srivathsan et al., 2018). The combination of both clustering and *de novo* alignment-based polishing with racon (Vaser et al., 2017) and medaka (https://nanoporetech.github.io/medaka/) has previously been used for the correction of metagenomes (Rodríguez-Pérez et al., 2020). In contrast, the DECONA pipeline combines similarity-based clustering based on short word tables instead of an alignment approach in combination with alignment-based polishing with racon and medaka, which further increases the sequence accuracies. Limitations of DECONA may lie in the necessity to cluster, which makes it possible that reads from genetically similar organisms end up in the same clusters, resulting in lower detection sensitivity than is actually sequenced. In addition, clustering with DECONA also disregards singletons, as such missing the rare reads in datasets. Fortunately, due to the fast development of Oxford Nanopore sequencing technologies, new sequencing chemistries with reduced sequencing error rates and basecalling algorithms are often released and accuracy is now at a 99.8% raw read

accuracy for model organisms (Srivathsan et al., 2021). Therefore, by using new chemistries it may become possible to skip the clustering and polishing process altogether and perform raw read identification using amplicon sequence variants (ASVs) in the near future, as is the standard for Illumina platforms (Van Der Reis et al., 2023).

Choices in bioinformatics influence the results and affect conclusions that can be drawn. Settings in DECONA should therefore be carefully considered and especially cluster similarity is of importance. Setting a high cluster similarity result in more clusters, but with the risk of obtaining more singletons that will then be discarded. Setting a low cluster similarity causes similar reads to be clustered together, reducing the observed diversity of a sample and especially prone to happen when closely related species are expected in the sample. The optimal cluster similarity changes with amplicon length. Therefore, it is important to test several settings of DECONA on a subset of each dataset to validate which settings give the most sensible results for the amplicon (length) of choice as well as the sequencing chemistry that is used.

In silico identification shows increased species level identification using longer DNA fragments

Alignment of species within the *Pleuronectidae, Ammodytidae* and *Raja* families showed a genetic variability insufficient to differentiate related species when aligning the MiFish target fragments. These assignment problems have already been reported for North Sea fish species (e.g. Barco et al., 2022). The 2kb target fragment alignment shows that for some species indeed the sequence dissimilarity increases to up to 2%. However, for *Hyperoplus lanceolatus* and *Ammodytes tobianus,* it remains impossible to distinguish on species level on the complete 2kb target region, which demonstrates that for some species it is needed to use an additional target region to adequately assign on species level. In addition, for *Pleuronectus platessa* and *Platichthys flesus* there are an additional 11 nucleotide differences found on the 2kb target region. The *in-silico* comparison therefore shows that taxonomic identification to species level can be obtained when using long read metabarcoding, but that there is a need to consider other fragments and lengths to be able to differentiate between genetically highly similar species.

Assignments from the aquarium samples show overlap between primer pairs, primer pair specific differences, and false positives.

Most species in the aquarium could be detected with both primer pairs used. The species *Scomber scombrus* was detected by both primer pairs but did not live in the aquarium. This species was used as feed for the different piscivorous animals in the aquaria, showing that both primer pairs are also able to pick up the signal of animals that are only temporarily present as part of the diet of the aquarium inhabitants (personal communication P. Bunskoek, Dolfinarium Harderwijk).

Analysis of the eDNA aquarium samples also detected unique species for each primer pair. For example, the sequences from the 2kb primer pair better represent the different ray and *Pleuronectidae* species, whereas the MiFish primer pair could capture DNA from *Pollachius pollachius*. As the 2kb primer pair was designed in such a way that it should amplify all *Gadiformes* species (See **S3**, Supplementary text 1), this finding suggests that, using multiple markers would improve the detection of the present species, and agrees with earlier findings illustrating that multiple markers give a better representation of the complete biodiversity (Cordier et al., 2019). For both primer pairs there is also false positive species assignment as is illustrated with *Mustelus mananzo*. Despite the careful choices made in this study for correct species identification, false positive species assignments can still arise. This may occur, since BLASTn assigns reads to top hit species while there may also have been a similar match with the correct species, but with a smaller alignment length. Taxonomy assigners that are currently used for Illumina MiFish metabarcoding make use of Naïve Bayesian classifiers such as RDP (Cole et al., 2003) that can do quick taxonomic assignment for ASV metabarcoding sequences and also assigns taxa to a higher taxonomic level when there are sequences with multiple hits. For Nanopore based consensus sequences such assigners are, to our knowledge, not yet applied. Therefore, it is still needed to manually adjust results based on *a priori* knowledge on the genetic similarity, despite thorough ruling-out of such events in data-processing.

There were also species present in the aquarium that were not detected by any primer pair. It is often observed that eDNA methods do not identify the complete biodiversity, despite using a multi-marker approach (Morey et al., 2020). *Scophthalmus rhombus* for example was not detected by any molecular method as there is no representation of its 12-16S frag-

ment in public databases, making it impossible to assign a read to this species. This further stresses the need to continue improving genetic reference databases both with short fragments as well as for complete (mitochondrial) genomes. Of the undetected species only one or two individuals were present in the aquarium, which suggests that the lack of detection is a result of low initial DNA concentration of those species. And since there are a total of 301 specimens in total in the aquarium (**S2**), it is possible that that the overrepresentation of DNA of other species have masked these detections. This is in line with inconsistent detection of rare taxa between filters described in previous reports (Evans et al., 2016; Kelly et al., 2014; Morey et al., 2020), and species detection could be improved by using more replicates (Beentjes et al., 2019; Evans et al., 2016) or collecting a larger volume of water where possible. An alternative explanation for the lack of detection of these low abundant species could come from the sequence processing. As it is necessary to cluster raw reads, rare reads can end up as singletons or in a cluster that is removed during further sequence processing. Overall, despite the detection of false positives, false negatives, and primer pair specific results, both primer pairs, and especially the 2kb primer pair could identify the majority of the marine vertebrates, identifying an additional 7 species, mostly due to increased possibility for species level identification.

Field samples show lower diversity in 2kb fragment length

The alpha diversity in both Shannon index and richness was overall higher for the MiFish results in the in the field samples, although not always significant. An explanation for the increase in alpha diversity could lie in the eDNA fragment length sampled. In aquaria it can be expected that the relative concentration of eDNA in the water and especially of freshly released long eDNA fragments in the water is high, hence more diversity could be found with the 2kb primer pair in the aquaria. Finding a lower alpha diversity in the field samples may be due to lower fish density, and potentially faster breakdown of free extracellular DNA (Seymour et al., 2018). Therefore, it is likely that the average size of DNA fragments present in the field eDNA samples is smaller and hence a smaller proportion can be successfully amplified with the 2kb primer pair, while amplification of short DNA fragments with the MiFish primer pairs is still possible. This is in line with the hypothesis that longer fragments of nucleic acids in the environment break down rapidly, and that therefore longer fragments be used to incorporate time-scale information into the eDNA analysis (Jo, 2023). Our results thus suggest that the MiFish primer pair approach can identify additional signals from taxa that released

their DNA longer ago, while the 2kb primer pair would provide temporal snapshots of species that have been present more recently. There is a need to further assess how DNA length is affected by degradation both intracellularly and extracellularly (Jo, 2023) to understand how read lengths could be exploited to obtain additional insight into diversity on a temporal scale.

Additionally, the species compositions between the locations and primer pair used was in most cases consistent between replicates (**S13**) which was especially apparent in local wreck sampling. In addition, the wreck samples were more consistent in terms of species compositions between primer pairs, as most of the species could be found with either primer pair. Primer specific observations, as consistent detections of *Limanda limanda* and several *Gadiformes* species as *Merlangius merlangus* and *Melanogrammus aeglefinus*, seem specific to the 2kb primer pair, likely because these species are genetically too similar for correct species level assignments with MiFish. This consistency was less obvious, however, in the Gemini/Borkum dataset and, especially with the 2kb primer pair. Different sampling methods have resulted in these findings. The wreck samples were taken while diving, which may have provided a more stable water column, allow for collection on a precise location on these local biodiversity hotspot (Fowler & Booth, 2012) whereas the Gemini/Borkum samples were taken less locally using a niskin bottle. Alternatively, due to the several rounds of revisions that this work has undergone, DNA extracts were partly re-analyzed years later (see Supplementary text 1) and may have resulted in degradation of the sample over time. Nevertheless, Nanopore based long read metabarcoding in combination with read processing with DECONA, can be utilized to find differences in diversities between ecologically relevant sites, albeit with lower alpha diversities than with MiFish primer pair. Thus, by carefully choosing the settings in DECONA, the combination of long and short reads enables assessing the fish biodiversity on species level at multiple different sample sites (e.g. shipwreck sampling), where short reads enhance detected alpha diversity and long reads additionally provides a species level assignment of genetically closely related species while possibly providing a temporal snapshot of the community.

Conclusion

This study demonstrates and validates an eDNA metabarcoding approach using Nanopore long read technology. To enable this approach, we present our Nanopore sequence processing pipeline DECONA. DECONA is bioinformatics pipeline that allows researchers to set the right cluster similarity and can be tailored to the amplicon length and ONT chemistry at hand. We demonstrate an increased species resolution due to the longer DNA fragments analyzed. We further show limitations such as false positive assignments and limited detection of rare species suggesting the importance of using multiple markers for increased detection resolution for fish. Further research should focus on exploring the use of long read metabarcoding to gain biodiversity information on a spatial-temporal scale to further understand the role of long reads for eDNA biodiversity assessments. In addition, studies should focus on the possibility to use Nanopore generated raw reads directly, to further implement Nanopore based (long read) metabarcoding as standard to the molecular ecology toolbox. Moreover, it is essential that addition of longer reference sequences to databases, preferably of full (mitochondrial) genomes, maintains a high priority in marine molecular ecology. Only then can long read based DNA metabarcoding and metagenomics develop to its full potential to serve as monitoring tool.

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AUTHORS CONTRIBUTIONS

KD, LJ and RN designed the experiment; LJ, PK, OB, RN, AM and EW were involved in sample collection and processing; KD, LJ and RN did the laboratory work; SO, KD and RN designed the bioinformatics pipeline DECONA; KD and RN conducted the data analysis; KD, LJ, SO, RN and AM interpreted the data; all authors wrote and revised the manuscript.

SUPPLEMENTARY TABLES

Table S1: The total amount of reads and species found for each control sample

Controls				
BC	CTRL	CTRL type	Total_reads	Species found
10	2kb run	PCR CTRL	163556	H. sapiens
11	2kbrun	PCRCTRL	0	No meaningfull cluster
81	2kb DOLF	BC PCR	0	No meaningfull cluster
71	2kb DOLF	Filter CTRL	0	No meaningfull cluster
72	2kb DOLF	Filter CTRL	9423	H. sapiens
30	NZ field	Filter CTRL	91	H. sapiens
16	Shipwrecks	Filter CTRL	10635	H. sapiens
44	Mifish gemini	Filter CTRL	0	No meaningfull cluster
45	Mifish gemini	PCR	0	No meaningfull cluster
34	Mifish wrecks	Filter CTRL	42	NA
34	Mifish wrecks	Filter CTRL	26030	Homo sapiens
35	Mifish wrecks	PCR	33782	Pomatoschistus microps
35	Mifish wrecks	PCR	8429	Homo sapiens
36	Mifish wrecks	BC PCR	1	clupea harengus
36	Mifish wrecks	BC PCR	35358	Homo sapiens
36	Mifish wrecks	BC PCR	24437	unclassified

Table S2: The species that are present in the ray reef aquarium

Species list of Ray reef aquarium		
Latin name	**Roggenrif bassin**	**Total species from aquaria in the same filter system**
Dicentrarchus labrax	15	58
Sparus aurata	3	9
Chelon labrosus	15	16
Labrus bergylta	4	4
Scopthalmus rhombus	2	2
Platichthys flesus	2	2
Pleuronectus platessa	5	9
Solea solea	0	5
Scopthalmus maximus	8	8
Anquilla anquilla	1	1
Raja undulata	17	17
Raja clavata	84	84
Raja microocellata	35	35
Raja montaqui	6	6
Dasyatis pastinaca	0	1
Mustelus asterias	6	6
Scyliorhinus canicula	79	83
Scyliorhinus stellaris	16	16
Gadus morhua	0	1
Chelidonichthys lucerna	1	1
Pollchius pollachius	2	2
Labrus mixtus	0	1

Table S3: The species of the North Sea and their representation of reference fragments present in the used database.

See supplements of online publication

Table S4: accession numbers of NCBI references for in silico comparison

Accesionnumbers of NCBI references for In sillico comparison				
Pleurinectidae				
Pleuronectes platessa	MN122873.1			
Plathichthys flesus	OX438740.1			
Clupeidea				
Sprattus sprattus	AP009234			
Clupea harengus	KC193765			
Ammodytidae				
Ammodytes personatus	KF672362			
Ammodytes tobianus	MN122889			
Ammodytes marinus	MN122922.1			
Hyperooplus lanceolatus	CM049395.1			
Raja				
Raja montagui	EF100188			
Raja micoostellata	EF081264			
Raja brachyura	MT850125.1			

Table S5: BLAST database entries North Sea

See online publication

Table S6: Clusters that are assigned to multiple species with the same e-value (>98%) per primer pair and cluster similarity setting, Aquarium samples



Table S7: Rarefaction curves

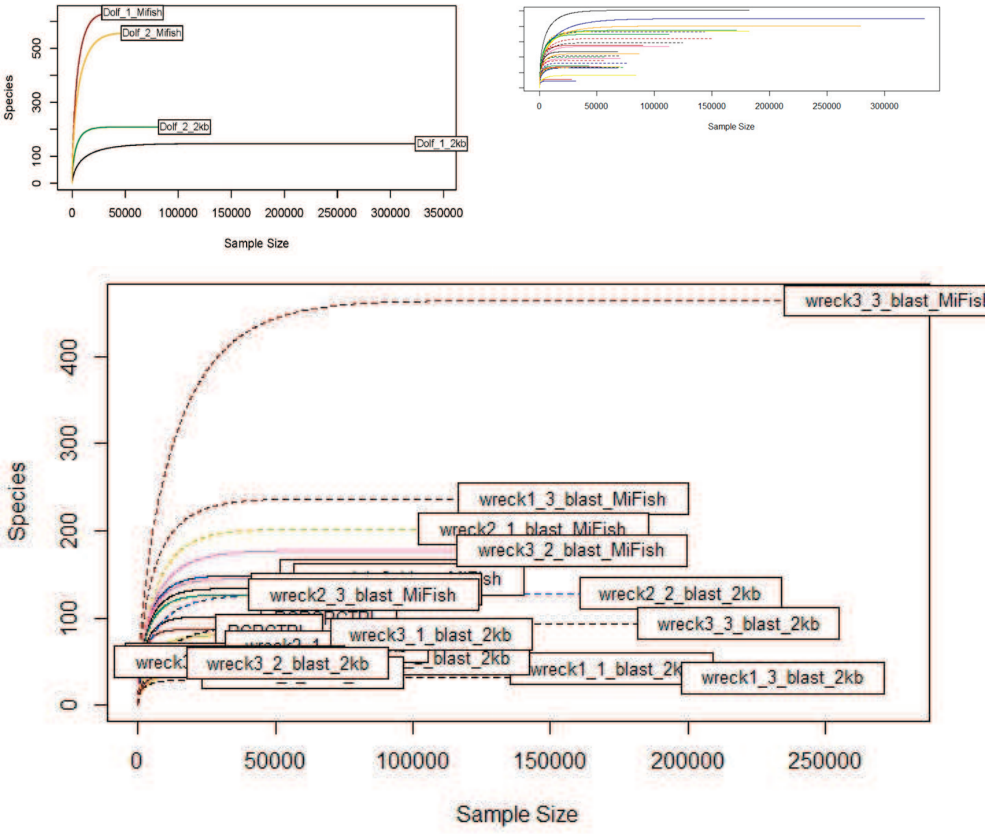


Table S7: Rarefaction curves

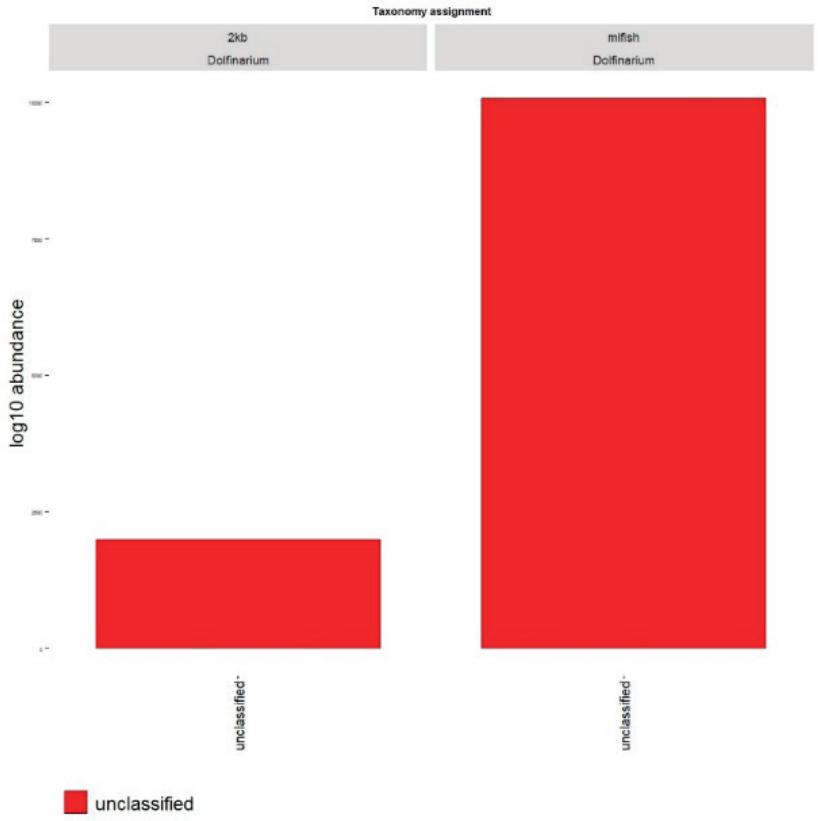


Table S8: Off target hits from the different datasets

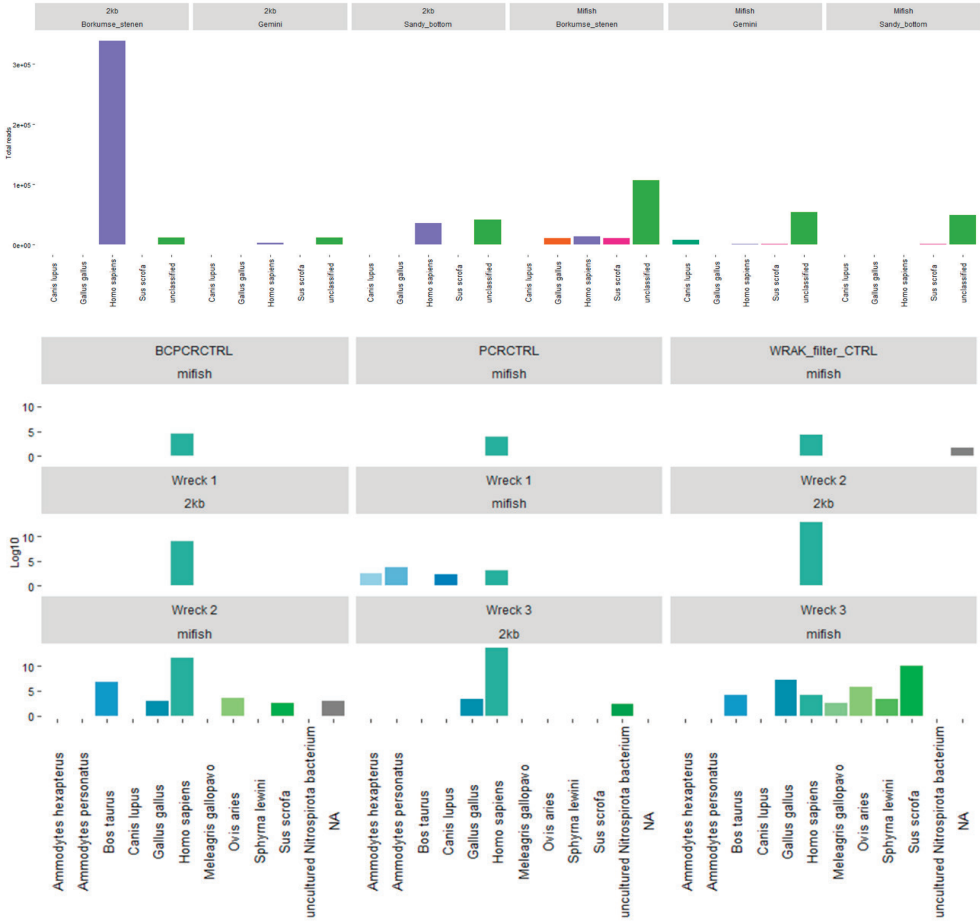


Table S9: Read counts after processing for each dataset

Read counts after processing

Ray reef aquarium

	Sample	raw	After Dec	Taxonomy to target species
	ray_aquarium_2kb_BC23	387762	347887	343883
	ray_aquarium_2kb_BC24	141302	105827	99537
	ray_aquarium_miFish_BC17	220963	56870	51984
	ray_aquarium_miFish_BC18	220963	75196	70010

North Sea

Barcode	Sample	raw	After Dec	Taxonomy to target species
04	NZ_2.7_BS1_2kb_BLAST	389758	348889	2973
25	NZ_2.7_BS1_Mifish	399710	117075	90280
05	NZ_2.7_BS2_2kb_BLAST	119253	90152	86545
26	NZ_2.7_BS2_Mifish	454771	86104	34896
06	NZ_2.7_BS3_2kb_BLAST	205824	1434	633
27	NZ_2.7_BS3_Mifish	241572	57477	27869
01	NZ_2.7_GE1_2kb_BLAST	111336	40107	35626
28	NZ_2.7_GE1_Mifish	142502	50544	32082
02	NZ_2.7_GE2_2kb_BLAST	220722	149979	140217
29	NZ_2.7_GE2_Mifish	87738	46356	23402
03	NZ_2.7_GE3_2kb_BLAST	104679	44351	43722
30	NZ_2.7_GE3_Mifish	70867	26397	13510
07	NZ_2.7_HW1_2kb_BLAST	170532	98227	59431
31	NZ_2.7_HW1_Mifish	89195	52813	22462
08	NZ_2.7_HW2_2kb_BLAST	148706	114628	112190
32	NZ_2.7_HW2_Mifish	30640	7335	5143
09	NZ_2.7_HW3_2kb_BLAST	156971	71771	35002
33	NZ_2.7_HW3_Mifish	100569	33151	16529

Shipwrecks

Barcode	Sample	raw	After Dec	Taxonomy to target species
12	wreck1_1_blast_2kb	195780	172161	93822
25	wreck1_1_blast_MiFish	109147	93765	38816
13	wreck1_2_blast_2kb	122354	105806	79526
26	wreck1_2_blast_MiFish	113255	98278	34189
14	wreck1_3_blast_2kb	259783	234478	229596
27	wreck1_3_blast_MiFish	179773	158256	33414
15	wreck2_1_blast_2kb	93631	68502	58152
28	wreck2_1_blast_MiFish	164339	143847	89121
16	wreck2_2_blast_2kb	231175	197324	132151
29	wreck2_2_blast_MiFish	96845	82974	39202
17	wreck2_3_blast_2kb	100413	59735	36455
30	wreck2_3_blast_MiFish	95946	81882	58719
18	wreck3_1_blast_2kb	315890	106563	67723
31	wreck3_1_blast_MiFish	39760	33224	27892
19	wreck3_2_blast_2kb	92726	54516	30921
32	wreck3_2_blast_MiFish	180862	157730	22725
20	wreck3_3_blast_2kb	266184	218412	78611
33	wreck3_3_blast_MiFish	321109	276793	91470

Table S10: Statistical analysis

Ray reef aquarium:

	Shannon	Richness
Shapiro wilk	p-value = 0.3246	p-value = 0.4064
T-test	0,5476	0,3499

Borkum/Gemini

	Shannon	Observed
Shapiro Wilk	p-value = 0.401	p-value = 0.6621

Observed

	Location	Primer	interaction
2 way anova	0,981	0,151	0,29

Shannon

	Location	Primer	interaction
2 way anova	0,7304	0,0685	0,4963

betadisper:

	Location	Primer
	0,424	

```
adonis2(formula = bray_dist ~ Name * Primer, data = sample_info, permutations
          Df SumOfSqs      R2      F Pr(>F)
Name       2   0.6009 0.1481 1.7578  0.019 *
Primer     1   0.8419 0.2075 4.9259  0.001 ***
Name:Primer  2   0.5636 0.1389 1.6487  0.031 *
Residual  12   2.0511 0.5055
Total     17   4.0575 1.0000
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

	pairs	Df	SumOfSqs	F.Model	R2	p.value	p.adjusted	sig
1	2kb.Borkumse_stenen vs Mifish.Borkumse_stenen	1	0.3891261	1.8179645	0.3124743	0.1	0.1250000	
2	2kb.Borkumse_stenen vs 2kb.Gemini	1	0.3323922	1.3608973	0.2538563	0.3	0.3214286	
3	2kb.Borkumse_stenen vs Mifish.Gemini	1	0.4346551	2.1702307	0.3517260	0.1	0.1250000	
4	2kb.Borkumse_stenen vs 2kb.Sandy_bottom	1	0.2074143	0.6866392	0.1465099	0.9	0.9000000	
5	2kb.Borkumse_stenen vs Mifish.Sandy_bottom	1	0.3740677	1.5344381	0.2772527	0.1	0.1250000	
6	Mifish.Borkumse_stenen vs 2kb.Gemini	1	0.5018524	4.4625039	0.5273267	0.1	0.1250000	
7	Mifish.Borkumse_stenen vs Mifish.Gemini	1	0.2904933	4.2410532	0.5146251	0.1	0.1250000	
8	Mifish.Borkumse_stenen vs 2kb.Sandy_bottom	1	0.4651122	2.7313482	0.4057654	0.1	0.1250000	
9	Mifish.Borkumse_stenen vs Mifish.Sandy_bottom	1	0.2604659	2.3256617	0.3676551	0.1	0.1250000	
10	2kb.Gemini vs Mifish.Gemini	1	0.4850736	4.9148502	0.5513105	0.1	0.1250000	
11	2kb.Gemini vs 2kb.Sandy_bottom	1	0.5361390	2.6741885	0.4006762	0.1	0.1250000	
12	2kb.Gemini vs Mifish.Sandy_bottom	1	0.4588566	3.2269206	0.4465139	0.1	0.1250000	
13	Mifish.Gemini vs 2kb.Sandy_bottom	1	0.6324941	4.0409191	0.5025444	0.1	0.1250000	
14	Mifish.Gemini vs Mifish.Sandy_bottom	1	0.1196215	1.2197792	0.2336841	0.3	0.3214286	
15	2kb.Sandy_bottom vs Mifish.Sandy_bottom	1	0.5313292	2.6563368	0.3990689	0.1	0.1250000	

shipwrecks

```
mean in group 2kb mean in group mifish
                18.5                17.0
```

	Shannon	Observed
Shapiro wilk	p-value = 0.6053	p-value = 0.2352

richness

```
> print(summary(anova_result))
                Df Sum Sq Mean Sq F value Pr(>F)
Location         1  32.00   32.00   6.128 0.0292 *
Primer           2  52.00   26.00   4.979 0.0266 *
Primer:Location  2   9.33    4.67   0.894 0.4347
Residuals       12  62.67    5.22
---
$Location
                diff       lwr      upr     p adj
Wreck 2-Wreck 1    1 -2.5199028 4.519903 0.7347365
Wreck 3-Wreck 1    4  0.4800972 7.519903 0.0261904
Wreck 3-Wreck 2    3 -0.5199028 6.519903 0.0986187
```

Shannon

```
> print(summary(anova_result))
                Df Sum Sq Mean Sq F value Pr(>F)
Primer           1 0.9203  0.9203   9.302 0.0101 *
Location         2 1.2061  0.6030   6.095 0.0149 *
Primer:Location  2 0.0278  0.0139   0.140 0.8705
Residuals       12 1.1872  0.0989
---
$Location
                diff       lwr      upr     p adj
Wreck 2-Wreck 1 0.3206851 -0.1638003 0.8051705 0.2222276
Wreck 3-Wreck 1 0.6340383  0.1495529 1.1185238 0.0114731
Wreck 3-Wreck 2 0.3133532 -0.1711322 0.7978386 0.2360135
```

betadisper 0,575

Permanova

adonis2(formula = bray, betadisper, p-value = 0.079

	Df	SumOfSc	Location
Name	2	1.5633	0.39896 9.1486 0.001 ***
Primer	1	0.8656	0.22091 10.1314 0.001 ***
Name:Primer	2	0.4642	0.11848 2.7168 0.007 **
Residual	12	1.0252	0.26165
Total	17	3.9184	1.00000

```
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Table S11: Read count per species and per sample from the aquarium dataset

Read count per species and sample aquarium

warning	Dolf_1_2kb	Dolf_1_Mifish	Dolf_2_2kb	Dolf_2_Mifish	Ray reef aquarium	detected 2kb	detected Mifish	detected c	total shared 3	shared 2kb en mifish	shared 2kb en aquarium	shared mi	undetecte	2kb only	mifish onl
Anquill Anquilla anquilla	0	0	0	0	100	0	0	1	no	no	no	no	yes	no	no
2 Chelidonichthys lucerna	0	0	188	0	100	1	0	1	no	no	yes	no	no	no	no
3 Chelon labrosus	81	2311	99	562	1600	1	1	1	yes	no	no	no	no	no	no
4 Chelon ramada	42	0	624	0	0	1	0	0	no	no	no	no	no	yes	no
5 Clupea harengus	4266	6396	689	12710	0	1	1	0	no	yes	no	no	no	no	no
6 Dicentrarchus labrax	766	2746	3148	1166	5800	1	1	1	yes	no	no	no	no	no	no
7 Eutrigla gurnardus	0	123	0	14	0	0	1	0	no	no	no	no	no	no	yes
8 Labrus bergylta	38	0	289	0	400	1	0	1	no	no	yes	no	no	no	no
9 Mustelus asterias	146	0	1710	0	600	1	0	1	no	no	yes	no	no	no	no
10 Mustelus manazo	0	867	0	25	0	0	1	0	no	no	no	no	no	no	yes
11 Platichthys flesus	0	0	1318	0	200	1	0	1	no	no	yes	no	no	no	no
12 Pleuronectes platessa	104	0	0	0	900	1	0	1	no	no	yes	no	no	no	no
13 Pleuronectidae	0	1651	0	99	0	0	1	0	no	no	no	no	no	no	yes
14 Pollachius pollachius	0	11	0	0	200	0	1	1	no	no	no	yes	no	no	no
15 Raja	0	3069	0	301	0	0	1	0	no	no	no	no	no	no	yes
17 Raja clavata	7110	5682	23748	1733	8400	1	1	1	yes	no	no	no	no	no	no
18 Raja microocellata	845	0	25869	0	3500	1	0	1	no	no	yes	no	no	no	no
19 Raja montagui	38	0	726	0	600	1	0	1	no	no	yes	no	no	no	no
20 Raja undulata	377	1160	3833	113	1700	1	1	1	yes	no	no	no	no	no	no
21 Scomber scombrus	139	7126	1094	664	0	1	1	0	no	yes	no	no	no	no	no
22 Scophthalmus maximus	329349	11476	14812	52116	800	1	1	1	yes	no	no	no	no	no	no
23 Scopthalmus rhombus	0	0	0	0	200	0	0	1	no	no	no	no	yes	no	no
24 Scyliorhinus canicula	378	4262	5926	340	8300	1	1	1	yes	no	no	no	no	no	no
25 Scyliorhinus stellaris	101	684	1493	107	1600	1	1	1	yes	no	no	no	no	no	no
26 Solea solea	47	4335	13797	35	0	1	1	0	no	yes	no	no	no	no	no
27 Sparus aurata	56	70	171	25	900	1	1	1	yes	no	no	no	no	no	no
28 Taurulus bubalis	0	15	0	0	0	0	1	0	no	no	no	no	no	no	yes
29 unclassified	3988	4886	5494	5186	0	1	1	0	no	yes	no	no	no	no	no

Table S12: Read count per species and per sample from the Borkum/Gemini dataset

Read count per species and sample Borkum/Gemini

species	NZ_2.7_BS1_2kb_BLAST	NZ_2.7_BS	NZ_2.7_BS	NZ_2.7_BS2_Mifish	NZ_2.7_BS	NZ_2.7_BS	NZ_2.7_GI	NZ_2.7_GI	NZ_2.7_GI	NZ_2.7_GI	NZ_2.7_GI	NZ_2.7_H	NZ_2.7_H	NZ_2.7_H	NZ_2.7_H	NZ_2.7_H	NZ_2.7_H
1 Ammodyt	0	0	6705	0	0	0	18853	0	121411	0	12042	0	20	0	0	0	0
2 Ammodyt	0	0	0	69	0	15	0	0	0	143	0	0	0	0	0	0	0
3 Anguilla a	0	0	71	0	0	0	0	0	0	0	0	0	0	78361	2806	0	0
4 Arnogloss	0	522	13209	262	0	280	2458	0	558	0	0	0	1331	35	0	22	0	178
5 Callionym	0	0	18130	0	65	0	703	12	0	0	0	0	0	0	3586	0	0	10
6 Clupea ha	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4470
7 Ctenolabr	0	0	0	0	0	0	0	0	666	0	0	0	0	0	0	0	0	0
8 Engraulis	0	0	3734	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
9 Eutrigla gu	0	0	42	0	0	0	1128	1757	12126	3883	0	3283	0	792	0	219	0	2494
10 Gadus mo	0	0	45	0	0	0	0	100	0	0	0	0	0	2549	0	0	0	0
11 Hippoglos	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	52
12 Limanda li	0	0	19	0	287	0	0	0	1033	0	0	0	0	0	8776	0	2642	0
13 Microston	0	0	30	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
14 Mullus su	0	0	55	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
15 Phocoena	0	0	0	6	0	0	0	496	0	49	0	0	0	0	0	0	0	0
16 Pleuronec	0	1633	0	0	0	0	0	0	0	0	0	0	0	6	0	0	0	821
17 Pomatosc	0	10998	44279	8254	190	8675	0	2360	0	645	0	1696	0	272	7778	1276	4768	2105
18 Raja micr	0	0	0	0	0	0	11	0	0	0	0	0	0	0	0	0	0	0
19 Sardina pi	2973	39217	50	8608	0	0	340	4731	1623	1549	29726	4258	0	5276	13689	439	0	2342
20 Scomber s	0	8090	10	12326	91	5965	8755	8864	2800	1799	1954	1291	0	557	0	96	0	2216
21 Scophthal	0	0	25	0	0	5	0	0	0	0	0	0	0	0	0	0	0	0
22 Solea sole	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	796
23 Sprattus s	0	0	141	26	0	0	3378	0	0	0	0	10	58080	58	0	0	27592	0
24 Trachurus	0	0	0	3058	0	8368	0	2731	0	185	0	2062	0	4738	0	193	0	885
25 Trisopteru	0	0	0	0	0	0	0	382	0	0	0	335	0	3255	0	66	0	0
26 unclassifie	345916	56615	3607	52862	801	33699	4481	18907	9762	32260	629	13424	38796	31027	2438	2218	36769	16782

Table S13: Read count per species and per sample from the shipwrecks dataset

Read count per species and sample shipwrecks

species	wreck1_1_blast_2	wreck1_1_blast_Mifi	wreck1_2_	wreck1_2_	wreck1_3_	wreck1_3_	wreck2_1_	wreck2_1_	wreck2_2_	wreck2_2_	wreck2_3_	wreck2_3_	wreck3_1_	wreck3_1_	wreck3_2_	wreck3_2_	wreck3_3_	wreck3_3_
1 Ammodytes hexapterus	0	13	0	0	0	28	0	0	0	0	0	0	0	0	0	0	0	0
2 Ammodytes marinus	35915	0	64740	0	168969	0	0	0	0	0	0	0	0	0	4370	0	38673	0
3 Ammodytes personatus	0	59	0	0	0	76	0	0	0	0	0	0	0	0	0	0	0	0
4 Anguilla anguilla	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	7505
5 Arnoglossus laterna	0	0	0	0	0	0	74	0	0	0	491	0	0	0	0	0	0	54
6 Callionymus lyra	0	0	0	0	0	0	0	0	23028	31	0	0	8986	65	0	0	0	852
7 Chirolophis ascanii	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1748	0
8 Clupea harengus	0	1527	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
9 Ctenolabrus rupestris	0	2924	0	0	0	0	0	0	2648	0	0	0	0	0	0	0	0	2883
10 Eutrigla gurnardus	0	0	0	0	0	0	1671	8121	41479	17185	0	3397	5596	6781	12706	8940	5898	13563
11 Gadus morhua	38804	19259	3662	28410	34743	23843	0	4447	0	0	469	13426	8188	12400	9375	6203	2511	33163
12 Hippoglossoides platessoide	0	13	0	0	0	0	0	116	0	46	0	0	0	0	0	0	0	0
13 Limanda limanda	0	0	0	0	0	0	6867	0	0	0	0	0	0	0	3930	0	0	0
14 Melanogrammus aeglefinus	0	0	0	0	0	0	0	0	0	0	0	0	12424	0	0	0	0	0
15 Merlangius merlangus	0	0	0	0	12089	0	0	0	0	0	0	0	0	0	0	0	0	0
16 Microstomus kitt	0	0	4374	0	0	0	10140	0	31383	0	11623	0	0	0	0	0	0	0
17 Molva dypterygia	0	0	5915	0	13795	0	0	0	0	0	0	0	0	0	0	0	0	0
18 Molva molva	0	2098	0	2303	0	4310	0	0	0	0	0	0	0	0	0	1171	0	958
19 Mullus surmuletus	0	0	0	0	0	0	22855	31272	17622	5373	17302	24276	10724	1768	0	0	0	0
20 Myoxocephalus scorpius	0	0	0	0	0	0	0	0	0	0	0	0	0	412	0	472	0	2612
21 Pholis gunnellus	0	3004	0	313	0	1248	0	0	0	0	0	0	0	732	0	0	0	0
22 Platichthys flesus	0	0	0	3163	0	1492	0	5454	0	5522	0	6276	0	443	0	0	0	1863
23 Pleuronectes platessa	0	0	448	0	0	0	12653	36343	15991	9379	7061	9351	0	0	0	1017	0	1650
24 Pollachius pollachius	0	0	0	0	0	0	0	0	0	1059	0	0	12771	0	0	0	0	0
25 Pomatoschistus minutus	0	0	0	0	0	0	0	0	0	0	0	1502	0	0	0	0	0	1547
26 Raja clavata	0	0	387	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
27 Raniceps raninus	0	0	0	0	1136	0	0	0	0	0	0	0	0	0	0	325	0	0
28 Salmo salar	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	24174	3692
29 Scomber scombrus	19103	9919	0	0	1281	0	3294	0	0	0	0	0	0	1193	0	0	3707	8424
30 Scophthalmus maximus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4068
31 Sparus aurata	0	0	0	0	0	0	0	0	0	607	0	0	0	0	0	0	0	0
32 Sphyrna lewini	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1973
33 Sprattus sprattus	0	0	0	0	0	0	0	0	0	0	0	0	1935	0	0	0	0	0
34 Trachinus draco	0	0	0	0	0	0	3966	0	0	0	0	0	0	0	0	0	0	0
35 Trachurus trachurus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2522
36 Trisopterus luscus	0	0	0	0	0	0	0	0	0	0	0	0	7099	3156	540	3709	1900	3705
37 Trisopterus minutus	0	0	0	0	0	0	0	0	0	0	0	0	0	942	0	888	0	436
38 unclassified	78339	52974	26280	57882	4882	116391	10350	53290	65173	38432	23280	19730	38840	153	23595	122715	139801	164462

Table S14: Mock community findings and read counts

Mock community			
Mock community:			
	Species present in community	**reads found per species (2 samples)**	
1	Arnoglossus laterna	37551	
2	Chelon labrosus	100917	
3	Chelon ramada	9160	
4	Gadus morhua	69355	
5	Gasterosteus aculeatus	94192	
6	Petromyzon marinus	532287	
7	Neogobius melanostomus	85413	
8	Phycis blennoides	32563	
9	Salmo trutta	185827	
10	Scophthalmus rhomus	30973	
11	Solea solea	44138	
12	Trisopterus luscus	36578	
13	unclassified	138062	

Chapter 4

The potential of eRNA to assess the local biodiversity in North Sea bivalve reefs

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ABSTRACT

North Sea reefs are becoming increasingly rare, and monitoring their biodiversity is crucial for effective conservation strategies for the remaining ones. Using non-invasive methods such as environmental DNA to study biodiversity is powerful; however, transport by currents and the presence of preserved DNA fragments can overestimate biodiversity in fluctuating or dynamic environments. Environmental (e)RNA and longer eDNA fragments are linked to the active and local community due to their increased lability and consequently reduced detectability. Nonetheless, how they can be used for monitoring the marine vertebrate community in natural reefs remains untested.

We studied the community composition of a natural *Modiolus modiolus* reef in the marine protected area (MPA) Noss Head, in the Scottish North Sea, by comparing metabarcoding of short fragment eDNA, long fragment eDNA, and short fragment eRNA data collected from sites with varying *M. modiolus* coverage. Additionally, we evaluated the relationship between metabarcoding results and Baited Remote Underwater Video (BRUV) findings to explore the added value of an integrated approach combining BRUV and molecular analyses. Vertebrate species diversity was consistently higher in eDNA than in eRNA datasets. Using eRNA fragments rarely yielded detections of large, high-trophic-level animals. Long fragment detections consistently showed the lowest diversity, likely due to reduced detectability. Only short fragment eDNA enabled differentiation of species communities between high and low *M. modiolus* coverage sites, and a positive relationship was observed between species communities characterised with BRUVs and eRNA. Overall, this suggests that eRNA fragments mainly capture the temporally active community rather than spatial variation. These findings offer practical insights into integrating eRNA metabarcoding into the molecular marine monitoring toolkit and highlight its potential as a complementary tool for detecting changes in marine reef systems.

Keywords: eDNA, eRNA, Fish, North Sea, Reefs, vertebrates, community ecology, biodiversity, nucleic acids

INTRODUCTION

North Sea biogenic reefs, particularly bivalve reefs, are vital in supporting biomass production by providing food, shelter, and nursery habitats for various fish and mammal species (Kent, Mair, et al., 2017; Seitz et al., 2014; Støttrup et al., 2014). However, the North Sea is heavily utilised for resource extraction, including sand and mineral dredging and extensive bottom trawling. (Porz et al., 2024; Thurstan et al., 2024). These activities resulted in a dramatic decline of bivalve reefs in the North Sea region (Thurstan et al., 2024). Fortunately, the European Union adopted significant environmental policies such as the Marine Strategy Framework Directive (MSFD, 2008/56/EC), the European Water Framework Directive (WFD, 2000/60/EC) and the European network (Natura 2000 network, Habitat Directive) to protect vulnerable habitats and species. These policies initiated routine monitoring of biodiversity dynamics and environmental impact of industrial developments (Carvalho et al., 2019; Coolen et al., 2022; Fowler et al., 2020; Ibanez-Erquiaga et al., 2025), thereby improving conservation management strategies (Borja et al., 2010; Kraan et al., 2024; Pikesley et al., 2021).

Conventional monitoring methods typically involve netting and trapping, which may result in considerable disturbance of the often-vulnerable habitat (Murphy & Jenkins, 2010). Therefore, non-invasive observatory techniques like camera-based visual imaging (Langlois et al., 2020; Mallet & Pelletier, 2014), acoustics and soundscapes (Ibanez-Erquiaga et al., 2025) are often the favoured option, but analysing these data is time-consuming and, depending on visibility and sound pollution, may not reach the preferred taxonomic resolution (Anderson et al., 2008; Connolly et al., 2023). Environmental DNA (eDNA), another non-invasive technique, may serve as a cost-effective and time-effective alternative (Dukan et al., 2024; Mauffrey et al., 2021) and a complementary signal to camera data (Clark et al., 2024; Jeunen et al., 2020; Stat et al., 2019).

Although eDNA-based approaches yielded promising observations of both vertebrate (Barco et al., 2022; Dukan et al., 2024; van Bleijswijk et al., 2020) and invertebrate communities (Cornelis et al., 2024; Mauffrey et al., 2021), they also have limitations. These limitations are linked to the amplification and annealing biases that reduce the possibility of obtaining reliable (relative) abundance data (Beng & Corlett, 2020). In addition, most metabarcoding studies rely on short fragment amplicons (Miya et al., 2015), which often lack taxonomic resolution and may lead

to false-negative and/or false-positive detection (Beng & Corlett, 2020). Moreover, the combination of waterbody movements, differential DNA decay, and contamination with old, preserved DNA in the water column further obscures the reliability of eDNA findings (T. Jo & Minamoto, 2021; Sassoubre et al., 2016; Zanni et al., 2025).

Several alternatives exist to circumvent some of these biases, including the use of multiple markers (Ferreira et al., 2024; Fontes et al., 2024) and using primers that amplify longer DNA fragments for improved taxonomic resolution (Doorenspleet et al., 2025; S. Zhang et al., 2020). Moreover, the use of qPCR methods to progress abundance estimations (Rourke et al., 2022; Urban et al., 2024) and the use of spatial-temporal sampling (Courtaillac et al., 2024; Dukan et al., 2024) are all presented to improve eDNA-based monitoring.

Previously, little attention was paid to using eRNA for biodiversity monitoring, primarily because of the assumption that the RNA molecule is too unstable to persist in the environment (Cristescu, 2019). Despite its unstable nature, the eRNA signal was proposed to reduce false positive detection, to improve spatiotemporal resolution due to its higher degradation rate and faster decay (Cristescu, 2019; T. Jo & Minamoto, 2021) and could additionally be used to assess the physiological and health status of an ecosystem (Ramón-Laca et al., 2025). Indeed, the detectability of eRNA was found to persist for days, compared with weeks for eDNA in microcosms (Kagzi et al., 2022). In addition, eRNA metabarcoding is a robust signal for biodiversity assessment in freshwater Arthropoda (Kagzi et al., 2023). eRNA metabarcoding also appears to reduce false-positive detection rates moderately (Li et al., 2024; Littlefair et al., 2022). Nevertheless, biodiversity assessments show conflicting results, where in some cases the eRNA signal results in a higher overall species richness of freshwater fish (Li et al., 2024) and meiofauna (Giroux et al., 2022) but also resulted in lower diversity in other cases (An et al., 2023; Y. Zhang et al., 2024). Consequently, it is still unclear what the exact value of the eRNA signal is in biodiversity assessments. To our knowledge, no study has assessed the marine vertebrate community in North Sea bivalve reefs using eRNA, and the added value of eRNA metabarcoding compared to short- and long-fragment eDNA metabarcoding in such habitats remains unstudied. It also remains unclear whether these different signals might reflect distinct spatial-temporal snapshots of the reef vertebrate community.

We evaluated biodiversity using a multi-metabarcoding approach on a *Modiolus modiolus* reef in the MPA Noss Head in the Scottish North Sea. We compared it to high *M. modiolus* coverage (>50%), medium coverage (<50%), no coverage (~0%) and an area 5km away from the reef. In this study, we 1) compare the species richness and community composition of each marker and evaluate how this represents the fish community on and around the biogenic reef 2) discuss the complementary value of short and long amplicon eDNA metabarcoding in combination with eRNA metabarcoding and BRUV findings. We hypothesise that long-fragment metabarcoding and eRNA metabarcoding lead to reduced diversity compared to short-fragment eDNA metabarcoding, but that the techniques target and detect similar species.

MATERIALS AND METHODS

Study area

The Marine Protected Area (MPA) Noss Head is situated off the coast of Scotland and encompasses a 3.85 km2 horse mussel (M. modiolus) bed, which lies at approximately 35-45 m depth (Figure 1, S8). Sampling took place onboard the RV Pelagia from 3rd to 10th October 2023. Areas for sampling were chosen based on ground truth imaging using an HD video hopper frame (Coral et al., in review; Figure S10) deployed from the vessel. Video footage was live-streamed onboard, allowing for real-time assessment of the seabed. The video survey covered the southern part of the mussel bed, as previous surveys confirmed high-coverage patches of M. modiolus in the northern area (Figure 1A). area (Hirst et al., 2012; Moore, C. G. and Roberts, 2011; Figure 1A).

Sampling design

Overall, the sampling design was limited by restricted access to areas containing static fishing gear. Nonetheless, three biotopes were chosen based on the video surveys (Figure 1C): high-density mussel bed (>50% *M. modiolus* coverage), medium-density mussel bed (<50% *M. modiolus* coverage), and absence mussel bed (~0% *M. modiolus* coverage). Different stations were sampled within each biotope (Table S1). Due to limited ship time and to reduce processing costs, not all habitats (High coverage, Medium coverage, Low coverage) were evenly sampled (1 to 3 stations per habitat), and one additional station, located 5 km from the reef edge, was included to evaluate the reef effect (Table S1, Figure 1C). Neverthe-

less, the same stations and sampling times were consistently used for comparisons between fragment choices.

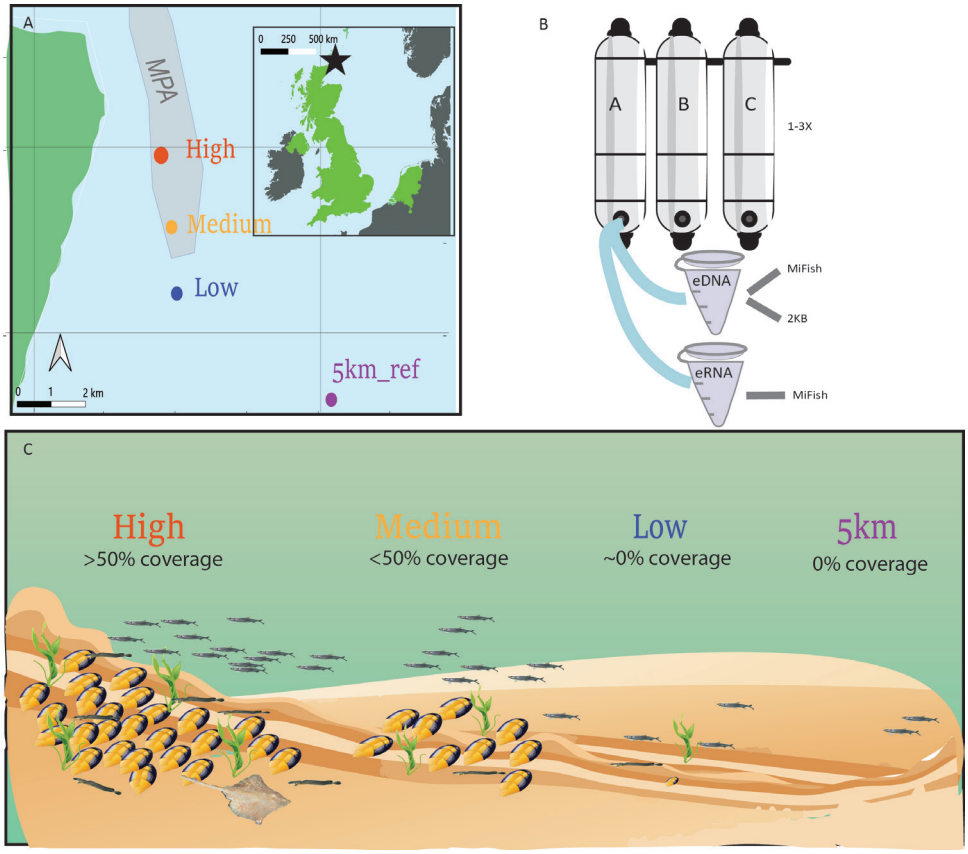


Figure 1: Study area, locations, habitat and sampling strategy, showing A) the location of the M. modiolus reef at Noss Head, in the North Sea along the Scottish coast. B) Positioning of the used sampling stations as described by Coral et al. (under review). Colors indicate the location of the station representing either high (>50%), medium (<50%), low (~0%) or 5 km (0%) M. modiolus coverage. C) Visual representation of the different modiolus coverage based on ground truth imaging as described in Coolen J.W.P et al. (2024). D) Cartoon of the sampling method, design and replication strategy of the different eNA samples.

Water sampling

Water sampling for eDNA and eRNA was conducted using six 25 L Niskin bottles mounted on a rosette frame, with three replicates collected from different bottles. Samples were collected near the seabed. For each sample analysis replicate (eDNA or eRNA), water from the same Niskin bottle was used. Niskin bottles were rinsed with fresh water between

water sampling events to avoid contamination. Water was transferred into separate 2 L plastic bottles for each replicate (one for eDNA and one for eRNA from each Niskin). Clean gloves were worn and changed between samples while handling and processing to prevent human DNA contamination. For each replicate, 2000 ml of seawater (or until clogged, minimum 1300 ml) was filtered through a 1.2 µm cellulose nitrate filter using filtration units connected to a vacuum pump (Rocker, USA). Filters were folded with chloride-cleaned tweezers prior to storage in a 2 ml screw cap containing 400 µl RNA/DNA shield (Zymo, USA). The filter equipment (filter holders, plastic bottles, and tweezers) were rinsed and sterilised using a 10-minute 0.5% bleach bath and subsequently rinsed with MilliQ water between samples. Field controls were taken before and after each filtering session using approximately 1 litre of MilliQ water. Samples were immediately stored in the freezer at -20 °C for eDNA, or snap-frozen in liquid nitrogen and stored at -80 °C for eRNA, for further processing. All samples were collected during the same tide.

DNA and RNA extraction

DNA was extracted using the DNeasy Blood and Tissue extraction kit (Qiagen, USA) following the manufacturer's protocol for tissue samples, except ATL was replaced with 400 µl RNA/DNA shield, which was already in the filter, and the volumes of AL buffer and ethanol were doubled to maintain a 1:1:1 ratio. DNA was eluted in 50 µl AE buffer, and concentrations were measured with a DeNovix DS-11 (DeNovix Inc., USA). Extraction controls were included in each round of extractions.

RNA was extracted using the RNeasy Micro kit (Qiagen, USA), with some adaptations to the manufacturer's protocol made, as detailed in the complete protocol (Supplementary file 1). RNA concentrations were quantified using a DeNovix DS-11 (DeNovix Inc., USA), as well as with a Qubit RNA High Sensitivity (HS) kit (Invitrogen, USA) and a Qubit 2.0 Fluorometer (Invitrogen, USA).

cDNA synthesis

To enable amplification, eRNA was first reverse transcribed into single-stranded (ss) complementary DNA (cDNA). The SensiFAST cDNA synthesis kit was used (Meridian Bioscience, USA) with an input of 100 ng to 500 ng of RNA. Multiple negative controls were included in the process, including one negative cDNA synthesis control with all chemicals, one cDNA synthesis control minus reverse transcriptase with 1 µL of RNA added to verify the absence of DNA, and all cDNA synthesis chemicals

with the negative RNA extraction blank. The cDNA synthesis protocol was carried out on a PCR machine (T100 Thermal Cycler, Bio-Rad) as follows: 10 minutes at 25°C, 10 minutes at 42°C, 15 minutes at 85°C, then held at 4°C until further processing.

PCR

DNA and cDNA were both amplified using short fragment MiFish primers (Miya et al., 2015) in a final reaction volume of 10 µL, containing 5 µL of 2x Phire Tissue Direct PCR Master Mix, 4.3 µL nuclease-free water (NFW), 0.2 µL of mixed forward and reversed unique tagged MiFish primers (adapted from Srivathsan et al., 2018) and 0.5 µL of template (c) DNA (Table S3). Three PCR replicates were performed to minimise the stochastic effects of PCR. The PCR program for amplification was 98°C for 180 seconds, followed by 35 cycles of 98°C for 10 seconds, 59 °C for 10 seconds, 72°C for 10 seconds, and a final step at 72°C for 180 seconds. DNA extractions were also amplified using a long fragment 2kb primer that spans both 12S and 16S rRNA (Table S3). The PCR conditions for this primer set were 98°C for 180 seconds, 35 cycles of 98°C for 10 seconds, 57 °C for 10 seconds, 72°C for 30 seconds, followed by a final extension at 72°C for 180 seconds. PCR products were analysed by 1.5% agarose gel electrophoresis, run for 40 minutes at 120v. Successful PCR replicates were pooled, and negative PCR controls were included in each PCR plate.

Barcoding PCR

For the 2kb amplicons, an additional barcoding PCR was required. For the MiFish primers, this step was skipped due to the use of tagged primers. PCR amplification was carried out in a final reaction volume of 15 µL, consisting of 7.5 µL LongAmp polymerase (New England Biolabs, USA), 6.7 µL NFW, 0.3 µL barcode (EXP-PBC096 kit, Oxford Nanopore Technologies, UK) and 0.5 µL first round PCR product. The PCR program was as follows: 95°C for 180 seconds, followed by 13 cycles of 95°C for 15 seconds, 62°C for 15 seconds, 65°C for 150 seconds, then a final step of 65°C for 300 seconds. PCR products were verified using 1.5% agarose gel electrophoresis, run for 40 minutes at 120V, and then pooled at equimolar concentrations. Negative PCR controls were included in both PCR and pooling steps. After pooling, AMPure XP beads clean-up was performed following the Oxford Nanopore Technologies protocol for end-prep (version: ligation-sequencing-amplicons-sqk-lsk114-ACDE_9163_v114_revH_29Jun2022-minion), with two modifications: twice the volume of beads was added relative to the total pool volume (2:1 ratio), and DNA

was eluted in 51 µL NFW. DNA was quantified using the HS dsDNA kit (Invitrogen, USA) and the Qubit 2.0 Fluorometer (Invitrogen, USA).

Gel extraction of the short fragment (MiFish) amplicons

To remove bacterial DNA amplified by MiFish primers, a gel extraction of the target amplicon (~150 bp) was performed. First, MiFish PCR products were pooled at equal molar concentrations. The pooled product was loaded onto a 1.5% agarose gel and run for 90 minutes at 100v. After visualisation using blue light from a Bluegel (miniPCR bio, USA), the target band was excised and collected into an Eppendorf tube. Gel purification was then performed using the QIAquick gel extraction kit (Qiagen, USA) following the manufacturer's protocol. After gel purification, an AMPure XP beads clean-up was performed as described previously.

Nanopore sequencing

End-prep and adapter ligation were performed using the SQK-LSK114 kit (Oxford Nanopore Technologies Ltd., UK) (protocol version: ligation-sequencing-amplicons-sqk-lsk114-ACDE_9163_v114_revH_29Jun2022-minion). Purified DNA was quantified with the HS dsDNA kit (Invitrogen, USA) and Qubit 2.0 Fluorometer (Invitrogen, USA). To prevent overload, less than 100 ng DNA was loaded onto a primed R10.4.1 flow cell, and sequencing was carried out using a MinION Mk1C. Sequencing speed was 450 bases/sec, with an average sequence depth of 200.000 reads per sample (~20-25 million reads per run). All sequencing runs were performed on separate flow cells to avoid cross-contamination between runs.

Bioinformatics

Base-calling of the raw POD5 files was conducted using Dorado (Version 0.5.3, Oxford Nanopore Technologies Ltd., UK) in super high accuracy (SUP) mode. Decona (version 0.1.4) was utilised for bioinformatic analysis. (Doorenspleet et al., 2025). SUP base called reads were filtered based on read length, only keeping reads from 150 bp to 240 bp for the MiFish amplicons and 1800 bp to 2350 bp for 2kb amplicons. A cluster similarity of 96% was chosen for MiFish and 95% for 2kb, based on careful examination of different clustering percentages. (Doorenspleet et al., 2025) with a minimum of 10 reads per cluster. (MiFish command: decona -T 30 -f -q 10 -l 150 -m 240 -g "GTYGGTAAAWCTCGTGCCAGC;max_error_rate=0.1;min_overlap=17...CAAACTRGGATTAGATACCCCACTAT;max_error_rate=0.1;min_overlap=21" -c 0.96 -n 10 -r -R 500 -k 6 -M -b /home /Blast_database/nt, 2kb command: decona -T 30 -f -q 10 -l 1800

-m 2350 -g "GGATTAGATACCCYACTATGY;max_error_rate=0.1;min_overlap=17...CYAGGGATAACAGCGCAATC;max_error_rate=0.1;min_overlap=17" -c 0.95 -n 10 -r -R 500 -k 6 -M -b /home /Blast_database/core_nt).

Taxonomic identification of the sequences was performed using the Basic Local Alignment Search Tool for nucleotides (BLASTn) with the nucleotide core (nt) database (last updated August 2024), integrated within Decona (https://github.com/Saskia-Oosterbroek/DECONA; Doorenspleet et al. 2025). Assignments at the species level were accepted if the top hit e-value was higher than that of the second hit, and/or when the second or third hit identified the same species. Additionally, species-level assignments were only accepted if the minimum alignment length was 100 bp and >99% identity. If this criterion was not met, the lowest common ancestor (LCA) was assigned.

The Decona output was further curated using RStudio v4.2.2 (RStudio Team, 2020). To minimise the effect of possible contamination, MicroDecon (McKnight et al., 2019) was used for decontamination of the samples. Contaminant sequences were identified and subtracted, sequentially by control type (PCR, (cDNA), extraction, field), with thresholds optimised by control type. Read removal (Table S4) and species richness (Figure S1) were quantified to ensure adequate decontamination. OTUs were further curated using LULU (Frøslev et al., 2017) to merge potential redundancies based on sequencing similarity (default settings, >0.84) and co-occurrence patterns. The curated OTU table was compared to the original, and no changes were made to the data.

To validate the species list obtained from BLASTn taxonomic assignments, species were detected through the WoRMS database (World Register of Marine Species) and non-marine species were excluded from the analysis when absent on WoRMS or when they belonged to the *Aves* class. Species occurrence data were retrieved from OBIS (www.obis.org) using the robis() function. Occurrence data was retrieved using a 2-degree radius, but the species list was corrected by retaining species observed within the 2-degrees radius. Based on the uncertainty of species level identification using the chosen markers, identifications were further manually checked and renamed, either with LCA or by adding an asterisk if the identification returned two or more species (example: hit with *Clupea harengus* and *Clupea pallasi* becomes *Clupea harengus** because only *harengus* is present in the North Sea and Atlantic, but *Ammodytes*

marinus and *Ammodytes tobianus* become *Ammodytes sp* because there are multiple *Ammodytes* species in the North Sea) to indicate a certain level of uncertainty.

Statistical analyses and visualization

Further data analysis was executed in RStudio v4.2.2 (RStudio Team, 2020). Species richness and Shannon diversity index were determined using the diversity() function (v*egan* v2.6.4) (Oksanen et al., 2022) and visualised using a boxplot (ggplot2, v3.4.0; Table S5) (Hadley Wickham, 2016). Abundance data were calculated as relative read count (0–1), as these data were only used for between-sample comparison (Kelly et al. 2019). The significance of the difference in variances between species richness and diversity indices across habitats was tested between target fragments (eDNA-MiFish, eDNA-2kb, eRNA-MiFish) and among reef locations (high coverage, medium coverage, low coverage, and 5km away from the reef. For the analysis, a generalized linear model (GLM) was fitted using mass() (Brian et al., 2024; Table S6,S7). Species richness was modelled using a Poisson distribution, the Shannon diversity index with a gamma distribution, and abundance with a negative binomial distribution of the residuals. Subsequently, an analysis of variance (ANOVA) was used to assess the significant differences among fragment types and reef locations using the aov() function (package stats, v3.6.2; R Core Team, 2024) after confirming Levene's and Shapiro's tests for homogeneity of variance and normal distribution. Pairwise comparisons were performed using a post hoc Tukey test (HSD) with the TukeyHSD function (stats, v3.6.2; R Core Team, 2024) to identify which groups differed significantly.

To visualise the community composition differences between fragment types and locations (figure 2, 2,3, and S7), a Non-metric Multidimensional Scaling (NMDS) with Bray-Curtis dissimilarity ('bray') was applied. The species composition was visualised using a stacked bar plot with ggplot() (package ggplot2, v4.0.0; Figure S6, S7). A PERMANOVA was used (adonis2(), v2.4.6) to analyse whether there was a significant difference between fragment choice and reef location (Table S8). Unique and overlapping species were compared between fragment types and reef locations using an Euler diagram (package eulerr, v7.0.2).

To compare species-specific occurrences of the 35 most abundant species between fragment types and reef location, presence-absence matrices were constructed for each sample. Relative contribution was calculated

by summing the total detections from each replicate per location, normalised to 100% and visualised using a stacked barplot with ggplot() (package ggplot2, v4.0.0).

To compare the species composition with BRUV observations, the BRUV data used are described in detail in Coolen et al. (2023) and Coral et al. (in review; Supplementary text 2). Since the BRUV data was not collected simultaneously with water sampling, we used the overall species presence/absence data from the BRUV footage (from all samples) as an indication of local presence. These were matched to the molecular detections. For each fragment type, the relative proportion based on read counts was calculated after excluding hits that were renamed to 'unclassified' or 'non-target'. A binomial logistic regression was employed to assess the relationship between BRUV presence (0/1) and the relative abundance of each species for each fragment type (Table S9). Odds ratios and 95% confidence intervals were derived from the model output and visualised using a forest plot. Predicted probabilities of BRUV presence were further modelled across the observed range of relative abundances of each fragment type (Table S9). All analyses and visualisations were carried out in RStudio (v4.3.0) using the dplyr, tidyr, ggplot2, broom, and forcats packages.

RESULTS

Comparisons between read processing strategies and taxonomic assignments of datasets

After processing a total of 2.598.798 (eDNA - MiFish), 1.567.589 (eDNA - 2kb), and 554.384 (eRNA – MiFish) reads, these were assigned to species (table S4). Variation in final read depth across fragments did not affect species richness, as no correlation was observed between sequencing depth and species richness (Figure S2). Therefore, no additional correction for sequencing depth was performed. A considerable number of non-marine species were identified, primarily birds, cattle, or freshwater organisms. While these findings are quite interesting, they were considered non-targets for this research. Decontamination of reads with Microdecon caused only a slight decrease in the total number of reads (Table S4) and species richness (Figure S1). Extraction and PCR controls contained only reads from non-target taxa, which were not further considered for this study (Figure S3). The cDNA negative control and the eDNA field control

showed minor contamination after decontamination, with very low read counts, and were therefore not further curated (Figure S4).

Overall impact of fragment choice on diversity metrics, richness, and taxonomic composition

In general, eDNA-MiFish were comparable to the eDNA-2kb and eRNA-MiFish fragment amplicons and yielded similar numbers of taxa, with the eDNA MiFish (53 species) community being less diverse than the eRNA MiFish community (57 species). The eDNA 2kb marker produced the lowest taxa diversity (27 species) (Table S2, Figure 2C). This trend was inconsistent with diversity across samples, as there was a significant difference in species richness and Shannon diversity between fragment types (eDNA-MiFish: $p < 0.001$; eDNA-2kb: $p < 0.001$; eRNA-MiFish: $p < 0.001$, Figure 2A, Table S5, S6). An Euler diagram showed considerable overlap among all three methods, with most overlapping species found between eDNA-MiFish and eRNA-MiFish (Figure 2C). eRNA detected the highest number of unique species (15 out of 71). PERMANOVA and NMDS indicated a significant difference in community composition between all fragment choices (eDNA-MiFish: $p < 0.001$; eDNA-2kb: $p < 0.001$; eRNA-MiFish: $p < 0.001$, Figure 2A, Figure S7, Table S6), but optically the NMDS showed the eDNA - 2kb fragment was more isolated compared to the eRNA and eDNA MiFish communities (Figure 2B). Additionally, species composition was more similar between eDNA-MiFish and eRNA-MiFish than between the MiFish and 2kb fragments (Figure 2D, Figures S5, S6).

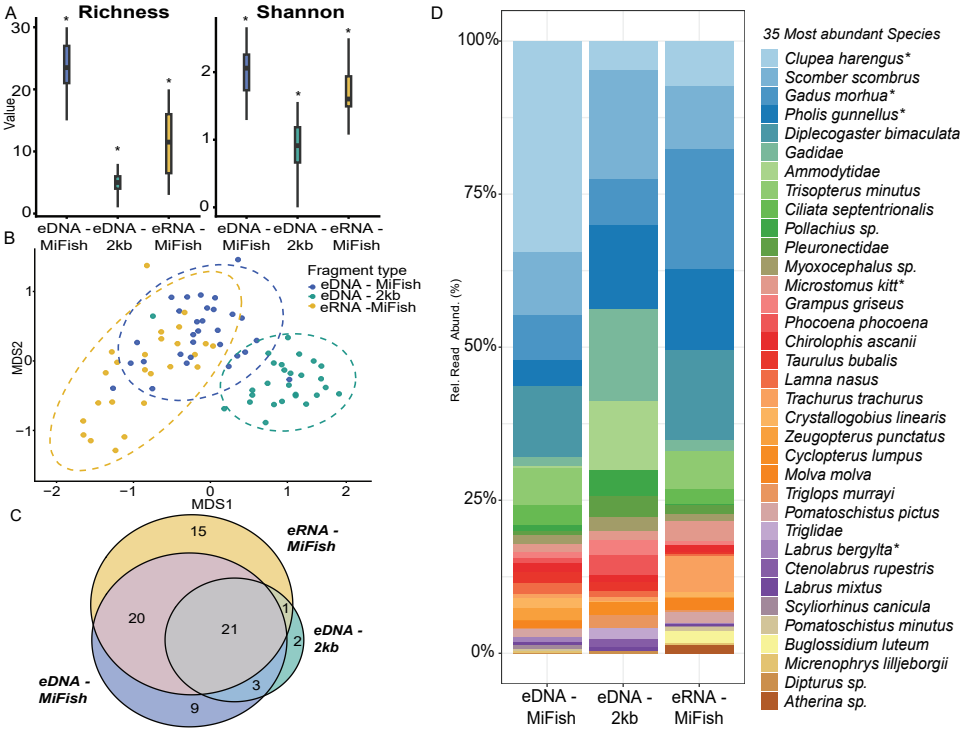


Figure 2: Comparison of the observed biodiversity of the vertebrate community of the M. modiolus reef between different marker and eNA choices. In A) boxplots of the biodiversity in terms of species richness per sample and shannon diversity index per sample, representing the amount and diversity of species between marker and eNA choice. Boxplots display the median, interquartile range (between 25th to 75th percentiles), minimum and maximum values (whiskers). Significant differences (GLM, Tukey post hoc comparisons) are indicated by asterisk. B) non-metric multidimensional scaling (NMDS) representing the overlap in community compositions. C) Overview of the 35 most abundant species each fragment type represented as a stacked bar plot where the average relative read abundance for each species is displayed in percentage. D) Euler diagram showing the number of overlapping and differences of observed species for each fragment type.

Location-specific impact of fragment choice on diversity and taxonomic composition

As previously reported by Coral et al. (in review), the diversity of fish communities was significantly higher on the reef than outside it, with a decreasing trend observed. However, in this study, the decreasing trend was less pronounced and not statistically significant for species richness ($p = 0.86$; Table S7). A significant difference was observed only in the Shannon diversity index for eDNA samples at 5 km from the reef. Interestingly, a significant interaction effect was detected ($p = 0.016$),

mainly driven by the notable difference in the eDNA-MiFish between 5 km from the reef and the other locations (Table S7). Consistent significant differences among all primer choices were observed across all locations (Figures 3A and 3B). Conversely, 2 kb and eRNA data showed no significant differences across locations, indicating consistency, likely reflecting the reduced sample size in this study. Nonetheless, the targeted eDNA-MiFish fragment detected most species at each location, with the majority of species identified through the eRNA-MiFish fragment overlapping with those detected by both eDNA markers (Figure 3B). Notably, the eDNA 2 kb marker identified substantially fewer species compared to the MiFish-eDNA and eRNA targeted fragments.

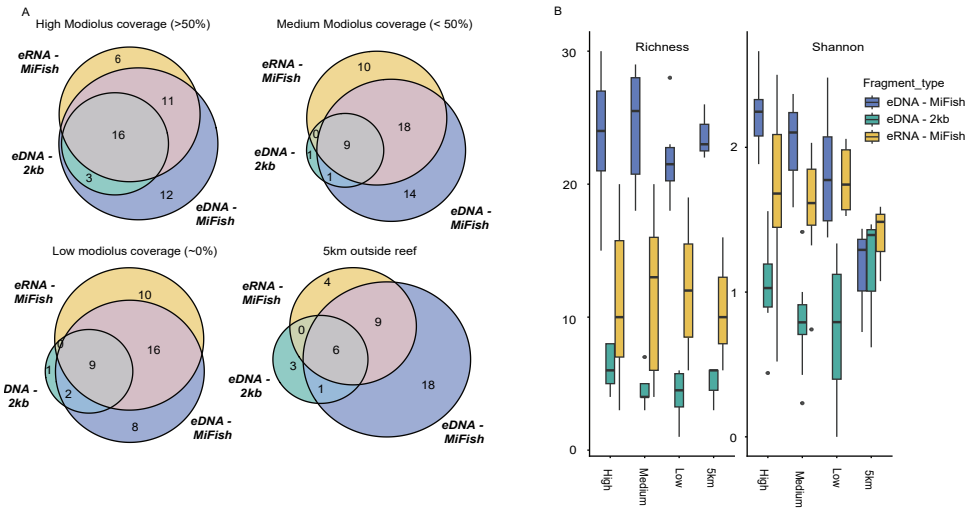


Figure 3: Comparison of the biodiversity of the vertebrate community between fragment type in different locations of the M. modiolus reef. Where A) Euler diagram showing overlap and differences in number of species of different marker and fragment choices between locations. B) Boxplots representing the biodiversity in terms of richness and Shannon index per sample between markers and eNA choice within different locations. Boxplots show median, interquartile range (between 25th to 75th percentiles), minimum and maximum values (whiskers) and outliers (closed circles). All comparisons were significantly different.

Species-specific variation between fragment types.

There was significant species-specific variation in the diversity and community composition between fragment types and reef locations (Figure 4, S6). Most taxa were present in the eDNA – MiFish fragment samples, including higher trophic taxa such as elasmobranchs like *Lamna nasus*, *Dipturus sp.*, and *Scyliorhinus canicula*, as well as marine mammals as *Pho-*

coena phocoena, *Grampus griseus*, and *Halichoerus grypus*. These taxa were largely absent or minimally represented in the eRNA and 2kb datasets. In contrast, several benthic species such as *Diplemogaster bimaculata*, *Pholis gunnellus*, *Microstomus kitt*, and *Molva molva*, along with demersal and pelagic fish species as *Trachurus trachurus*, *Clupea harengus*, and *Trisopterus minutus*, were more consistently detected across all fragment types. Overall relative contribution was roughly similar between locations, except at the 5 km site, which aligned with significant Shannon diversity results for that location (Figure 3A).

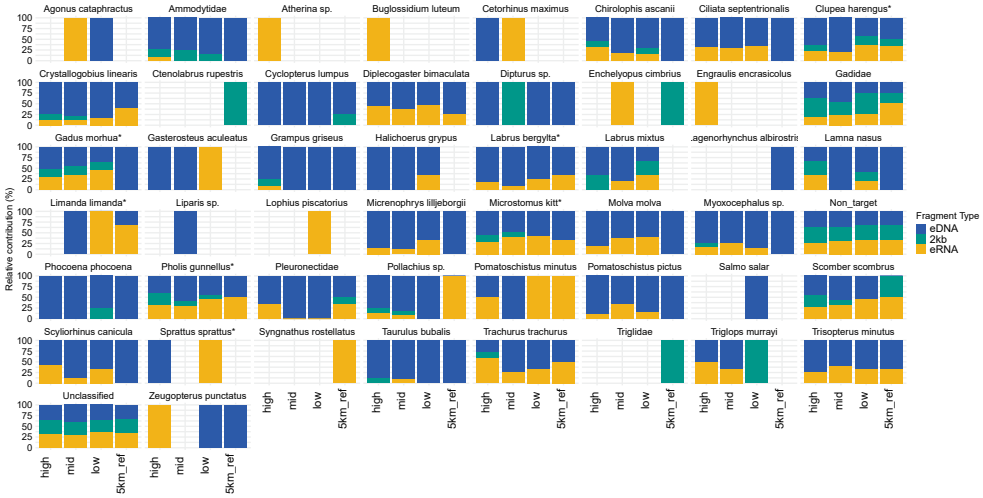


Figure 4: Relative contribution of each fragment type (eDNA – MiFish, eDNA – 2kb, and eRNA – MiFish) for the most abundant species in the complete dataset. Relative contribution is calculated as the proportion of samples that the given species was detected in, at a certain reef location, with 100% representing total number of detections. Each bar plot represents one of the locations (high, medium, low, 5 km). Colors represent the different fragment types (eDNA – MiFish, eDNA – 2kb, and eRNA – MiFish).

Relationship between BRUV and molecular findings

Overall, there is a positive relationship between BRUV detections (Figure S9) and detections from both eDNA and eRNA (Figure 5, Table S9). To test this, an odds ratio was calculated (>1) and showed that the odds ratio was most positive for the eRNA – MiFish fragment, although the effect was only marginally significant (odds ratio 1.16; p= 0.056). Nevertheless, overlapping intervals indicate similarity in patterns between target fragments (Figure 5A, Table S9). For all markers, increasing relative read

abundance was positively associated with a higher detection probability in BRUV data (Figure 5B).

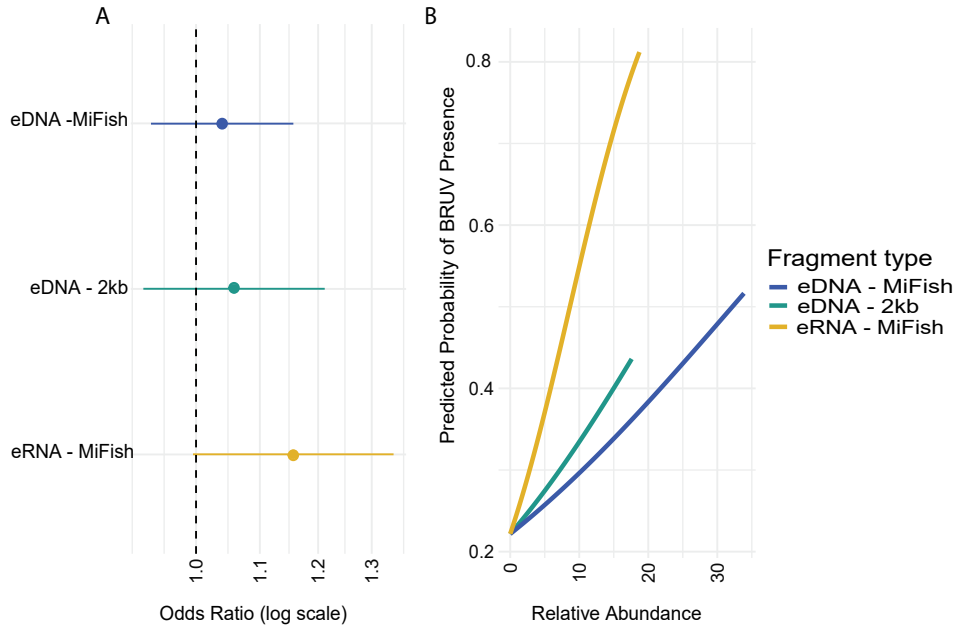


Figure 5: Relationship between detection by different fragments and BRUV observations. Where A) a forest plot shows the estimated odds ratios (log) from a logistic regression of species presence in BRUV data as a function of relative abundance of different fragment types (eDNA – MiFish, eDNA – 2kb, and eRNA – MiFish). Each point represents the odds ratio for a fragment type, with lines reflecting the 95% confidence interval. Odds ratios >1 indicate higher relative abundance increase chances of BRUV detection. B) Predicted probability curves of BRUV detection. Y-axis represents the calculated probability of detection and the X-axis represents the relative read abundance.

DISCUSSION

We compared three different types of nucleic acid fragments: a 12S eDNA - MiFish fragment, an eDNA - 2kb fragment spanning parts of the 12S and 16S regions, and a 12S eRNA - MiFish fragment. We evaluated the diversity of these fragments based on species richness, the Shannon diversity index, community composition, and their relationship with BRUV findings. Additionally, we examined whether different *M. modiolus* reef densities at various locations affected diversity and species-specific composition among the fragments. Our results demonstrated that the eDNA-MiFish fragment exhibited the highest species richness and Shannon

diversity index, particularly when comparing samples from reefs with different densities. The eRNA-MiFish fragment showed lower species richness but similar community composition compared to the eDNA-MiFish data. The 2kb fragment exhibited the lowest diversity and the most divergent species composition relative to the other types. Interestingly, eRNA and 2kb fragments detected only a few elasmobranch and mammal species in visits and had a stronger positive correlation with BRUV findings. To our knowledge, this study is the first to demonstrate the potential of eRNA metabarcoding to capture local and recent biodiversity signals, thereby enhancing molecular monitoring of marine vertebrate biodiversity in the North Sea.

The eRNA fraction was significantly less diverse compared to the eDNA MiFish fraction; fragment-specific observations need to be considered.

Data from the eRNA-MiFish fragments showed a significantly lower species richness and Shannon diversity index than the eDNA-MiFish fragments across samples. However, across all samples, similar species were found in reads of both eRNA and eDNA fragments, and both contained the same number of unique species, although the overall species richness and Shannon index were notably lower. Ordination analysis revealed a small yet significant difference in community composition between the eRNA and eDNA fragments. This supports the hypothesis that eRNA degrades more quickly and is therefore less prevalent in the water column, reflecting a less diverse community (Cristescu, 2019; T. S. Jo, 2023). Previous studies investigating the degradation rates of various fragment types have confirmed that mitochondrial RNA degrades faster than eDNA (Brandão-Dias et al., 2025; Marshall et al., 2021) in freshwater systems and are in line with our findings. These results show that, for example, in a lake system with a well-known fish species community, the eRNA metabarcoding data yielded slightly more true positives compared to eDNA data (Littlefair et al., 2022). Other aquatic studies also reported reduced diversity with eRNA metabarcoding in a range of aquatic taxonomic groups (Kagzi et al., 2023; Miyata et al., 2022; S. Zhang et al., 2020). Nevertheless, other comparisons show a more mixed image in which the eRNA signal has resulted in a higher alpha diversity and a different community composition in marine (Giroux et al., 2022) and other aquatic systems (Greco et al., 2022; Li et al., 2024; Macher et al., 2024; Pochon et al., 2017). These studies often report that elevated eRNA diversity reflects higher concentrations of eRNA from living and active organisms, which can lead to an overestimation of the alive community, relative to what

is detected with eDNA. Notably, many of these studies are conducted in mesocosms or other controlled settings where the overall nucleic acid concentration considerably higher than in open-water systems. In open, well mixed systems, as was the case for this study, eRNA degrades faster and/or is diluted more rapidly which reduces the probability of detection, hence the diversity (Scriver et al., 2023). Therefore, the reduced eRNA diversity observed around the *M. modiolus* reef likely represents the local and short lived eRNA fragments that characterizes the local and active community.

Low read abundance amplicons in the cDNA was also found after the decontamination using Microdecon and is likely introduced during reverse transcriptase step using DNAse 1. Although DNAse 1 is very effective and now PCR amplicon was found on gel, DNAse digestion might not have been complete (Hashemipetroudi et al., 2018). Additional DNAse treatments may improve DNA degradation but also risk the increased RNA degradation (Paska et al., 2019). Nevertheless the detected reads in the cDNA control were in order of magnitudes lower than biological samples and are therefore not likely to have influenced the community composition of the eRNA fragment samples.

Both eRNA and eDNA produced unique results, and although it is expected for eDNA, which likely detects both local and transported materials from surrounding areas, the unique eRNA is less in line with the hypothesis. eDNA and eRNA metabarcoding are prone to several technical biases that may occur during sampling and bioinformatics processing, leading to small deviations and stochastic effects between samples (van der Loos & Nijland, 2021) and maybe especially the case for low-abundant, single-sample findings. In this study, a separate filter was used for eRNA sampling, which was snap frozen in liquid nitrogen and stored at -80 degrees instead of at -20 as was the case for the eDNA samples. Although this was initially done to protect eRNA from degradation, it might have skewed degradation rates between eDNA and eRNA (Yates et al., 2021). In addition, some clear, unique signals in eRNA samples, as was the case for *Buglossidium luteum* and *Lophius piscatorius*, might be due to the localised activity of these species. As RNA primarily derived from active cells, it produces a relatively high concentration signal, whereas DNA is often extracellular, persisting in the water column and is therefore likely present in lower concentrations (Cristescu, 2019; Littlefair et al., 2022; Pochon et al., 2017). Additionally, eRNA associated with living cells or organelles can also affect extraction efficiency, potentially leading to taxa

being detected only with eRNA when the corresponding eDNA is not successfully extracted (Pochon et al., 2017; Yates et al., 2021).

The 2kb fragment shows less diversity than the eRNA and eDNA MiFish fragment.

The eDNA 2kb fragment analysis detected the fewest species and was also significantly lower than the eRNA MiFish fragment. Several studies have explored and shown the differential decay of nucleic acid fragments, demonstrating that RNA, especially mitochondrial RNA, degrades the most rapidly, followed by longer DNA fragments, and lastly short DNA fragments (Brandão-Dias et al., 2025; T. S. Jo, 2023; Marshall et al., 2021). Therefore, it is surprising that the 2kb DNA fragment showed the least biodiversity and a different community composition. One of the most obvious explanations would be primer bias, as mismatches in primers can produce different species lists (Miya et al., 2015; Polanco F. et al., 2021) and maybe especially so for long fragment metabarcoding (Deiner et al., 2017; Elbrecht et al., 2018; Elbrecht & Leese, 2015). Nevertheless, in this study, the 2kb primers were used and extensively tested (Doorenspleet et al., 2025); therefore, it is unlikely that this is the case for this primer set. Nevertheless, environmental long fragment amplification is particularly challenging and less efficient because they are more prone to incomplete extension, chimeras, and inhibitors that are more likely to affect amplification (Elbrecht et al., 2018; Fonseca et al., 2012; Śpibida et al., 2017). Additionally, long fragment metabarcoding requires sufficient high-quality templates, as longer templates degrade more quickly or may originate from other organic sources (active cells, shed cells, free-living; Brandão-Dias et al., 2025; T. Jo et al., 2017, 2020). Indeed, several studies on long fragment metabarcoding show that longer eDNA amplicons are less likely to be detected under environmental conditions, especially over time (Bista et al., 2017; Doorenspleet et al., 2025; Maggini et al., 2024). Thus, depending on the question and environment, it may be useful to use the 2kb fragment, especially as it can recover haplotypes (Feist & Lance, 2021; Karin et al., 2023) and improve species-level taxonomy assignments (Doorenspleet et al., 2025). Nevertheless, compared to eRNA species shown in this study, it is likely that the 2kb primer set will not serve as a reliable intermediate for improved spatial-temporal resolution. Mainly, because reduced quality and concentration in open water systems in combination with amplification restrictions are likely to overshadow the possibility to adequately pick up the long fragment eDNA signal.

Reduced location effect may be linked to molecular stability and sensitivity

In this study, there was no significant difference in species diversity between high, medium, and low M. modiolus coverage in the eRNA MiFish and eDNA 2kb data; only for the eDNA – MiFish samples, the 5 km reference location data showed a significantly lower species richness and Shannon index than the locations on the reef. Previously, studying the complete set of eDNA – MiFish samples across many more locations on the reef, a significant decrease in alpha diversity was found with decreasing density of M. modiolus coverage (Coral et al. in review). Using a subset of those samples likely reduced statistical power, which reduced the detectability of the observed reef effect. This observation is in line with studies suggesting that field and technical replications, especially across locations and gradients, are key to improving spatial resolution (Beentjes et al., 2019; Macher et al., 2021; Seymour et al., 2021). Therefore, it is likely that the reduced sample size has contributed to a reduced detection of the spatial biodiversity differences among locations with varying *M. modiolus* densities. Nevertheless, eDNA MiFish data detected a significant difference in diversity between inside and 5 km outside the reef, which was not observed with eRNA- MiFish data. Several mechanisms likely explain this discrepancy. As eRNA is generally less stable, it reduces detection stability, leading to more stochastic effects, increased variance, zero-inflation, and skewed spatial community patterns (Littlefair et al., 2022; Marshall et al., 2021). Thus, although the relatively small sample size likely played a significant role, the lack of detection of larger mobile species in most eRNA-MiFish samples, together with the absence of considerable location effects, likely reflects the stability of eRNA, suggesting that the observed signal primarily reflects improved temporal rather than spatial resolution.

eRNA species-specific detection sensitivity reflects the species-specific behaviour on the reef and has the strongest relationship with BRUV values

The species-specific detection sensitivity comparison showed that several higher trophic taxa, as *Lamna nasus*, *Dipturus sp.* and *Phocoena phocoena*, are well represented in the eDNA MiFish samples but absent or minimally represented in the eRNA MiFish data. This suggests that eDNA is particularly sensitive to residual and/or transported material from larger mobile species that do not permanently reside in the reef (Balasingham et al., 2017; Dunn et al., 2023; Heessen et al., 2023). In addition, there was more consistent detection of benthic and demersal species, such as

Diplemogaster bimaculata, Pholis gunnellus, and *Clupea harengus*, across the fragment types. These species are often associated with *Modiolus* reefs and other biogenic reefs and are more permanent residents of reef systems (Dickson et al., 2023; Kent, Mair, et al., 2017; van der Reijden et al., 2019), therefore shedding eDNA and eRNA more locally and continuously. Thus, these results further support that eRNA detects the living and active community, whereas eDNA also captures the residual and/or transported community. Additionally, our findings are consistent with previous research showing that eRNA signals from larger animals are linked to higher turnover rates due to their lower abundance (Scriver et al., 2023; Y. Zhang et al., 2024), further suggesting the potential role for eRNA to provide a snapshot of the active community.

A positive relationship was also observed between the relative read abundance of eDNA and eRNA and the probability of BRUV detection. In other words, molecular methods significantly contribute to visual surveys. The strongest association between BRUV and the eRNA fragment suggests that eRNA may slightly better represent the more active local community. However, overlapping confidence intervals indicate that the statistical power to support this claim is too low. Additionally, extensive BRUV surveys also have limitations, as they mainly focus on scavenging and hunting species and are restricted by view, visibility, and behaviour. (Cappo et al., 2004; Roberts et al., 2016; Sherman et al., 2020). Thus, although limited, taxon-specific observations in both eDNA and eRNA MiFish fragment types, combined with a positive relationship between BRUV results, suggest the potential of eRNA as a complementary signal for marine biodiversity monitoring in natural reef systems.

Although the results are promising, it remains debated whether eRNA metabarcoding can be easily integrated into routine molecular monitoring, as costs are higher and sample handling in the field is more complicated, mainly due to the less stable nature of eRNA. For example, eRNA requires rapid sample processing and lower storage temperatures to minimise degradation, as well as specialised extraction kits and a reverse transcriptase kit, all of which increase costs (Macher et al., 2024). eDNA, on the other hand, is more stable and is used more routinely, which considerably reduces laboratory processing costs (Deiner et al., 2017). Thus, although eRNA metabarcoding is a promising method for improved detection of the active community, this advantage must be weighed against the increased costs and challenges of field sampling. Nevertheless, improving the molecular toolbox by adding eRNA-based

analysis of biodiversity to detect the living, active community is vital for detecting short-term ecological dynamics, such as after a heatwave or during recruitment or spawning events. In addition to biodiversity detection, the eRNA signal can also be used to identify the functional or eco-physiological status of the community, including responses to stress or environmental change. Therefore, despite additional costs, eRNA provides a powerful complement that can significantly improve the detectability of ecosystem function in reefs in the North Sea and beyond.

Conclusion and future perspectives

In this study, we have demonstrated that eRNA and eDNA provide complementary signals, with distinct perspectives on the *M. modiolus* reef. Data from the eRNA short fragment showed lower diversity than eDNA, while the overall community composition remained constant, likely reflecting the local, metabolically active community associated with this eRNA signal. eDNA, on the other hand, may indicate a more regional signal, reflecting the diversity of transported and decaying material. This is further supported by consistent signals of more permanent benthic species, whereas larger species were mostly undetected with eRNA. eRNA was also most strongly positively associated with BRUV findings. These results suggest that differences between eDNA and eRNA signals are not solely due to nucleic acid persistence but are also influenced by ecological context, such as species activity, habitat complexity, and methodological constraints, including amplification efficiency and biases. Nonetheless, using eRNA metabarcoding as a complementary method for monitoring marine vertebrates adds value by enhancing temporal sensitivity and increasing confidence in detecting the local community, while eDNA provides a broader view of reef and surrounding biodiversity.

Future research should focus on standardising eRNA sampling protocols, especially for preservation, to minimise degradation and processing costs. Additionally, comparative experiments under controlled and field conditions could enhance understanding of degradation rates and detection windows among different signals, which is particularly relevant when considering degradation times at various temperatures, water chemistries, and habitats. Studies linking biomass and abundance data to eRNA detection rates will improve understanding of what the eRNA signal actually reflects. Since eRNA can be very useful for understanding physiological and functional responses, future research should also explore environmental genomics and transcriptomics to better comprehend natural reef responses to both natural and human-induced disturbances.

Incorporating eRNA into the molecular toolkit for monitoring marine life will significantly improve temporal resolution in biodiversity assessments and serve as a foundation for better understanding rapid shifts and stress responses following heatwaves, storms, and pollution events.

ACKNOWLEDGEMENTS

We are grateful to all students who helped with the analysis of the multitude of this data including the BRUV video analysis and processing of the eDNA samples. We also would like to thank all students and scientists on board the R/V Pelagia who helped with the sample collection, especially Daniel van Berkel, Loran Kleine Schaars, Leandra Kornau and Rob Witbaard. We would warmly like to thank the crew of R/V Pelagia for their priceless contribution and assistance on board for operating fieldwork equipment, as well as for the amount of care given to our well-being.

FUNDING STATEMENT

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DATA AVAILABILITY

The sequence data used for writing this research are uploaded to the European Nucleotide Archive (ENA) under project accession PRJEB105274 for the data.

SUPPLEMENTARY MATERIAL

Supplementary figures

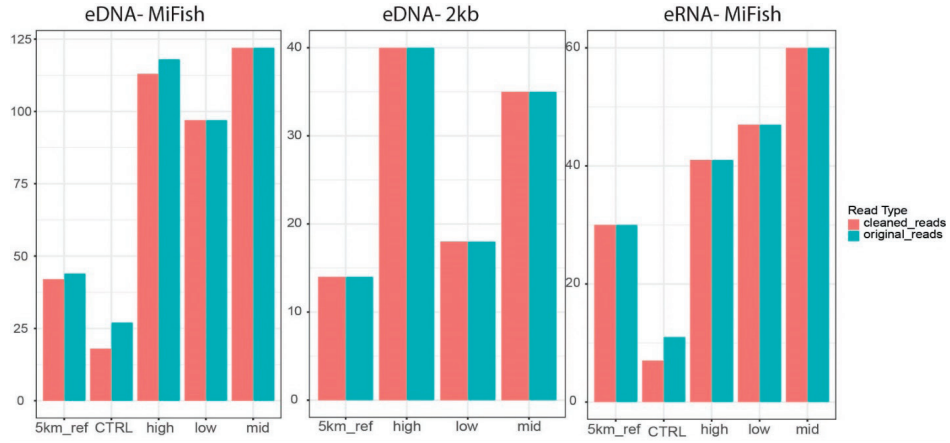


Figure S1: Species richness before and after decontamination using Microdecon, for eDNA–MiFish, eDNA– 2kb and eRNA – MiFish.

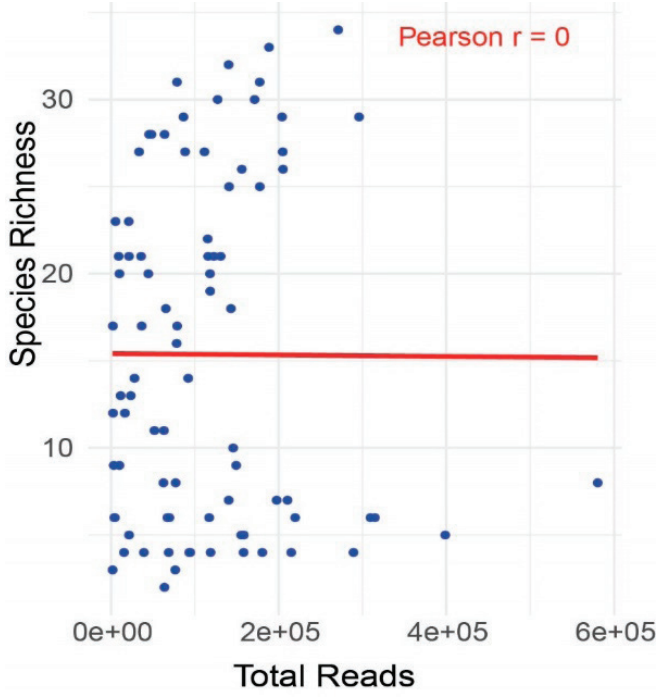


Figure S2: correlation between sequencing depth and species richness using a pearson correlation coefficient. Figure shows no correlation between sequencing depth and richness.

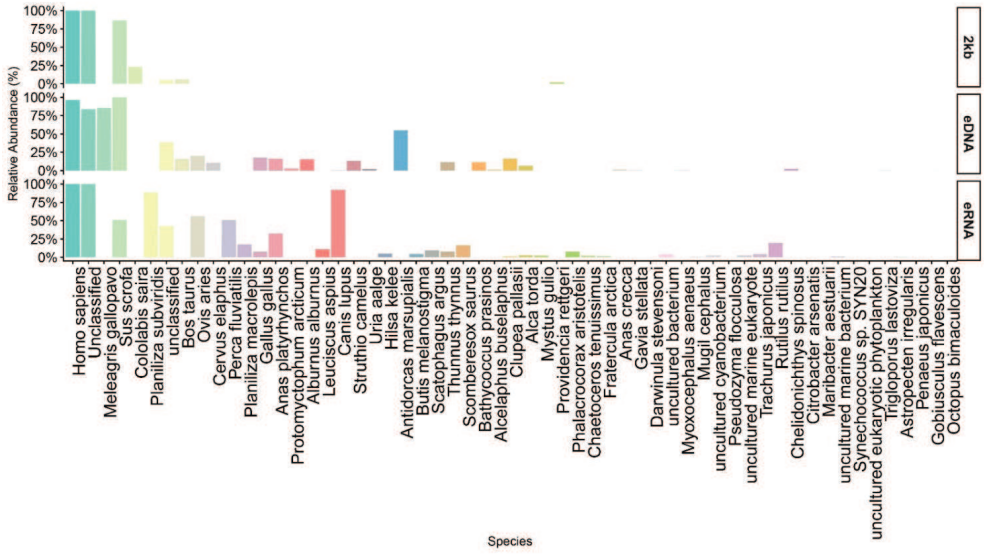


Figure S3: Relative read abundance of the species identified as Non-target for each Fragment type.

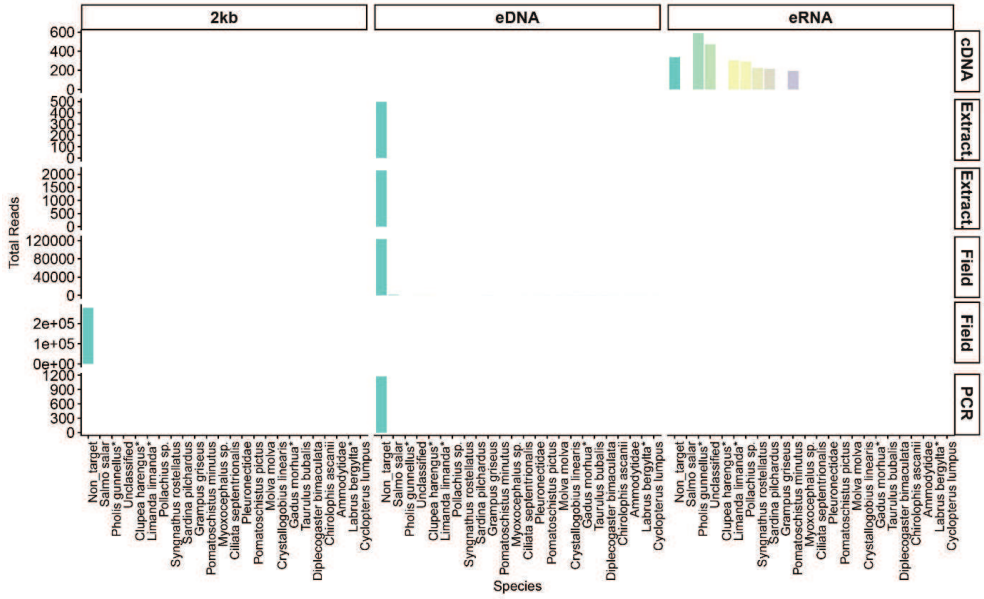


Figure S4: Present reads in the different negative controls (Field, extraction, PCR and cDNA controls) from the Fragment types.

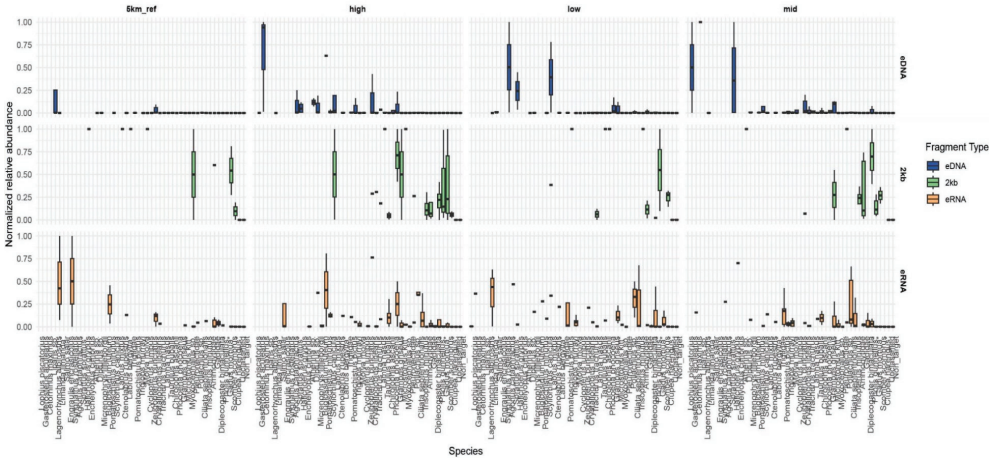


Figure S5: Boxplot of the normalized (log10) relative read abundance of each species for each fragment type and each Reef location. Colors indicate the different fragment types and facet the different reef locations.



Figure S6: Stacked barplot of the community composition for each sample. Colors indicate the relative read abundances of each species

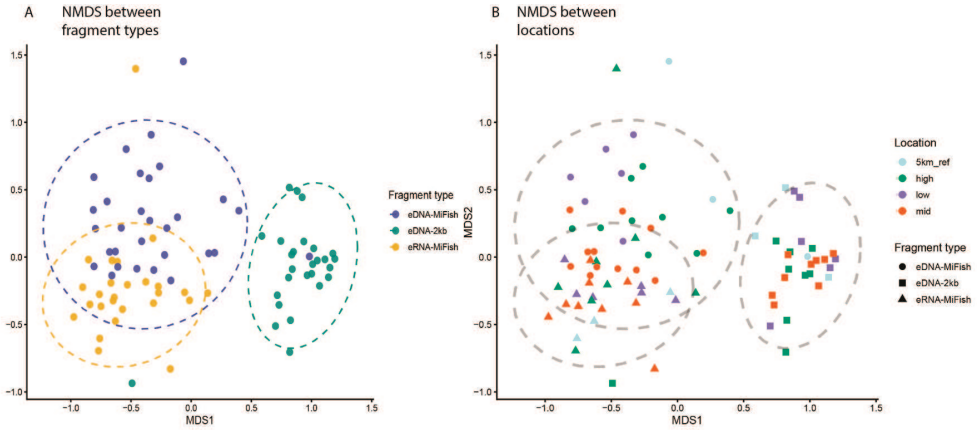


Figure S7: NMDS plots of the community composition between fragments and location on the reef. A) between fragment types where the colors indicate the different fragment types. Eclipses indicate de spread between samples of the same fragment type. B) between locations and fragment types where colors indicate the different locations and the shapes the different fragment types. Eclipses indicate de spread between samples of the same fragment type.



Figure S8: Box core from three different perspectives of the *M. modiolus* reef of the high location.

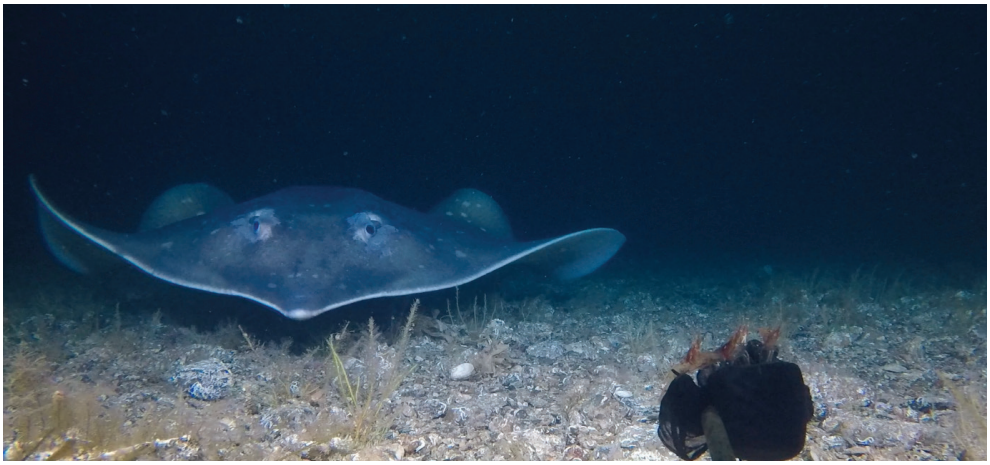


Figure S9: Still of footage of BRUV detection in this case showing the detection of a *D.batis* individual.

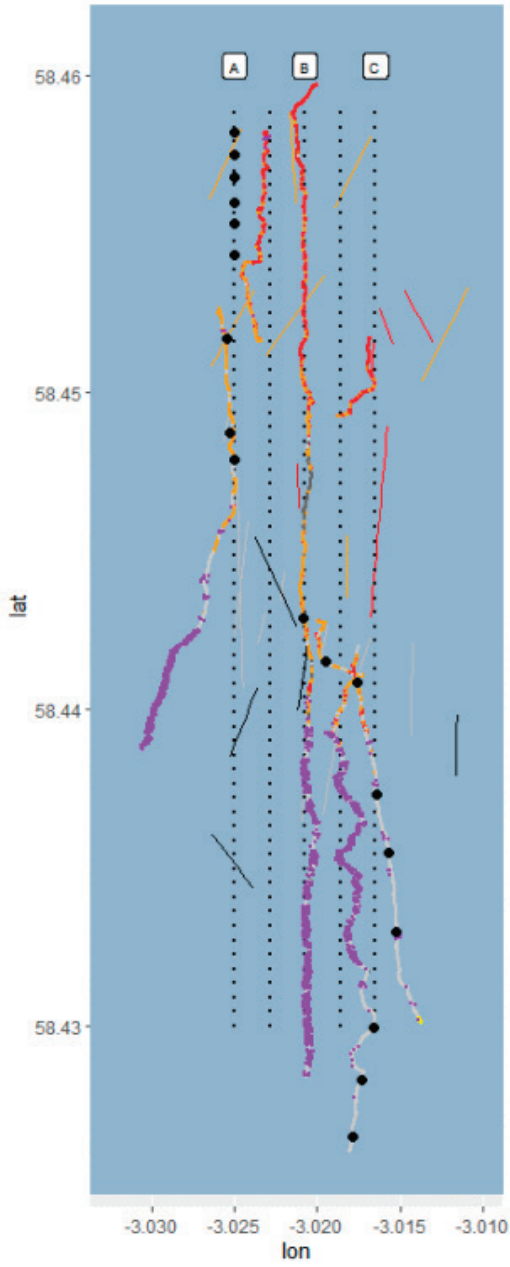


Figure S10: Video analysis of M. modiolus coverage around the Noss Head reef with seabed in sight and scored value indicated in color (yellow: sand, orange: 50% modiolus, dark grey: course ground no small rocks, light grey: course ground with small rocks, purple: rocks >30 cm). Small black dots indicate transect lines and large black dots indicate sample stations. Stripes (in color) indicate previous data.

Supplementary tables

Table S1: overview of the samples per fragment type and location on the reef, including longitude, latitude, depth and total number of replicates per sample.

Fragment_type	*Location_reef*	*latitude*	*longitude*	*Depth*	*NR_samples*
2kb	*5km_ref*	*58,4077*	*-2,9406*	*~66m*	*3*
2kb	*high*	*58,4567*	*-3,0253*	*~45m*	*6*
2kb	*high*	*58,4582*	*-3,0257*	*~45m*	*3*
2kb	*low*	*58,4267*	*-3,018*	*~60m*	*6*
2kb	*mid*	*58,441*	*-3,018*	*~48m*	*3*
2kb	*mid*	*58,448*	*-3,025*	*~48m*	*6*
eDNA	*5km_ref*	*58,4077*	*-2,9406*	*~66m*	*3*
eDNA	*high*	*58,4567*	*-3,0253*	*~45m*	*6*
eDNA	*high*	*58,4582*	*-3,0257*	*~45m*	*3*
eDNA	*low*	*58,4267*	*-3,018*	*~60m*	*6*
eDNA	*mid*	*58,441*	*-3,018*	*~48m*	*3*
eDNA	*mid*	*58,448*	*-3,025*	*~48m*	*6*
eRNA	*5km_ref*	*58,4077*	*-2,9406*	*~66m*	*3*
eRNA	*high*	*58,4567*	*-3,0253*	*~45m*	*6*
eRNA	*high*	*58,4582*	*-3,0257*	*~45m*	*2*
eRNA	*low*	*58,4267*	*-3,018*	*~60m*	*6*
eRNA	*mid*	*58,441*	*-3,018*	*~48m*	*3*
eRNA	*mid*	*58,448*	*-3,025*	*~48m*	*6*

Table S2: *overview of* read counts for each species and individual samples
See excel

Table S3: Primer sequences used for this study.

		Sequence
2kb_fw	Fish_12S_fw1-ONT	GGATTAGATACCCYACTATGY
2kb_rev	Fish_16S_rv1-ONT	GATTGCGCTGTTATCCCTRG
MiFish_fw	MiFish_U/E_V1 Forward	GTYGGTAAAWCTCGTGCCAGC
MiFish_rev	MiFish_U/E_V1 Reverse	ATAGTGGGGTATCTAATCCYAGTTTG

† Calculations by ThermoFisher Scientific Tm calculator for Phire DNA polymerase, for the sequence specific part only

Table S4: Read count from each sequencing run and after each processing step

Seq run	Raw reads	Base-called reads	Decona reads	After_decon	species	Species After Final_taxonomy assignment
eDNA_MiFIsh	5.96E6	4.9E6	4.827.478	4.190.837	3.973.773	2.589.798
eDNA_2kb	17.47E6	11.7E6	4.989.135	4.989.135	4.281.501	1.567.589
eRNA_MiFish	23.6E6	15.7E6	1.271.568	1.034.786	982.747	554.384

Table S5: Mean ecological indices per Fragment_type (eDNA_MiFish, eDNA_2kb and eRNA_MiFish), Location on the reef (hig, medium low), shown as mean ± SEM."

Fragment_type	*Location_reef*	*Diversity_Index*	*mean_value*	*se*	*n*	*mean_se*
eDNA_MiFish	*high*	*richness*	*23.556*	*1.717*	*9*	*23.556 ± 1.717*
eDNA_MiFish	*high*	*shannon*	*2.216*	*0.079*	*9*	*2.216 ± 0.079*
eDNA_MiFish	*mid*	*richness*	*24.417*	*1.138*	*12*	*24.417 ± 1.138*
eDNA_MiFish	*mid*	*shannon*	*2.046*	*0.075*	*12*	*2.046 ± 0.075*
eDNA_MiFish	*low*	*richness*	*22.000*	*1.390*	*6*	*22 ± 1.39*
	low	*shannon*	*1.830*	*0.174*	*6*	*1.83 ± 0.174*
eDNA_MiFish	*5km_ref*	*richness*	*23.667*	*1.202*	*3*	*23.667 ± 1.202*
eDNA_MiFish	*5km_ref*	*shannon*	*1.151*	*0.218*	*3*	*1.151 ± 0.218*
eDNA_2kb	*high*	*richness*	*6.000*	*0.553*	*9*	*6 ± 0.553*

Table S5: Mean ecological indices per Fragment_type (eDNA_MiFish, eDNA_2kb and eRNA_MiFish), Location on the reef (hig, medium low), shown as mean ± SEM." *(continued)*

Fragment_type	*Location_reef*	*Diversity_Index*	*mean_value*	*se*	*n*	*mean_se*
eDNA_2kb	*high*	*shannon*	*1.074*	*0.113*	*9*	*1.074 ± 0.113*
eDNA_2kb	*mid*	*richness*	*4.333*	*0.408*	*9*	*4.333 ± 0.408*
eDNA_2kb	*mid*	*shannon*	*0.789*	*0.112*	*9*	*0.789 ± 0.112*
eDNA_2kb	*low*	*richness*	*4.167*	*0.792*	*6*	*4.167 ± 0.792*
eDNA_2kb	*low*	*shannon*	*0.737*	*0.212*	*6*	*0.737 ± 0.212*
eDNA_2kb	*5km_ref*	*richness*	*5.000*	*1.000*	*3*	*5 ± 1*
eDNA_2kb	*5km_ref*	*shannon*	*1.160*	*0.272*	*3*	*1.16 ± 0.272*
eRNA_MiFish	*high*	*richness*	*11.000*	*2.204*	*8*	*11 ± 2.204*
eRNA_MiFish	*high*	*shannon*	*1.668*	*0.217*	*8*	*1.668 ± 0.217*
eRNA_MiFish	*mid*	*richness*	*12.000*	*1.972*	*9*	*12 ± 1.972*
eRNA_MiFish	*mid*	*shannon*	*1.602*	*0.133*	*9*	*1.602 ± 0.133*
eRNA_MiFish	*low*	*richness*	*12.167*	*2.040*	*6*	*12.167 ± 2.04*
eRNA_MiFish	*low*	*shannon*	*1.774*	*0.097*	*6*	*1.774 ± 0.097*
eRNA_MiFish	*5km_ref*	*richness*	*10.667*	*2.906*	*3*	*10.667 ± 2.906*
eRNA_MiFish	*5km_ref*	*shannon*	*1.383*	*0.157*	*3*	*1.383 ± 0.157*

Table S6: Significant differences in richness and Shannon between fragment choices using GLM and tukey post hoc comparisons

Richness	**LR Chisq**	**Df**	**Pr(>Chisq)**				
Fragment_ type	*386.82*	*2*	*< 2.2e-16 ****				
Index	**Shapiro_W**	**Shapiro_P**	**Levene_F**	**Levene_P**			
Shannon	*0.97*	*0.05*	*0.006*	*0.99*			
Shannon	**Df**	**Sum sq**	**Mean sq**	**F value**	**Pr(>F)**		
Fragment_ type	*2*	*16.201*	*8.1006*	*45.8*	*5.532e-14 ****		
Residuals	*80*	*14.150*	*0.1769*				
contrast	**estimate**	**SE**	**df**	**z.ratio**	**p.value**	**Index**	**t.ratio**
eDNA - 2kb	*1,57*	*0,097*	*Inf*	*16,5783*	*0*	*richness*	
eDNA - eRNA	*0,717*	*0,069*	*Inf*	*10,35092*	*2,78E-14*	*richness*	
2kb - eRNA	*-0,857*	*0,104*	*Inf*	*-8,20689*	*3,52E-14*	*richness*	
eDNA - 2kb	*1,057*	*0,111*	*80*		*0*	*shannon*	*9,41*

Table S7: Significant differences in richness and Shannon between fragment choice within different locations using GLM and tukey post hoc comparisons

Richness	**LR Chisq**	**Df**	**Pr(>Chisq)**				
Fragment_type	*384.19*	*2*	*< 2e-16 ****				
Location_reef	*0.74*	*3*	*0.86*				
Fragment_type: Location_reef	*4.41*	*6*	*0.62*				
Contrast	**estimate**	**SE**	**df**	**z.ratio**	**P.value**	**index**	**location**
eDNA-2kb	*1.555*	*0.284*	*Inf*	*5.471*	*<.0001*	*richness*	*5km_ref*
eDNA-eRNA	*0.797*	*0.213*	*Inf*	*3.743*	*0.0005*	*richness*	*5km_ref*
2kb-eRNA	*-0.758*	*0.313*	*Inf*	*-2.421*	*0.0409*	*richness*	*5km_ref*
eDNA-2kb	*1.368*	*0.152*	*Inf*	*8.972*	*<.0001*	*richness*	*High*
eDNA-eRNA	*0.761*	*0.127*	*Inf*	*6.005*	*<.0001*	*richness*	*High*
2kb-eRNA	*-0.606*	*0.173*	*Inf*	*-3.506*	*0.0013*	*richness*	*High*
eDNA-2kb	*1.664*	*0.218*	*Inf*	*7.629*	*<.0001*	*richness*	*Low*
eDNA-eRNA	*0.592*	*0.146*	*Inf*	*4.061*	*<.0001*	*richness*	*Low*
2kb-eRNA	*-1.072*	*0.232*	*Inf*	*-4.624*	*0.0001*	*richness*	*Low*

Table S7: Significant differences in richness and Shannon between fragment choice within different locations using GLM and tukey post hoc comparisons *(continued)*

Richness	**LR Chisq**	**Df**	**Pr(>Chisq)**				
eDNA-2kb	*1.729*	*0.170*	*Inf*	*10.143*	*<.0001*	*richness*	*Mid*
eDNA-eRNA	*0.710*	*0.113*	*Inf*	*6.310*	*<.0001*	*richness*	*Mid*
2kb-eRNA	*-1.019*	*0.187*	*Inf*	*-5.452*	*<.0001*	*richness*	*Mid*
Index	**Shapiro_W**	**Shapiro_P**	**Levene_F**	**Levene_P**			
Shannon	*0.97*	*0.05*	*0.006*	*0.99*			
Shannon	**Df**	**Sum sq**	**Mean sq**	**F value**	**Pr(>F)**		
Fragment_type	2	16.20	8,100641	55.62	2.93E-15		
Location_reef	3	1.34	0.48	3.07	0.033		
Fragment_type: Location_reef	6	2.47	0.41	2.82	0.016		
Residuals	71	10.34	0.15				
contrast	**estimate**	**SE**	**df**	**t.ratio**	**p.value**	**Index**	**Location_reef**
eDNA - 2kb	-0.009	0.312	71	-0.029	0.999	*shannon*	5km_ref
eDNA - eRNA	-0.2324	0.312	71	-0.746	0.737	*shannon*	5km_ref
2kb - eRNA	-0.223	0.312	71	-0.716	0.755	*shannon*	5km_ref
eDNA - 2kb	1.141	0.180	71	6.345	5.44E-08	*shannon*	high
eDNA - eRNA	0.548	0.185	71	2.955	0.011	*shannon*	high
2kb - eRNA	-0.594	0.185	71	-3.201	0.005	*shannon*	high
eDNA - 2kb	1.093	0.220	71	4.961	1.37E-05	*shannon*	low

Table S7: Significant differences in richness and Shannon between fragment choice within different locations using GLM and tukey post hoc comparisons *(continued)*

Richness	LR Chisq	Df	Pr(>Chisq)				
eDNA - eRNA	0.056	0.220	71	0.253	0.965	*shannon*	low
2kb - eRNA	-1.037	0.220	71	-4.707	3.56E-05	*shannon*	low
eDNA - 2kb	1.257	0.168	71	7.471	4.03E-10	*shannon*	mid
eDNA - eRNA	0.444	0.168	71	2.638	0.027	*shannon*	mid
2kb - eRNA	-0.813	0.180	71	-4.520	7.09E-05	*shannon*	mid

contrast	Fragment_type	estimate	SE	df	t.ratio	p.value	Index
5km_ref - high	eDNA	-1.065	0.254	71	-4.185	0.0004	Shannon_ across
5km_ref - low	eDNA	-0.679	0.270	71	-2.515	0.066	Shannon_ across
5km_ref - mid	eDNA	-0.895	0.246	71	-3.634	0.002	Shannon_ across
high - low	eDNA	0.386	0.201	71	1.919	0.230	Shannon_ across
high - mid	eDNA	0.169	0.168	71	1.006	0.746	Shannon_ across
low - mid	eDNA	-0.217	0.191	71	-1.135	0.669	Shannon_ across
5km_ref - high	2kb	0.086	0.254	71	0.338	0.987	Shannon_ across
5km_ref - low	2kb	0.424	0.270	71	1.569146	0.403	Shannon_ across

Table S7: Significant differences in richness and Shannon between fragment choice within different locations using GLM and tukey post hoc comparisons *(continued)*

Richness	LR Chisq	Df	Pr(>Chisq)				
5km_ref - mid	2kb	0.371	0.254	71	1.459039	0.468	Shannon_ across
high - low	2kb	0.338	0.201	71	1.67795	0.343	Shannon_ across
high - mid	2kb	0.285	0.180	71	1.585679	0.393	Shannon_ across
low - mid	2kb	-0.052	0.201	71	-0.25968	0.994	Shannon_ across
5km_ref - high	eRNA	-0.284	0.258	71	-1.10065	0.690	Shannon_ across
5km_ref - low	eRNA	-0.390	0.270	71	-1.44708	0.475	Shannon_ across
5km_ref - mid	eRNA	-0.219	0.254	71	-0.86013	0.825	Shannon_ across
high - low	eRNA	-0.106	0.206	71	-0.51494	0.955	Shannon_ across
high - mid	eRNA	0.066	0.186	71	0.353408	0.985	Shannon_ across
low - mid	eRNA	0.172	0.201	71	0.853478	0.829	Shannon_ across

Table S8: Permanovas of fragment choice, Location and interaction.

Analysis of variance Fragment_type	**Df**	**Sum sq**	**Mean sq**	**F value**	**Pr(>F)**
Groups	*2*	*0.082*	*0.041*	*2.350*	*0.101*
Residuals	*80*	*1.402*	*0.018*		
Perm. Test for homogeneity	**Df**	**Sum sq**	**Mean sq**	**F value**	**Pr(>F)**
Groups	*2*	*0.082*	*0.041*	*2.350*	*0.121*
Residuals	*80*	*1.402*	*0.018*		
Fragment_type Permanova	**Df**	**sumofsqrs**	**R2**	**F**	**Pr(>F)**
Model	*2*	*8.160*	*0.409*	*27.657*	*1e-04 ****
Residual	*80*	*11.802*	*0.591*		
Total	*82*	*19.963*	*1.000*		
Analysis of variance Location_reef	**Df**	**Sum sq**	**Mean sq**	**F value**	**Pr(>F)**
Groups	*3*	*0.018*	*0.006*	*0.3878*	*0.762*
Residuals	*79*	*1.216*	*0.015*		
Perm. Test for homogeneity	**Df**	**Sum sq**	**Mean sq**	**F value**	**Pr(>F)**
Groups	*3*	*0.018*	*0.006*	*0.3878*	*0.79*
Residuals	*79*	*1.216*	*0.015*		
Location_reef Permanova	**Df**	**sumofsqrs**	**R2**	**F**	**Pr(>F)**
Model	*3*	*1.225*	*0.061*	*1.722*	*0.057 .*
Residual	*79*	*18.737*	*0.939*		
Total	*82*	*19.963*	*1.000*		
Analysis of variance Location_reef* Fragement_type	**Df**	**Sum sq**	**Mean sq**	**F value**	**Pr(>F)**
Groups	*11*	*0.096*	*0.009*	*0.397*	*0.953*
Residuals	*71*	*1.553*	*0.022*		
Perm. Test for homogeneity	**Df**	**Sum sq**	**Mean sq**	**F value**	**Pr(>F)**
Groups	*11*	*0.096*	*0.009*	*0.397*	*0.95*
Residuals	*71*	*1.553*	*0.022*		
Location_reef* Fragment_type Permanova	**Df**	**sumofsqrs**	**R2**	**F**	**Pr(>F)**

Table S8: Permanovas of fragment choice, Location and interaction. *(continued)*

Analysis of variance Fragment_type	Df	Sum sq	Mean sq	F value	Pr(>F)
Model	*11*	*10.726*	*0.537*	*7.495*	*1e-04 ****
Residual	*71*	*9.237*	*0.463*		
Total	*82*	*19.963*	*1.000*		

Table S9: Logistic regression of the odds ratios and predicted probabilities including mcFadden R2.

Regression (binominal logistic) odds ratio							
term	**Estimate**	**Std. err.**	**Statistic**	**P. value**	**Odds**	**lower**	**upper**
intercept	*-1.26*	*0.232*	*-5.41*	*6.7 e-7*	*0.285*	*0.181*	*0.449*
eDNA	*0.039*	*0.054*	*0.719*	*0.472*	*1.04*	*0.935*	*1.16*
eRNA	*0.145*	*0.076*	*1.9*	*0.057*	*1.16*	*0.995*	*1.34*
2kb	*0.057*	*0.069*	*0.821*	*0.412*	*1.06*	*0.924*	*1.21*
(binominal logistic) Regression predicted probability							
Intercept	*eDNA*	*eRNA*	*2kb*				
-1.255	*0.039*	*0.145*	*0.056*				
Df	*82 (total)*	*80 residual*					
Null deviance	*150*						
residual	*145.3*	*AIC*	*153.3*				
R2_eDNA	**R2_eRNA**	**R2_2kb**					
0.054	*0.788*	*0.065*					

Chapter 5

Integrating current and future molecular tools for standardised monitoring of North Sea biodiversity and ecosystem dynamics

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ABSTRACT

The North Sea is among the largest and most productive shallow marine ecosystems, heavily influenced by historical and ongoing exploitation. Monitoring of North Sea biodiversity is vital for a better understanding of ecosystem responses to ongoing anthropogenic pressures and for informing effective management and conservation strategies.

Molecular techniques provide a cost and time-efficient alternative to traditional biodiversity assessment methods and are increasingly employed in marine monitoring. In this review we explore the current molecular-based monitoring approaches used to assess marine biodiversity in the North Sea, including species-specific assays, eDNA metabarcoding, and metagenomics, by highlighting their opportunities and areas for improvement. Furthermore, we address the potential for integrating novel DNA and RNA-based methods, such as epigenetics and metatranscriptomics, into standardised monitoring for marine conservation and restoration.

We propose that further integration of a broader omics-based approach may enhance the overall understanding of ecosystem structure, function, and health. We then move beyond molecular tools and examine the challenges and opportunities associated with nucleotide reference databases, data curation, and data archiving. We further address how to improve the harmonisation and standardisation of marine biodiversity data. Finally, we provide three suggestions for integrating molecular methods in a standardised manner to enhance the assessment of North Sea ecosystem biodiversity, dynamics, and functioning.

Keywords: Biodiversity, Ecosystem dynamics, eDNA, eRNA, Molecular monitoring, multi-omics, North Sea

1. INTRODUCTION

1.1 Biodiversity and conservation status of the North Sea

The North Sea is one of the world's largest and most productive shallow marine ecosystems, subject to historical and ongoing exploitation by multiple industries (e.g. fisheries, oil and gas drilling, renewable energy production, shipping, and tourism) (Emeis et al. 2015). Historically, this marine basin was covered with extensive hard substrates, such as shellfish reefs and rocks, which supported high levels of benthic and pelagic biodiversity (Olsen 1883). However, many of these habitats have been degraded due to centuries of intensive bottom-trawling fisheries, targeting a wide variety of demersal fish, crustaceans, and bivalves (Dickey-Collas et al. 2010, Ter Hofstede and Rijnsdorp 2011, Engelhard et al. 2014). Bottom-trawling can have long-lasting adverse effects on ecosystem functioning by reducing habitat complexity, altering the benthic community composition, and diminishing the overall productivity of benthic ecosystems (Jennings et al. 2001, Kaiser et al. 2006, Hiddink et al. 2011).

Anthropogenic activities can also give rise to opportunities for the recovery of nature and biodiversity, as is the case with offshore wind farms (OWFs). OWFs create local no-take zones where fishing is prohibited. Additionally, the scour protection surrounding the turbine base provides a hard settlement substrate that facilitates growth of reef-building organisms, and is demonstrated to increase local biodiversity and recruitment of commercial fish species (Scheidat et al. 2011, Reubens et al. 2013b, 2013a, 2014). However, several studies also address the potential of OWFs, and associated epifaunal communities, serving as stepping stones for non-indigenous species (De Mesel et al., 2015). The facilitated expansion of non-indigenous species may be further intensified by climate-related pressures, threatening North Sea ecosystem functioning (Beaugrand and Kirby 2010, Dickey-Collas et al. 2010, Huserbråten et al. 2018). Although North Sea ecosystems have thus far demonstrated an ability to adapt to various stressors, the extent to which ongoing and intensifying pressures will alter ecosystem dynamics remains unclear (Wiltshire et al. 2008, Dickey-Collas et al. 2010, Coates et al. 2015, Brooks et al. 2017). Hence, effective biodiversity monitoring is crucial to improve our understanding of how various stressors and opportunities alter ecosystem processes. Consequently, monitoring of marine biodiversity will provide the scientific foundation for conservation and restoration strategies in a changing North Sea.

Conservation and restoration are increasingly being prioritised in countries bordering the North Sea, but nation-specific regulatory frameworks for marine management can be inconsistent or even conflicting between countries (Grip and Blomqvist 2020, Kusters et al. 2025). An example is the Doggersbank, which is a sandbank in the North Sea that borders five countries with distinct regulatory frameworks in place for conservation of the shallow area (Mackelworth et al. 2019). At the European level, several policy guidelines and frameworks are employed to guide the development and implementation of biodiversity monitoring systems for such transboundary marine areas. These include the United Nations guidelines for establishing national biodiversity monitoring programmes, and the Marine Strategy Framework Directive (MSFD), which aims to protect the marine environment by achieving "good environmental status" (GES). In addition, guidelines have been formulated that focus specifically on habitat protection and restoration. For example, as part of the EU biodiversity strategy for 2030, biogenic reefs and their associated biodiversity are protected under the EU habitats directive. Here, an interpretation manual was developed that more specifically explains which habitats, ranging from sand beds and reefs to sand dunes and grasslands, and their associated species require protection, restoration, and conservation. This further emphasises the need for routine biodiversity monitoring of ecosystems in the North Sea region to assess the effectiveness of restoration and conservation efforts, and improve them when necessary.

1.2 From morphological to molecular monitoring of North Sea marine biodiversity

Conventional monitoring approaches that are currently applied in biodiversity assessments heavily rely on morphological species identification, which is often invasive and time-consuming (Leray and Knowlton 2016). Additionally, the lack of expertise on specific taxonomic groups can pose constraints when discriminating between cryptic species or upon identification of non-indigenous species (Danovaro et al. 2016, Duarte et al. 2021). Molecular based techniques, as an alternative to conventional taxonomic identification, have revolutionised biodiversity monitoring by greatly improving cost- and time efficiency, while often providing more accurate and reliable taxonomic classifications (Thomsen and Willerslev 2015, Goodwin et al. 2017, Thompson and Thielen 2023).

Molecular monitoring using environmental DNA (eDNA) is emerging as a powerful approach to assess biodiversity and ecosystem dynamics in the North Sea. With relatively little sampling effort, species can be identified

from the genetic material that is released into the environment, from various sources (e.g. skin, mucous, scales, faeces, gametes) or whole organisms such as bacteria and plankton (Thomsen and Willerslev 2015, Holman et al. 2019, Rodríguez-Ezpeleta et al. 2021, Suarez-Bregua et al. 2022). Depending on the objective, monitoring using eDNA sampling can involve species-specific assays, to detect the presence of an individual target species, or metabarcoding, to characterise ecosystem community compositions and assess broader ecosystem dynamics (Ruppert et al. 2019, Guri et al. 2024b). The possibility to use more in-depth analyses to study the composition of environmental nucleic acids (eNA), accompanied by (meta-)omics approaches, are presenting themselves as a valuable asset to the molecular toolbox (Garlapati et al. 2019, Littlefair et al. 2022, Miya 2022). While these molecular methods hold promise for elaborate analysis of ecosystem dynamics, such as species interactions, functional responses to environmental change, and life-history characteristics, there are several challenges to be addressed prior to implementation in standardised monitoring of North Sea ecosystems (Bowers et al. 2021, Bunholi et al. 2023). Among those challenges are the methodological limitations that arise from sample filtration and preservation methods, which affect detection probability and comparability across replicates and studies. During water filtration, eNA molecule capture efficiency is strongly affected by filter pore size, membrane material, filtration volume, and preservation conservative (Majaneva et al. 2018, Bunholi et al. 2023). To address these limitations, recent studies have examined the use of natural eNA filters, such as sponges, which may offer an alternative as a passive eNA sampler, potentially expanding spatial coverage in species detection (Mariani et al. 2019, Cai et al. 2022, Weber et al. 2023).

Current opportunities for improvement include enhancing consistency and reproducibility between sampling efforts and combining tools used in molecular analyses (Bowers et al. 2021), advancing methods for quantifying species abundance (Gold et al. 2023), and expanding reference databases to enable species-level identification (van der Loos and Nijland 2020). Furthermore, standardisation of metadata organisation across projects and countries would facilitate more detailed comparisons among experiments (Fediajevaite et al. 2021). As such, continued efforts are needed to further incorporate, improve, and standardise emerging methods while improving reference databases and harmonising data management. Additionally, advances in machine learning and artificial intelligence are expected to further improve automation and comparability in molecular North Sea monitoring efforts.

In this review, we explore the potential of the molecular toolbox to assess North Sea marine biodiversity and ecosystem dynamics. We summarise current and emerging molecular techniques, highlighting their application, not only for biodiversity assessment, but also for understanding broader ecosystem functioning and dynamics. We discuss the challenges that currently hamper the integration of these molecular methods into standardised marine monitoring, alongside the opportunities presented for future research to facilitate their incorporation. Additionally, we address the role of genetic databases, as well as issues related to data curation and archiving which prevent consistent and harmonised marine biodiversity monitoring in a transboundary water basin. Finally, we make three recommendations to improve transparency and facilitate reproducibility between molecular studies. While this review focuses on the North Sea as an illustrative case study, recommendations presented here are applicable to improve molecular biodiversity monitoring in other marine ecosystems.

2. CURRENT MOLECULAR TOOLS FOR MARINE BIODIVERSITY MONITORING

Sampling in the North Sea is often challenging due to its complex hydrography and intensive anthropogenic use. Therefore, monitoring requires demanding field campaigns, which can be compromised in terms of sampling coverage and consistency. Monitoring approaches that use eDNA are promising alternatives to conventional tools as these methods require minimal sampling efforts and can greatly reduce time spent in the field (Kopp et al. 2023). Initially, these sampling efforts relied on molecular assays targeting species-specific occurrences, but community-based metabarcoding was soon adopted and showed the potential of using eDNA to study North Sea biodiversity (Miya 2022). In this section, we summarise the current molecular techniques used for biodiversity assessments in the North Sea and highlight the advantages and limitations of these approaches (Fig. 1).

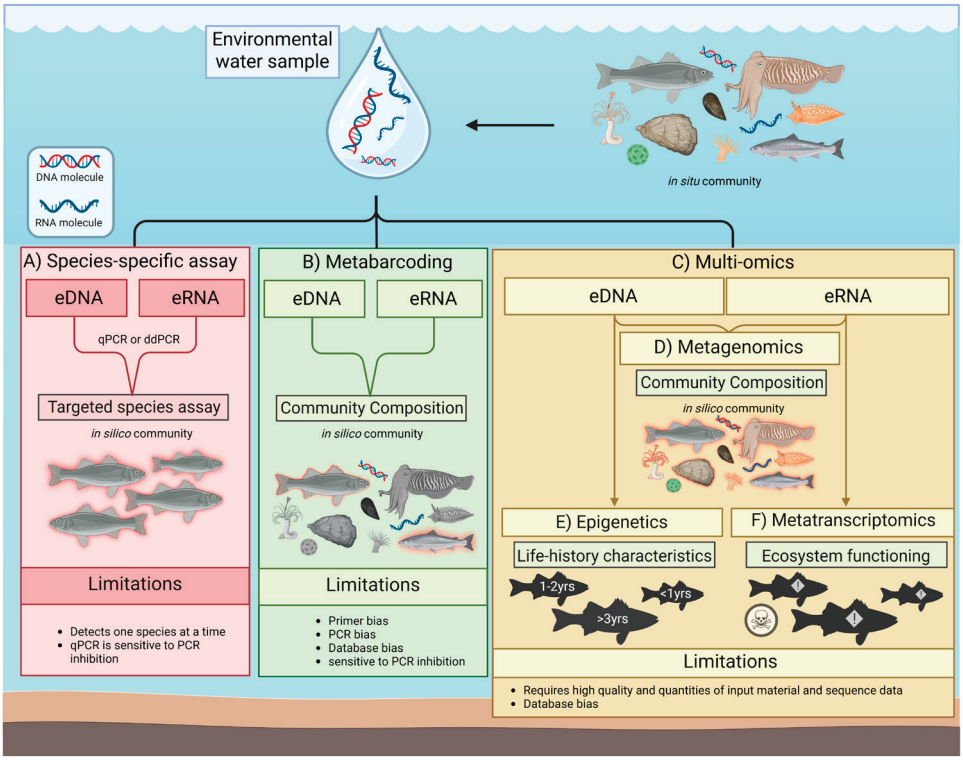


Figure 1. Schematic overview of the types of molecular workflows used in assessing biodiversity and ecosystem dynamics from an environmental water sample. The figure illustrates the *in situ* biological community and the corresponding *in silico* communities generated by each workflow, focussing either on specific species in species-specific assays (A), groups of species or taxa through metabarcoding (B), or the entire community or selected taxa using multi-omics approaches (C).

2.1 Species-specific assays

Over the past decades, molecular species detection from non-invasively collected environmental samples has revolutionised North Sea marine monitoring. The application of species-specific assays has enabled the detection of a wide variety of rare and non-native species in the North Sea from environmental samples, underscoring their potential to complement and enhance conventional monitoring approaches (Engesmo et al. 2018, Keller et al. 2022, Shelton et al. 2022, Morin et al. 2023). Detecting the presence of a target species in environmental samples is most often carried out by real-time PCR, also known as quantitative PCR (qPCR), or by digital-droplet PCR (ddPCR) (Guri et al. 2024b). Currently, qPCR is the golden standard for species-specific detection because this method is highly sensitive to low DNA concentrations. Theoretically, species de-

tection by qPCR relies on the presence of a single target DNA molecule, making it a suitable application for environmental samples with generally low concentrations of target DNA. Despite its high sensitivity, qPCR can be inhibited by contaminants originating from the environment, such as salts (Lance and Guan 2020). The alternative approach, ddPCR, is less affected by PCR inhibition as DNA molecules are divided over individual oil droplets where amplification takes place separately within each droplet. The main advantage of both species-specific approaches is that they are quantitative, enabling not only the detection of a species' presence but also quantifying relative differences in occurrence across samples (Guri et al. 2024b). To this extent, species-specific assays are widely applied in various contexts in the North Sea, from analysis of fish communities (Van Bleijswijk et al. 2020, Guri et al. 2024b), to detecting cryptic and non-indigenous species (Laakmann et al. 2017, Mauger et al. 2021, Morin et al. 2023), to the detection of toxic microalgae, antimicrobial resistance in flatfish (Bourdonnais et al. 2024), and the monitoring of parasitic infections in aquaculture systems (Krolicka et al. 2022). Despite the high sensitivity of qPCR and ddPCR for DNA detection, both assays are inherently species-specific and therefore have limited applicability in comprehensive biodiversity assessments. Therefore, most biodiversity studies rely on broader molecular approaches that target all DNA fragments present within an environmental sample (e.g. metabarcoding).

2.3 Metabarcoding

Currently, metabarcoding is the most used molecular tool for identifying species compositions from DNA fragments in an eDNA sample (Fig. 1B; Cordier et al., 2019; Miya, 2022; Staehr et al., 2022; Y. Zhang et al., 2020). Metabarcoding, which is inherently coupled with next-generation sequencing (NGS), facilitates the simultaneous taxonomic identification of multiple species across numerous samples, providing an affordable and rapid alternative to conventional biodiversity assessment methods (Ferreira et al., 2024; Scarano et al., 2024; Singer et al., 2019; van der Loos & Nijland, 2020; Fig. 1B). Depending on the objective, metabarcoding can be applied to various sample types, such as sediment, water, or bulk samples (Van den Bulcke et al. 2024; van der Loos and Nijland 2020; Staehr et al. 2022). For the purpose of this review, we only discuss metabarcoding of marine environmental samples.

In the North Sea ecosystem, metabarcoding is widely adopted to study marine biodiversity of different taxonomic groups, such as marine mammals (Boyse et al. 2024), fish (Van Bleijswijk et al. 2020), crustaceans

(Shum et al. 2023), phytoplankton (Martens et al. 2024), zooplankton (Ohnesorge et al. 2024), and meiofauna (Klunder et al. 2020). Beyond characterising biodiversity, eDNA metabarcoding can monitor changes in community composition, detect shifts in interactions, reveal hidden trophic links, and assess the impacts of human activities, thereby providing insights into the integrity and functioning of ecological systems (Cordier et al. 2017, Ruppert et al. 2019, Käse et al. 2021, Li et al. 2024b). For example, eDNA metabarcoding has been used to examine connectivity patterns and co-occurrence networks along with establishing trophic interactions in the North Sea (Boyse et al. 2025). Further, eDNA metabarcoding applied to OWFs and oil platforms in the North Sea also demonstrated horizontal and vertical community patterns and revealed composition shifts of fish and invertebrate communities upon the introduction of artificial structures (Dukan et al. 2024, Cornelis et al. 2024, Mauffrey et al. 2021). Moreover, a comparison of species community composition between natural and artificial oyster reefs demonstrated similar occurrences of non-indigenous species on both reefs (Jansen et al. 2025). These examples illustrate the broadened scope provided by eDNA metabarcoding, which enhances our understanding of ecosystem dynamics and functioning.

Although metabarcoding is currently the most widely adopted molecular approach for assessing marine biodiversity, the method is subject to several biases and limitations. Biases may be introduced during laboratory processing such as primer choice, amplification efficiency, and stochasticity during PCR (Alberdi et al. 2019, Piñol et al. 2019, van der Loos and Nijland 2020, Gold et al. 2023). Additionally, computational sequence processing may further limit reliable interpretation, (Hunter et al. 2017, Sigsgaard et al. 2020a, van der Loos and Nijland 2020, Suarez-Bregua et al. 2022). Where metabarcoding typically targets a universal genetic region that is conserved across taxa (e.g. COI, 18S, or 12S), the universality of the targeted region often compromises taxonomic resolution. This issue can be mitigated by using a multi-marker approach or by amplifying longer DNA fragments (Stoeck et al. 2010, Leray et al. 2013, Alberdi et al. 2018, Zhang et al. 2018, Cordier et al. 2019, Ferreira et al. 2024, Doorenspleet et al. 2025a). Without a clear relationship between (relative) read count and biomass, inferring species' abundance remains a significant challenge, limiting the use of eDNA in quantitative ecological assessments (Garlapati et al. 2019, Beng and Corlett 2020, Guri et al. 2024b). Although various approaches have been proposed to improve quantitative accuracy in metabarcoding (Schenk et al. 2019, Di

Muri et al. 2020, Ershova et al. 2021, Guri et al. 2024a), whether eDNA can ultimately provide reliable abundance estimates remains to be determined. Metabarcoding studies are further limited by the completeness of nucleotide reference databases, where the lack of species-specific sequences restricts species-level identification (van der Loos and Nijland 2020, Zhang et al. 2020a, Ferreira et al. 2024).

Integrating metabarcoding into existing management frameworks can improve the capacity to detect, conserve, and restore species and habitats. Its wider adoption into international monitoring projects (e.g. GEANS and MARCO-BOLO) is already being extensively explored, and these projects have begun to demonstrate the feasibility of implementing metabarcoding as a standardised approach for routine biodiversity monitoring in the North Sea.

2.4 Metagenomics

Unlike targeted approaches that focus on specific taxa or genomic regions, metagenomic approaches encompass all available DNA nucleotides contained in a sample, thereby achieving broader taxonomic resolution (Fig. 1D) (Serite et al. 2023). As metagenomics relies on sequencing all DNA molecules from a sample, the approach omits potential primer and PCR biases (Crampton-Platt et al. 2016, Serite et al. 2023, Callens et al. 2025). The applications of metagenomics in studying North Sea biodiversity from eDNA samples is still in its infancy but shows considerable potential for achieving high taxonomic resolution while retaining a broad ecosystem-wide perspective (Bista et al. 2018, Doorenspleet et al. 2025b, Holman et al. 2025). Beyond facilitating marine biodiversity assessments, metagenomic approaches enable the analysis of population genetics, thereby providing non-invasive strategies for elucidating population dynamics within marine ecosystems (Crowley et al. 2025, Serivichyaswat et al. 2025). Interestingly, viruses lack universal genetic markers, restricting their detection through a metabarcoding approach. Metagenomic approaches, however, provide valuable opportunities to characterise DNA-based viral communities in marine environments, offering deeper insights into ecosystem structure, function, and overall health (Garin-Fernandez et al. 2018). The advent of third-generation sequencing techniques has enabled a more comprehensive molecular characterisation of DNA present within environmental samples, including base modifications, such as DNA methylation, alongside canonical sequences . (Rang et al. 2018, Sigurpalsdottir et al. 2024). These epigenetic modifications provide the opportunity to analyse species' ontogeny

or population demographics (Anastasiadi and Piferrer 2020, Wang et al. 2021). The utilisation, application, and implementation of these approaches are further discussed in section 3.1: Epigenetics.

While metagenomics has considerable advantages over metabarcoding, it's incorporation into standardised monitoring is still in its infancy as the approach exhibits notable limitations. As whole-genomic DNA is sequenced rather than a targeted amplified region, more data is required to ensure sufficient coverage for accurate taxonomic identification of all species in a sample (Manu and Umapathy 2023). Further, analysis of metagenomics data requires a nucleotide reference database containing whole genome data, bioinformatic expertise, and substantial computing power (Garlapati et al. 2019, Holman et al. 2025). Although metagenomics circumvents quantification issues that arise in metabarcoding from primer or PCR biases, challenges in accurate quantification of species abundance (e.g. due to copy number variation) remain, emphasising the need for further research to enable standardised implementation (Krehenwinkel et al. 2017, Di Muri et al. 2020, Serite et al. 2023). Nevertheless, approaches capable of simultaneously assessing biodiversity and relative biomass from environmental samples are currently under development (Callens et al. 2025). Consequently, expected continued reductions in sequencing costs and expansion of reference databases should further enhance the application of metagenomics for investigating marine biodiversity and ecosystem dynamics from environmental samples, and facilitate its integration into standardised ecosystem monitoring.

3. EMERGING MOLECULAR TOOLS AND APPROACHES FOR MONITORING SPECIES DYNAMICS

It can be argued that the health and functioning of ecosystems are not solely determined by biodiversity, but are also fundamentally influenced by ecosystem complexity. Ecosystem complexity, encompassing inter- and intraspecies interactions, and structural organisation within (e.g. age or sex cohorts) and between species (trophic niches), plays a critical role in shaping ecosystem resilience, productivity, and overall stability (Farnsworth et al. 2012, Tilman et al. 2014, Murgier et al. 2021). While currently used molecular tools have proven useful to assess marine biodiversity, the potential to assess population status, species' demographics, or ecosystem health is generally limited. As molecular technologies

advance beyond metabarcoding and metagenomics, more research questions can be addressed by following a multi-omics based approach (Fig. 1C; "eDNA Approaches for Ecosystem Health Monitoring," 2025). Integration of these novel tools could offer new opportunities to enhance our understanding of North Sea ecosystem dynamics, functioning, and health. Here, we discuss several novel molecular tools for monitoring marine ecosystem dynamics and evaluate their potential for integration within current North Sea biodiversity monitoring frameworks.

3.1 Epigenetics

Epigenetics represent chemical modifications to DNA, RNA, chromatins, and histones that have the potential to influence gene regulatory mechanisms without changing the coding sequence (Jones 2012). As such, these modifications are involved in important regulatory processes, such as growth, development, and reproduction (Duncan et al. 2014, Liu et al. 2022). Environmental conditions can modulate epigenetic programming, thereby providing the molecular machinery to facilitate phenotypic plasticity in a wide variety of organisms (Bogan & Yi, 2024; Bonasio, 2015; Roberts & Gavery, 2012, Fig 1, E). Patterns of DNA methylation, currently the most studied epigenetic mechanism, can vary in response to environmental conditions, such as changes in nutrition, temperature, and acidification (Eirin-Lopez and Putnam 2019, Bock et al. 2022, Sun et al. 2023). Accordingly, within biodiversity research, the detection of such epigenetic changes or reprogramming can serve as a valuable indicator of environmental change (Fig. 1E; Dang et al., 2023; Gombeau et al., 2016; Lee et al., 2020; F. Liu et al., 2023). For example, alterations in DNA methylation patterns facilitate thermal tolerance to rising temperatures *in Acartia tonsa,* an abundant copepod in the North Sea (Janssens et al. 2024). As DNA methylation changes often follow a predictable correlation with life-history characteristics, intraindividual and intraspecific differences in DNA methylation patterns can help to understand other aspects of population demography in changing environments (e.g. sex and reproductive behaviour, diseases, pathogen response, and species' welfare and resilience) (Ortega-Recalde et al. 2020, Sigsgaard et al. 2020b, Silliman et al. 2023, Belenguer et al. 2024, Brink et al. 2024, Viña-Feás et al. 2024, Saito et al. 2025, Valk et al. 2025). In addition to studying epigenetic mechanisms from tissue samples, methylation profiles derived from environmental samples (epi-eDNA) show substantial potential in understanding population demographics in marine ecosystems (Sigsgaard et al. 2020b, Balard et al. 2024). For example, DNA methylation was proven a useful indicator for inferring the age of fish and inverte-

brates from eDNA samples (Zhao et al. 2023, Ruiz et al. 2025). However, despite early successes in using epi-eDNA, studying such modifications requires collecting well-preserved and large quantities of genomic DNA (Li and Tollefsbol 2021). Moreover, it was shown that biases introduced by sample characteristics and methodology complicate comparability between studies (Shao et al. 2014, Trigg et al. 2022, Rahman and Lozier 2023, Balard et al. 2024). As such, it is pivotal to expand our knowledge of epigenetic signals and markers in different species, life-stages, and environmental conditions to effectively integrate epigenetic and epi-eDNA data into biodiversity monitoring of North Sea ecosystems (Liu et al. 2025).

3.2 Environmental RNA and meta-transcriptomics

In addition to eDNA, samples can contain environmental RNA (eRNA), encompassing mRNA, tRNA, rRNA, and miRNA. Due to the higher instability of RNA, eRNA exhibits a faster degradation rate than eDNA and is therefore considered to provide a more accurate representation of recent biodiversity (Giroux et al. 2022, Kagzi et al. 2022, Li et al. 2024a, Macher et al. 2024). Accordingly, eRNA detection is proposed to complement eDNA analyses and thereby improve the spatio-temporal accuracy of biodiversity assays (Fig. 1F; W. Li et al., 2024; Marshall et al., 2021; Miyata et al., 2022). An additional opportunity offered by eRNA in comparison to eDNA, is the possibility to differentiate between viable and non-viable organisms, improving pathogen detection sensitivity and biosecurity surveillance (Darling and Frederick 2018, Mérou et al. 2020, Miyata et al. 2022, Bass et al. 2023). These applications highlight the potential of eRNA research in assessing ecosystem dynamics and health, and offer promising possibilities for their expansion into studying marine systems (Fig. 1). The complementary application of eDNA and eRNA approaches, collectively referred to as eNA, in metabarcoding studies has been demonstrated to reduce false positives and subsequently provide a more accurate representation of overall biodiversity (Littlefair et al. 2022, Miyata et al. 2022, 2025, Kagzi et al. 2023, Li et al. 2024a). Consequently, the integrated use of eDNA and eRNA detection can expand the resolution of eNA-based monitoring and broaden our understanding of North Sea dynamics and other marine ecosystems (Fig. 1C).

In addition to its use in biodiversity monitoring through eRNA metabarcoding (Fig. 1, B), eRNA-derived metatranscriptomic analysis, which targets transcriptional signals (Fig. 1F). provides the opportunity to assess species' ontogeny, population structure and dynamics, as well as

organismal responses to environmental change (Tsuri et al. 2021, Hechler et al. 2023, Sutherland et al. 2023, Parsley and Goldberg 2024, Miyata et al. 2025). Beyond biodiversity assessments, metatranscriptomics has the potential to enhance understanding of ecosystem functioning by identifying differential gene expression between individuals, species, or environments (Sutherland et al. 2023, He et al. 2025). For example, sequencing of all available transcripts from an environmental sample was demonstrated to elucidate stress responses in aquatic organisms (Hechler et al. 2023, Miyata et al. 2025). However, current applications of eRNA-based approaches are often applied to enclosed systems targeting a single species (Hechler et al. 2023, Miyata et al. 2025). A more comprehensive understanding of eRNA characteristics is required prior to its effective integration into biodiversity monitoring frameworks. Future research could explore eRNA degradation rates, shedding characteristics, or gene transcript quantities and variety in environmental samples (Wood et al. 2020, Giroux et al. 2022, Kagzi et al. 2022, 2023, Stevens and Parsley 2023). Once these areas are better understood, eRNA metabarcoding and metatranscriptomics can be integrated with eDNA analysis to expand our understanding of North Sea ecosystem functioning and health (Fig. 1).

3.2 Environmental nucleic acids (eNA)

Recently, biodiversity assessment approaches are emerging that collectively target environmental nucleic acids (eNA), encompassing all nucleic acids (both eDNA and eRNA) released by organisms into the environment (Littlefair et al. 2022, Wang et al. 2025). The applications of analyses targeting eNA reach far beyond biodiversity assessments, where for example, the detection of microbial activity can be utilised as an indicator of ecosystem health (Fig. 1C; Cook et al., 2025). As such, eNA-based approaches represent the next step in molecular-based biodiversity monitoring by offering an integrated nucleic acid profile rather than utilising eDNA or eRNA approaches separately. These developments demonstrate the advancement of omics-based tools to not only identify taxonomic diversity but also biological activity within marine ecosystems. However, similar to the limitations faced by single-molecule (DNA or RNA-based) approaches, biodiversity assessments relying on eNA are limited by variability in the amount of eNA released by different individuals and species, as well as by differences in eNA integrity, where eNA degradation varies under different environmental conditions (Kagzi et al. 2022, 2023, Littlefair et al. 2022). We refer to other reviews for a more complete overview of the current challenges that arise from eNA shedding, decay, and deg-

radation in assessing species biodiversity (Collins et al. 2018, Kagzi et al. 2022, 2023, Lamb et al. 2022, Nagler et al. 2022, Wang et al. 2025).

With the ongoing improvement of sequencing technologies and bioinformatic analytical pipelines, it is only a matter of time before eDNA studies pave the way for broader eNA and omics-based approaches. In the meantime, further research is required to explore the complementary roles of eDNA and eRNA, and to understand how the integrated application of total eNAs can inform restoration and conservation efforts by elucidating ecosystem structure, stability, and function.

4. DATA CURATION AND STANDARDISATION

4.1 Current challenges in database management

Nucleotide reference databases are a crucial component of most molecular workflows, however, challenges persist during accurate taxonomic assignment. Robust and accurate species identification requires a comprehensive and up-to-date database, including a broad range of taxa identified to the lowest taxonomic rank. Such reference databases may be public (Table 1), such as those under the umbrella of the International Nucleotide Sequence Database Collaboration (INSDC, https://www.insdc.org/), for example, the European Nucleotide Archive (ENA, https://www.ebi.ac.uk/ena/browser/home), the National Centre for Biotechnology Information database (NCBI, https://www.ncbi.nlm.nih.gov/), and the DNA databank of Japan (DDBJ, https://www.ddbj.nig.ac.jp/index-e.html) or local, which are typically focused on specific geographic regions.

Table 1. Summary of established open-access nucleotide reference databases, detailing taxonomic scope, molecule types and data types accepted for submission.

Database	Taxonomic scope	Molecule types	Data types accepted	Release year	Recent citation	Link
GenBank (NCBI)	All domains of life	Gene regions such as COI, 12S/16S/18S, ITS. Proteins, whole genomes	Nucleotide sequences, assemblies (inc. mitogenomes and whole genomes. Uses FASTQ, BAM/CRAM or Genbank format.	1982	Sayers et al., 2025	https://www.ncbi.nlm.nih.gov/genbank/
SRA (NCBI)	All domains of life	Raw sequence data	Fastq, Fast5, BAM/CRAM, HDF and HDF5	2007	Katz et al., 2022	https://www.ncbi.nlm.nih.gov/sra/
European Nucleotide Archive (ENA)	All domains of life	Gene regions such as COI, 12S/16S/18S, ITS. Proteins, whole genomes	Raw reads (FASTQ, BAM/CRAM), assemblies, annotated sequences, metadata	1982	O'Cathail et al., 2025	https://www.ebi.ac.uk/ena/browser/
DNA Data Bank of Japan (DDBJ)	All domains of life	Gene regions such as COI, 12S/16S/18S, ITS. Proteins, whole genomes	Raw reads (FASTQ, BAM/CRAM), assemblies, annotated sequences, metadata	1987	Tateno & Gojobori, 1997	https://www.ddbj.nig.ac.jp/index-e.html
RefSeq (NCBI)	Curated representative sequences focused on model organisms	Genomes, transcripts, proteins (reference loci)	RefSeq data is derived from sequences curated from GenBank/ENA/DDBJ.	2000	Pruitt, 2004	https://www.ncbi.nlm.nih.gov/refseq/
BOLD Systems (Barcode of Life Data System)	Broad eukaryotes (animals, plants, fungi)	Genetic markers only such as COI, rbcL and ITS	FASTA	2005	Ratnasingham & Hebert, 2007	https://boldsystems.org/

Table 1. Summary of established open-access nucleotide reference databases, detailing taxonomic scope, molecule types and data types accepted for submission. (*continued*)

Database	Taxonomic scope	Molecule types	Data types accepted	Release year	Recent citation	Link
SILVA	Prokaryotes & eukaryotic rRNA	Ribosomal RNA markers only such as 16S, 18S and 23S rRNA (SSU/LSU)	FASTA, FASTA with metadata and ARB	2007	Quast et al., 2012	https://www.arb-silva.de/
UNITE (fungal ITS)	ITS database predominantly Fungi	Ribosomal ITS region	UNITE data is curated from INSDC	2003	Abarenkov et al., 2024	https://unite.ut.ee/index.php
PR2 (Protist Ribosomal Reference)	Protists / micro-eukaryotes	18S rRNA	Data extrapolated from genome assemblies on NCBI	2010	Krabberød et al., 2025	https://app.pr2-database.org/pr2-database/
			Marine focused			
Database	**Taxonomic scope**	**Molecule types**	**Data types accepted**	**Release year**	**Recent citation**	**Link**
AquaGene	**Fish species**	COI, CYTB and the nuclear genes myosin 6 (MYH6) and rhodopsin I (RHOD)	Does not accept public uploads	2013	*AquaGene*, n.d.	https://www.aquagene.org/
MarRef / MarDB / MarCat	Marine prokaryotes / metagenomes	Reference genomes	Uses genomes and metagenomes collated from ENA and NCBI	2017	Klemetsen et al., 2018	https://sfb.mmp2.sigma2.no/
MARES (pipeline)	Marine eukaryotes	COI	Reproducible pipeline to retrieve sequences from GenBank and BOLD	2020	Arranz et al., 2020	https://github.com/wpearman1996/MARES_database_pipeline

Table 1. Summary of established open-access nucleotide reference databases, detailing taxonomic scope, molecule types and data types accepted for submission. (*continued*)

Database	Taxonomic scope	Molecule types	Data types accepted	Release year	Recent citation	Link
MareMAGE (fish reference)	Marine fishes	Mitochondrial 12S rRNA (fish), COI	Sequences retrieved from GenBank, ENA, AquaGene and BOLD	2023 (publication / release)	Kasmi et al., 2023	https://maremage.weebly.com/
MetaZooGene Atlas (MZGdb)	Zooplankton, benthic invertebrates, fish, marine mammals and phytoplankton	COI, 12S, 16S, 18S and 28S	Sequences retrieved from GenBank and BOLD	2021	Christodoulou et al., 2025	https://metazoogene.org/database/

Despite the extensive taxonomic coverage of public repositories, the lack of curation, gaps in taxa representation, and irregular maintenance of these databases increases the risk of incomplete or erroneous taxonomic assignments (Hestetun et al. 2020, 2021, van der Loos and Nijland 2020). Consequently, numerous studies have highlighted the need to expand public nucleotide databases, including clearer metadata requirements to ensure consistency and reproducibility (Bucklin et al. 2016, Alberdi et al. 2018, 2019, Duarte et al. 2020). To address some of these challenges, the EU project eDNAquaplan has been working collaboratively across EU and international research institutions and data repository leaders to harmonise metadata standards for eDNA metabarcoding datasets, with a focus on nucleotide database submissions (https://ednaquaplan.com/). In addition, databases belonging to INSDC have taken steps towards these requirements through the sharing of nucleotide data once uploaded to one of these three databases and creating resources for INSDC members such as metadata structures and facilitating the submission of raw sequence reads. Local databases are typically more curated and, therefore, less likely to lead to erroneous species identification. However, non-indigenous, rare, or cryptic species are easily overlooked in local databases, thereby emphasising the need to combine local and public databases for molecular biodiversity analysis (Duarte et al. 2020). Furthermore, by maintaining "local" or private databases derived from publicly-funded

research raises ethical as well as practical concerns regarding data equity, reproducibility and transparency. Limiting the sharing of such resources can impact international collaboration and the wider advancement of molecular knowledge, particularly in shared ecosystems such as the North Sea. Currently, there are several initiatives that aim to harmonise database curation and data sharing being implemented globally (e.g. FAANG, GEANS, OBON, and ELIXIR (*FAANG Data Portal*, https://data.faang.org; *GEANS*, https://www.geans.eu/;; Samuel et al., 2021; Waterhouse et al., 2022). Beyond database accessibility, there are wider debates in the molecular ecology field over whether efforts should be made to transition away from targeted gene regions (e.g. COI, 12S, 18S) towards complete mitochondrial or whole-genome reference sequences (Dziedzic et al. 2023). This shift in direction could improve taxonomic resolution from eNA samples and facilitate a multi-target or target-free (multi-omics) approach to eDNA sequencing.

4.2. Standardisation and implementation of molecular data for routine North Sea marine monitoring

Molecular ecological methodologies, such as species-specific assays and eDNA metabarcoding, have become increasingly popular in recent years (Derycke et al. 2021, Cornelis et al. 2024, Moutinho et al. 2024). However, the uptake of these approaches into routine biodiversity monitoring has been hindered by a lack of standardisation across sampling techniques, metadata collection, and subsequent inconsistencies in publications (Veilleux et al. 2021, Shea et al. 2023, Klymus et al. 2024). A clear overview on the reported use of monitoring methods demonstrated that over 20 different methods are used as part of monitoring programmes in European regional sea conventions (Korpinen et al. 2022). Despite the spatial and temporal expansion of marine monitoring, the integration and harmonisation across these methods in standardised monitoring programmes remains uncoordinated. Recently, formal standards for eDNA-based approaches using metabarcoding were published as the "Minimum Information for reporting of Environmental Metabarcoding data" (MIEM), and is similar to the existing guidelines for species-specific assays (Bustin et al. 2009, Hunter et al. 2017, Loeza-Quintana et al. 2020, The dMIQE Group et al. 2020, Borchardt et al. 2021, Langlois et al. 2021). However, subsequent reviews of similar guidelines found a lack of uptake and reporting in published articles (Bustin et al. 2009, Bustin and Nolan 2017, Bustin 2024). This inconsistency, compounded by heterogeneous or incomplete metadata in public nucleotide repositories limits reproducibility and long-term data use (Gilbert et al. 2012, Pope et al. 2015).

The aforementioned eDNAquaplan project aims to address some of these issues by setting clear definitions to vocabulary, generating mandatory metadata fields and creating consistent bioinformatic reporting protocols that align with MIEM and the FAIRe (Findable, Accessible, Interoperable, Reusable eDNA data) data principles. A fundamental component of eDNAquaplan is to federate access to as many reference datasets and physical reference materials as possible, therefore ensuring that the data and metadata standards developed facilitate interoperability and accessibility of these resources. Achieving these objectives is essential for improving comparability of eDNA data across research institutes and countries and to ultimately facilitate integration with marine biodiversity frameworks such as the Marine Strategy Framework Directive (MSFD) and OSPAR monitoring frameworks. Furthermore, an online repository that consists of molecular data complemented by ecological and geographical data would be an asset for biodiversity monitoring and analysis. To this end, the Global Biodiversity Information Facility (GBIF) has recently established the metabarcoding data toolkit (https://mdt.gbif.org/) to facilitate the standardised submission, curation, and integration of metabarcoding datasets with existing species occurrence records, thereby bridging the gap between molecular biodiversity data and traditional ecological observations (GBIF Secretariat 2024). Another initiative that focuses on linking genetic sequence material to ecological metadata and geographical species occurrence databases (e.g. WORMS or OBIS), is the Genomic Observatories Metadatabase (*Ocean Biodiversity Information System*, 2022.; *WoRMS - World Register of Marine Species*, 2023; Riginos et al., 2020). To further promote data sharing, raw sequences that would otherwise be filtered out during bioinformatic workflows could be archived in open-access repositories. This approach aligns with FAIR principles and would facilitate reproducibility, support the maintenance of bioinformatic pipelines, and improve future taxonomic assignment and database expansion.

4.3 Improving data processing and standardisation for North Sea biodiversity assessment using machine learning

The integration of artificial intelligence and machine learning approaches into routine environmental biodiversity monitoring has the potential to facilitate faster and more accurate species identification, remote sample collection, and predictive modelling using environmental parameters (Ditria et al. 2022, Stefanni et al. 2022a). In the broader context of marine biodiversity, machine learning models have been applied to investigate the effects of abiotic environmental conditions on North Sea cod stocks (Kühn et al. 2021), map plankton distributions to define

ecological niches (Plonus and Floeter 2024), and to assess the health of marine mammals (IJsseldijk et al. 2024) Machine learning is increasingly applied to monitor zooplankton (Van Walraven et al. 2025), invertebrates (Sokolova et al. 2021), birds (Sommer et al. 2025), and pollution in the North Sea (Kaandorp et al. 2022), using automated species detection from high-resolution imagery and videos. For a complete overview of all the current applications of machine learning in marine ecology, we refer to the review of Rubbens et al., 2023. Another application of machine learning is facilitating continuous and automated sample collection based on forecasted environmental conditions, thereby minimising labour and enabling access to remote areas, such as offshore wind farms (Stefanni et al. 2022b).

There are numerous possibilities for integrating machine learning tools into data collection, processing, and archiving, to facilitate biodiversity monitoring. For example, the utilisation of neural network algorithms to curate molecular reference libraries has been explored and can greatly reduce the laborious efforts that come with manual curation (Fontes et al. 2024). Furthermore, the application of machine learning models for predicting the ecological quality status of marine environments, using eDNA metabarcoding data, has also been demonstrated (Cordier et al. 2017). This highlights machine learning as a promising tool to model non-linear complex ecological relationships that enhance ecological assessments. These models also show potential to be utilised to organise and autonomously maintain large reference databases that integrate data from different sources (Stefanni et al. 2022b, Yang et al. 2024). Automated algorithms have the potential to assist in continuous monitoring and updating data records by cross-referencing changes in databases, ensuring data accuracy and reducing inconsistencies. This would reduce the likelihood of nomenclature errors as seen in current nucleotide reference databases, which often contain misidentifications and incomplete taxonomic coverage (Mugnai et al. 2021). Implementing automated systems requires agreement on standardised data formats and collaboration between host organisations of existing databases. The Global Biodiversity Information Facility (GBIF) has been a significant step towards creating a single platform where data from many different sources can be accessed (*GBIF*, https://www.gbif.org/). An integrated framework that combines imaging, sound, habitat assessment, molecular data, and behavioural analysis into one autonomously managed database would significantly enhance biodiversity monitoring and our understanding of ecosystem function (Heberling et al. 2021, Stefanni et al. 2022b). Consequently,

it is strongly recommended that researchers publish comprehensive metadata alongside environmental or molecular datasets to enrich data repositories and comply with the FAIRe principles (https://ednaquaplan.com/). Such complete datasets can be utilised in modelling approaches or incorporated into machine learning algorithms aimed at estimating ecosystem-related indices informing on ecosystem functioning, health, and overall stability.

The formulation of clear data-sharing guidelines, coupled with the support of machine learning in automated data curation and archiving, is expected to enhance the harmonisation of marine datasets across experiments, thereby improving the understanding of marine ecosystem dynamics and biodiversity by increasing interpretability across studies.

5. CONCLUSION AND RECOMMENDATIONS

With the current intensity of anthropogenic exploitation in the North Sea, an accurate and more extensive understanding of ecosystem health and functioning is pivotal for management and conservation. Above, we have summarised current methods and highlighted novel molecular approaches that could be adopted into standard monitoring frameworks for assessing the biodiversity and functioning of the North Sea. Upscaling and standardising the utilisation of these multi-omics tools for robust ecosystem biodiversity monitoring requires a collaborative approach among researchers, conservationists, and policymakers. This would not only require data sharing and integrating various monitoring technologies, but also active pursuance ensuring that these frameworks and guidelines are adhered to. This multifaceted approach will facilitate the identification of biodiversity dynamics, assess the impacts of environmental stressors, and inform management and conservation. Finally, we have highlighted how current limitations in reference databases, as well as challenges in data curation and archiving, can hamper harmonisation across experiments. To this end, we provide three suggestions for integrating molecular methods in a standardised manner to improve assessment of ecosystem biodiversity, function, and structure in the dynamic North Sea:

(i) **Comprehensive archiving of metadata alongside biodiversity data to improve data curation, facilitate integration across experiments and enhance the interoperability and reproducibility between molecular and conventional monitoring of marine biodiversity.** This approach, integrating molecular and conventional monitoring methods with comprehensive metadata curation, will improve understanding of ecosystem functioning by providing a multi-disciplinary perspective of different aspects and interactions within an ecosystem.

(ii) **Establish data-sharing guidelines to enable standardisation and comparison between studies.** Once data collection and curation become more standardised, this will allow for comprehensive comparisons between studies, by using data collected through different approaches and on different scales.

(iii) **Make all molecular data publicly available to enable comprehensive analysis of ecosystem biodiversity and function.** Publishing all sequence data, instead of only the subset used for publication, is expected to improve understanding of ecosystem functioning by providing a more detailed image of molecular biodiversity in a spatial-temporal context.

6. LIST OF ABBREVIATIONS

OWF	– Offshore wind farm
MSFD	– Marine Strategy Framework Directive
GES	– Good Environmental Status
eDNA	– environmental DNA
eRNA	– environmental RNA
eNA	– environmental Nucleic Acids
qPCR	– quantitative PCR
ddPCR	– digital-droplet PCR
NGS	– Next Generation Sequencing
epi-eDNA	– epigenetic patterns derived from eDNA samples
FAIR	– Findable, Accessible, Interoperable, Reusable

7. DECLARATIONS

Ethics approval and consent to participate
Not applicable

Consent for publication
Not applicable

Availability of data and materials
Not applicable

Competing interests
The authors declare that they have no competing interests.

Author's Contributions
KD, ASH, and SV wrote the first draft of the manuscript. All authors contributed to writing and reviewing of the final manuscript.

Chapter 6

Shellfish reefs as Essential Fish Habitats: complementary insights from eDNA and BRUVs in the North Sea.

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ABSTRACT

Biogenic reefs, shaped by organisms like corals, tubeworms and bivalves, have faced extensive degradation worldwide, resulting in a vast reduction of their extent. The remaining reefs are essential habitat for marine vertebrates, providing food, shelter, and nursery grounds. However, the role of natural shellfish reefs as essential fish habitats is poorly understood, particularly in temperate regions. This study assessed the effect of reef presence on marine vertebrate diversity and composition and compared traits for habitat and feeding strategy at two shellfish reefs in the North Sea: a mixed shellfish reef and a horse mussel reef. We also assessed the complementarity of two non-invasive techniques: eDNA metabarcoding and baited camera analysis (BRUVs). Stations inside and outside reefs were compared. Vertebrate diversity was consistently higher within both reefs than in their surroundings, declining with increasing distance from the reefs up to 400–1500m from the reefs' border. Community composition varied, with several species, such as gobies, gunnels, clingfish, and sculpins, occurring more frequently within the reefs. Commercial species like cod, pollock, and mullet, also occurred more often in reef areas, with reefs likely serving as juvenile habitats for species like ling. Functional diversity showed that reefs attracted mostly invertivores and pelagic species adapting their diet to food availability. eDNA identified more species overall, while BRUVs detected both adult and juvenile stages, highlighting the value of complementary methods in biodiversity assessments. These results emphasize the importance of temperate reefs as essential fish habitats and inform more effective conservation and fisheries management strategies.

Keywords: Fishes, vertebrate communities, mobile communities, biogenic reefs, shellfish, Modiolus, eDNA, BRUVs, North Sea

1. INTRODUCTION

From tropical to temperate waters, marine reefs represent biodiversity hotspots for both benthic and mobile faunal communities worldwide (Holt et al. 1998, Spalding et al. 2002, Sobha et al. 2023). They enhance resources and habitat availability for species, offering grounds for attachment, foraging, spawning, or nursing (Spalding et al. 2002, Hunter & Sayer 2009, Ponti et al. 2021). Despite their importance, these fundamental habitats are facing a dramatic global decline due to anthropogenic pressures, particularly in temperate marginal seas such as the North Sea (Munday 2004, Thurstan et al. 2024, zu Ermgassen et al. 2024). For this reason, in the last decade growing attention has been directed toward the remaining temperate reefs, for which literature is relatively limited in comparison to that on tropical coral reefs.

The North Sea is one of the most intensively used marine ecosystems globally. Resource extraction, including dredging sand and minerals, hydrocarbons, shipping, and chronic bottom trawling, has severely impacted fish stocks and led to significant habitat losses (Thurstan et al. 2024, Porz et al. 2024). Critical ecosystems, such as biogenic reefs built by ecosystem engineers like mussels (e.g. *Modiolus modiolus*), oysters (e.g. *Ostrea edulis*) and certain species of tube-building worms (e.g. *Sabellaria spinulosa*), have suffered dramatic declines, with some nearing functional extinction (e.g. flat oysters in Europe; Beck et al. 2001, Thurstan et al. 2024). These habitats, which enhance biodiversity and biomass production, play an essential role in supporting benthic and mobile species, including commercially important fish (Støttrup et al. 2014, Seitz et al. 2014, Kent et al. 2017b,c, van der Reijden et al. 2019).

Reefs are essential for the spawning, feeding, and maturation of numerous species (e.g. whelks: Kent et al. 2016, 2017; octopus: Garofalo et al. 2011; Fluharty 2000). Various species, including elasmobranchs, gadoids, seabasses, and commercially important species of crabs (*Cancer pagurus*) and whelks (*Buccinum undatum*), aggregate around natural and artificial reef habitats (Krone & Schröder 2011, Krone et al. 2013, 2017, Kent et al. 2017b,c, Flávio et al. 2023, Ibanez-Erquiaga et al. 2024). In addition, monitoring around artificial reefs has shown aggregation of several pelagic species, including Atlantic cod *Gadus morhua*, pouting *Trisopterus luscus*, mackerel *Scomber scombrus,* and seabass *Dicentrarchus labrax* (Reubens et al. 2011, 2014, van Hal et al. 2017, Flávio et al. 2023). Limited studies targeted natural biogenic reefs in the North Sea (Seitz et al. 2014,

Kent et al. 2017b, Flávio et al. 2023). For example, reef-associated species have been identified within *S. spinulosa* reefs, such as dragonets (*Callionymus spp.*), small-spotted catshark (*Scyliorhinus canicula*), and rock gunnel (*Pholis gunnellus*) (van der Reijden et al. 2019).

The loss of habitat has been linked to the reduced effectiveness of fish stock management strategies, as habitat availability is a key determinant of population resilience (Mangel 2000, Brown et al. 2019). Selecting essential fish habitats (EFH) is crucial for making informed fishery management decisions to protect and restore these habitats (US Fishery Act, 1996). EFHs are defined as habitats with a critical role in supporting commercial species at various life stages (e.g. as feeding or nursery grounds), ultimately sustaining fish stocks (USDOC, 1997) and emphasise the importance of an ecosystem-based management approach (e.g. The Habitats Directive, Council Directive 92/43/EEC). Temperate reefs are gaining increasing attention for their key role in biodiversity provision and are regarded as EFH, although their specific role in supporting mobile vertebrate communities has yet to be fully explored (Coen et al. 1999). Detailed knowledge of the ecological association between species and their environment, including habitat use across all life stages, is needed to define temperate reefs as EFH (Coen et al. 1999, Levin & Stunz 2005). Particularly, emphasis should be placed on understanding the specific function of temperate reefs within the life cycle of mobile species, such as feeding, spawning, or living grounds (Johnson et al. 2013, Kent et al. 2017b, Brown et al. 2019). Despite their ecological significance, these relationships remain poorly understood, including in the North Sea (zu Ermgassen et al. 2016a, Kent et al. 2017b, Smaal et al. 2018).

Several methods exist for monitoring biodiversity of marine mobile communities, both extractive and observational. Extractive techniques like fishing, netting or trapping are most commonly used, however are often dependent on the species catchability, and can cause significant damage to the specimens and the seabed (Murphy & Jenkins 2010, Mallet & Pelletier 2014). Conversely, observational methods like underwater visual census, videos and acoustic techniques, though more labour-intensive compared to fishing related methods, are generally preferred when monitoring occurs in sensitive habitats (e.g. reefs or marine protected areas (Murphy & Jenkins 2010). Among these, the combined use of baited remote underwater video systems (BRUVs) and environmental DNA (eDNA) has increased in the recent years, as it offers a valid alternative that minimise habitat disturbance (Rees et al. 2014, Langlois et al. 2020). BRUVs

and eDNA are complementary, with BRUVs providing visual identification of species and eDNA offering broader taxonomic scope (Stat et al. 2019, Jeunen et al. 2020, Mirimin et al. 2021, Clark et al. 2024). However, their combined application to natural biogenic reefs has not been thoroughly explored, especially between contrasting locations at small spatial scales (>100 – 1,000 meters).

In this study, we 1) evaluate the effects of biogenic reefs on mobile vertebrate communities in terms of diversity and community composition; 2) compare habitat preference and feeding strategy based on functional traits of the vertebrate communities; and 3) determine the complementarity value of eDNA and BRUVs in terms of richness and detected species. We evaluate the biodiversity of fish and other marine vertebrates at two distinct shellfish reefs: a small shallow (approx. 4 m depth) mixed shellfish reef in the Dutch North Sea, and a large deep (approx. 40 m depth) *M. modiolus* reef in the Scottish North Sea. Using BRUVs and eDNA, we assessed the mobile vertebrate communities on the reefs and compared them to nearby sandy seabed areas. We hypothesize that reefs have distinct communities with distinct habitat preferences and feeding strategies depending on reef density as this determines food availability, habitat complexity and predator presence. In addition, we hypothesize that adding eDNA monitoring to BRUVs application provides a more comprehensive picture of fish communities, including cryptic or nocturnal species, better captured with genetic methods (Watson et al. 2005, Stat et al. 2019).

2. MATERIALS AND METHODS

Two study areas were selected for this study: the Voordelta and the Noss Head, both featuring a natural bivalve reef in an environment of sandy seabed (without reef). These areas were chosen due to their contrasting environmental conditions. The Voordelta was selected as a small shallow reef in the southern North Sea, characterized by higher turbidity and a composition of mixed bivalves (blue mussels, pacific and European oysters). The Noss Head was selected as a larger and deeper reef in the Northern North Sea, with lower turbidity and solely composed of horse mussels. Both sites are located within a marine protected area where bottom trawling is prohibited, ensuring minimal disturbance. However, some anthropogenic activities (e.g. regulated whelk fishery, tourism) still occur across both reef and non-reef areas, potentially effecting fish

distribution and applying a relatively uniform random noise to the background. As we lack the data needed to explicitly quantify these effects, they were not included in the analyses.

At both sites, seabed was first ground-truthed with a towed camera to locate the reef areas and plan the sampling stations within and outside reefs. Then, at selected sampling stations, water sampling was performed to retain eDNA samples and BRUVs were deployed to acquire footage of fish specimens (Tables S1, S2). Due to the differing conditions of the two study areas, sampling was conducted onboard two different vessels; hence, field methods are described separately for each area. In contrast, onshore procedures (e.g. laboratory work, video and statistical analysis) were identical for all samples and are therefore described together for both areas.

2.1 Field sampling: Voordelta

2.1.1 The Voordelta

The Voordelta is a coastal area in the southern North Sea, near the coast of Zeeland, in the Netherlands (Figure 1A). It is designated as a Natura 2000 site under the EU Birds and Habitats Directives, which aims at protecting and restoring habitats and species of special conservation interest (European Union, 1992, 2009). This area includes a bivalve reef composed of a mix of Pacific oysters (*Magallana gigas*), European flat oysters (*Ostrea edulis*), and blue mussels (*Mytilus edulis*; Figure 1A) at a depth of approximately 3-6m and covering ~0.54km^2 (Christianen et al. 2018, Kamermans et al. 2023).

Due to its depths, the Voordelta is reachable only by limited sized research vessels especially designed for research in shallow areas, such as the RV Navicula used for this study (26m). Therefore, due to ship time and space limitations, different fieldwork activities (ground truthing, water sampling and BRUVs deployments) were performed during three separated expeditions, conducted between May 2021 and August 2022.

2.1.2 Gound-truthing and sampling design

To determine the precise location of the reef and estimate its boundaries, the area was ground truthed using the towed camera system BRUCE Tow Cam (van der Reijden et al. 2019) between May 27th and June 4th, 2021. During the survey, the video was live-streamed on board, enabling real-time assessment of the seabed for the presence or absence of the reef

while collecting time and position data (cable payout length and direction corrected) for each observation. Based on the collected data, 60 sampling stations were selected along six transects, including stations within the reef and increasing distance from the reef edge, to assess how far the reef effect extended beyond its border (A–K; Figure 1B; Figure S1, Table S1). Of A–F, 16 stations were located within the reef area, 25 at relatively close distances from the reef edge (25–200m), and 24 stations were placed 250–1000m from the edge. Furthermore, an extra transect K (1–10km from the reef edge) was designed to control the eDNA background conditions. Along this transect, stations 10, 9, and 8 were sampled during ebb tide, while stations 1 through 7 during flow tide, in order to minimize any potential background eDNA signal from the nearby water sluice connecting the area to lake Grevellingen.

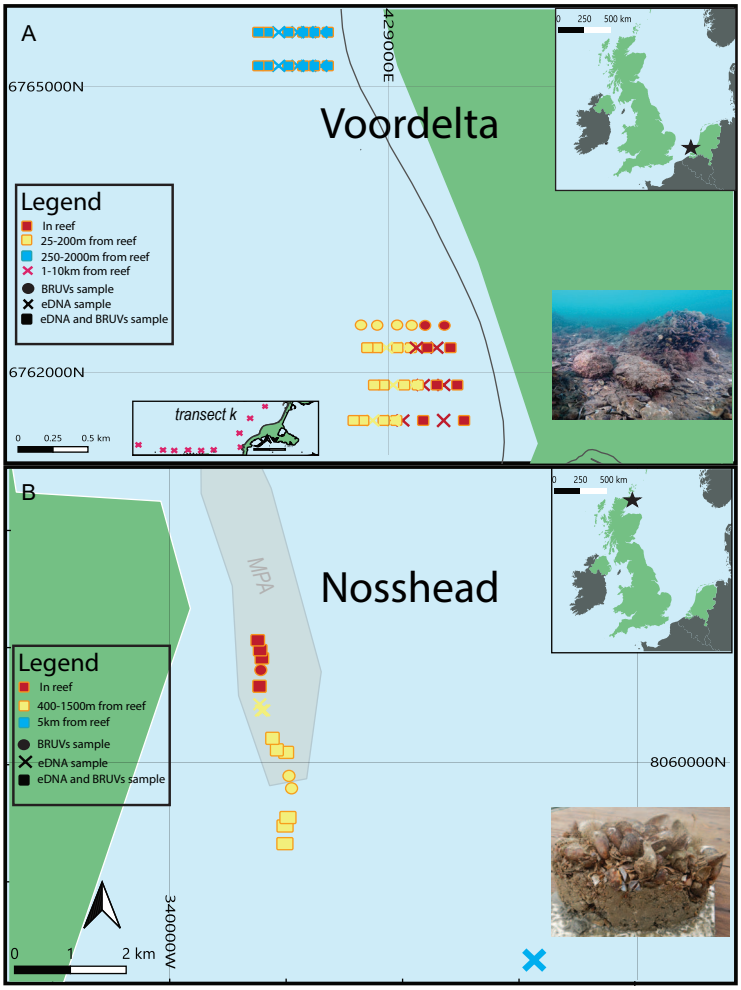


Figure 1: Study area locations and habitat pictures, showing A) the mixed bivalve reef at Voordelta including a Close-up view of the position of the sampling locations at Voordelta (for details of transect K and sampling locations see Supplementary figure 1) and a Seafloor picture (picture by Peter van Rodijnen). B) The Modiolus modiolus reef at Noss Head, in the North Sea and Close-up view of the position of the sampling locations and a picture from a sediment sample collected at Noss Head (picture by Joop Coolen) . Colours indicate the different biotopes; open circles represent stations sampled with the BRUVS method, crosses indicate those sampled with the eDNA method, and open squares show stations sampled with both techniques. The brown shape at Noss Head marks the area protected under the Noss Head MPA. The map is presented in the WGS84 Geographic Coordinate System.

2.1.3 Water sampling

Water sampling for eDNA was conducted between October 6th and October 10th, 2021, using a 10L Niskin bottle lowered to approximately 50cm above the seafloor and rinsed between stations. During high tide, three 2L pseudo-replicates were collected from the same Niskin at each station (Table S2). Water was transferred into 2L plastic bottles previously rinsed with milliQ water. Gloves were worn throughout sample handling and processing to prevent contamination and were changed at each station. For each replicate, ~2000-1300ml of seawater (depending on when the filter clogged) was filtered through 0.45µm cellulose nitrate filter using a vacuum pump (Rocker, Taiwan), directly shipboard in a laboratory setting. Filters were then folded with chloride-cleaned tweezers and stored in a 2 ml screw cap containing 400ul RNA/DNA shield (Zymo, USA). The filter holders, plastic bottles and tweezers were rinsed between samples then sterilized using a 10-minute 1:10 litres commercial bleach bath and followed by MilliQ water rinsing. Field controls were taken on decontaminated filter equipment before and after each day of filtering using ~1L MilliQ water. Samples were immediately stored in the freezer at -20 °C. Samples were immediately stored in the freezer at -20 °C.

2.1.4 Video collection using BRUVs

Video collection through BRUVs deployment was conducted between 2-9 August 2022. 12 camera systems were used and each consisted of a GoPro digital camera (Hero 8 or Hero 9) mounted on a frame of PVC pipes filled with gravel. As natural light reached the seafloor at all sampling stations, artificial lighting was not used. All systems were baited using frozen mackerel (*Scomber scombrus*) wrapped in a nylon mesh and attached to the frame through a 45-50cm bamboo stick. During sampling, 6 or 12 BRUV camera systems were deployed simultaneously, with one camera placed

at each sampling station. Sampled stations during each deployment were evenly distributed across biotopes to achieve a balanced design (Tables S1, S2). Each camera recorded for 45–90 minutes, depending on battery life. In total, 90 videos were obtained.

2.2 Field sampling: Noss Head

2.2.1 The Noss Head

The Noss Head is a Marine Protected Area (MPA) located off the coast of Scotland (Figure 1B). It encompasses a horse mussel (*Modiolus modiolus*) reef, which lies at around 35–50m depth and covers an estimated 3.85km^2 (Hirst et al. 2012, Moore 2019). Fisheries management measures are enforced within the Noss Head MPA, restricting activities to controlled static gear only, which must be further limited in areas where horse mussels are present.(Hirst et al. 2012, NatureScot 2024). All fieldwork activities (ground-truthing, water sampling, BRUVs deployment) were performed onboard the RV Pelagia between October 3rd and 10th, 2023. The presence of multiple static fishing gear (i.e. whelk fykes) in multiple locations within the reef area restricted RV Pelagia's transit and sampling zones to gear-free areas, which constrained the overall sampling design. As a result, the sampling design differed slightly between Voordelta and Noss Head, leading to a lower resolution of the distance gradient at Noss Head, where sampling intervals were wider (In reef, 400-1500m, 5km) compared to Voordelta (In reef, 25-200m, 250-2000m, 1-10 km).

2.2.2 Gound-truthing and sampling design

Previous surveys at the Noss Head confirmed the presence of high-density patches of *M. modiolus* in the northern part of the area (Moore and Roberts 2011, Hirst et al. 2012). Consequently, the video survey focused on the southern part of the reef to complete the seabed characterization and locate the reef's southern boundary. Ground truthing was performed using an HD video hopper frame (Coolen et al. 2023) towed from the side of the vessel while live-streamed on board, registering observations in real-time. Based on the observed seabed, 18 stations were selected across three biotopes, with six stations per biotope (Figure 1B, Figure S1, Table S2): high-density reef (>50% of alive *M. modiolus* cover), low-density reef (<50% of alive *M. modiolus* cover), and no reef (0% of alive *M. modiolus*). The southernmost station within the low-density reef was selected as a proxy of the reef edge to assess distances from stations outside the reef ranging between 400 and 1500m from such edge. Moreover, an extra

station, situated 5 km from the reef edge, was included to control for the eDNA background conditions.

Due to limited ship time and technical constraints (e.g. a single crane was available for deploying either the BRUVs or the rosette for eDNA water sampling), it was not possible to collect eDNA samples on the same day as the BRUV deployments (Tables S1, S2). Instead, all eDNA samples were collected during the same tidal phase to at least minimize variability and obtain a more reliable dataset.

2.2.3 Water sampling

Water was collected using six 25 L Niskin bottles mounted on a rosette frame, with three replicates taken 1 m above the seafloor and three replicates 1 m below the surface, each from a distinct bottle. To minimize contamination, the Niskin bottles were rinsed with freshwater between samples, and gloves were worn throughout sample handling and processing, changing to a new pair at each station. For each replicate, the water was transferred into 2L plastic bottles, then ~2000- 1300ml (depending on when the filter clogged) was filtered through a 1.2µm cellulose nitrate filter using a vacuum pump (Rocker, Taiwan). Filtration was performed in the dry lab onboard the research vessel. Filters were then folded using chloride-cleaned tweezers and stored in 2 mL screw-cap tubes containing 400 µL RNA/DNA Shield (Zymo, USA). Filter holders, plastic bottles, and tweezers were rinsed between samples and sterilized in a 1:10 litre commercial bleach bath for 10 minutes, followed by rinsing with Milli-Q water. Field controls were taken on decontaminated filter equipment before and after each filtering session using ~1L of MilliQ water. All samples were immediately stored at -20 °C onboard.

2.2.4 Video collection using BRUVs

BRUV sampling was conducted using eight systems, each consisting of a camera (GoPro Hero 8 or Hero 9) mounted on a 1.5 m^2 metal cage used as a lander (Coolen et al. 2023). Also, two LetonPower BB27-1/S high-power 20.000 lm LED flood lights were attached to the cage and angled downward at 45° to ensure adequate illumination of the seafloor. Each system was baited with frozen mackerel (*Scomber scombrus*), wrapped in nylon mesh and mounted on the cage at the bottom centre of the camera's field of view using a 45–50 cm bamboo stick. The eight BRUVs were deployed twice over consecutive days, with one system per sampling station, and were evenly distributed across biotopes to achieve a balanced design

(Table S2). Each camera recorded 45-90 minutes, depending on battery life. In total, 14 videos were acquired.

2.3 DNA processing: Voordelta and Noss Head

2.3.1 DNA extraction

Samples with filters and DNA/RNA shield were defrosted at room temperature prior to extraction. DNA was extracted in batches of 24 samples using the DNeasy Blood and Tissue extraction kit (Qiagen, USA) following the manufacturer's protocol for tissue samples. Samples were pre-processed by adding 20µl Proteinase K and 400µl lysis buffer, followed by incubation for one hour at room temperature. DNA concentrations were measured using a Qubit 2.0 Fluorometer (Invitrogen, USA). Extraction controls were taken within each round of extractions.

2.3.2 DNA amplification and gel extraction

DNA extracts were amplified in a 10µL reaction volume consisting of 5µL of 2x Phire Tissue Direct PCR Master Mix, 2µL of template DNA, 0.2µL (10 µM) of each primer, and nuclease-free water (NFW) to a final volume of 10µL. Three PCR replicates were used to reduce the stochastic effect of PCR. Short read Mifish primers (Miya et al. 2015) were used for the amplification. In addition, the Mifish primers were manufactured (Eurofins, NL) with an addition of 13bp tags to avoid an extra barcoding of index PCR cycle, as adapted from Srivathsan et al. (2018) (Table S3 primers with 96 individual tags). PCR settings for amplification were 98°C 180s, 98° 10s, 59.6°C for 8s, 72°C 10s, and 72°C 3min with 35 cycles. Three PCR replicates were pooled, and PCR controls were also taken during each PCR experiment (96 reactions in total).

Amplification was confirmed and pooled to semi- equimolar concentration using gel electrophoresis (1.5% TAE agarose gel). To reduce the amount of bacterial amplicon and enhance the sequence efficiency of the target amplicons, the pooled product was run on a 2% agarose gel for 90 minutes at 100V. After detection using blue light from a Bluegel (mini-PCR bio, USA), the target band was cut out, and the DNA was extracted using a gel extraction kit (MinElute Gel, Qiagen, USA) according to the manufacturer's protocol.

2.3.3 Nanopore sequencing

End prep and adapter ligation were performed using the SQK-LSK114 kit (Oxford Nanopore Technologies Ltd., UK). The total purified DNA con-

centration was estimated using the Qubit HS kit. A maximum of 100ng DNA was loaded on a primed flow cell to prevent overload. A total of 96 samples was sequenced on a Mk1c with an R10.4.1 flow cell with a sequencing speed of 450 bases/sec, run with an average sequence depth of 200K reads per sample (~20–25 million reads per run). All sequencing runs were performed on separate flow cells to avoid cross-contamination between runs.

2.3.4 Bioinformatics

Base-calling of the pod5 files was performed using Dorado (Version 0.5.3, Oxford Nanopore Technologies Ltd., UK) in super high accuracy (SUP) mode. Decona was used for the bioinformatic analysis (Doorenspleet et al. 2025). The raw base called reads were filtered for length in a window from 150 to 240bp. A cluster similarity at 96% was chosen based on carefully checking different clustering percentages (Doorenspleet et al. 2025) with a minimum of 5 reads per sequence. (decona -f – T 20 -l 150 -m 240 -c 0.94 -g "GTYGGTAAAWCTCGTGCCAGC; max_error_rate=0.1; min_overlap=20...CAAACTYGGA TACCCCACTAT; max_error_rate=0.1; min_overlap=20" -n 5 -r 0.99= -R 500 -k 6 -M).

Taxonomic identification of the sequences was performed using the Basic Local Alignment Search Tool for nucleotides (BLASTn) using the Chordata nucleotide (nt) database (last updated April 2024), integrated within DECONA (https://github.com/Saskia-Oosterbroek/DECONA; Doorenspleet et al. 2025). Singletons were not taken into account. Assignments on species level were accepted if the top hit e-value was higher than the second hit, and/or when the second or third hit assigned the same species. If this criterion was not met, the lowest common ancestor (LCA) was assigned.

To validate the species list obtained from BLASTn taxonomic assignments, the online repositories WoRMS (World Register of Marine Species) and GBIF were used. First, marine species were detected using *pyworms*, the Python client for WORMS REST service, and consequently non-marine species were excluded from the analysis. For each marine species the number of occurrences were then extracted from the GBIF using the Python client *pygbif*. Occurrences were counted within a radius of 2 degrees, representative of local species, and 10 degrees, representative of rare or potentially visiting species, around the sample locations (https://github.com/cld321/ednavalid). The BLASTn species list was corrected by elevating species not observed within the 2-degree radius. These identifica-

tions were manually checked and replaced to their LCA, or species names were replaced with the North Sea species, and marked with an asterisk to indicate a certain level of uncertainty. All results were manually checked; the LCA was assigned if species from the same genus had one or fewer mismatches. To correct for sequencing depth, species occurring less than <0.1% in each barcode were removed to correct for PCR stochasticity and tag jumping ((Wang et al. 2022, Skelton et al. 2023). To correct for possible contamination, the uncorrected reads of the dataset were also tested using Microdecon and LULU (Frøslev et al. 2017, McKnight et al. 2019).

2.4 Video analysis: Voordelta and Noss Head

Analysis of the baited videos was done by manual visual observations using the VLC software (version 3.0.21). Starting when the camera reached the bottom, the number of species and individuals per species were registered for each second for the entire length of each video. Individuals were identified to the lowest possible taxonomic level using relevant literature (i.e. Heessen et al. 2015, Maitland et al. 2009, Wood 2018) and with assistance from experts at NIOZ and WUR. The maximum observed number of individuals per species in a single frame (MaxN) was used to measure abundance to avoid double counting (e.g., when a fish swam in and out of the video frame multiple times (Langlois et al. 2018, Clark et al. 2024). As video length varied with camera battery life (45-90 minutes), the maximum number of individuals (MaxN) for each species was divided by video duration (in hours) to calculate sightings per unit effort (SPUE; Sherman et al. 2020), rather than analysing only the first 45 minutes. This approach corrected for variation in recording time while retaining all data for species richness. As recording time was not standardised among deployments and no consistent relationship between species richness and video length was detected, the full recording duration was retained for analyses of species richness. Moreover, MaxN values were not used for direct biomass comparisons among stations, but only to calculate ecological indices within stations. The visibility of each video was evaluated on a scale of 0 to 5, based on the visibility of the bait. Recordings rated with a visibility of 0 or those where the camera didn't land horizontally on the seafloor were excluded from the analysis.

2.5 Traits collection: Voordelta and Noss Head

From the list of species obtained from eDNA, functional traits (feeding strategy and habitat preference) were collected using literature and online resources (Table S4, Supplementary data file). Each trait was categorized, and species were scored for each category using fuzzy coding (Chevene et

al. 1994), up to a total score of 1 per species per trait. Subsequently, community weighted means were calculated per each eDNA sample, using eDNA relative read abundance per each species.

2.6 Statistical analysis: Voordelta and Noss Head

The data analysis was executed in RStudio v4.2.2 (RStudio Team, 2020). Taxa richness and the Shannon ecological index were determined using the diversity() function (v*egan* v2.6.4) Oksanen et al. 2022) and visualized using a violin plot (*ggplot2*, v3.4.0) Wickham, 2016). The significance of the difference in variance between richness and diversity indices across habitats was tested for both sampling locations (Noss head and Voordelta) and methods (BRUVs, eDNA). As there was no significant difference between bottom and surface samples in the eDNA Noss Head dataset, all samples were used from each area for analysis. Abundance from BRUVs data was also tested. First, a generalized linear model (GLM) was applied (package *mass*, Brian et al. 2024). Richness was fitted with a Poisson distribution, the Shannon index with a gamma distribution, and Simpson's and evenness indices with beta distributions. To calculate ecological indices for the eDNA dataset, the relative read abundance was preferred over the eDNA index (Kelly et al. 2019), because the analysis compared diversity between samples. Abundance was fitted with a negative binomial distribution. Model selection was based on the lowest Akaike information criterion (AIC; Akaike, 1973), and residuals were checked for overdispersion. Subsequently, an analysis of variance (ANOVA) was conducted on the resulting models to assess the significance of differences among habitats using the *aov* function (package *stats*, v3.6.2; R Core Team, 2024). A post hoc Tukey test (HSD) was then performed using the TukeyHSD function (*stats*, v3.6.2; R Core Team, 2024) for pairwise comparison. Finally, to analyse differences between community composition across habitats, a PERMANOVA was conducted with 999 permutations and Bray-Curtis similarity, using the function adonis from the *vegan* package (v2.6.4; Oksanen et al. 2022). Non-metric multidimensional scaling (NMDS) with Bray-Curtis dissimilarity ('bray') was applied to visualize clustering among habitats for both locations and methods (Figure S6, S7).

To test for differences between habitats in the presence of specific taxa in the eDNA samples, generalized linear latent variable models were applied using the gllvm package v2.0 (Niku et al. 2024) following methods by Zuur & Ieno (2025). The eDNA data were converted to presence-absence per sample by assigning 1 to read counts >0. Observations per cast

were aggregated as counts when a taxon was detected in multiple (sub) samples. Confounding effects of the distance from the coast at Noss Head with the reef density challenged the interpretation of the gllvm results. Therefore, the Noss Head data was not further analysed using gllvm. In the Voordelta, the models included area type (reef/non-reef) and distance from the coast as explanatory variables. Sampling date and cast number were included as random effects. Taxa occurring in less than 10 samples (33 taxa) were excluded from the analysis. *Phocoena phocoena* (250-2000 m, 1-10 km from reef), *Gobius paganellus* (In reef, 25-200 m, 250-2000 m from reef), and Clupeidae (In reef, 25-200 m, 1-10 km from reef) were almost exclusively present in a sub-set of the areas and caused poor model fit. Thus, they were excluded from the model. The final model included 34 species. The model family was selected by generating multiple models with 0, 1, or 2 latent variables and different distributions (Poisson, negative binomial, zero-inflated Poisson, and zero-inflated negative binomial). From these 12 models, the best fitting model (Poisson distribution with zero latent variables) was selected using the AIC (Akaike, 1973). After model formulation, post hoc contrasts based on the estimated marginal means between all area combinations within each species were generated using the *emmeans* and *pairs* functions of the emmeans package (Lenth, 2025).

3. RESULTS

3.1 Read processing comparison and taxonomic assignment between datasets.

Overall, more taxa were found with eDNA compared to BRUVs (Table 1, Table S6, Figure S8). At the Voordelta, a total of seventy-two marine vertebrate taxa were identified using the eDNA method, while ten taxa could be identified through BRUVs. Sequencing of the Voordelta eDNA reads yielded 40 710 000 reads, where 16 140 460 could be taxonomically assigned to target taxa (marine vertebrates, Table S5). At the Noss Head, ninety-three different taxa were found using the eDNA method and eleven taxa using BRUVs. A total of 45 160 000 reads were sequenced from the Noss Head eDNA samples, where 19 191 294 could be taxonomically assigned to target taxa (Table S5). Species accumulation curves indicated that enough sequencing depth was reach for the samples (Figure S4). Field negative extraction or negative PCR controls (31 in total) mostly contained unassigned or non-target DNA. Nevertheless, field control samples (Figure S3) showed low read counts on a reduced amount of

marine vertebrate species for both areas. Indeed, decontamination let to the reduction of richness in the control samples without affecting the diversity patterns between samples, indicating that although some contamination occurred, the influence on the results is likely limited.

3.2 The effect of reefs on the diversity indices and community compositions

Overall, the diversity of fish communities was significantly higher on reefs than away from the reefs at both Voordelta (Figure 2, Figure S5, Table S6, A1) and Noss Head (Figure 2, Figure S6). In addition, there was a decreasing trend at short distance intervals from the reef in both Voordelta and Noss Head. However, these differences were not significant. At the Voordelta, both richness and Shannon index were significantly higher in the reef stations compared to stations >250m from the reef for both eDNA and BRUVs (eDNA: $p < 0.001$; BRUVs: $p < 0.001$), but not with 25-200m distance from the reef. Similarly, at the Noss Head, the richness and Shannon indexes were significantly higher in the reef than at >400m from the reef with eDNA ($P < 0.001$ (richness), $P < 0.001$ (Shannon). However, these differences were non-significant for the BRUVs data. PERMANOVA and NMDS showed a significant difference in the community composition between habitats at Voordelta (eDNA, $P < 0.01$, BRUVs, $P < 0.01$) and Noss Head (eDNA, $P < 0,01$, BRUVs, $P < 0.01$), based on the relative read abundance (eDNA) and maxN (BRUVs) (Table A1, Figure S7, S8). Nevertheless, NMDS plots showed a greater and clearer differentiation among stations within eDNA samples compared to BRUVs, suggesting that the two methods detect community differences at different spatial scales.

Table 1: Full list of taxa detected at both study areas, Noss Head and Voordelta, through eDNA and BRUVs. The presence of a taxa within an habitat is depicted by a P.

	Voordelta		Noss Head			Voordelta		Noss Head			Voordelta		Noss Head	
Taxon	eDNA	BRUVS	eDNA	BRUVS	Taxon	eDNA	BRUVS	eDNA	BRUVS	Taxon	eDNA	BRUVS	eDNA	BRUVS
Agonus cataphractus	P		P		Gadus morhua*	P		P	P	Pollachius sp.	P		P	
Alosa sp.	P				Gasterosteus aculeatus	P		P		Pomatomidae			P	
Ammodytidae	P		P		Gobiesocidae			P	P	Pomatoschistus microps	P			
Anarhichadidae			P		Gobiidae		P	P	P	Pomatoschistus minutus	P		P	
Anguilla anguilla*	P		P		Gobius niger	P				Pomatoschistus pictus	P		P	
Aphia minuta	P				Gobius paganellus	P				Raja clavata*			P	
Arnoglossus laterna			P		Gobiusculus flavescens			P		Raja montagui			P	
Atherina sp.	P	P			Grampus griseus	P		P		Raniceps raninus			P	
Balaenoptera acutorostrata			P		Halichoerus grypus	P		P		Salmo salar	P		P	
Balistes capriscus			P		Hippocampus hippocampus	P				Salmo trutta*	P		P	
Belone belone	P		P		Hippoglossoides platessoides*	P				Sardina pilchardus	P		P	
Brosme brosme			P		Labrus bergylta*	P		P		Scomber scombrus	P		P	
Buglossidium luteum	P		P		Labrus mixtus	P		P		Scombridae			P	
Callionymus lyra			P	P	Lagenorhynchus albirostris			P		Scophthalmus maximus	P			
Callionymus reticulatus			P		Lamna nasus			P		Scophthalmus rhombus	P		P	
Callyonimus sp		P			Leuciscidae			P		Scyliorhinus canicula			P	
Carangidae			P		Leucoraja naevus			P		Sebastidae			P	
Centrolabrus exoletus*			P		Limanda limanda*	P		P		Sepiia officinalis		P		
Cetorhinus maximus			P		Liparidae			P		Solea solea	P		P	
Chelon auratus	P				Liparis montagui	P		P		Sparus aurata	P		P	
Chelon sp.	P				Liparis sp.	P		P		Sprattus sprattus*	P		P	
Chirolophis ascanii			P		Lipophrys pholis	P		P		Spinachia spinachia			P	
Ciliata mustela	P		P		Lophius piscatorius			P		Squalidae			P	
Ciliata septentrionalis	P		P		Melanogrammus aeglefinus				P	Syngnathus acus	P	P		

Ciliata septentrionalis	P		P		Melanogrammus aeglefinus				P	Syngnathus acus	P	P		

Clupea harengus*	P		P		Merluccius merluccius			P		Syngnathus rostellatus	P			
Clupeidae	P	P	P		Micrenophrys lilljeborgii			P		Taurulus bubalis	P		P	
Conger conger			P		Microstomus kitt*	P		P		Thunnus thynnus*	P			
Cottidae	P		P		Molva molva	P		P	P	Trachinotus ovatus	P			
Crystallogobius linearis			P		Mugilidae	P		P		Trachurus trachurus	P		P	P
Ctenolabrus rupestris	P		P		Mullus surmuletus	P		P		Triglidae	P		P	
Cyclopterus lumpus			P		Myctophidae	P		P		Triglops murrayi			P	
Delphinidae	P		P		Myoxocephalus sp.	P		P	P	Triglops pingelii			P	
Dicentrarchus labrax		P	P		Neogobius melanostomus	P		P		Trisopterus esmarkii			P	
Diplecogaster bimaculata	P		P		Nerophis lumbriciformis			P		Trisopterus luscus	P		P	P
Dipturus sp.			P	P	Osmerus eperlanus	P				Trisopterus minutus	P		P	
Echiichthys vipera	P		P		Parablennius gattorugine	P				Zeugopterus sp.	P		P	
Engraulidae			P		Phoca vitulina*	P		P		Zeus faber			P	
Engraulis encrasicolus	P				Phocoena phocoena	P		P		Zoarces viviparus*	P		P	
Eutrigla gurnardus				P	Pholis gunnellus*	P	P	P						
Gadidae	P		P		Pleuronectidae	P	P	P	P					

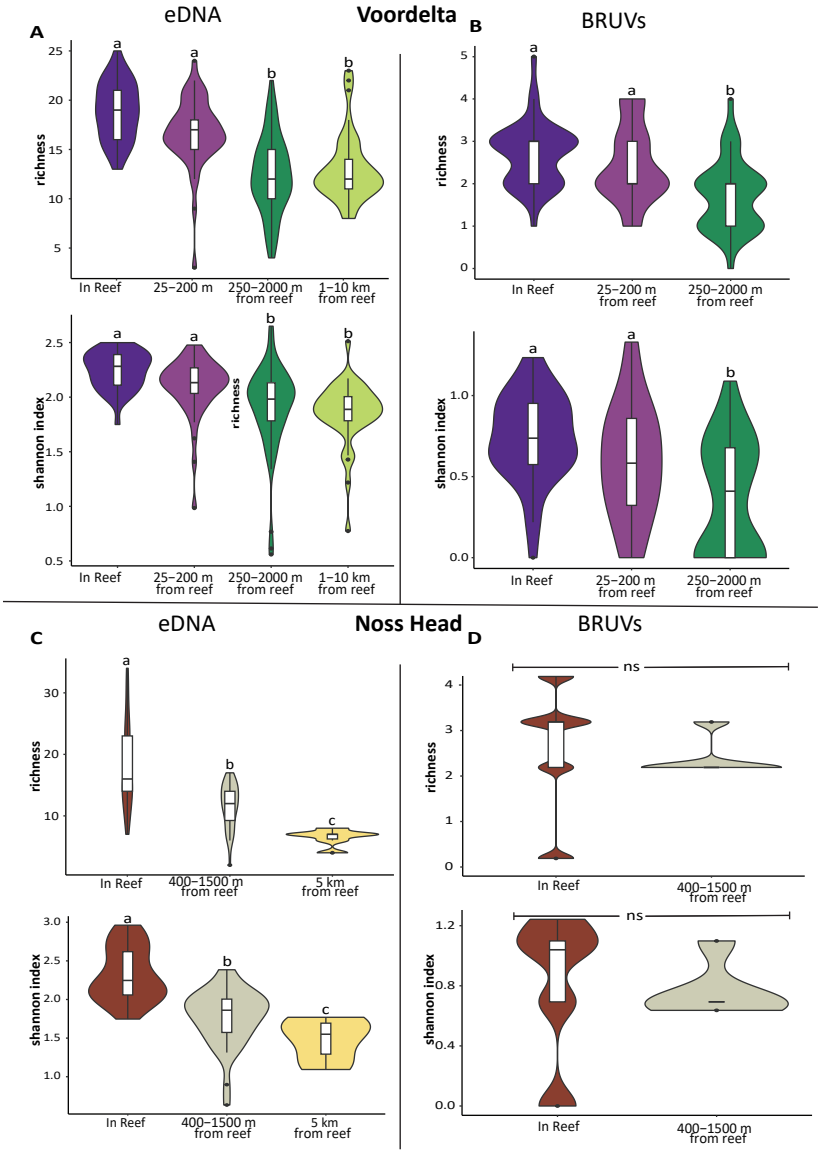


Figure 2: Comparison of vertebrate communities between reef habitats and increasing distances from their edges, at the mixed bivalve reef at Voordelta and the Modiolus modiolus reef at Noss Head. Biodiversity is compared in terms of richness (number of taxa) per sample and Shannon diversity index per sample representing taxa distribution within the community. Biodiversity indices are exhibited for eDNA data (A, C) and BRUVS data (B, D). Data are presented as violin plots with embedded boxplot. Violin plots illustrate the distribution of the data points, while boxplots display the median, interquartile range (between 25th to 75th percentiles), and minimum and maximum values (whiskers). Significant differences, determined using a generalized linear model with Tukey post hoc comparisons, are indicated by different letters.

3.3 Species-specific findings

A generally consistent species composition was observed throughout habitats at both locations, although some species were observed more frequently in or near the reefs. Post hoc comparison of the estimated marginal means from the GLLVM at Voordelta showed that the number of observations was significantly higher within the reef habitat for several species, such as sand smelt (*Atherina* sp.; $P < 0.05$, Table S7, Figure 3), five-bearded rockling (*Ciliata mustela*; $P<0.05$) and black goby (*Gobius niger*; $P < 0.05$). Contrastingly, the common seal *Phoca vitulina* was significantly more present in the areas outside the reef surroundings. There were less reads of common goby (*Pomatochistus microps*) and butterfish (*Pholis gunnellus*) in the outside areas, although this was not significant (Table S7). For the Noss Head dataset, boxplots showed that many commercially important species, such as cod (*Gadus morhua*), mackerel (*Scomber scombrus*), and ling (*Molva molva*), as well as many reef-associated species, such as pouting (*Trisopterus minutus*), *P. gunnellus*, and clingfish (*Diplecogaster bimaculata*), were more present inside the reef than outside the reef.

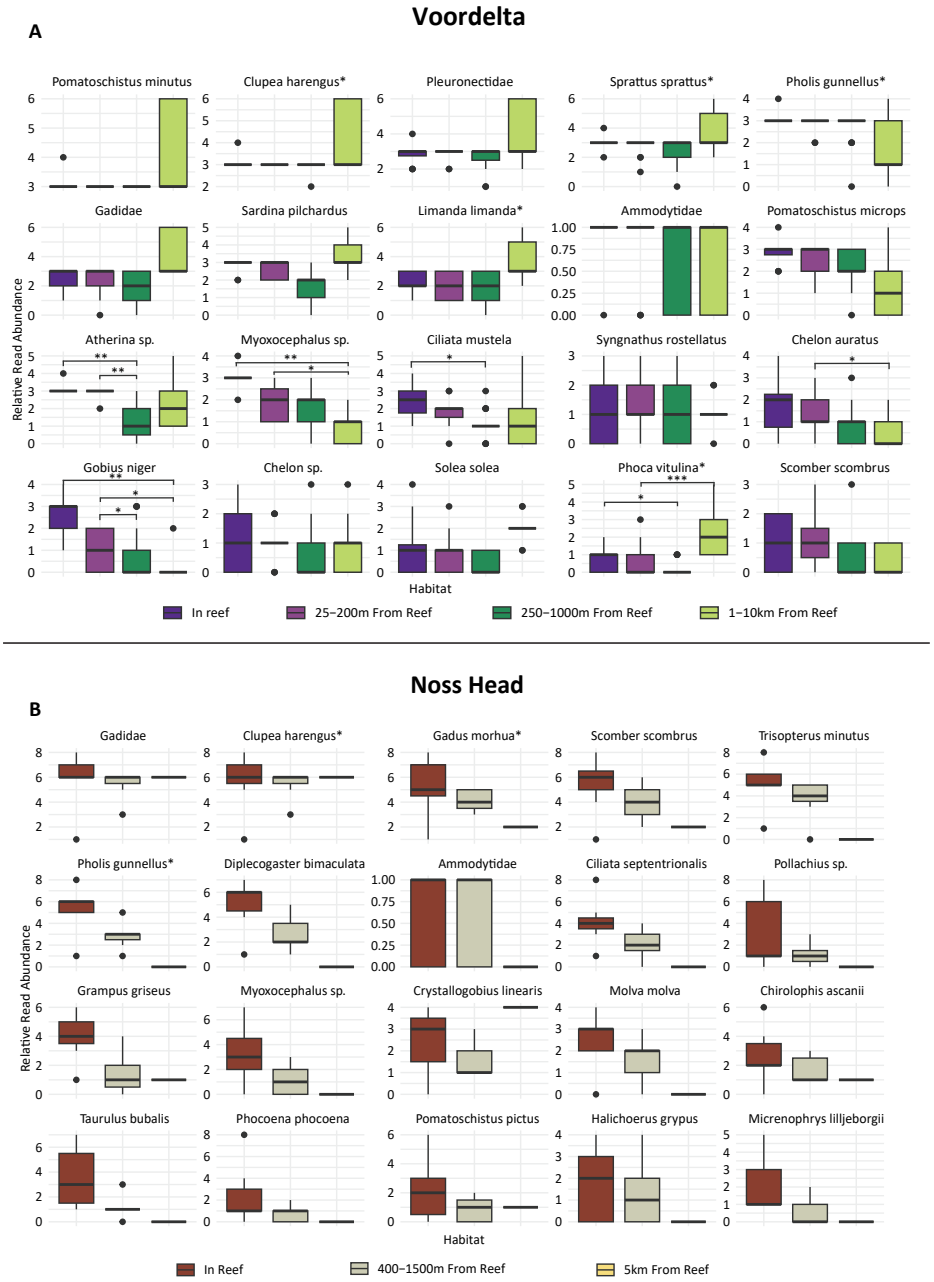


Figure 3: Comparison of the 20 most frequently recurring species from eDNA dataset between reef habitats and increasing distances from their edges, at the mixed bivalve reef at Voordelta (A) and the Modiolus modiolus reef at Noss Head (B). Data are presented as boxplots displaying the median, interquartile range (between 25th to 75th percentiles), minimum and maximum values (whiskers) and outliers (closed circles). Significant differences, identified using GLLVMs, are indicated by asterisk

3.4 Reef effect on trait composition

Reef presence slightly influenced functional diversity for both feeding strategy and habitat preference, with this effect also evident at stations at close proximity to the reef (Voordelta: 25 -200 m from the reef edge; Figure 4, Table S8). Regarding habitat preferences, the reef at the Voordelta showed a negative trend of pelagic taxa occurrences with increasing distance from the reef. This trend was opposite for sand and mud taxa, which increased with distance to the reef. Within the Noss Head community, the proportion of pelagic taxa decreased with distance from the reef. With regards to the feeding strategy, at Voordelta, reef communities were primarily composed of invertivores and planktivorous species, with a minor proportion of piscivores, which was relatively higher off the reef. There was a clear difference in the reef at the Noss Head, with a higher proportion of planktivorous and piscivorous fish outside of the reef. In contrast, the proportion of invertivores fish was higher inside the reef.

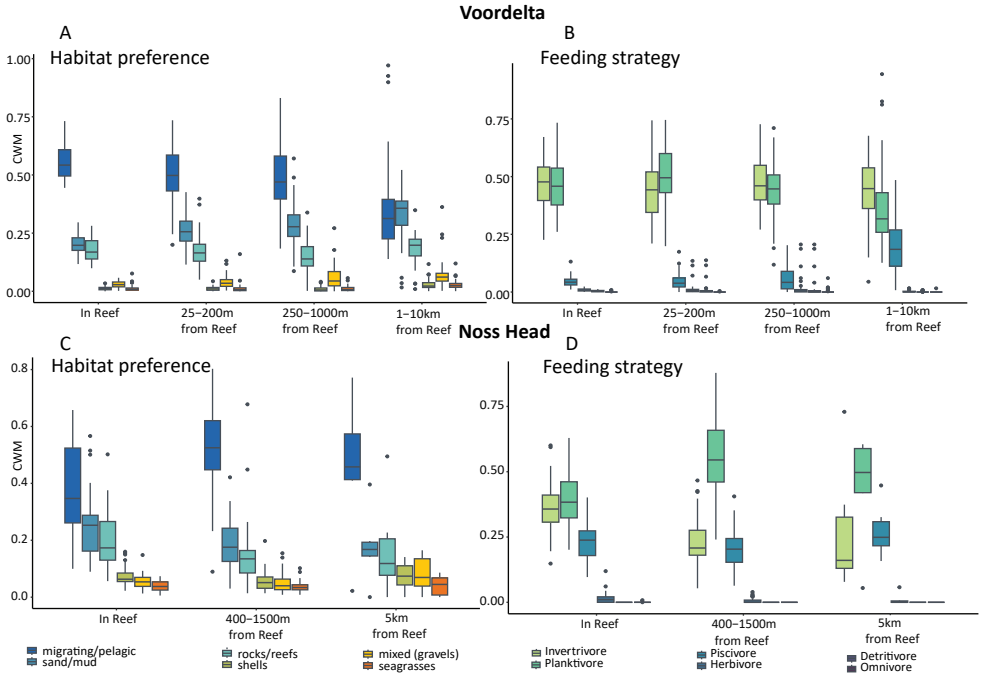


Figure 4: Comparison of functional diversity from eDNA dataset between reef habitats and increasing distances from their edges, at the mixed bivalve reef at Voordelta and the Modiolus modiolus reef at Noss Head. Different habitat preferences (A,C) and feeding strategies (B, D) of the species found within the communities at the different habitats are compared within habitats. Data are displayed as community weighted means per sample. Boxplots display the median, interquartile range (between 25th to 75th percentiles), minimum and maximum values (whiskers) and outliers (closed circles).

3.5 General considerations on the complementary value of eDNA and BRUVs

Although differences in sampling seasons, times and sample sizes prevent a direct comparison between the two methods, some general conclusions can still be drawn from their overall findings. Overall, eDNA identified more taxa than BRUVs, with seventy-two vs nine at Voordelta and ninety-three vs twelve at Noss Head (Table 1, Figure S9). Most species identified using BRUVs were also identified with eDNA; however, three additional taxa were found with BRUVs at Voordelta (*Callyonimus* sp., *Dicentrarchus labrax*, and *Sepia officinalis*) and two at Noss Head (*Eutrigla gurnardus* and *Melanogrammus aeglefinus*). Moreover, video analysis of BRUVs data could identify species at different developmental stages, as for example ling (*M. molva)* and bull-rout (*Myoxocephalus scorpius)* (Figure S10). In some instances, BRUVs data could identify taxa on a lower taxonomic level than eDNA, such as *Melanogrammus aeglefinus*. In other cases, however, eDNA could identify taxa to a lower taxonomic level than BRUVs, such as for Gobiidae and Pleuronectiformes.

4. DISCUSSION

We investigated the effect of reefs on marine vertebrate communities by comparing areas with and without a reef presence, and at increasing distance from the reef, across two distinct systems in the North Sea: a mixed bivalve reef in the Dutch MPA Voordelta and a *Modiolus modiolus* reef in the Scottish MPA Noss Head. To achieve this, we used two non-invasive complementary techniques: environmental DNA (eDNA) and baited remote underwater video systems (BRUVs). We found that reefs enhanced species richness and diversity, which were found decreasing with distance from the reef. Reefs also influenced species-specific distribution (i.e. *Atherina* sp., *Ciliata mustela, Gobius niger)*, resulting in slightly distinct community compositions characterized by differences in habitat use and feeding strategies. Notably, eDNA detected a broader range of species than BRUVs, whereas BRUVs provided information on species life stages, confirming the complementary value of the two approaches. However, a direct comparison between the methods was not possible due to differences in sample size and timing resulting from survey conditions. In addition, this study further suggests the sensitivity of eDNA for biodiversity assessments and its ability to capture fine-scale spatial patterns in community structure.

4.1 Reef effect on species richness and diversity

An increase in biodiversity within the reef habitats compared to off reefs was found at both locations. This included reduced taxa and community evenness, with off-reef sites being less diverse and increasingly dominated by fewer species. These results are in accordance with already published studies for natural reefs (Peterson et al. 2003, Coolen et al. 2015, zu Ermgassen et al. 2016b, Kent et al. 2017b, van der Reijden et al. 2019), recovered biogenic reefs (Kristensen et al. 2015, Gilby et al. 2019, 2021), and artificial reefs (Reubens et al. 2013, Coolen et al. 2020, Dickson et al. 2023, Flávio et al. 2023, Ibanez-Erquiaga et al. 2025). This increase in biodiversity is associated with reefs enhancing habitat complexity and availability, as well as the diversity and abundance of benthic fauna (Rees et al. 2008, Coolen et al. 2015, van der Reijden et al. 2019), which represents a food source for fish and is positively linked to their abundance (Kaiser et al. 2006, Sell & Kröncke 2013, Eggleton et al. 2018). At both locations, the number of species identified through eDNA in the reef habitats was comparable to other studies on natural and artificial structures within the North Sea (e.g.; Dahlgren et al. 2023, Ibanez-Erquiaga et al. 2024, Doorenspleet et al. 2025). In some cases, artificial structures may have a stronger influence on fish communities than natural reefs, as they provide greater habitat complexity, expanding habitat availability higher in the water column (Hunter & Sayer 2009, Claisse et al. 2014, Paxton et al. 2020, Flávio et al. 2023). Often, they are specifically designed and deployed to increase fishing yield (Hylkema et al. 2021). Nevertheless, our findings indicate that shellfish reefs that offer relatively limited 3D complexity and size compared to artificial structures, do enhance overall marine vertebrate biodiversity.

4.2 Reef effect on surrounding areas

At both locations, we observed a decline in fish biodiversity with increasing distance from the reefs, suggesting that the influence of reef presence may extend up to a few hundred meters outside the reef area. Reefs facilitate fish diversity, abundance and production through different mechanisms. They enhance fish biomass as nursery and spawning grounds for several species, thereby increasing larval production and survival and leading to a net species spillover from the reef (Gell & Roberts, 2003). Additionally, the structural complexity of reefs provides foraging opportunities that attract adult fish. Restored reefs can increase fish attraction even within the first few months and have long served as a management tool to support fish stock recovery (Fernandes & Cook 2013, Gilby et al. 2019, 2021, Knoester et al. 2023). In our study,

we cannot determine with certainty whether the observed patterns reflect attraction, spillover effects, or a combination of both. However, the habitats studied here are long-established natural reefs, persisting for several years (Voordelta: Christianen et al. 2018, Witbaard et al. 2020; Noss Head: personal observation, R. Witbaard, Coolen et al. 2023), rather than newly introduced structures. Therefore, we hypothesize a combined attraction and production effect. Investigating this effect further—particularly by assessing the presence of juveniles and larvae—could provide deeper knowledge on the mechanisms behind temperate biogenic reefs effect on fish populations.

Studies conducted on natural and artificial reefs have shown that fish distributions are influenced up to approximately 90 meters from the reef, which is somewhat less than what we observed (dos Santos et al. 2010, Claire Rosemond et al. 2018). However, our study examined fish distribution over a distance gradient with a relatively low resolution, spanning from 25-200 m (Voordelta) and 400-1500 m (Noss Head). We therefore recommend that future research investigate this gradient at finer spatial scales, highlighting the 90 m threshold. Additionally, reef characteristics such as composition, size, and depth could influence the extent of the gradient, and should be considered to better understand how these features could shape fish distribution. Moreover, future studies could also benefit from sampling a greater portion of the water column both horizontally and vertically to gain better understanding of the impact of shellfish reefs to the surrounding areas.

4.3 Reef effect on community composition

The community composition of the *M. modiolus* reef was fundamentally different from the surrounding area at 400-1500m and 5 km distance, although limited replication at 5 km prevents firm conclusions. At the mixed bivalve reef at Voordelta, species composition showed more overlap close to the reef, suggesting an influence of the reef to locations at relatively close distances (25-200m). Reef-associated species that declined away from the reef edge included rockling (*Ciliata mustela*) at Voordelta and the northern rockling (*Ciliata septentrionalis*) at Noss Head. Both species are known to prefer complex habitats like *Sabellaria spinulosa* reefs (Pearce et al. 2013, van der Reijden et al. 2019), rocky or bivalve reefs (Gonçalves et al. 2002, Heessen et al. 2015, Dye et al. 2024) or experimental reef structures like tree-reefs (Dickson et al. 2023), or other hard substrates such as rocks, wood, reef paste, etc (Watson et al. 2025). In these habitats, fish find shelter from predators and forage for their op-

portunistic diet (Badsha & Sainsbury 1978). Other typical reef-associated species included gobies (*Pomatoschistus* spp., *Gobius niger*), clingfish (*Diplecogaster bimaculata*) and gunnels (*Pholis gunnellus*). These species too are known to use reefs for refuge and to feed on their rich and abundant benthic communities (Heessen et al. 2015). Sculpins (*Myoxocephalus* spp.) and scorpion fish (*Taurulus bubalis*) also occurred more on the reef habitats in our study. These benthic predators are common within reefs where they ambush crustaceans (i.e. shrimps, small crabs, amphipods etc), polychaetes, molluscs (including gastropods and bivalves), or other fish including gobies (Heessen et al. 2015, Ivanova et al. 2018). These medium sized species can represent important food sources for larger fish and other predators like seabirds and marine mammals (Divoky & Tremblay 2013, Camphuysen & Henderson 2017, Aarts et al. 2019, Dye et al. 2024). Thus, our findings are in line with expected habitat preferences and highlight that bivalve reefs support a wide range of fish species, offering refuges, habitat and forage, hence potentially playing an important role in marine food webs.

Several commercial species were found more frequently in reef habitats at both locations. These included cod (*Gadus morhua*), pollack (*Pollachius* sp.), poor cod (*Trisopterus minutus*) and ling (*Molva molva*) at Noss Head, and golden grey mullet (*Chelon auratus*) at Voordelta. Juveniles of cod are often associated with reefs, where habitat complexity increases their survival rate (Lindholm et al. 2001, Wieland et al. 2009, Dunlop et al. 2022), while adults appear to save up to 20% energy by using rocky reefs for shelter and predation, which become more efficient due to the higher prey availability (Schwartzbach et al. 2020). Gobies, abundant in reef habitats, may also attract cod (Fjøsne & Gjøsœter 1996, Wennhage & Pihl 2002, Almqvist et al. 2010, Kristensen et al. 2015). Juvenile pollacks and poor cod adults may also benefit by feeding within reefs as their diet include benthic preys like small crabs, worms and small fish (Dunlop et al. 2022, Heessen et al. 2015 and references within).

Ling was detected with both eDNA and BRUVs within the reef at Noss Head (30-60 m depth). Although adults typically occur at depths of 200–1000m (Svetovidov 1986, Heessen et al. 2015), juveniles are more commonly found at shallower depths of <100 m (Bergstad & Gordon 1994, Heessen et al. 2015), and interestingly, the BRUVS video data confirmed the presence of juvenile ling individuals. This suggests that mussel beds could serve as a nursery habitat for this species and warrants further investigation.

With their filtration activity, bivalves enhance water quality and favour algal growth if in the photic zone (Riemann et al. 1988, Newell 2004, Nielsen & Maar 2007, Kristensen et al. 2015), while also increasing the nutritional value of the sediments with their bio deposits (Rees et al. 2008, Kent et al. 2017a). This could explain the presence of grey mullets, which feed by scraping mud or other surfaces to feed on diatoms, algae, and meiofauna (Heessen et al. 2015). The association between these species and natural, healthy reefs is nevertheless noteworthy, reinforcing the role of bivalve reefs in enhancing fish habitats and supporting commercial stocks.

4.4 Reef relevance for functional diversity

The high proportion of invertivores in reef habitats suggests that rich benthic communities attract fish that feed on benthos or have opportunistic diet, like mid-sized species. However, scoring trait data based on literature rather than empirical observations has limitations. As we did not account for possible juveniles occurrences, this may have resulted in over or underrepresentation of certain traits, as juveniles primarily rely on phyto- and zooplankton as a food source (Nunn et al. 2012). Additionally, gadoids shift their diet based on food availability, from pelagic to benthic sources, which may have influenced the proportion of invertivores (Nunn et al. 2012 and reference within).

The proportion of planktivorous fish was higher with increasing distance to the reefs, particularly at Noss Head. This may be related to the depleting effect of bivalve filtration on plankton concentrations and community structure (Petersen et al. 2013, Kristensen et al. 2015, Tan et al. 2024).

As expected, the proportion of rock/shell habitats preferring species decreased with distance from the reef. At Noss Head, the no-reef areas were characterized by coarse sediments with sparse cobbles, as observed during the ground-truthing survey. This may explain the great proportion of species with mixed sediment habitat preference and with sand/mud preference found there, as they might benefit from the muddy bio deposits produced by the horse mussels.

4.5 Complementarity of monitoring methods

Using eDNA metabarcoding, we detected up to 6 times more fish and other vertebrate species than with BRUVs. However, these results were likely influenced by differences in sampling size, timing and season (Voordelta) between the two methods and must be interpreted with cau-

tion. Nevertheless, the magnitude of the observed differences was consistent with previous studies reporting approximately 5-10 times higher species detection with eDNA than with BRUVs (Gold et al. 2023, Clark et al. 2024). Other studies have reported greater diversity per BRUVs deployment (Stat et al. 2019, Gold et al. 2023, Clark et al. 2024) than we found, which could be related to our limited number of deployments, especially at Noss Head. At the Voordelta, the low visibility and high number of swimming tourists during the sampling period could have reduced the number of recorded fish. Low individual counts from BRUVs prevented species biomass estimation and comparison among habitats. As fish aggregation is a known reef effect, we recommend future studies incorporate more efficient biomass-targeting methods, such as acoustic detections. Despite limitations, this study suggests that BRUVs and eDNA can be used complementarily if supported by an appropriate sampling design, as both methods picked up a unique set of species (Clark et al. 2024). In particular, we observed that BRUVs allowed the distinction between juvenile and adult fish, as shown in our study for ling and bull-rout, and reported by Clark et al. (2024).

Our results demonstrate that eDNA can detect differences in community composition at fine horizontal spatial scales, even across distances of just a few hundred meters. This aligns with previous studies that have also reported variation in community composition at both horizontal and vertical scales (Dukan et al. 2024). Developing a proxy for biomass or individual abundance of different species from eDNA measurements remains an important goal for future research (Urban et al. 2023, Dukan et al. 2024). In addition to often-unknown dilution factors, eDNA persistence in the water column depends on chemical, physical (Collins et al. 2018, McCartin et al. 2022) and biological (Joseph et al. 2022) degradation processes, which are, among other factors, temperature-dependent. At species levels, cell shedding rates per kg of animal differs depending on traits such as size, age, season, and diet (Sassoubre et al. 2016). Additionally, methodological biases may be introduced during laboratory processing, such as amplification bias, primer binding efficiency, and sequencing data processing biases (van der Loos & Nijland 2021). Moreover, avoid contamination can be quite challenging within field settings or on board research vessels, due to suboptimal laboratory conditions. Thereby, this study further stresses the importance of complementary methods for better-informed biodiversity assessment studies.

4.6 Conclusions and future perspectives

In this study, we demonstrated that eDNA is a sensitive method to identify habitat-specific species composition over scales of few hundred meters. Combined with BRUVs, it increases taxonomic resolution and enables identification of developmental stages of the detected species. Using both methods, we found that biogenic shellfish reefs in the North Sea positively affect the biodiversity of mobile fish community, increasing species richness. Reef effects were detectable even a few hundred meters from the reef borders, due to the spill-over or the attraction effect of reefs. Mid-sized invertivores like gobies, rocklings, and clingfish, were more frequent on reefs, where they can hide and feed opportunistically on benthic fauna. Planktivorous species, in contrast, were higher further from the reef, possibly due to the plankton-depleting of filtering bivalves. Commercial species like cod and ling were also attracted by the reefs, either as adults seeking preys or as juveniles using the reef as habitat. Future research should focus on the role of temperate reefs as nursery areas and further expand on the horizontal and vertical spatial effects of reefs on their surroundings.

Ultimately, our study contributes to a better understanding and quantification of the effects of natural reefs on vertebrate communities and surrounding areas, highlighting their ecological importance and role as EFHs. Protecting and restoring these habitats not only promotes nature conservation but also supports the productivity of commercial fish stocks. By officially designating temperate reefs as EFHs, their protection and restoration can be more effectively integrated into fisheries policies and management frameworks. Remaining temperate reefs should be mapped and protected. Our results indicate that these reefs can influence fish communities beyond their physical boundaries, suggesting that a buffer zone of at least 200 m should be established around protected reef areas. However, further research is needed to clarify how reef characteristics affect the extent of this influence. Currently, many restoration projects focus on bivalves such as European and Pacific oysters. We recommend expanding restoration efforts to include other bivalves, such as horse mussels, which can provide with additional habitats. Moreover, incorporating shellfish reef restoration into the nature-inclusive design of artificial infrastructures, such as offshore wind farms, could enhance their potential as fish habitats and aggregators. Ultimately, our findings provide a valuable foundation for future strategies that align ecological restoration with fishery management, offering important perspectives for future nature conservation projects.

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Data availability

The sequence data used for writing this manuscript have been uploaded to the European Nucleotide Archive (ENA) under project accession PRJEXXX. The code for the detection of non-target organisms is available through https://github.com/cld321/ednavalid. BRUVs videos are available upon request by the authors.

SUPPLEMENTARY MATERIAL

Appendices

Table A1: Summary of the statistical analysis performed on the data, or biodiversity, diversity indices, community composition, and abundance.

Voordelta

	Mean values ± SE				Statistics		
	In reef (R)	25 - 200 m (P)	250 - 2000 m (O)	1 - 10 km (B)	F	p	Post hoc groups (R, P, O, B)
eDNA: diversity indices							
Taxa richness (sample)	18.89 ± 0.48	16.71 ± 0.52	12.41 ± 0.47	13.05 ± 0.55	32.26	<0.001	a, a, b, b
Shannon	2.24 ± 0.03	2.10 ± 0.04	1.92 ± 0.05	1.86 ± 0.05	12.64	<0.001	a, a, b, b
Simpson's	0.85 ± 0.01	0.82 ± 0.01	0.79 ± 0.01	0.77 ± 0.02	7.29	<0.001	a, b, b, c
Evenness	0.77 ± 0.01	0.76 ± 0.01	0.78 ± 0.01	0.74 ± 0.02	0.07	ns	
BRUVS: diversity indices							
Taxa richness (sample)	2.69 ± 0.12	2.44 ± 0.22	1.71 ± 0.14	-	13.1	<0.001	a, a, b
Abundance (Max N/hours per sample)	8.48 ± 1.16	10.28 ± 1.74	6.96 ± 0.95	-	1.52	ns	
Shannon	0.74 ± 0.05	0.59 ± 0.09	0.36 ± 0.06	-	8.61	<0.001	a, a, b
Simpson's	0.44 ± 0.03	0.35 ± 0.05	0.36 ± 0.07	-	1.41	ns	
Evenness	0.75 ± 0.03	0.62 ± 0.07	0.45 ± 0.07	-	1.02	ns	
PERMANOVA							
	DF	**Sum of squares**	**R^2**	**Pr (>F)**			
eDNA	8	4.08448	0.22327	<0.001			
BRUVS	5	1.949	0.12941	<0.01			

Noss Head

	Mean values ± SE				Statistics		
	High density reef (H)	Low density reef (L)	400 - 1500 m (N)	5 km (B)	F	p	Post hoc groups (H, L, N, B)
eDNA: diversity indices							
Taxa richness (sample)	17.95 ± 1.09	14.82 ± 0.46	11.68 ± 0.57	6.50 ± 0.56	21.23	<0.001	a, b, c, d
Shannon	2.32 ± 0.05	2.09 ± 0.04	1.77 ± 0.06	1.49 ± 0.11	22.88	<0.001	a, b, c, d
Simpson's	0.86 ± 0.01	0.81 ± 0.01	0.74 ± 0.02	0.72 ± 0.03	17.81	<0.001	a, b, c, c
Evenness	0.82 ± 0.01	0.79 ± 0.01	0.75 ± 0.02	0.80 ± 0.04	4.9	0.01	a, a, b, a
BRUVS: diversity indices							
Taxa richness (sample)	2.40 ± 0.68	3.00 ± 0.41	2.20 ± 0.20	-	0.66	ns	
Abundance (Max N per sample)	5.21 ± 2.06	10.06 ± 2.01	4.34 ± 0.81	-	2.61	ns	
Shannon	0.81 ± 0.22	0.87 ± 0.15	0.69 ± 0.08	-	0.11	ns	
Simpson's	0.69 ± 0.08	0.50 ± 0.07	0.52 ± 0.04	-	1.08	ns	
Evenness	0.77 ± 0.19	0.80 ± 0.05	0.98 ± 0.02	-	2.66	ns	
PERMANOVA							
	DF	**Sum of squares**	**R^2**	**Pr (>F)**			
eDNA	4	5.1209	0.17253	<0.001			
BRUVS	2	0.74752	0.337	<0.01			

Table S1: Overview of the sampling stations of each location at Voordelta, with their rationale and indication of distance from reef. Distance from coast was calculated from a map in qGIS. Number of replicate per each station and method are also indicated.

Sampling station	Latitude	Longitude	Rationale	Distance gradient class	Distance from coast (m)	Method applied	N replicates BRUV	Dates BRUVs collection	N replicates eDNA	Dates eDNA samples collection
A01	51.7835	3.862	In reef	Reef	294	BRUV, eDNA	6	04-08 Aug 2022	3	06-10 Oct 2021
A02	51.7835	3.8605	In reef	Reef	397	eDNA	-	-	3	06-10 Oct 2021
A03	51.7835	3.859	In reef	Reef	501	BRUV, eDNA	6	04-08 Aug 2024	3	06-10 Oct 2021
A04	51.7835	3.8575	Reef edge	Reef	645	eDNA	-	-	3	06-10 Oct 2021
A05	51.7835	3.8571	25 m distance from A04	25-200m from reef edge	631	BRUV, eDNA	2	04-08 Aug 2026	3	06-10 Oct 2021
A06	51.7835	3.8568	50 m distance from A04	25-200m from reef edge	653	eDNA	-	-	3	06-10 Oct 2021
A07	51.7835	3.8561	100 m distance from A04	25-200m from reef edge	701	BRUV, eDNA	2	04-08 Aug 2028	3	06-10 Oct 2021
A08	51.7835	3.8553	150 m distance from A04	25-200m from reef edge	756	eDNA	-	-	3	06-10 Oct 2021
A09	51.7835	3.8546	200 m distance from A04	25-200m from reef edge	805	BRUV, eDNA	2	04-08 Aug 2030	3	06-10 Oct 2021
A10	51.7835	3.8539	250 m distance from A04	250-2000m from reef edge	853	BRUV, eDNA	6	04-08 Aug 2031	3	06-10 Oct 2021
B01	51.7854	3.8615	In reef	Reef	305	BRUV, eDNA	5	04-08 Aug 2032	3	06-10 Oct 2021

Table S1: Overview of the sampling stations of each location at Voordelta, with their rationale and indication of distance from reef. Distance from coast was calculated from a map in qGIS. Number of replicate per each station and method are also indicated. (*continued*)

Sampling station	Latitude	Longitude	Rationale	Distance gradient class	Distance from coast (m)	Method applied	N replicates BRUV	Dates BRUVs collection	N replicates eDNA	Dates eDNA samples collection
B02	51.7854	3.8605	In reef	Reef	374	eDNA	-	-	3	06-10 Oct 2021
B03	51.7854	3.86	In reef	Reef	409	BRUV, eDNA	5	04-08 Aug 2034	3	06-10 Oct 2021
B04	51.7854	3.859	Reef edge	Reef	478	eDNA	-	-	3	06-10 Oct 2021
B05	51.7854	3.8586	25 m distance from B04	25-200m from reef edge	505	BRUV, eDNA	1	04-08 Aug 2036	3	06-10 Oct 2021
B06	51.7854	3.8583	50 m distance from B04	25-200m from reef edge	526	eDNA	-	-	3	06-10 Oct 2021
B07	51.7854	3.8576	100 m distance from B04	25-200m from reef edge	574	BRUV, eDNA	1	04-08 Aug 2038	3	06-10 Oct 2021
B08	51.7854	3.8568	150 m distance from B04	25-200m from reef edge	629	eDNA	-	-	3	06-10 Oct 2021
B09	51.7854	3.8561	200 m distance from B04	25-200m from reef edge	678	BRUV, eDNA	1	04-08 Aug 2040	3	06-10 Oct 2021
B10	51.7854	3.8554	250 m distance from B04	250-2000m from reef edge	726	BRUV, eDNA	5	04-08 Aug 2041	3	06-10 Oct 2021
C01	51.7874	3.861	In reef	Reef	298	BRUV, eDNA	5	04-08 Aug 2042	3	06-10 Oct 2021

Table S1: Overview of the sampling stations of each location at Voordelta, with their rationale and indication of distance from reef. Distance from coast was calculated from a map in qGIS. Number of replicate per each station and method are also indicated. (*continued*)

Sampling station	Latitude	Longitude	Rationale	Distance gradient class	Distance from coast (m)	Method applied	N replicates BRUV	Dates BRUVs collection	N replicates eDNA	Dates eDNA samples collection
C02	51.7874	3.86	In reef	Reef	367	eDNA	-	-	3	06-10 Oct 2021
C03	51.7874	3.8593	In reef	Reef	415	BRUV, eDNA	5	04-08 Aug 2044	3	06-10 Oct 2021
C04	51.7874	3.8585	Reef edge	Reef	470	eDNA	-	-	3	06-10 Oct 2021
C05	51.7874	3.8581	25 m distance from C04	25-200m from reef edge	498	BRUV, eDNA	1	04-08 Aug 2046	3	06-10 Oct 2021
C06	51.7874	3.8578	50 m distance from C04	25-200m from reef edge	518	eDNA	-	-	3	06-10 Oct 2021
C07	51.7874	3.8571	100 m distance from C04	25-200m from reef edge	567	BRUV, eDNA	1	04-08 Aug 2048	3	06-10 Oct 2021
C08	51.7874	3.8563	150 m distance from C04	25-200m from reef edge	622	eDNA	-	-	3	06-10 Oct 2021
C09	51.7874	3.8556	200 m distance from C04	25-200m from reef edge	670	BRUV, eDNA	1	04-08 Aug 2050	3	06-10 Oct 2021
C10	51.7874	3.8549	250 m distance from C04	250-2000m from reef edge	719	BRUV, eDNA	5	04-08 Aug 2051	3	06-10 Oct 2021
D01	51.7886	3.8605	In reef	Reef	303	BRUV, eDNA	1	04-08 Aug 2052	-	
D02	51.7886	3.8598	In reef	Reef	351	eDNA	-	-	-	

Table S1: Overview of the sampling stations of each location at Voordelta, with their rationale and indication of distance from reef. Distance from coast was calculated from a map in qGIS. Number of replicate per each station and method are also indicated. (*continued*)

Sampling station	Latitude	Longitude	Rationale	Distance gradient class	Distance from coast (m)	Method applied	N replicates BRUV	Dates BRUVs collection	N replicates eDNA	Dates eDNA samples collection
D03	51.7886	3.8591	In reef	Reef	400	BRUV, eDNA	1	04-08 Aug 2054	–	
D04	51.7886	3.8585	Reef edge	Reef	441	eDNA	–	–	–	
D05	51.7886	3.8581	25 m distance from D04	25-200m from reef edge	469	BRUV, eDNA	1	04-08 Aug 2056	–	
D06	51.7886	3.8578	50 m distance from D04	25-200m from reef edge	489	eDNA	–	–	–	
D07	51.7886	3.8571	100 m distance from D04	25-200m from reef edge	538	BRUV, eDNA	1	04-08 Aug 2058	–	
D08	51.7886	3.8563	150 m distance from D04	25-200m from reef edge	593	eDNA	–	–	–	
D09	51.7886	3.8556	200 m distance from D04	25-200m from reef edge	641	BRUV, eDNA	1	04-08 Aug 2060	–	
D10	51.7886	3.8549	250 m distance from D04	250-2000m from reef edge	690	BRUV, eDNA	1	04-08 Aug 2061	–	
E01	51.8025	3.852	No reef control site north	250-2000m from reef edge	240	BRUV, eDNA	1	04-08 Aug 2062	3	06-10 Oct 2021
E02	51.8025	3.8515	No reef control site north	250-2000m from reef edge	274	eDNA	–	–	3	06-10 Oct 2021
E03	51.8025	3.851	No reef control site north	250-2000m from reef edge	309	BRUV, eDNA	1	04-08 Aug 2064	3	06-10 Oct 2021

Table S1: Overview of the sampling stations of each location at Voordelta, with their rationale and indication of distance from reef. Distance from coast was calculated from a map in qGIS. Number of replicate per each station and method are also indicated. (*continued*)

Sampling station	Latitude	Longitude	Rationale	Distance gradient class	Distance from coast (m)	Method applied	N replicates BRUV	Dates BRUVs collection	N replicates eDNA	Dates eDNA samples collection
E04	51.8025	3.8505	No reef control site north	250-2000m from reef edge	343	eDNA	-	-	3	06-10 Oct 2021
E05	51.8025	3.8502	No reef control site north	250-2000m from reef edge	364	BRUV, eDNA	1	04-08 Aug 2066	3	06-10 Oct 2021
E06	51.8025	3.8498	No reef control site north	250-2000m from reef edge	392	eDNA	-	-	3	06-10 Oct 2021
E07	51.8025	3.8491	No reef control site north	250-2000m from reef edge	440	BRUV, eDNA	1	04-08 Aug 2068	3	06-10 Oct 2021
E08	51.8025	3.8484	No reef control site north	250-2000m from reef edge	488	eDNA	-	-	3	06-10 Oct 2021
E09	51.8025	3.8476	No reef control site north	250-2000m from reef edge	543	BRUV, eDNA	1	04-08 Aug 2070	3	06-10 Oct 2021
E10	51.8025	3.8469	No reef control site north	250-2000m from reef edge	592	BRUV, eDNA	1	04-08 Aug 2071	3	06-10 Oct 2021
F01	51.8043	3.852	No reef control site north	250-2000m from reef edge	184	BRUV, eDNA	4	04-08 Aug 2072	3	06-10 Oct 2021
F02	51.8043	3.8515	No reef control site north	250-2000m from reef edge	218	eDNA	-	-	3	06-10 Oct 2021
F03	51.8043	3.851	No reef control site north	250-2000m from reef edge	253	BRUV, eDNA	5	04-08 Aug 2074	3	06-10 Oct 2021

Table S1: Overview of the sampling stations of each location at Voordelta, with their rationale and indication of distance from reef. Distance from coast was calculated from a map in qGIS. Number of replicate per each station and method are also indicated. (*continued*)

Sampling station	Latitude	Longitude	Rationale	Distance gradient class	Distance from coast (m)	Method applied	N replicates BRUV	Dates BRUVs collection	N replicates eDNA	Dates eDNA samples collection
F04	51.8043	3.8505	No reef control site north	250–2000m from reef edge	287	eDNA	–	–	3	06–10 Oct 2021
F05	51.8043	3.8502	No reef control site north	250–2000m from reef edge	308	BRUV, eDNA	1	04–08 Aug 2076	3	06–10 Oct 2021
F06	51.8043	3.8498	No reef control site north	250–2000m from reef edge	335	eDNA	–	–	3	06–10 Oct 2021
F07	51.8043	3.8491	No reef control site north	250–2000m from reef edge	384	BRUV, eDNA	1	04–08 Aug 2078	3	06–10 Oct 2021
F08	51.8043	3.8484	No reef control site north	250–2000m from reef edge	432	eDNA	–	–	3	06–10 Oct 2021
F09	51.8043	3.8476	No reef control site north	250–2000m from reef edge	487	BRUV, eDNA	1	04–08 Aug 2080	3	06–10 Oct 2021
F10	51.8043	3.8469	No reef control site north	250–2000m from reef edge	535	BRUV, eDNA	5	04–08 Aug 2081	3	06–10 Oct 2021
K01	51.7504	3.7274	Background eDNA	1–10km from reef edge		eDNA	–	–	3	06–10 Oct 2021
K02	51.7468	3.75	Background eDNA	1–10km from reef edge		eDNA	–	–	3	06–10 Oct 2021
K03	51.7468	3.7731	Background eDNA	1–10km from reef edge		eDNA	–	–	3	06–10 Oct 2021

Table S1: Overview of the sampling stations of each location at Voordelta, with their rationale and indication of distance from reef. Distance from coast was calculated from a map in qGIS. Number of replicate per each station and method are also indicated. (*continued*)

Sampling station	Latitude	Longitude	Rationale	Distance gradient class	Distance from coast (m)	Method applied	N replicates BRUV	Dates BRUVs collection	N replicates eDNA	Dates eDNA samples collection
K04	51.7469	3.7966	Background eDNA	1-10km from reef edge		eDNA	-	-	3	06-10 Oct 2021
K05	51.7488	3.8215	Background eDNA	1-10km from reef edge		eDNA	-	-	3	06-10 Oct 2021
K07	51.7488	3.8215	Background eDNA	1-10km from reef edge		eDNA	-	-	3	06-10 Oct 2021
K08	51.7586	3.8203	Background eDNA	1-10km from reef edge		eDNA	-	-	3	06-10 Oct 2021
K09	51.769	3.8301	Background eDNA	1-10km from reef edge		eDNA	-	-	3	06-10 Oct 2021
K10	51.7773	3.8438	Background eDNA	1-10km from reef edge		eDNA	-	-	3	06-10 Oct 2021

Table S2: Overview of the sampling stations of each location at Noss Head, with their rationale and assigned biotope for the analysis. Distance from coast was calculated from a map in qGIS. Number of replicate per each station and method are also indicated.

Station	Latitude	Longitude	Rationale	Distance gradient class	Distance from coast (m)	Method applied	N replicates BRUV	Dates BRUV collection	N replicates eDNA	Dates eDNA collection
5km_ref	58.4077	-2.9408	Background eDNA	5 km from reef	7849	eDNA	-	-	6	02 Oct 2023
high1	58.4582	-3.0257	High density reef	Reef	2644	eDNA, BRUVS	1	04/08 Oct 2023	6	04/06/08/09 Oct 2023
high2	58.4577	-3.0250	High density reef	Reef	2638	eDNA, BRUVS	1	04/08 Oct 2023	6	04/06/08/09 Oct 2023
high3	58.4567	-3.0253	High density reef	Reef	1513	eDNA, BRUVS	1	04/08 Oct 2023	6	04/06/08/09 Oct 2023
high5	58.4562	-3.0252	High density reef	Reef	1852	BRUVS	1	04/08 Oct 2023	6	04/06/08/09 Oct 2023
high6	58.4543	-3.0252	High density reef	Reef	1965	BRUVS	1	04/08 Oct 2023	6	04/06/08/09 Oct 2023
low1	58.4373	-3.0165	No reef	300-1500 m from reef	3151	BRUVS	1	04/08 Oct 2023	6	04/06/08/09 Oct 2023
low2	58.4353	-3.0157	No reef	300-1500 m from reef	3293	BRUVS	1	04/08 Oct 2023	6	04/06/08/09 Oct 2023
low4	58.4300	-3.0172	No reef	300-1500 m from reef	2822	eDNA, BRUVS	1	04/08 Oct 2023	6	04/06/08/09 Oct 2023
low5	58.4283	-3.0173	No reef	300-1500 m from reef	3113	eDNA, BRUVS	1	04/08 Oct 2023	6	04/06/08/09 Oct 2023
low6	58.4267	-3.0178	No reef	300-1500 m from reef	3234	eDNA, BRUVS	1	04/08 Oct 2023	6	04/06/08/09 Oct 2023

Table S2: Overview of the sampling stations of each location at Noss Head, with their rationale and assigned biotope for the analysis. Distance from coast was calculated from a map in qGIS. Number of replicate per each station and method are also indicated. (*continued*)

Station	Latitude	Longitude	Rationale	Distance gradient class	Distance from coast (m)	Method applied	N replicates BRUV	Dates BRUV collection	N replicates eDNA	Dates eDNA collection
mid1	58.4515	-3.0258	Low density reef	Low density reef	2211	eDNA, BRUVS	1	04/08 Oct 2023	6	04/06/08/09 Oct 2023
mid2	58.4487	-3.0252	Low density reef	Low density reef	1534	eDNA	-	04/08 Oct 2023	6	04/06/08/09 Oct 2023
mid3	58.4480	-3.0250	Low density reef	Low density reef	1548	eDNA	-	04/08 Oct 2023	6	04/06/08/09 Oct 2023
mid4	58.4330	-3.0212	Low density reef	Low density reef	2791	eDNA, BRUVS	1	04/08 Oct 2023	6	04/06/08/09 Oct 2023
mid5	58.4413	-3.0198	Low density reef	Low density reef	2315	eDNA, BRUVS	1	04/08 Oct 2023	6	04/06/08/09 Oct 2023
mid6	58.4405	-3.0173	Low density reef	Low density reef / Edge proxy	2367	eDNA, BRUVS	1	04/08 Oct 2023	6	04/06/08/09 Oct 2023

Table S3 Designed tagged primers as adapted from (Srivathsan et al. 2018) with a primer pair specific 13bp tag (black) attached to the MiFish Primer (Miya et al. 2015) as exemplary indicated in red in the first six primer pairs. Every two columns (FwMIFV, RvMIFV1001..1096) correspond to a unique primer pair.

List of used primers	
1	FwMIFV1001 ATC CGG TCG GAG AGT YGG TAA AWC TCG TGC CAG C
2	RvMIFV1001 ATC CGG TCG GAG AAT AGT GGG GTA TCT AAT CCY AGT TTG
3	FwMIFV1002 CTG AGG TGA TCA GGT YGG TAA AWC TCG TGC CAG C
4	RvMIFV1002 CTG AGG TGA TCA GAT AGT GGG GTA TCT AAT CCY AGT TTG
5	RvMIFV1003 AGT GTC CTG CTA GAT AGT GGG GTA TCT AAT CCY AGT TTG
6	FwMIFV1003 AGT GTC CTG CTA GGT YGG TAA AWC TCG TGC CAG C
7	FwMIFV1004 ATA AGC AAT TCG AGT YGG TAA AWC TCG TGC CAG C
8	RvMIFV1004 ATA AGC AAT TCG AAT AGT GGG GTA TCT AAT CCY AGT TTG
9	FwMIFV1005 ATC TAA CAG GAC CGT YGG TAA AWC TCG TGC CAG C
10	RvMIFV1005 ATC TAA CAG GAC CAT AGT GGG GTA TCT AAT CCY AGT TTG
11	FwMIFV1006 CAT TAT ATA GCC AGT YGG TAA AWC TCG TGC CAG C
12	RvMIFV1006 CAT TAT ATA GCC AAT AGT GGG GTA TCT AAT CCY AGT TTG
13	FwMIFV1007 CCT GAT TAC GTA AGT YGG TAA AWC TCG TGC CAG C
14	RvMIFV1007 CCT GAT TAC GTA AAT AGT GGG GTA TCT AAT CCY AGT TTG
15	FwMIFV1008 CTA ATA CAC ACC GGT YGG TAA AWC TCG TGC CAG C
16	RvMIFV1008 CTA ATA CAC ACC GAT AGT GGG GTA TCT AAT CCY AGT TTG
17	FwMIFV1009 TTA TGA CCG TTG CGT YGG TAA AWC TCG TGC CAG C
18	RvMIFV1009 TTA TGA CCG TTG CAT AGT GGG GTA TCT AAT CCY AGT TTG
19	FwMIFV1010 TTC TCG GCC GCA TGT YGG TAA AWC TCG TGC CAG C
20	RvMIFV1010 TTC TCG GCC GCA TAT AGT GGG GTA TCT AAT CCY AGT TTG
21	FwMIFV1011 TTG GCG GTA AGA AGT YGG TAA AWC TCG TGC CAG C
22	RvMIFV1011 TTG GCG GTA AGA AAT AGT GGG GTA TCT AAT CCY AGT TTG
23	FwMIFV1012 ACT TGT GCA CCT GGT YGG TAA AWC TCG TGC CAG C
24	RvMIFV1012 ACT TGT GCA CCT GAT AGT GGG GTA TCT AAT CCY AGT TTG
25	RvMIFV1013 CAT GCC TTG ATA CAT AGT GGG GTA TCT AAT CCY AGT TTG
26	FwMIFV1013 CAT GCC TTG ATA CGT YGG TAA AWC TCG TGC CAG C
27	RvMIFV1014 CTG ACG TTC GGT AAT AGT GGG GTA TCT AAT CCY AGT TTG
28	FwMIFV1014 CTG ACG TTC GGT AGT YGG TAA AWC TCG TGC CAG C
29	FwMIFV1015 GAG GAG AGA CAC AGT YGG TAA AWC TCG TGC CAG C
30	RvMIFV1015 GAG GAG AGA CAC AAT AGT GGG GTA TCT AAT CCY AGT TTG
31	RvMIFV1016 GCC GGT CCA AGT GAT AGT GGG GTA TCT AAT CCY AGT TTG
32	FwMIFV1016 GCC GGT CCA AGT GGT YGG TAA AWC TCG TGC CAG C

Table S3 Designed tagged primers as adapted from (Srivathsan et al. 2018) with a primer pair specific 13bp tag (black) attached to the MiFish Primer (Miya et al. 2015) as exemplary indicated in red in the first six primer pairs. Every two columns (FwMIFV, RvMIFV1001..1096) correspond to a unique primer pair. (*continued*)

List of used primers	
33	RvMIFV1017 TTG CGT CTC ACG CAT AGT GGG GTA TCT AAT CCY AGT TTG
34	FwMIFV1017 TTG CGT CTC ACG CGT YGG TAA AWC TCG TGC CAG C
35	RvMIFV1018 TTA TTC GCA CAC TAT AGT GGG GTA TCT AAT CCY AGT TTG
36	FwMIFV1018 TTA TTC GCA CAC TGT YGG TAA AWC TCG TGC CAG C
37	RvMIFV1019 TGC ACA GGC GGC AAT AGT GGG GTA TCT AAT CCY AGT TTG
38	FwMIFV1019 TGC ACA GGC GGC AGT YGG TAA AWC TCG TGC CAG C
39	RvMIFV1020 AAC ATG TGG TAA GAT AGT GGG GTA TCT AAT CCY AGT TTG
40	FwMIFV1020 AAC ATG TGG TAA GGT YGG TAA AWC TCG TGC CAG C
41	FwMIFV1021 ACG CGC TCT TAT AGT YGG TAA AWC TCG TGC CAG C
42	RvMIFV1021 ACG CGC TCT TAT AAT AGT GGG GTA TCT AAT CCY AGT TTG
43	RvMIFV1022 CAA TGT TAA TGG TAT AGT GGG GTA TCT AAT CCY AGT TTG
44	FwMIFV1022 CAA TGT TAA TGG TGT YGG TAA AWC TCG TGC CAG C
45	RvMIFV1023 CTA GGA TAT AGT TAT AGT GGG GTA TCT AAT CCY AGT TTG
46	FwMIFV1023 CTA GGA TAT AGT TGT YGG TAA AWC TCG TGC CAG C
47	RvMIFV1024 CTG TAG GCG AGG AAT AGT GGG GTA TCT AAT CCY AGT TTG
48	FwMIFV1024 CTG TAG GCG AGG AGT YGG TAA AWC TCG TGC CAG C
49	RvMIFV1025 CTG TCG AGG CGA CAT AGT GGG GTA TCT AAT CCY AGT TTG
50	FwMIFV1025 CTG TCG AGG CGA CGT YGG TAA AWC TCG TGC CAG C
51	FwMIFV1026 GAC TAA TGA GGA AGT YGG TAA AWC TCG TGC CAG C
52	RvMIFV1026 GAC TAA TGA GGA AAT AGT GGG GTA TCT AAT CCY AGT TTG
53	FwMIFV1027 TAT CCG TAA TCA TGT YGG TAA AWC TCG TGC CAG C
54	RvMIFV1027 TAT CCG TAA TCA TAT AGT GGG GTA TCT AAT CCY AGT TTG
55	FwMIFV1028 TCC TGG AGG ATA AGT YGG TAA AWC TCG TGC CAG C
56	RvMIFV1028 TCC TGG AGG ATA AAT AGT GGG GTA TCT AAT CCY AGT TTG
57	FwMIFV1029 TCT ACT GTT GTG CGT YGG TAA AWC TCG TGC CAG C
58	RvMIFV1029 TCT ACT GTT GTG CAT AGT GGG GTA TCT AAT CCY AGT TTG
59	RvMIFV1030 TGT ATA TTC AGC GAT AGT GGG GTA TCT AAT CCY AGT TTG
60	FwMIFV1030 TGT ATA TTC AGC GGT YGG TAA AWC TCG TGC CAG C
61	RvMIFV1031 ACA TCA GTA GTT CAT AGT GGG GTA TCT AAT CCY AGT TTG
62	FwMIFV1031 ACA TCA GTA GTT CGT YGG TAA AWC TCG TGC CAG C
63	RvMIFV1032 AGC GCT GGA CCA AAT AGT GGG GTA TCT AAT CCY AGT TTG

Table S3 Designed tagged primers as adapted from (Srivathsan et al. 2018) with a primer pair specific 13bp tag (black) attached to the MiFish Primer (Miya et al. 2015) as exemplary indicated in red in the first six primer pairs. Every two columns (FwMIFV, RvMIFV1001..1096) correspond to a unique primer pair. (*continued*)

List of used primers	
64	FwMIFV1032 AGC GCT GGA CCA AGT YGG TAA AWC TCG TGC CAG C
65	RvMIFV1033 CAA GAT CGG TAC CAT AGT GGG GTA TCT AAT CCY AGT TTG
66	FwMIFV1033 CAA GAT CGG TAC CGT YGG TAA AWC TCG TGC CAG C
67	FwMIFV1034 CCA GCC TGA CGG TGT YGG TAA AWC TCG TGC CAG C
68	RvMIFV1034 CCA GCC TGA CGG TAT AGT GGG GTA TCT AAT CCY AGT TTG
69	RvMIFV1035 CCG AAG CCG GTT CAT AGT GGG GTA TCT AAT CCY AGT TTG
70	FwMIFV1035 CCG AAG CCG GTT CGT YGG TAA AWC TCG TGC CAG C
71	FwMIFV1036 CCG AGC AGC TGT TGT YGG TAA AWC TCG TGC CAG C
72	RvMIFV1036 CCG AGC AGC TGT TAT AGT GGG GTA TCT AAT CCY AGT TTG
73	FwMIFV1037 CCT CCG CAC AAT CGT YGG TAA AWC TCG TGC CAG C
74	RvMIFV1037 CCT CCG CAC AAT CAT AGT GGG GTA TCT AAT CCY AGT TTG
75	FwMIFV1038 CTC TAA CCT ATT AGT YGG TAA AWC TCG TGC CAG C
76	RvMIFV1038 CTC TAA CCT ATT AAT AGT GGG GTA TCT AAT CCY AGT TTG
77	RvMIFV1039 GCA CCT TGA CAT AAT AGT GGG GTA TCT AAT CCY AGT TTG
78	FwMIFV1039 GCA CCT TGA CAT AGT YGG TAA AWC TCG TGC CAG C
79	FwMIFV1040 TAA GCG TGC TGA AGT YGG TAA AWC TCG TGC CAG C
80	RvMIFV1040 TAA GCG TGC TGA AAT AGT GGG GTA TCT AAT CCY AGT TTG
81	RvMIFV1041 TTC GGC ACA GGA GAT AGT GGG GTA TCT AAT CCY AGT TTG
82	FwMIFV1041 TTC GGC ACA GGA GGT YGG TAA AWC TCG TGC CAG C
83	RvMIFV1042 AGA CTC GAT TGA GAT AGT GGG GTA TCT AAT CCY AGT TTG
84	FwMIFV1042 AGA CTC GAT TGA GGT YGG TAA AWC TCG TGC CAG C
85	FwMIFV1043 ATC CTT CTG TTC TGT YGG TAA AWC TCG TGC CAG C
86	RvMIFV1043 ATC CTT CTG TTC TAT AGT GGG GTA TCT AAT CCY AGT TTG
87	FwMIFV1044 CGT TAC TTA GGT CGT YGG TAA AWC TCG TGC CAG C
88	RvMIFV1044 CGT TAC TTA GGT CAT AGT GGG GTA TCT AAT CCY AGT TTG
89	FwMIFV1045 CTG GTT GGC TAT CGT YGG TAA AWC TCG TGC CAG C
90	RvMIFV1045 CTG GTT GGC TAT CAT AGT GGG GTA TCT AAT CCY AGT TTG
91	FwMIFV1046 GCA CCA ACG ATT GGT YGG TAA AWC TCG TGC CAG C
92	RvMIFV1046 GCA CCA ACG ATT GAT AGT GGG GTA TCT AAT CCY AGT TTG
93	RvMIFV1047 TCT TAT GCA TAA CAT AGT GGG GTA TCT AAT CCY AGT TTG
94	FwMIFV1047 TCT TAT GCA TAA CGT YGG TAA AWC TCG TGC CAG C

Table S3 Designed tagged primers as adapted from (Srivathsan et al. 2018) with a primer pair specific 13bp tag (black) attached to the MiFish Primer (Miya et al. 2015) as exemplary indicated in red in the first six primer pairs. Every two columns (FwMIFV, RvMIFV1001..1096) correspond to a unique primer pair. (*continued*)

List of used primers	
95	FwMIFV1048 TTA GGT GTG GAC CGT YGG TAA AWC TCG TGC CAG C
96	RvMIFV1048 TTA GGT GTG GAC CAT AGT GGG GTA TCT AAT CCY AGT TTG
97	FwMIFV1049 TTC TTC TGG AAC AGT YGG TAA AWC TCG TGC CAG C
98	RvMIFV1049 TTC TTC TGG AAC AAT AGT GGG GTA TCT AAT CCY AGT TTG
99	FwMIFV1050 AGC AAG TGG CTC TGT YGG TAA AWC TCG TGC CAG C
100	RvMIFV1050 AGC AAG TGG CTC TAT AGT GGG GTA TCT AAT CCY AGT TTG
101	FwMIFV1051 TCA AGT CAC TAA TGT YGG TAA AWC TCG TGC CAG C
102	RvMIFV1051 TCA AGT CAC TAA TAT AGT GGG GTA TCT AAT CCY AGT TTG
103	FwMIFV1052 ATG AAG ACT ACT GGT YGG TAA AWC TCG TGC CAG C
104	RvMIFV1052 ATG AAG ACT ACT GAT AGT GGG GTA TCT AAT CCY AGT TTG
105	RvMIFV1053 CAC CGG TAG AAC CAT AGT GGG GTA TCT AAT CCY AGT TTG
106	FwMIFV1053 CAC CGG TAG AAC CGT YGG TAA AWC TCG TGC CAG C
107	FwMIFV1054 CAG AAC AAC GCA TGT YGG TAA AWC TCG TGC CAG C
108	RvMIFV1054 CAG AAC AAC GCA TAT AGT GGG GTA TCT AAT CCY AGT TTG
109	RvMIFV1055 CCT GCC GAG AAC GAT AGT GGG GTA TCT AAT CCY AGT TTG
110	FwMIFV1055 CCT GCC GAG AAC GGT YGG TAA AWC TCG TGC CAG C
111	RvMIFV1056 GTG ATG GAG CGG TAT AGT GGG GTA TCT AAT CCY AGT TTG
112	FwMIFV1056 GTG ATG GAG CGG TGT YGG TAA AWC TCG TGC CAG C
113	RvMIFV1057 TAC GAA CTA TAG CAT AGT GGG GTA TCT AAT CCY AGT TTG
114	FwMIFV1057 TAC GAA CTA TAG CGT YGG TAA AWC TCG TGC CAG C
115	RvMIFV1058 TAC GGA CCG CTC AAT AGT GGG GTA TCT AAT CCY AGT TTG
116	FwMIFV1058 TAC GGA CCG CTC AGT YGG TAA AWC TCG TGC CAG C
117	RvMIFV1059 TGG ACG CTA GAT CAT AGT GGG GTA TCT AAT CCY AGT TTG
118	FwMIFV1059 TGG ACG CTA GAT CGT YGG TAA AWC TCG TGC CAG C
119	FwMIFV1060 AGT CTT CAC CTC CGT YGG TAA AWC TCG TGC CAG C
120	RvMIFV1060 AGT CTT CAC CTC CAT AGT GGG GTA TCT AAT CCY AGT TTG
121	RvMIFV1061 AGT GCT TAT CCG TAT AGT GGG GTA TCT AAT CCY AGT TTG
122	FwMIFV1061 AGT GCT TAT CCG TGT YGG TAA AWC TCG TGC CAG C
123	RvMIFV1062 ATC GCT GCC TGG CAT AGT GGG GTA TCT AAT CCY AGT TTG
124	FwMIFV1062 ATC GCT GCC TGG CGT YGG TAA AWC TCG TGC CAG C
125	FwMIFV1063 ATG CTT GCG ACA GGT YGG TAA AWC TCG TGC CAG C

Table S3 Designed tagged primers as adapted from (Srivathsan et al. 2018) with a primer pair specific 13bp tag (black) attached to the MiFish Primer (Miya et al. 2015) as exemplary indicated in red in the first six primer pairs. Every two columns (FwMIFV, RvMIFV1001..1096) correspond to a unique primer pair. (*continued*)

List of used primers	
126	RvMIFV1063 ATG CTT GCG ACA GAT AGT GGG GTA TCT AAT CCY AGT TTG
127	FwMIFV1064 CAA TCC TCA AGA GGT YGG TAA AWC TCG TGC CAG C
128	RvMIFV1064 CAA TCC TCA AGA GAT AGT GGG GTA TCT AAT CCY AGT TTG
129	FwMIFV1065 CAT GCA CAC CTC TGT YGG TAA AWC TCG TGC CAG C
130	RvMIFV1065 CAT GCA CAC CTC TAT AGT GGG GTA TCT AAT CCY AGT TTG
131	FwMIFV1066 CGG AGA ATA TTA TGT YGG TAA AWC TCG TGC CAG C
132	RvMIFV1066 CGG AGA ATA TTA TAT AGT GGG GTA TCT AAT CCY AGT TTG
133	RvMIFV1067 GAG CGA AGG TAG TAT AGT GGG GTA TCT AAT CCY AGT TTG
134	FwMIFV1067 GAG CGA AGG TAG TGT YGG TAA AWC TCG TGC CAG C
135	RvMIFV1068 GGC GAC TGT TCC TAT AGT GGG GTA TCT AAT CCY AGT TTG
136	FwMIFV1068 GGC GAC TGT TCC TGT YGG TAA AWC TCG TGC CAG C
137	RvMIFV1069 GTC AGT TAA CGA CAT AGT GGG GTA TCT AAT CCY AGT TTG
138	FwMIFV1069 GTC AGT TAA CGA CGT YGG TAA AWC TCG TGC CAG C
139	RvMIFV1070 TCC ACA GAT ACA GAT AGT GGG GTA TCT AAT CCY AGT TTG
140	FwMIFV1070 TCC ACA GAT ACA GGT YGG TAA AWC TCG TGC CAG C
141	RvMIFV1071 TCG AGT TGT AAC TAT AGT GGG GTA TCT AAT CCY AGT TTG
142	FwMIFV1071 TCG AGT TGT AAC TGT YGG TAA AWC TCG TGC CAG C
143	FwMIFV1072 TCG GTC TTA GAC GGT YGG TAA AWC TCG TGC CAG C
144	RvMIFV1072 TCG GTC TTA GAC GAT AGT GGG GTA TCT AAT CCY AGT TTG
145	FwMIFV1073 TCT AAG GAG TTA TGT YGG TAA AWC TCG TGC CAG C
146	RvMIFV1073 TCT AAG GAG TTA TAT AGT GGG GTA TCT AAT CCY AGT TTG
147	FwMIFV1074 TCT ATC TCT CGC TGT YGG TAA AWC TCG TGC CAG C
148	RvMIFV1074 TCT ATC TCT CGC TAT AGT GGG GTA TCT AAT CCY AGT TTG
149	RvMIFV1075 TGT GAA GTT GCC AAT AGT GGG GTA TCT AAT CCY AGT TTG
150	FwMIFV1075 TGT GAA GTT GCC AGT YGG TAA AWC TCG TGC CAG C
151	RvMIFV1076 TTA CCA TTC CTC AAT AGT GGG GTA TCT AAT CCY AGT TTG
152	FwMIFV1076 TTA CCA TTC CTC AGT YGG TAA AWC TCG TGC CAG C
153	FwMIFV1077 AGA TTC TAC ACA AGT YGG TAA AWC TCG TGC CAG C
154	RvMIFV1077 AGA TTC TAC ACA AAT AGT GGG GTA TCT AAT CCY AGT TTG
155	RvMIFV1078 ATG CGA TTA ATT GAT AGT GGG GTA TCT AAT CCY AGT TTG
156	FwMIFV1078 ATG CGA TTA ATT GGT YGG TAA AWC TCG TGC CAG C

Table S3 Designed tagged primers as adapted from (Srivathsan et al. 2018) with a primer pair specific 13bp tag (black) attached to the MiFish Primer (Miya et al. 2015) as exemplary indicated in red in the first six primer pairs. Every two columns (FwMIFV, RvMIFV1001..1096) correspond to a unique primer pair. (*continued*)

List of used primers	
157	RvMIFV1079 CCG TAG TGT TGA TAT AGT GGG GTA TCT AAT CCY AGT TTG
158	FwMIFV1079 CCG TAG TGT TGA TGT YGG TAA AWC TCG TGC CAG C
159	FwMIFV1080 CCT GTA CCA TTG TGT YGG TAA AWC TCG TGC CAG C
160	RvMIFV1080 CCT GTA CCA TTG TAT AGT GGG GTA TCT AAT CCY AGT TTG
161	RvMIFV1081 GGC TGT TAC AAC AAT AGT GGG GTA TCT AAT CCY AGT TTG
162	FwMIFV1081 GGC TGT TAC AAC AGT YGG TAA AWC TCG TGC CAG C
163	RvMIFV1082 GGT TCA ATG ACG CAT AGT GGG GTA TCT AAT CCY AGT TTG
164	FwMIFV1082 GGT TCA ATG ACG CGT YGG TAA AWC TCG TGC CAG C
165	FwMIFV1083 GGT TCC TGC ACT TGT YGG TAA AWC TCG TGC CAG C
166	RvMIFV1083 GGT TCC TGC ACT TAT AGT GGG GTA TCT AAT CCY AGT TTG
167	FwMIFV1084 GTC CAA CTT CAG TGT YGG TAA AWC TCG TGC CAG C
168	RvMIFV1084 GTC CAA CTT CAG TAT AGT GGG GTA TCT AAT CCY AGT TTG
169	RvMIFV1085 GTC CAG GCC TTC TAT AGT GGG GTA TCT AAT CCY AGT TTG
170	FwMIFV1085 GTC CAG GCC TTC TGT YGG TAA AWC TCG TGC CAG C
171	FwMIFV1086 GTG ACG CCG TAT TGT YGG TAA AWC TCG TGC CAG C
172	RvMIFV1086 GTG ACG CCG TAT TAT AGT GGG GTA TCT AAT CCY AGT TTG
173	RvMIFV1087 TGC AGC AAC CAG CAT AGT GGG GTA TCT AAT CCY AGT TTG
174	FwMIFV1087 TGC AGC AAC CAG CGT YGG TAA AWC TCG TGC CAG C
175	RvMIFV1088 TGG ACA TGG CAT TAT AGT GGG GTA TCT AAT CCY AGT TTG
176	FwMIFV1088 TGG ACA TGG CAT TGT YGG TAA AWC TCG TGC CAG C
177	RvMIFV1089 TGG TGG TCA TTC AAT AGT GGG GTA TCT AAT CCY AGT TTG
178	FwMIFV1089 TGG TGG TCA TTC AGT YGG TAA AWC TCG TGC CAG C
179	RvMIFV1090 TTA CCA GAG CCT GAT AGT GGG GTA TCT AAT CCY AGT TTG
180	FwMIFV1090 TTA CCA GAG CCT GGT YGG TAA AWC TCG TGC CAG C
181	RvMIFV1091 TTC CGC GGC ATA GAT AGT GGG GTA TCT AAT CCY AGT TTG
182	FwMIFV1091 TTC CGC GGC ATA GGT YGG TAA AWC TCG TGC CAG C
183	FwMIFV1092 ACA ACC ATT GGC AGT YGG TAA AWC TCG TGC CAG C
184	RvMIFV1092 ACA ACC ATT GGC AAT AGT GGG GTA TCT AAT CCY AGT TTG
185	FwMIFV1093 AGC CTA GCC AAC TGT YGG TAA AWC TCG TGC CAG C
186	RvMIFV1093 AGC CTA GCC AAC TAT AGT GGG GTA TCT AAT CCY AGT TTG
187	FwMIFV1094 ATA ATC GAG GCT TGT YGG TAA AWC TCG TGC CAG C

Table S3 Designed tagged primers as adapted from (Srivathsan et al. 2018) with a primer pair specific 13bp tag (black) attached to the MiFish Primer (Miya et al. 2015) as exemplary indicated in red in the first six primer pairs. Every two columns (FwMIFV, RvMIFV1001..1096) correspond to a unique primer pair. (*continued*)

List of used primers	
188	RvMIFV1094 ATA ATC GAG GCT TAT AGT GGG GTA TCT AAT CCY AGT TTG
189	RvMIFV1095 CTG AGT CAG AAG TAT AGT GGG GTA TCT AAT CCY AGT TTG
190	FwMIFV1095 CTG AGT CAG AAG TGT YGG TAA AWC TCG TGC CAG C
191	RvMIFV1096 GAT AGG CGC CTT GAT AGT GGG GTA TCT AAT CCY AGT TTG
192	FwMIFV1096 GAT AGG CGC CTT GGT YGG TAA AWC TCG TGC CAG C

Table S4: Table depicting the category for the selected traits (Feeding strategy and Habitat preference) with their descriptions.

Trait category	Description
	Feeding strategy
Detritivores	Diet includes sediments and organic matter within sediments
Invertivores	Diet includes benthic invertebrates of different sizes (i.e. amphipods, crabs, polychaetes)
Piscivore	Diet includes fish
Herbivores	Diet includes algae, seagrasses
Planktivores	Diet includes plankton
Omnivores	Generalist diet
	Habitat preference
Soft sediments	Preference for soft sediments like sand, mud, etc
Mixed sediments	Preference for mixed sediments like gravel, shells interspersed with soft sediments
Hard substrates	Preferences for hard substrates like reefs, rocks, bedrocks, artificial reefs, boulders etc
Pelagic	Migrating species, species living predominantly in the water column
Seagrasses	Preference for seagrasses
Shells	Preference for shelly sediments

Table S5: Sequencing depth of the raw sequenced reads from each sequencing run (three different runs for Noss Head and 2 different runs for Voordelta). Additionally, the remaining sequencing depth after each processing step (Basecalling, Bioinformatics processing with DECONA, Taxonomic assignment and Nontarget removal) was also reported in this table.

Seq run	Raw reads	Basecalled reads	Decona reads	Taxonomic assignment (Species)	After non target removal and final taxonomy assignment (including unclassified)
Nosshead 1	24.230.000	15.196.000	14.508.164	13.607.465	19.191.294
Nosshead 2	12.810.000	12.368.000	9.783.369	9.128.254	
Voordelta/ Nosshead	16.340.000	16.212.000	8.945.310	639.480 + 6.152.865	16.140.460
Voordelta 1	17.760.000	15.959.000	8.179.220	6.452.591	
Voordelta 2	14.830.000	13.236.000	7.599.669	6.176.241	

Table S6: Mean calculated ecological indices per habitat calculated at both locations. Values are expressed as mean values and standard error (SEM)

eDNA									
Area	**Biotope**	Richness		Shannon (H)		Simpson's (1 – D)		Evenness (J)	
		Mean	**± SE**	**Mean**	**± SE**	**Mean**	**± SE**	**Mean**	**± SE**
Voordelta	In reef	18.89	0.48	2.24	0.03	0.85	0.01	0.77	0.01
	25–200 m from reef edge	16.71	0.52	2.10	0.04	0.82	0.01	0.76	0.01
	250–2000 m from reef edge	12.41	0.47	1.92	0.05	0.79	0.01	0.78	0.01
	1–10 km from reef edge	13.05	0.55	1.86	0.05	0.77	0.02	0.74	0.02
Noss Head	High density reef	17.95	1.09	2.32	0.05	0.86	0.01	0.82	0.01
	Low density reef	14.82	0.46	2.09	0.04	0.81	0.01	0.79	0.01
	400–1500 m from reef edge	11.68	0.57	1.77	0.06	0.74	0.02	0.75	0.02
	5km from reef edge	6.50	0.56	1.49	0.11	0.72	0.03	0.80	0.04
BRUVS									
Area	**Biotope**	Richness		Shannon (H)		Simpson's (1 – D)		Evenness (J)	
		Mean	**± SE**	**Mean**	**± SE**	**Mean**	**± SE**	**Mean**	**± SE**

Table S6: Mean calculated ecological indices per habitat calculated at both locations. Values are expressed as mean values and standard error (SEM) (*continued*)

BRUVS									
Voordelta	In reef	2.69	0.12	0.74	0.05	0.44	0.03	0.75	0.03
	25–200 m from reef edge	2.44	0.22	0.59	0.09	0.35	0.05	0.62	0.07
	250–2000 m from reef edge	1.71	0.14	0.36	0.06	0.26	0.04	0.45	0.07
Noss Head	High density reef	2.40	0.68	0.81	0.22	0.69	0.08	0.77	0.19
	Low density reef	3.00	0.41	0.87	0.15	0.50	0.07	0.80	0.05
	400–1500 m from reef edge	2.20	0.20	0.76	0.08	0.52	0.04	0.98	0.02

Table S7: Results of the generalized linear latent variable model (GLLVM) applied to eDNA data from the Voordelta, showing significant and non-significant differences in species occurrence frequencies among habitats. Station names along the distance gradient were shortened in the analysis for practicality (background = 1–10 km from reef, off reef = 250–1000 m from reef, close to reef = 25–200 m from reef, in reef = In reef).

Contrast	Species	Estimate	SE	z.ratio	p.value
1_background - 4_reef	Ammodytidae	-0.711	0.347	-2.050	0.170
1_background - 3_close to reef	Ammodytidae	-0.555	0.305	-1.818	0.265
2_off reef - 4_reef	Ammodytidae	-0.360	0.230	-1.567	0.398
1_background - 2_off reef	Ammodytidae	-0.351	0.338	-1.038	0.727
2_off reef - 3_close to reef	Ammodytidae	-0.204	0.240	-0.850	0.830
3_close to reef - 4_reef	Ammodytidae	-0.156	0.253	-0.618	0.926
2_off reef - 3_close to reef	*Anguilla anguilla**	-2.408	0.805	-2.989	0.015
2_off reef - 4_reef	*Anguilla anguilla**	-2.258	0.776	-2.910	0.019
1_background - 3_close to reef	*Anguilla anguilla**	-1.083	0.647	-1.675	0.337
1_background - 2_off reef	*Anguilla anguilla**	1.324	0.960	1.379	0.512
1_background - 4_reef	*Anguilla anguilla**	-0.934	0.713	-1.309	0.557
3_close to reef - 4_reef	*Anguilla anguilla**	0.150	0.488	0.308	0.990
2_off reef - 4_reef	*Atherina* sp.	-0.818	0.246	-3.325	0.005
2_off reef - 3_close to reef	*Atherina* sp.	-0.823	0.252	-3.262	0.006

Table S7: Results of the generalized linear latent variable model (GLLVM) applied to eDNA data from the Voordelta, showing significant and non-significant differences in species occurrence frequencies among habitats. Station names along the distance gradient were shortened in the analysis for practicality (background = 1–10 km from reef, off reef = 250–1000 m from reef, close to reef = 25–200 m from reef, in reef = In reef). (*continued*)

Contrast	Species	Estimate	SE	z.ratio	p.value
1_background - 3_close to reef	*Atherina* sp.	-0.502	0.275	-1.829	0.260
1_background - 4_reef	*Atherina* sp.	-0.497	0.314	-1.584	0.388
1_background - 2_off reef	*Atherina* sp.	0.321	0.326	0.984	0.758
3_close to reef - 4_reef	*Atherina* sp.	0.005	0.239	0.021	1.000
1_background - 3_close to reef	*Chelon auratus*	-1.455	0.545	-2.672	0.038
1_background - 4_reef	*Chelon auratus*	-1.384	0.588	-2.353	0.086
2_off reef - 3_close to reef	*Chelon auratus*	-0.668	0.349	-1.916	0.221
2_off reef - 4_reef	*Chelon auratus*	-0.597	0.315	-1.896	0.230
1_background - 2_off reef	*Chelon auratus*	-0.787	0.592	-1.330	0.544
3_close to reef - 4_reef	*Chelon auratus*	0.071	0.345	0.207	0.997
2_off reef - 4_reef	*Chelon* sp.	-0.714	0.387	-1.847	0.251
2_off reef - 3_close to reef	*Chelon* sp.	-0.549	0.403	-1.361	0.524
1_background - 4_reef	*Chelon* sp.	-0.621	0.530	-1.172	0.645
1_background - 3_close to reef	*Chelon* sp.	-0.456	0.465	-0.981	0.761
3_close to reef - 4_reef	*Chelon* sp.	-0.165	0.395	-0.418	0.975
1_background - 2_off reef	*Chelon* sp.	0.093	0.536	0.174	0.998
2_off reef - 4_reef	*Ciliata mustela*	-0.764	0.278	-2.750	0.030
2_off reef - 3_close to reef	*Ciliata mustela*	-0.723	0.308	-2.346	0.088
1_background - 4_reef	*Ciliata mustela*	-0.633	0.377	-1.678	0.336
1_background - 3_close to reef	*Ciliata mustela*	-0.592	0.358	-1.653	0.349
1_background - 2_off reef	*Ciliata mustela*	0.131	0.389	0.337	0.987
3_close to reef - 4_reef	*Ciliata mustela*	-0.041	0.294	-0.139	0.999

Table S7: Results of the generalized linear latent variable model (GLLVM) applied to eDNA data from the Voordelta, showing significant and non-significant differences in species occurrence frequencies among habitats. Station names along the distance gradient were shortened in the analysis for practicality (background = 1–10 km from reef, off reef = 250–1000 m from reef, close to reef = 25–200 m from reef, in reef = In reef). (*continued*)

Contrast	Species	Estimate	SE	z.ratio	p.value
1_background - 2_off reef	*Clupea harengus**	0.077	0.241	0.318	0.989
1_background - 3_close to reef	*Clupea harengus**	0.058	0.228	0.253	0.994
1_background - 4_reef	*Clupea harengus**	0.045	0.262	0.171	0.998
2_off reef - 4_reef	*Clupea harengus**	-0.032	0.207	-0.153	0.999
2_off reef - 3_close to reef	*Clupea harengus**	-0.019	0.205	-0.092	1.000
3_close to reef - 4_reef	*Clupea harengus**	-0.013	0.230	-0.056	1.000
2_off reef - 4_reef	*Engraulis encrasicolus*	-1.415	0.871	-1.626	0.364
1_background - 4_reef	*Engraulis encrasicolus*	-1.536	1.238	-1.241	0.601
3_close to reef - 4_reef	*Engraulis encrasicolus*	-0.750	0.749	-1.002	0.748
1_background - 3_close to reef	*Engraulis encrasicolus*	-0.786	1.040	-0.756	0.874
2_off reef - 3_close to reef	*Engraulis encrasicolus*	-0.665	0.882	-0.754	0.875
1_background - 2_off reef	*Engraulis encrasicolus*	-0.120	1.273	-0.095	1.000
1_background - 2_off reef	Gadidae	0.507	0.269	1.886	0.234
2_off reef - 4_reef	Gadidae	-0.361	0.240	-1.501	0.437
2_off reef - 3_close to reef	Gadidae	-0.265	0.240	-1.108	0.684
1_background - 3_close to reef	Gadidae	0.241	0.244	0.986	0.758
1_background - 4_reef	Gadidae	0.146	0.280	0.519	0.954
3_close to reef - 4_reef	Gadidae	-0.096	0.253	-0.377	0.982
3_close to reef - 4_reef	*Gadus morhua**	-0.624	0.764	-0.817	0.846
2_off reef - 4_reef	*Gadus morhua**	-0.461	0.607	-0.759	0.873
1_background - 4_reef	*Gadus morhua**	-0.350	0.808	-0.434	0.973
1_background - 3_close to reef	*Gadus morhua**	0.274	0.826	0.332	0.987
2_off reef - 3_close to reef	*Gadus morhua**	0.164	0.746	0.219	0.996

Table S7: Results of the generalized linear latent variable model (GLLVM) applied to eDNA data from the Voordelta, showing significant and non-significant differences in species occurrence frequencies among habitats. Station names along the distance gradient were shortened in the analysis for practicality (background = 1–10 km from reef, off reef = 250–1000 m from reef, close to reef = 25–200 m from reef, in reef = In reef). (*continued*)

Contrast	Species	Estimate	SE	z.ratio	p.value
1_background - 2_off reef	*Gadus morhua**	0.110	0.793	0.139	0.999
2_off reef - 4_reef	*Gobius niger*	-1.346	0.309	-4.352	0.000
1_background - 4_reef	*Gobius niger*	-2.390	0.739	-3.233	0.007
1_background - 3_close to reef	*Gobius niger*	-2.472	0.847	-2.917	0.019
2_off reef - 3_close to reef	*Gobius niger*	-1.427	0.494	-2.890	0.020
1_background - 2_off reef	*Gobius niger*	-1.044	0.748	-1.396	0.502
3_close to reef - 4_reef	*Gobius niger*	0.081	0.412	0.197	0.997
1_background - 2_off reef	*Halichoerus grypus*	2.878	1.089	2.643	0.041
2_off reef - 3_close to reef	*Halichoerus grypus*	-2.630	1.056	-2.490	0.061
2_off reef - 4_reef	*Halichoerus grypus*	-2.435	1.081	-2.253	0.109
1_background - 4_reef	*Halichoerus grypus*	0.443	0.595	0.744	0.879
1_background - 3_close to reef	*Halichoerus grypus*	0.247	0.471	0.525	0.953
3_close to reef - 4_reef	*Halichoerus grypus*	0.196	0.532	0.368	0.983
1_background - 2_off reef	*Limanda limanda**	0.418	0.264	1.579	0.390
1_background - 3_close to reef	*Limanda limanda**	0.387	0.257	1.508	0.433
1_background - 4_reef	*Limanda limanda**	0.288	0.288	1.000	0.749
2_off reef - 4_reef	*Limanda limanda**	-0.130	0.246	-0.527	0.953
3_close to reef - 4_reef	*Limanda limanda**	-0.099	0.274	-0.362	0.984
2_off reef - 3_close to reef	*Limanda limanda**	-0.031	0.247	-0.124	0.999
2_off reef - 3_close to reef	*Lipophrys pholis*	-2.121	0.863	-2.456	0.067
2_off reef - 4_reef	*Lipophrys pholis*	-1.353	0.868	-1.559	0.402
1_background - 3_close to reef	*Lipophrys pholis*	-1.089	0.795	-1.370	0.518

Table S7: Results of the generalized linear latent variable model (GLLVM) applied to eDNA data from the Voordelta, showing significant and non-significant differences in species occurrence frequencies among habitats. Station names along the distance gradient were shortened in the analysis for practicality (background = 1–10 km from reef, off reef = 250–1000 m from reef, close to reef = 25–200 m from reef, in reef = In reef). (*continued*)

Contrast	Species	Estimate	SE	z.ratio	p.value
3_close to reef - 4_reef	*Lipophrys pholis*	0.768	0.685	1.121	0.677
1_background - 2_off reef	*Lipophrys pholis*	1.032	1.035	0.997	0.751
1_background - 4_reef	*Lipophrys pholis*	-0.321	0.895	-0.359	0.984
1_background - 2_off reef	Mugilidae	-1.330	1.241	-1.072	0.707
2_off reef - 3_close to reef	Mugilidae	0.831	0.859	0.967	0.768
2_off reef - 4_reef	Mugilidae	0.612	0.803	0.763	0.871
1_background - 4_reef	Mugilidae	-0.718	1.377	-0.521	0.954
1_background - 3_close to reef	Mugilidae	-0.499	1.266	-0.394	0.979
3_close to reef - 4_reef	Mugilidae	-0.219	1.046	-0.209	0.997
1_background - 4_reef	*Mullus surmuletus*	-3.095	1.445	-2.142	0.140
3_close to reef - 4_reef	*Mullus surmuletus*	-1.499	0.758	-1.978	0.196
1_background - 2_off reef	*Mullus surmuletus*	-2.508	1.354	-1.853	0.249
2_off reef - 3_close to reef	*Mullus surmuletus*	0.912	0.711	1.283	0.574
1_background - 3_close to reef	*Mullus surmuletus*	-1.596	1.296	-1.232	0.607
2_off reef - 4_reef	*Mullus surmuletus*	-0.586	0.566	-1.036	0.728
1_background - 4_reef	*Myoxocephalus* sp.	-1.480	0.461	-3.210	0.007
1_background - 3_close to reef	*Myoxocephalus* sp.	-1.269	0.449	-2.827	0.024
2_off reef - 4_reef	*Myoxocephalus* sp.	-0.680	0.241	-2.826	0.024
1_background - 2_off reef	*Myoxocephalus* sp.	-0.800	0.468	-1.711	0.318
2_off reef - 3_close to reef	*Myoxocephalus* sp.	-0.469	0.292	-1.603	0.377
3_close to reef - 4_reef	*Myoxocephalus* sp.	-0.211	0.283	-0.748	0.878
1_background - 2_off reef	*Osmerus eperlanus*	0.589	0.679	0.868	0.822
2_off reef - 4_reef	*Osmerus eperlanus*	-0.460	0.607	-0.758	0.873

Table S7: Results of the generalized linear latent variable model (GLLVM) applied to eDNA data from the Voordelta, showing significant and non-significant differences in species occurrence frequencies among habitats. Station names along the distance gradient were shortened in the analysis for practicality (background = 1–10 km from reef, off reef = 250–1000 m from reef, close to reef = 25–200 m from reef, in reef = In reef). (*continued*)

Contrast	Species	Estimate	SE	z.ratio	p.value
2_off reef - 3_close to reef	*Osmerus eperlanus*	-0.327	0.635	-0.515	0.956
1_background - 3_close to reef	*Osmerus eperlanus*	0.263	0.643	0.408	0.977
3_close to reef - 4_reef	*Osmerus eperlanus*	-0.133	0.658	-0.202	0.997
1_background - 4_reef	*Osmerus eperlanus*	0.130	0.699	0.185	0.998
1_background - 2_off reef	*Phoca vitulina**	2.315	0.578	4.007	0.000
2_off reef - 4_reef	*Phoca vitulina**	-1.556	0.593	-2.626	0.043
2_off reef - 3_close to reef	*Phoca vitulina**	-1.432	0.592	-2.419	0.073
1_background - 3_close to reef	*Phoca vitulina**	0.883	0.383	2.308	0.096
1_background - 4_reef	*Phoca vitulina**	0.759	0.427	1.779	0.283
3_close to reef - 4_reef	*Phoca vitulina**	-0.124	0.447	-0.277	0.993
1_background - 3_close to reef	*Pholis gunnellus**	-0.714	0.297	-2.403	0.076
1_background - 4_reef	*Pholis gunnellus**	-0.677	0.329	-2.059	0.167
1_background - 2_off reef	*Pholis gunnellus**	-0.566	0.316	-1.793	0.277
2_off reef - 3_close to reef	*Pholis gunnellus**	-0.148	0.220	-0.673	0.907
2_off reef - 4_reef	*Pholis gunnellus**	-0.111	0.210	-0.527	0.953
3_close to reef - 4_reef	*Pholis gunnellus**	0.038	0.241	0.156	0.999
2_off reef - 4_reef	Pleuronectidae	-0.056	0.217	-0.258	0.994
2_off reef - 3_close to reef	Pleuronectidae	-0.053	0.212	-0.249	0.995
1_background - 3_close to reef	Pleuronectidae	-0.037	0.241	-0.153	0.999
1_background - 4_reef	Pleuronectidae	-0.040	0.280	-0.142	0.999
1_background - 2_off reef	Pleuronectidae	0.016	0.258	0.062	1.000
3_close to reef - 4_reef	Pleuronectidae	-0.003	0.239	-0.013	1.000

Table S7: Results of the generalized linear latent variable model (GLLVM) applied to eDNA data from the Voordelta, showing significant and non-significant differences in species occurrence frequencies among habitats. Station names along the distance gradient were shortened in the analysis for practicality (background = 1–10 km from reef, off reef = 250–1000 m from reef, close to reef = 25–200 m from reef, in reef = In reef). (*continued*)

Contrast	Species	Estimate	SE	z.ratio	p.value
1_background - 3_close to reef	*Pomatoschistus microps*	-0.712	0.324	-2.198	0.124
1_background - 4_reef	*Pomatoschistus microps*	-0.642	0.341	-1.880	0.237
2_off reef - 3_close to reef	*Pomatoschistus microps*	-0.318	0.247	-1.285	0.572
1_background - 2_off reef	*Pomatoschistus microps*	-0.394	0.333	-1.185	0.636
2_off reef - 4_reef	*Pomatoschistus microps*	-0.247	0.225	-1.101	0.689
3_close to reef - 4_reef	*Pomatoschistus microps*	0.071	0.260	0.272	0.993
1_background - 3_close to reef	*Pomatoschistus minutus*	0.052	0.229	0.226	0.996
1_background - 2_off reef	*Pomatoschistus minutus*	0.050	0.241	0.208	0.997
1_background - 4_reef	*Pomatoschistus minutus*	0.033	0.263	0.126	0.999
2_off reef - 4_reef	*Pomatoschistus minutus*	-0.017	0.207	-0.083	1.000
3_close to reef - 4_reef	*Pomatoschistus minutus*	-0.019	0.230	-0.081	1.000
2_off reef - 3_close to reef	*Pomatoschistus minutus*	0.002	0.204	0.007	1.000
2_off reef - 3_close to reef	*Salmo trutta**	1.668	1.077	1.548	0.409
1_background - 3_close to reef	*Salmo trutta**	1.404	1.155	1.215	0.617
3_close to reef - 4_reef	*Salmo trutta**	-1.114	1.176	-0.947	0.779
2_off reef - 4_reef	*Salmo trutta**	0.554	0.660	0.840	0.836
1_background - 2_off reef	*Salmo trutta**	-0.264	0.711	-0.372	0.982
1_background - 4_reef	*Salmo trutta**	0.289	0.853	0.339	0.987
2_off reef - 4_reef	*Sardina pilchardus*	-0.453	0.235	-1.932	0.214

Table S7: Results of the generalized linear latent variable model (GLLVM) applied to eDNA data from the Voordelta, showing significant and non-significant differences in species occurrence frequencies among habitats. Station names along the distance gradient were shortened in the analysis for practicality (background = 1–10 km from reef, off reef = 250–1000 m from reef, close to reef = 25–200 m from reef, in reef = In reef). (*continued*)

Contrast	Species	Estimate	SE	z.ratio	p.value
2_off reef - 3_close to reef	*Sardina pilchardus*	-0.409	0.236	-1.733	0.306
1_background - 2_off reef	*Sardina pilchardus*	0.357	0.281	1.267	0.584
1_background - 4_reef	*Sardina pilchardus*	-0.097	0.287	-0.337	0.987
1_background - 3_close to reef	*Sardina pilchardus*	-0.052	0.250	-0.209	0.997
3_close to reef - 4_reef	*Sardina pilchardus*	-0.044	0.244	-0.182	0.998
2_off reef - 3_close to reef	*Scomber scombrus*	-0.928	0.437	-2.123	0.146
1_background - 3_close to reef	*Scomber scombrus*	-1.101	0.565	-1.949	0.208
2_off reef - 4_reef	*Scomber scombrus*	-0.731	0.419	-1.744	0.301
1_background - 4_reef	*Scomber scombrus*	-0.904	0.633	-1.427	0.482
3_close to reef - 4_reef	*Scomber scombrus*	0.197	0.422	0.467	0.966
1_background - 2_off reef	*Scomber scombrus*	-0.173	0.645	-0.268	0.993
1_background - 2_off reef	*Solea solea*	1.778	0.474	3.752	0.001
2_off reef - 4_reef	*Solea solea*	-1.263	0.470	-2.686	0.036
2_off reef - 3_close to reef	*Solea solea*	-1.089	0.489	-2.226	0.116
1_background - 3_close to reef	*Solea solea*	0.689	0.376	1.834	0.257
1_background - 4_reef	*Solea solea*	0.515	0.394	1.308	0.558
3_close to reef - 4_reef	*Solea solea*	-0.175	0.413	-0.423	0.975
2_off reef - 4_reef	*Sprattus sprattus**	-0.146	0.214	-0.683	0.903
1_background - 2_off reef	*Sprattus sprattus**	0.109	0.255	0.426	0.974
3_close to reef - 4_reef	*Sprattus sprattus**	-0.079	0.238	-0.334	0.987
2_off reef - 3_close to reef	*Sprattus sprattus**	-0.067	0.216	-0.310	0.990
1_background - 3_close to reef	*Sprattus sprattus**	0.042	0.240	0.174	0.998

Table S7: Results of the generalized linear latent variable model (GLLVM) applied to eDNA data from the Voordelta, showing significant and non-significant differences in species occurrence frequencies among habitats. Station names along the distance gradient were shortened in the analysis for practicality (background = 1–10 km from reef, off reef = 250–1000 m from reef, close to reef = 25–200 m from reef, in reef = In reef). (*continued*)

Contrast	Species	Estimate	SE	z.ratio	p.value
1_background - 4_reef	*Sprattus sprattus**	-0.038	0.273	-0.138	0.999
2_off reef - 3_close to reef	*Syngnathus acus*	-1.207	0.904	-1.335	0.540
1_background - 3_close to reef	*Syngnathus acus*	-1.607	1.265	-1.270	0.582
1_background - 4_reef	*Syngnathus acus*	-1.045	1.581	-0.661	0.912
2_off reef - 4_reef	*Syngnathus acus*	-0.645	1.001	-0.644	0.918
3_close to reef - 4_reef	*Syngnathus acus*	0.562	0.913	0.615	0.927
1_background - 2_off reef	*Syngnathus acus*	-0.400	1.566	-0.256	0.994
1_background - 3_close to reef	*Syngnathus rostellatus*	-0.773	0.429	-1.799	0.274
1_background - 2_off reef	*Syngnathus rostellatus*	-0.641	0.483	-1.327	0.545
1_background - 4_reef	*Syngnathus rostellatus*	-0.666	0.519	-1.283	0.574
2_off reef - 3_close to reef	*Syngnathus rostellatus*	-0.132	0.311	-0.423	0.974
3_close to reef - 4_reef	*Syngnathus rostellatus*	0.106	0.361	0.294	0.991
2_off reef - 4_reef	*Syngnathus rostellatus*	-0.025	0.333	-0.076	1.000
2_off reef - 3_close to reef	*Trachurus trachurus*	-2.466	0.775	-3.180	0.008
2_off reef - 4_reef	*Trachurus trachurus*	-2.346	0.770	-3.048	0.012
1_background - 2_off reef	*Trachurus trachurus*	1.586	0.907	1.748	0.299
1_background - 3_close to reef	*Trachurus trachurus*	-0.880	0.543	-1.622	0.366
1_background - 4_reef	*Trachurus trachurus*	-0.760	0.641	-1.186	0.636
3_close to reef - 4_reef	*Trachurus trachurus*	0.120	0.436	0.276	0.993
1_background - 2_off reef	Triglidae	-1.666	1.318	-1.264	0.586
2_off reef - 3_close to reef	Triglidae	0.965	0.856	1.127	0.673

Table S7: Results of the generalized linear latent variable model (GLLVM) applied to eDNA data from the Voordelta, showing significant and non-significant differences in species occurrence frequencies among habitats. Station names along the distance gradient were shortened in the analysis for practicality (background = 1–10 km from reef, off reef = 250–1000 m from reef, close to reef = 25–200 m from reef, in reef = In reef). (*continued*)

Contrast	Species	Estimate	SE	z.ratio	p.value
1_background - 4_reef	Triglidae	-1.482	1.503	-0.986	0.757
3_close to reef - 4_reef	Triglidae	-0.781	1.038	-0.752	0.876
1_background - 3_close to reef	Triglidae	-0.701	1.294	-0.542	0.949
2_off reef - 4_reef	Triglidae	0.184	0.843	0.219	0.996
1_background - 4_reef	*Trisopterus luscus*	0.904	0.745	1.213	0.618
1_background - 2_off reef	*Trisopterus luscus*	0.702	0.610	1.151	0.658
3_close to reef - 4_reef	*Trisopterus luscus*	0.614	0.727	0.844	0.834
2_off reef - 3_close to reef	*Trisopterus luscus*	-0.412	0.586	-0.703	0.896
1_background - 3_close to reef	*Trisopterus luscus*	0.291	0.581	0.500	0.959
2_off reef - 4_reef	*Trisopterus luscus*	0.202	0.691	0.292	0.991
2_off reef - 4_reef	*Zoarces viviparus**	-2.895	1.125	-2.573	0.049
2_off reef - 3_close to reef	*Zoarces viviparus**	-4.136	1.614	-2.562	0.051
1_background - 3_close to reef	*Zoarces viviparus**	-2.477	1.589	-1.559	0.402
1_background - 2_off reef	*Zoarces viviparus**	1.659	1.237	1.341	0.537
1_background - 4_reef	*Zoarces viviparus**	-1.236	0.948	-1.303	0.561
3_close to reef - 4_reef	*Zoarces viviparus**	1.241	1.068	1.162	0.651

Table S8: Community weighted means for each trait category, comparing the proportions of different feeding strategies and habitat preferences of species among different habitat, at Voordelta and Noss Head. Community weighted means were calculated per sample and averaged to summarize results. Data are expressed as mean and standard error (SEM).

		Feeding strategy											
Area	**Biotope**	Detritivores		Invertivores		Piscivore		Herbivores		Planktivores		Omnivores	
		Mean	**± SE**	**Mean**	**± SE**	**Mean**	**± SE**	**Mean**	**± SE**	**Mean**	**± SE**	**Mean**	**± SE**
Voordelta	In reef	0.004062	0.000575	0.477981	0.016633	0.044655	0.003920	0.009392	0.001054	0.464254	0.017734	0.000434	0.000281
	25–200 m from reef edge	0.010290	0.004097	0.437450	0.018340	0.044983	0.005049	0.013532	0.003998	0.493597	0.019899	0.000148	0.000112
	250–2000 m from reef edge	0.010033	0.004270	0.478894	0.013559	0.054608	0.005785	0.014363	0.004292	0.444817	0.014449	0.002372	0.001001
	1–10 km from reef edge	0.000918	0.000361	0.443443	0.023358	0.186066	0.018055	0.002599	0.000684	0.366560	0.029221	0.000414	0.000414
Noss Head	High density reef	0.000000	0.000000	0.363988	0.014906	0.232003	0.011311	0.014981	0.003407	0.388821	0.016777	0.000208	0.000199
	Low density reef	0.000005	0.000005	0.342632	0.013506	0.298263	0.011642	0.007428	0.001450	0.351537	0.018302	0.000489	0.000278
	400–1500 m from reef edge	0.000000	0.000000	0.238109	0.016134	0.205912	0.011622	0.005985	0.001613	0.550214	0.022640	0.000000	0.000000
	5km back-ground	0.000000	0.000000	0.271686	0.100629	0.272941	0.041611	0.010809	0.009465	0.444564	0.085040	0.000000	0.000000

Table S8: Community weighted means for each trait category, comparing the proportions of different feeding strategies and habitat preferences of species among different habitat, at Voordelta and Noss Head. Community weighted means were calculated per sample and averaged to summarize results. Data are expressed as mean and standard error (SEM).

		Habitat preference											
Area	**Biotope**	Soft sediments		Mixed sediments		Hard substrates		Pelagic		Seagrasses		Shells	
		Mean	**± SE**	**Mean**	**± SE**	**Mean**	**± SE**	**Mean**	**± SE**	**Mean**	**± SE**	**Mean**	**± SE**
Voordelta	In reef	0.203934	0.007611	0.029824	0.002398	0.180550	0.008504	0.559490	0.013783	0.012719	0.002316	0.012242	0.001244
	25–200 m from reef edge	0.260375	0.010367	0.038141	0.004049	0.175142	0.010521	0.500801	0.018399	0.013608	0.003616	0.011175	0.001293
	250–2000 m from reef edge	0.288441	0.010489	0.057454	0.005697	0.149515	0.007668	0.484475	0.014419	0.010589	0.001326	0.008953	0.001152
	1–10 km from reef edge	0.324359	0.018759	0.074663	0.010911	0.182410	0.010760	0.359840	0.032268	0.029022	0.003461	0.029388	0.003632
Noss Head	High density reef	0.251067	0.016912	0.054837	0.003878	0.199658	0.014671	0.377829	0.024126	0.039819	0.002877	0.074518	0.005363
	Low density reef	0.278586	0.011808	0.079013	0.004535	0.186304	0.009572	0.299936	0.017370	0.060018	0.003311	0.095664	0.005501
	400–1500 m from reef edge	0.187896	0.013215	0.049935	0.005617	0.147631	0.018950	0.518936	0.024147	0.037310	0.003193	0.058256	0.005808
	5km from reef edge	0.178374	0.052072	0.081591	0.027218	0.171500	0.071582	0.453416	0.102222	0.041086	0.014972	0.074033	0.021279

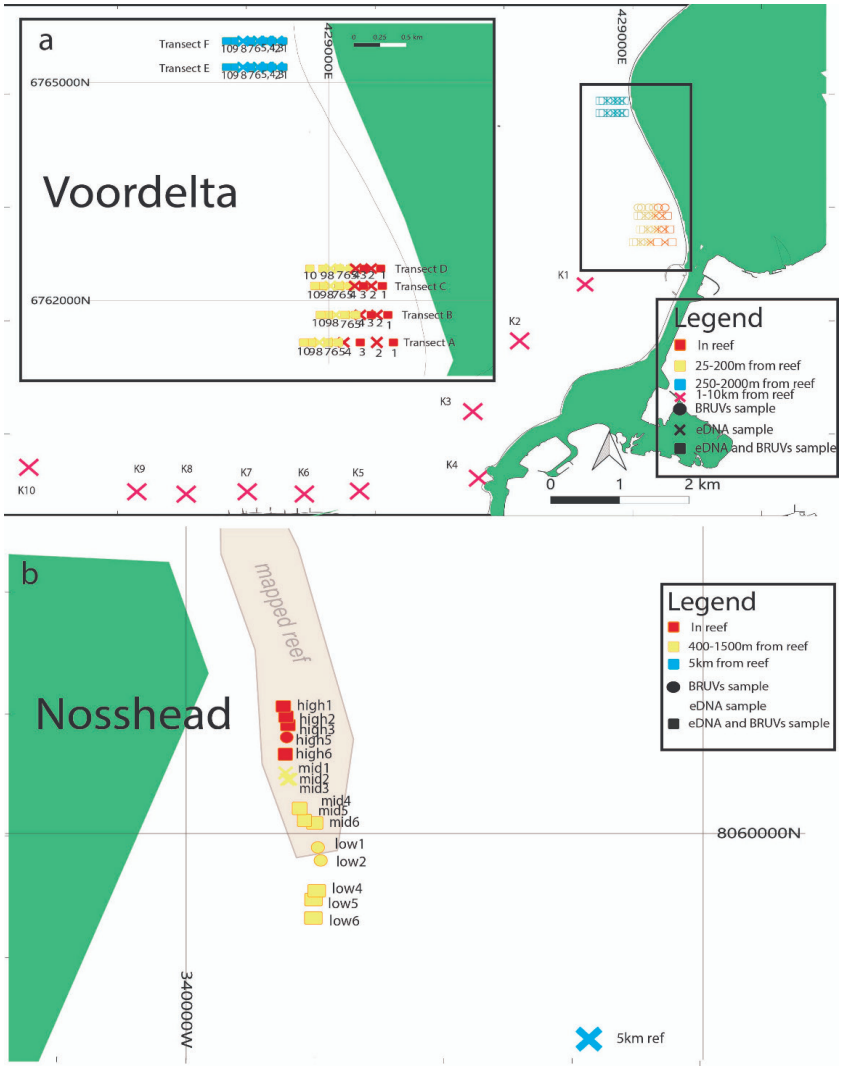


Figure S1. Details including station names of all sampling stations at a) the Voordelta and b) the Noss Head. Colours indicate the different biotopes; open circles represent stations sampled with the BRUVS method, crosses indicate those sampled with the eDNA method, and open squares show stations sampled with both techniques. The brown shape at Noss Head marks the area protected under the Noss Head MPA. The map is presented in the WGS84 Geographic Coordinate System.

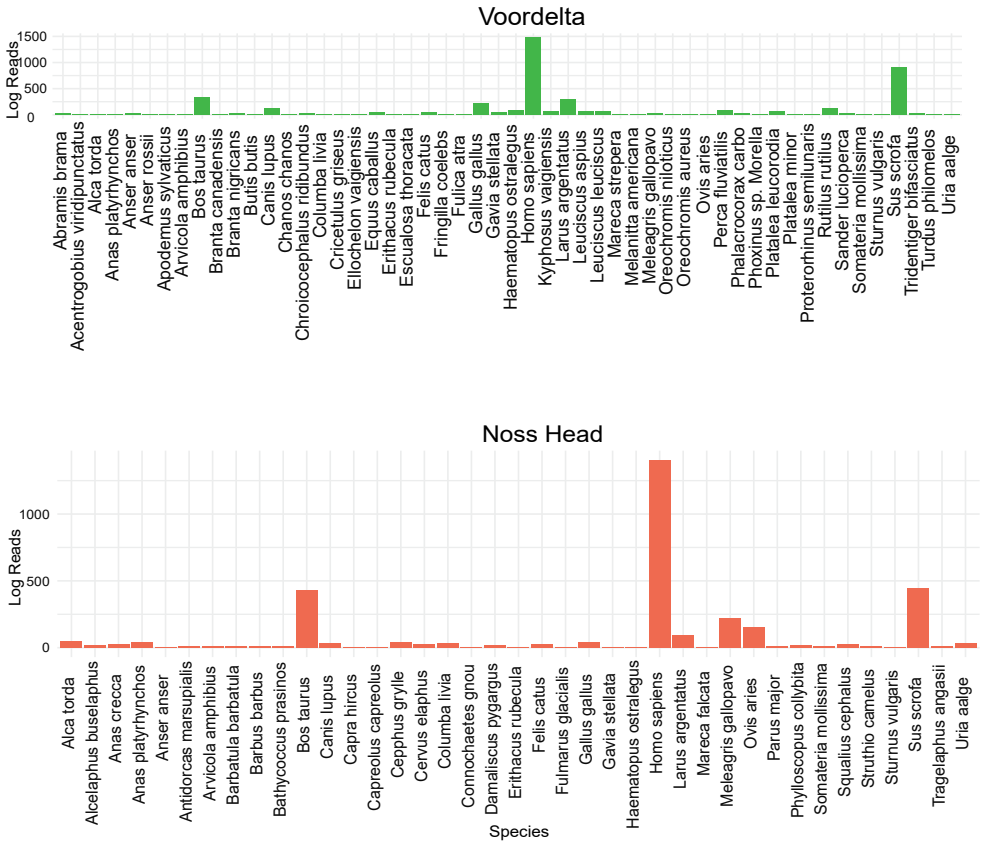


Figure S2: Bar plot of the species and log read count that were renamed to 'non-target'. This was chosen whenever a species was not a marine fish elasmobranch or mammal and resulted in the removal of terrestrial animals, freshwater fish species and birds.

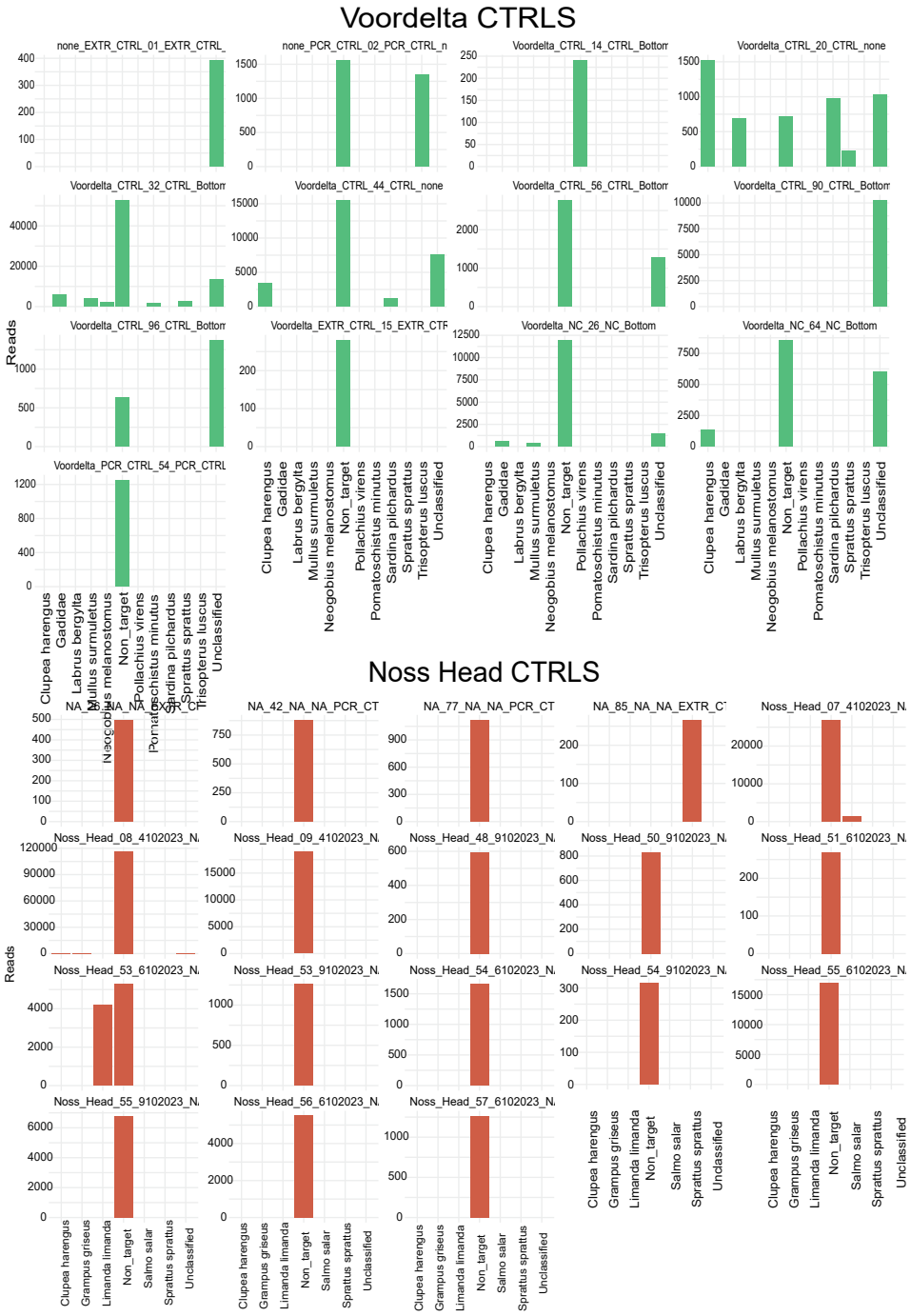


Figure S3 Control samples of from the Voordelta and Noss Head sampling and sample processing. Where PCR CTRL refers to the pooled negative controls taken after every round of PCR (96 samples). Extraction CTRL refers to the negative controls taken during extraction. Field CTRL refers to the controls taken before

(1, 2,3) and after a day of sample filtering (4,5,6). For Noss head CTRLS, days referred to the respective day that sampling and filtering took place. For Voordelta all sampling and filtering occurred on the same day and was not further indicated by date.

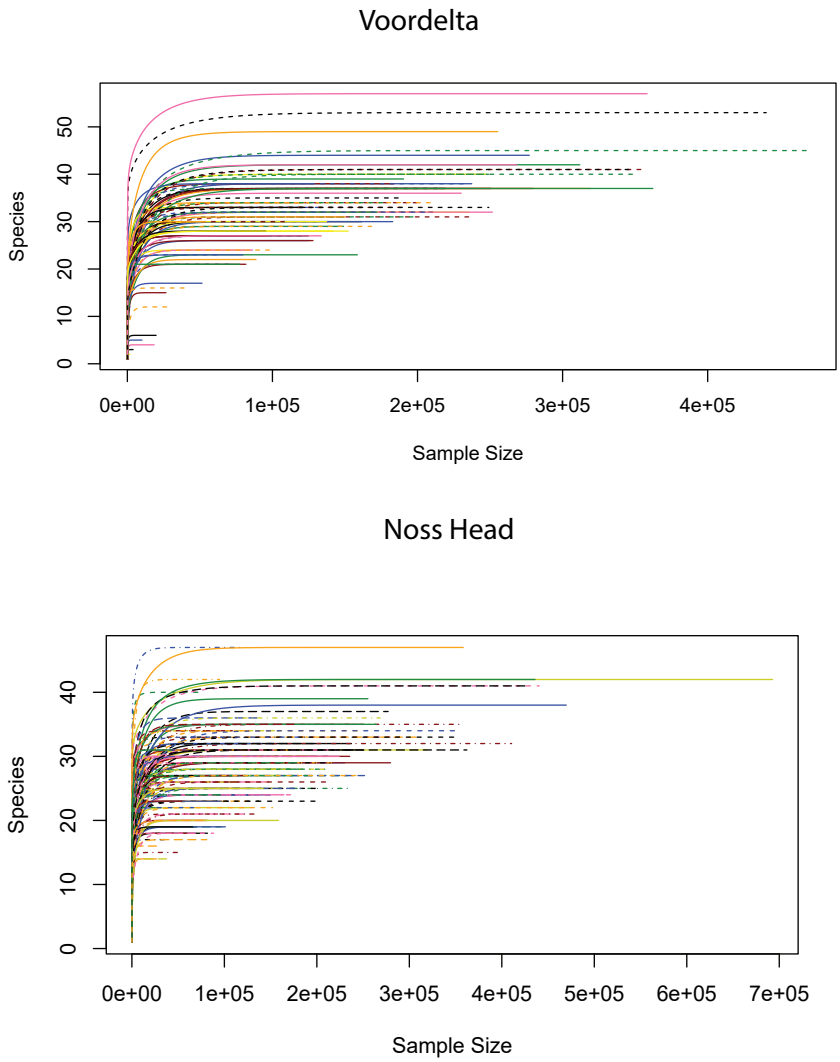


Figure S4: Species accumulation curve for every sample and sampling location seperetly. All samples show a flattening curve, indicating that the for that sample, sequencing depth was sufficient.

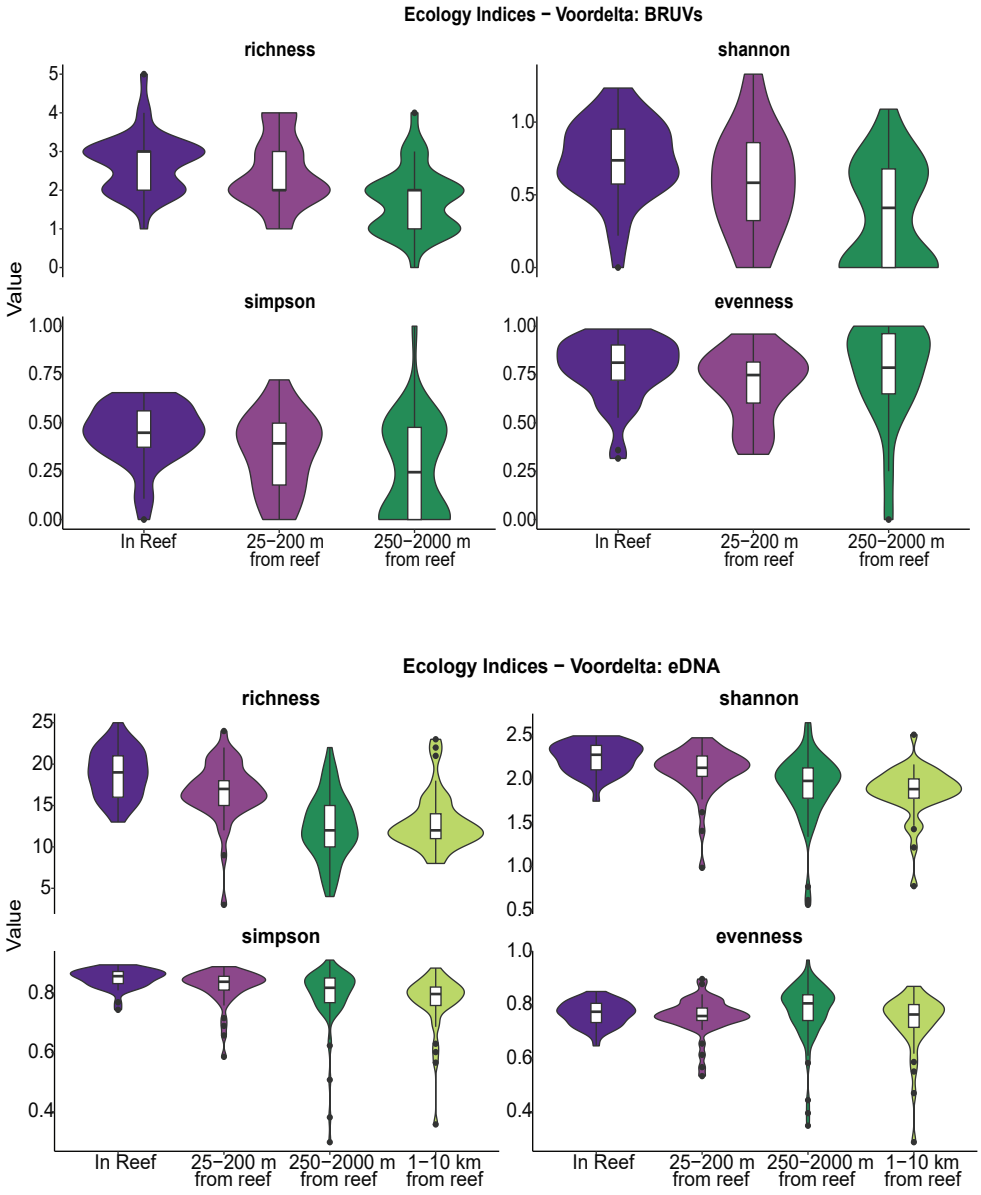


Figure S5: Main calculated biodiversity indices (richness, Shannon, Simpson, evenness) for eDNA data and BRUVS data at the Voordelta, presented as violin plots with embedded boxplot. Violin plots illustrate the distribution of the data points, while boxplots display the median, interquartile range (between 25th to 75th percentiles), and minimum and maximum values (whiskers).

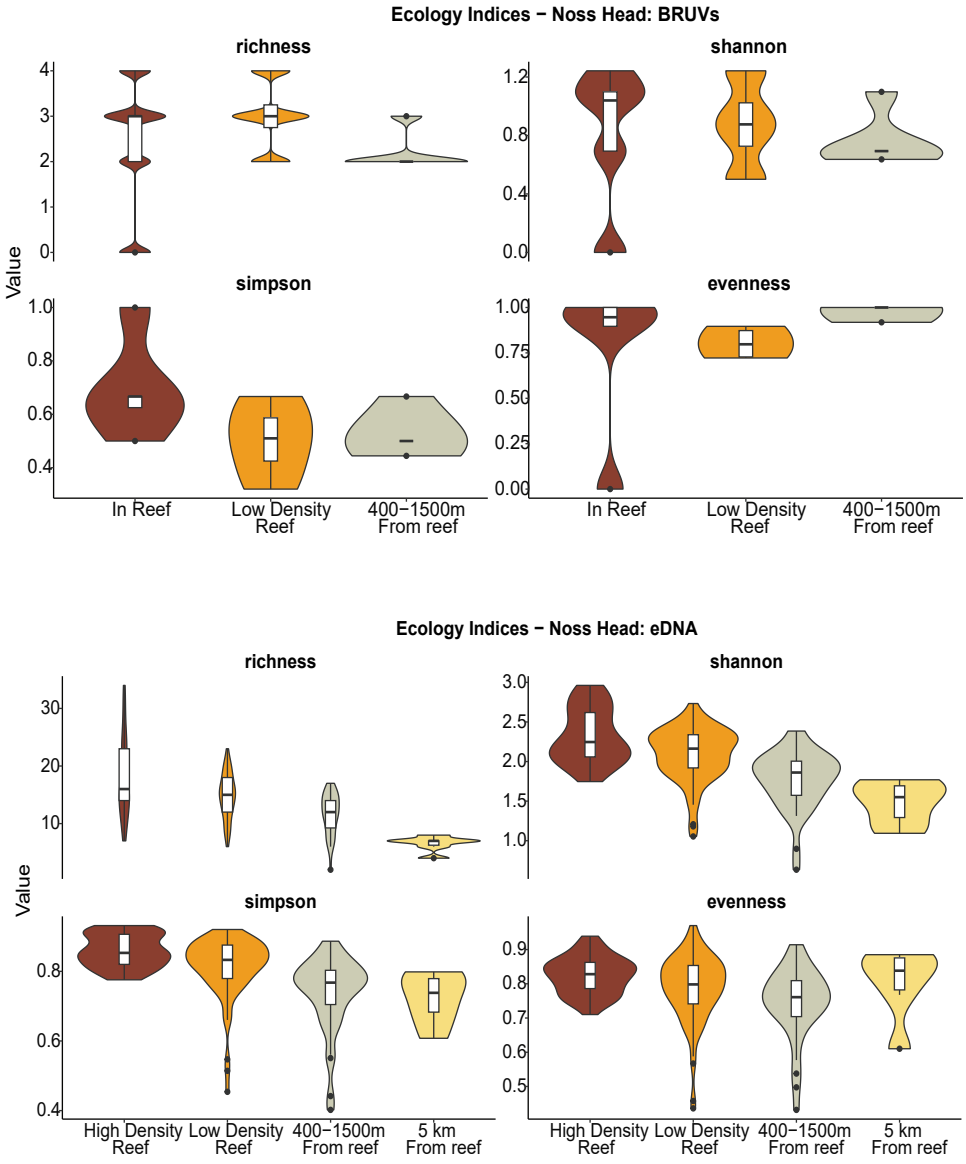


Figure S6: Main calculated biodiversity indices (richness, Shannon, Simpson, evenness) for eDNA data and BRUVS data at the Noss Head, including the low density reef habitat. Data are presented as violin plots with embedded boxplot. Violin plots illustrate the distribution of the data points, while boxplots display the median, interquartile range (between 25th to 75th percentiles), and minimum and maximum values (whiskers).

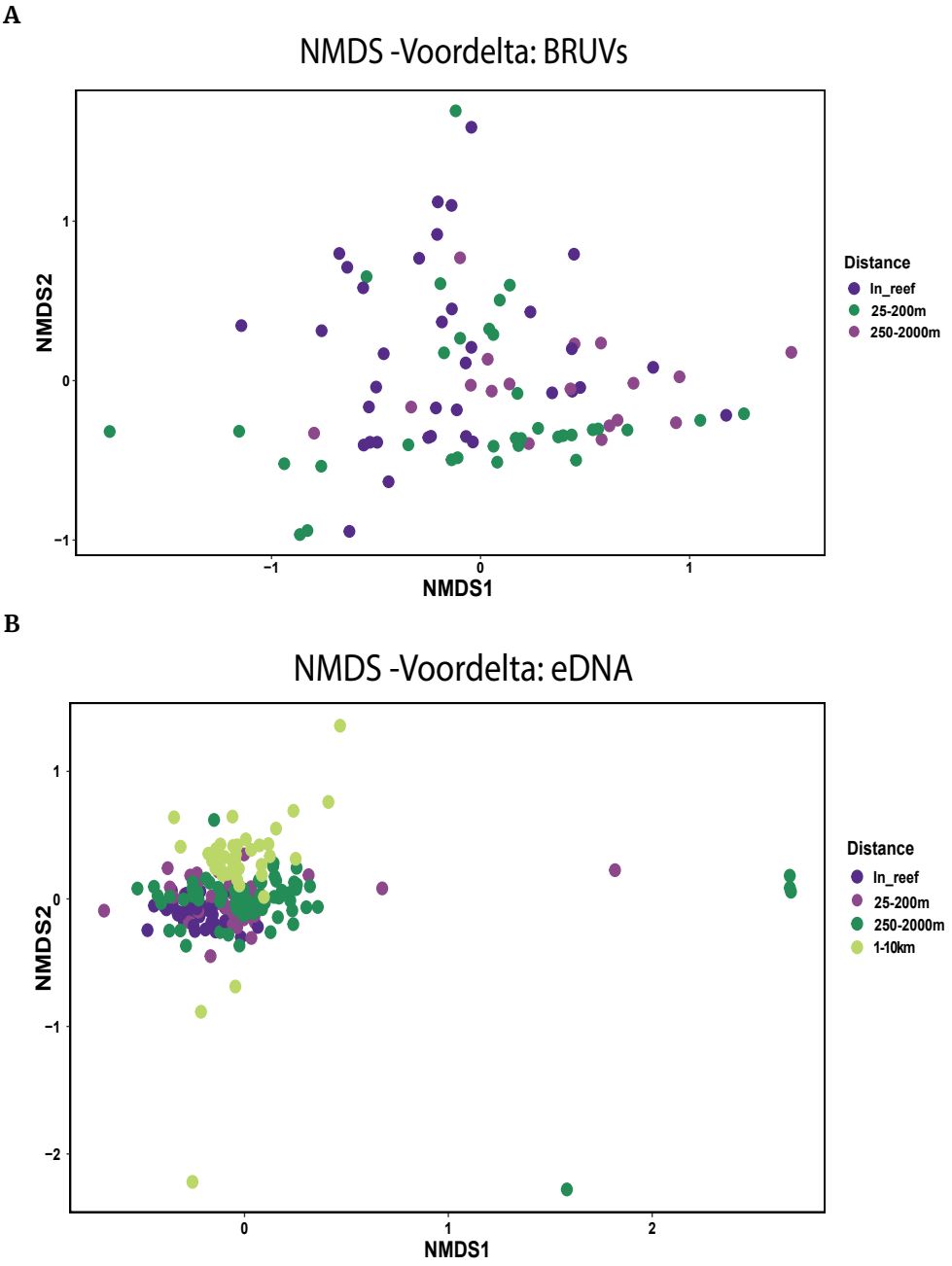


Figure S7: Biplots of the non-metric multidimensional scaling (nMDS) based on Bray-Curtis similarity of vertebrate communities using A) BRUVS and B) eDNA at the Voordelta. Points correspond to single samples (replicate) are coloured based on the habitat, comparing samples from the reefs and at increasing distance. The proximity of the points in the biplot is based on their similarity in terms of community composition of each sample, with closer points being more similar.

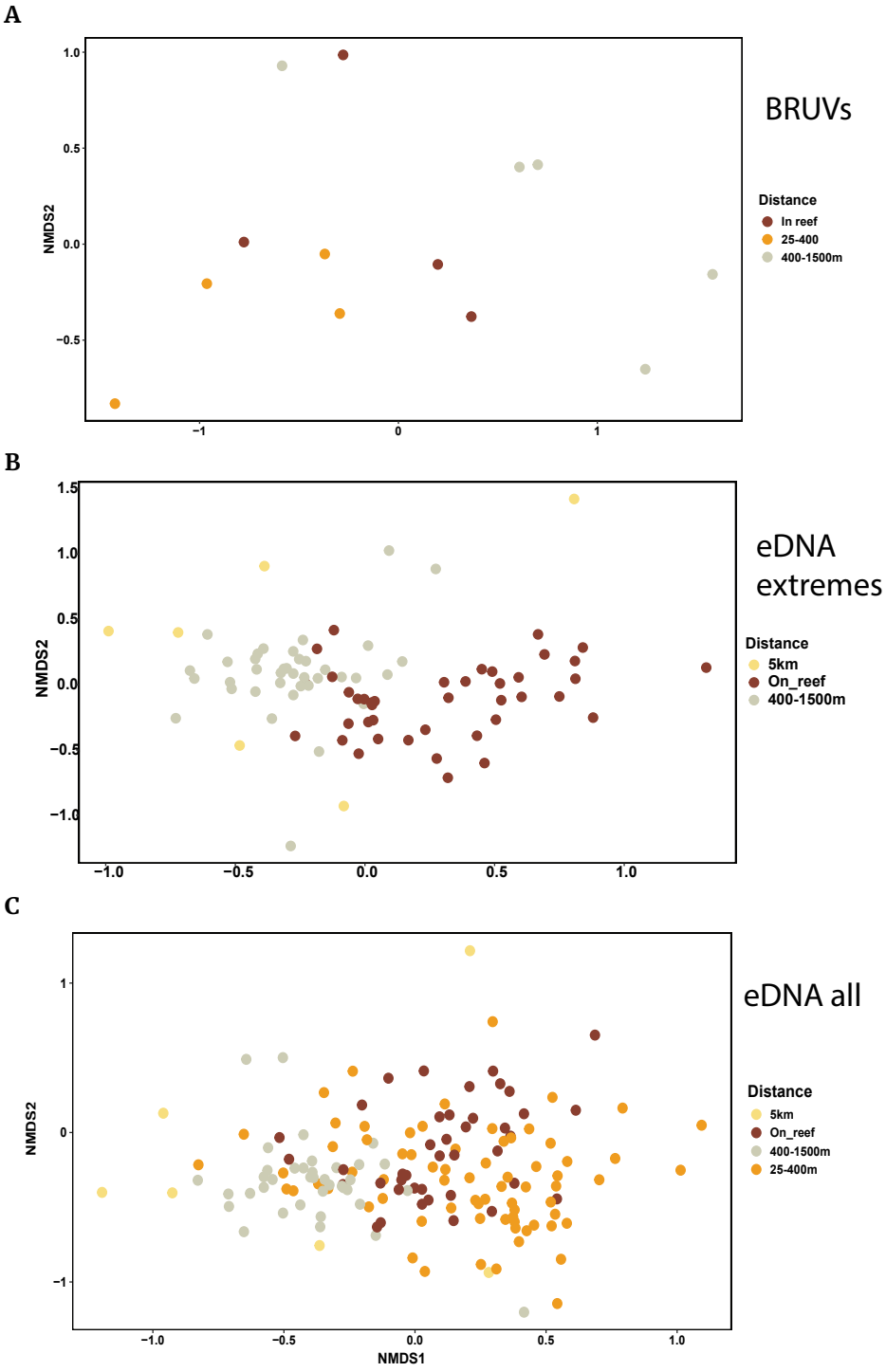


Figure S8 Biplots of the non-metric multidimensional scaling (nMDS) based on Bray-Curtis similarity of vertebrate communities using A) BRUVS and B)and C)

eDNA at the Noss Head. Points correspond to single samples (replicate) are coloured based on the habitat, comparing samples from the reefs and at increasing distance. The proximity of the points in the biplot is based on their similarity in terms of community composition of each sample, with closer points being more similar.

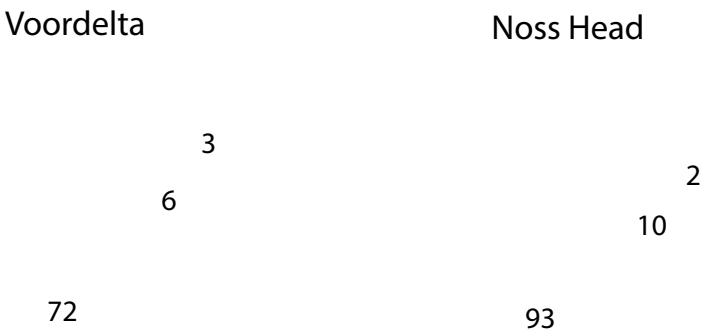


Figure S9: Venn diagrams illustrating the differences in species detected by eDNA and BRUVS at Voordelta and Noss Head, along with their overlap.

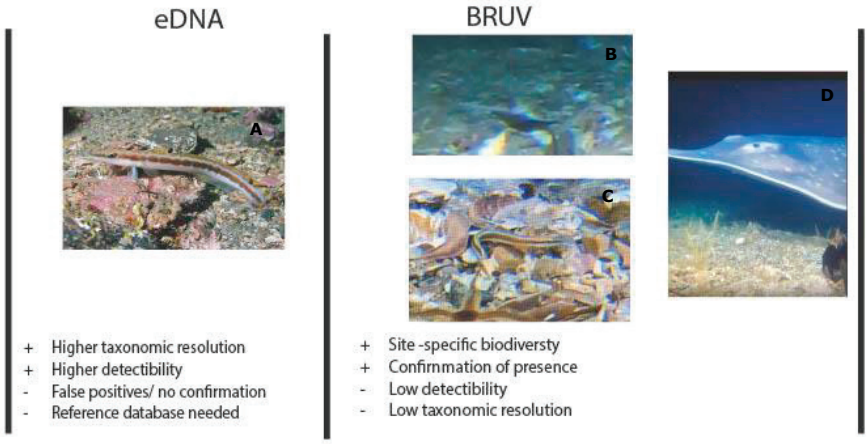


Figure S10: overall comparison among eDNA and BRUVS techniques, highlighting the main pros and cons of both. Example pictures display A) a juvenile individual of ling Molva molva (picture by Kare Telnes, https://www.seawater.no/fauna/chordata/molva.html); B) common dragonet Callyonimus lyra (screenshot from BRUVs video data at Noss Head, C) juvenile ling (screenshot from BRUVS video data at Noss Head) and D) blue skate Dipturus batis (screenshot from BRUVS video data at Noss Head).

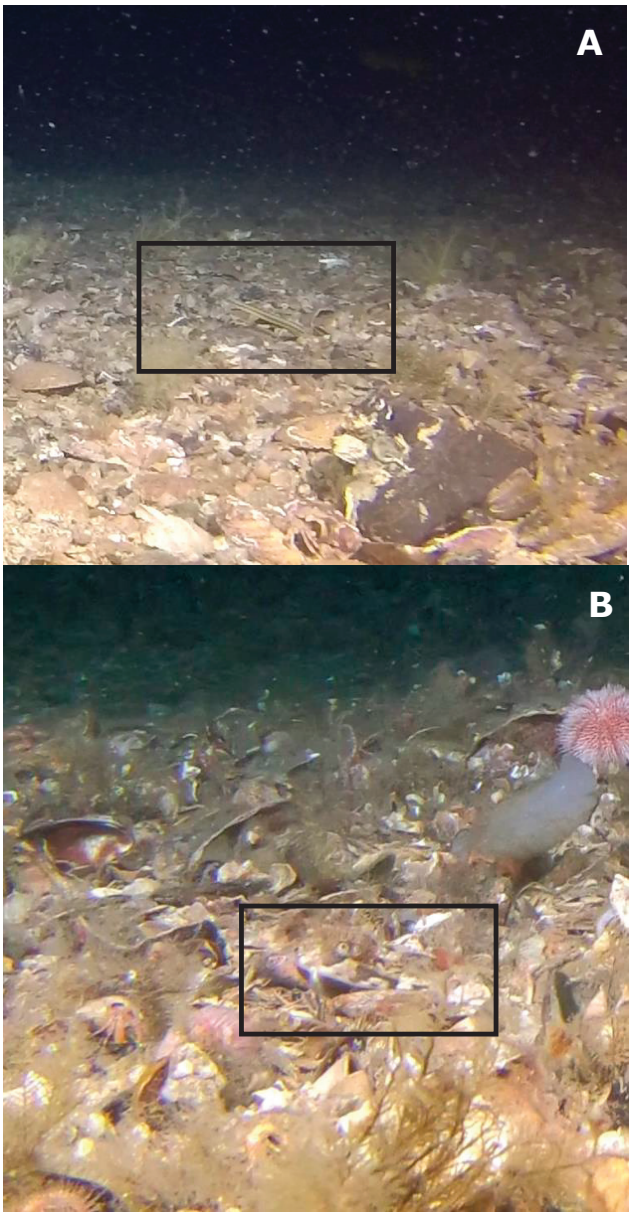


Figure S11: Screenshots from BRUVS video at Noss Head, depicting A) a juvenile individual of ling Molva molva, and B) a juvenile bullrout Myxocephalus scorpius.

Chapter 7

General Discussion

Karlijn Doorenspleet

7. GENERAL DISCUSSION

To meet European North Sea monitoring requirements and adequately inform industry and conservation strategies, there is a need for non-invasive, routine, cost-effective, and scalable monitoring methods. Molecular metabarcoding methods are highly sensitive and hold great potential for biodiversity monitoring. Before it can support management decisions, molecular methods need to be evaluated to determine whether they are sufficiently robust to serve as a standard monitoring tool. It is also important to evaluate whether technological advances can complement or improve existing molecular methods for studying and monitoring biodiversity in the North Sea. This thesis aimed to develop novel molecular methods and evaluate their potential for improved evidence-based monitoring in the North Sea. To do so, a metabarcoding workflow and bioinformatics pipeline were developed for the new Oxford Nanopore MinION platform, and the outputs were evaluated against conventional molecular and observational methods. The potential of using long-read molecular markers and eRNA metabarcoding was also examined to enhance species-level resolution and more accurately depict the recently active community. These methods were then applied in large-scale sampling campaigns on natural bivalve reefs to examine vertebrate biodiversity and functional trait patterns compared with non-reef areas.

First, the main findings of this thesis are summarised in relation to the three formulated objectives introduced in Chapter 1. The main findings are then discussed, focusing on how the choice of sequencing platforms and novel molecular methods affects the efficiency, quality, and flexibility of marine biodiversity monitoring in the North Sea. Finally, the latest advances, opportunities, and limitations of the molecular toolbox were explored, along with their influence on our understanding of North Sea ecosystems. Additionally, future research directions were outlined that can enhance monitoring efforts and provide timely information for nature-based management.

7.1 main findings of this thesis

Objective 1 is to validate a more flexible, cost-effective, and more straightforward sequencing platforms and methodological workflows by comparing them with established molecular and observational approaches, supporting future harmonised and standardised monitoring frameworks.

Validation was achieved in Chapter 2. Here, metabarcoding of the mitochondrial COI fragment of North Sea macrobenthos from the Belgian North Sea was compared across Oxford Nanopore, Illumina MiSeq, and NovaSeq shotgun sequencing. Highly similar performances in alpha and beta diversity were observed and were consistent with observed and historical location-specific community patterns. NovaSeq shotgun metagenomics showed slightly different community compositions compared to both metabarcoding results and morphology, and had the weakest correlation with size-corrected morphological abundance data. All methods showed mismatches with morphological identifications. This is partially due to primer biases and to incomplete reference databases. The results of this study show that nanopore metabarcoding is comparable to conventional Illumina MiSeq metabarcoding in bulk macrobenthos and thereby validates that a more flexible platform, such as Oxford nanopore, can support future standardised monitoring frameworks.

Objective 2 is to develop molecular monitoring approaches that enhance species-level resolution and provide improved spatial and temporal resolution, thereby advancing understanding of ecological patterns in North Sea ecosystems.

Novel molecular metabarcoding approaches were presented in Chapters 3, 4, and 5. Chapter 3 introduces a marine vertebrate-specific universal primer pair targeting a 2-kb region of mitochondrial 12S and 16S DNA, and compares it with MiFish primers. Tests with mock communities and aquarium DNA demonstrated improved species resolution with the 2kb primer. eDNA from natural and artificial North Sea reefs revealed distinct community compositions and lower richness, likely due to longer, degraded fragments, indicating a more accurate temporal signal. Chapter 4 examines how fragment length and type (eDNA vs eRNA) affect community detection, finding that short-read eDNA captures higher diversity, closely resembling eRNA results but with fewer large species such as sharks and rays, suggesting that eRNA reflects active benthic communities. Long-read metabarcoding showed lower detectability, probably due to rapid degradation, making it unreliable for community ecology. Chapter 5 reviews molecular tools for North Sea biomonitoring, discusses challenges, and recommends integrating novel methods into long-term, harmonised monitoring efforts.

Objective 3 is to apply these optimised tools to assess the biodiversity of natural reef systems in the North Sea and to complement detection

patterns obtained with other non-invasive observational surveys to gain a comprehensive understanding of how reef density influences species and trait composition.

In chapter 6, metabarcoding methods were applied to study the community ecology of a natural mixed bivalve reef in the Dutch Voordelta and an *M. modiolus* reef at Noss Head, Scotland. This chapter showed that eDNA metabarcoding consistently revealed higher vertebrate diversity within the reef and a distinct functional trait composition that decreased with increasing distance from the reef. eDNA detected the highest overall diversity compared to the non-invasive observational survey and baited remote underwater video (BRUVs), but BRUVs captured juvenile ling individuals that could be confirmed by eDNA detections, demonstrating the complementary value of using multiple methods simultaneously.

7.2 Oxford Nanopore is a promising sequencing platform that, due to its flexibility, cost-effectiveness, and quality, outperforms conventional platforms.

Over the past decade, high-throughput and next-generation sequencing have greatly advanced research into marine biodiversity (Hu et al., 2021; Mohrbeck et al., 2015). Illumina continues to be the gold standard for its precise short-read data, making it ideal for metabarcoding and metagenomics (Meyer & Kircher, 2010). PacBio is preferred for its ability to generate long reads with high consensus accuracy, which is useful for complex genomics (Hu et al., 2021). Oxford Nanopore provides a unique, portable solution for long reads at competitive prices, although it has historically been more prone to errors (Bleidorn, 2016; Wang et al., 2021). Nevertheless, ongoing improvements in chemistry and base-calling algorithms have significantly enhanced its accuracy throughout the years this PhD was conducted (Chang et al., 2024).

Between Nanopore and Illumina MiSeq metabarcoding, no differences in alpha or beta diversity were observed (Chapter 2), and both methods identified the same community composition, consistent with morphological observations at these locations (Chapter 2). Other comparisons of metabarcoding results between Illumina and Nanopore, for example, in a mixed food sample with six fish species, showed that all species were identified using Nanopore sequencing (Voorhuijzen-Harink et al., 2019). Other cases, such as a comparison between MiSeq and Nanopore conducted on the gut contents of juvenile lanternfish, also yielded similar community compositions (van der Reis et al., 2023). A sequencing platform

comparison of metabarcoding results from bulk zooplankton samples indicated that although Illumina MiSeq yielded approximately 100 more OTUs, this did not translate into higher species richness, and the species composition was comparable between methods (Chang et al., 2023). This aligns with other comparisons between these platforms in marine invasive species research (Egeter et al., 2022), arthropods from bat diets (Nolan et al., 2025) and protist communities (Bludau et al., 2025). This, together with the findings from chapter 2, shows an increasing consensus that Illumina and Nanopore indeed produce reproducible community profiles across eukaryotic taxonomic groups. Results from microbiome and fungal community ecology also align with this consensus of similar reproducibility (Heikema et al., 2020; Mittelstrass et al., 2025; Stevens et al., 2023a; Szoboszlay et al., 2023), further strengthening the accuracy of nanopore platforms across taxa. Nevertheless, robust bioinformatics pipelines, harmonised laboratory workflows, and up-to-date Nanopore chemistry are essential for obtaining comparable results (Chapter 2), (Chang et al., 2024) and platform-specific biases and differences in quantitative results between platforms remain (Mittelstrass et al., 2025).

The robust, user-friendly bioinformatics pipeline DECONA used throughout this thesis proved both accurate and ecologically informative, producing comparable benthic communities, correctly reconstructing an aquarium community, and observing increased richness on reefs. DECONA is unique because it is specifically designed to process Nanopore sequences through a pipeline that requires only a single line of code, offering adjustable clustering thresholds, flexible length filtering, and built-in consensus polishing. The choice of DECONA settings significantly influences results and depends on the Nanopore chemistry used, highlighting the importance of tuning parameters (Chapter 3). Although bioinformatics processing pipelines for Illumina sequencing are abundant and highly developed, such pipelines are optimised for Illumina-specific short reads and are hardly transferable to Nanopore long-read sequence processing (Hakimzadeh et al., 2024). Other user-friendly Nanopore bioinformatic pipelines have been developed, such as Nanopipe (Shabardina et al., 2019), PRONAME (Dubois et al., 2024), ASHURE (Baloğlu et al., 2021) and Minibarcoder (Srivathsan et al., 2019), which mostly support metabarcoding, while several studies also use separate tools for consensus OTU building (van der Reis et al., 2023; Voorhuijzen-Harink et al., 2019). As bioinformatics workflows rapidly develop, improved accessibility will likely increase the use of Nanopore metabarcoding. Nevertheless, because bioinformatics pipelines differ across settings and philosophies,

it is important to thoroughly compare their outputs. Most importantly, current Nanopore sequencing outputs yield much lower raw read error rates, going from 85% to over 99%, which enable amplicon sequence variant (ASV)-level resolution (Cousson et al., 2025) and require comparisons and likely updated bioinformatics pipelines that efficiently handle ASV-level data. Thus, although sequencing platforms can reach similar conclusions, each platform has distinct advantages and limitations as summarised in table 1.

For Nanopore, key advantages are its portability, rapid processing and minimal lab infrastructure needed, which enables sequencing in remote places such as in polar regions (Johnson et al., 2017), on a ship, in space (Castro-Wallace et al., 2017) and areas with limiting genomic technologies (Gygax et al., 2025). Using nanopore, may improve engagement with local high biodiversity by authorities that take conservation decisions (L. Urban et al., 2023). And although limitations remain in error rates and biases in quantitative results, the ongoing development of robust bioinformatics pipelines and improvements in pore chemistry overcome these. Therefore, Nanopore is slowly but steadily preferred over conventional sequencing platforms and can readily be used for marine molecular monitoring practices.

Table 1: Practical comparison of Nanopore compared to other sequencing platforms as Illumina and Pacbio.

	Oxford Nanopore	Other platforms	Notes
Cost	Low initial costs per sample, is competitive	Low per base cost, but the instrument is expensive	Price of Nanopore consumables is still considerable
Workflow accessibility	Very flexible, portable setups	More fixed lab infrastructure required more complex preps	More accessible for field use (educational purposes)
Autonomy	Real-time sampling: stop when enough data	Fixed running schedules	Sequencing can be done under own management
Accuracy	In recent years improved, comparable after polishing, but raw reads still 0-1% lower	High raw read accuracy and established error profile	
Flexibility	Very flexible, long reads, Native DNA/RNA, structural variants, modification	Short reads; limited for long range, RNA and variants	

Table 1: Practical comparison of Nanopore compared to other sequencing platforms as Illumina and Pacbio. (*continued*)

	Oxford Nanopore	Other platforms	Notes
Timeframe	Fast, quick sample prep, real-time sequencing and processing can be done in hours to days	Longer sample prep and run time, waiting time for facilities (weeks).	
Field diagnostics	Great, portable sequencer, minimal lab infrastructure required.	Hardly possible: lab required, sequencing in specialized labs	
Accepted	Emerging, increasingly accepted, but historical accuracy concerns are still catching up.	Widely established, also clinical.	
access bioinformatics tools	Many pipelines, testing and comparisons are still preliminary; fewer widely used pipelines and long reads need specialized processing.	Strong, widely established, and many well-tested pipelines for amplicon and metagenomics	Nanopore technology is continually innovating, and the varied outputs make it difficult to integrate with established pipelines from other platforms.

7.3 Using novel metabarcoding practices, such as longer fragments and RNA fragment metabarcoding, can improve marine biodiversity monitoring

With eDNA metabarcoding, it has become possible to detect species in a non-invasive, highly sensitive manner that can simultaneously identify species from different taxonomic groups (e.g., macrofauna, zooplankton, microbes), different trophic levels (e.g., gut content), cryptic symbionts and across spatial scales (Deiner et al., 2017). This added value for biodiversity assessments has so far been inaccessible with conventional techniques such as trawling and grab surveys. Therefore, eDNA-based biodiversity assessments became a popular research topic that is quickly adopted in North Sea biodiversity studies (Boyse et al., 2024; Dukan et al., 2024). For example, coastal North Sea eDNA sampling demonstrates how spatial community patterns correspond with beam trawl surveys (Maggini et al., 2024), and additionally reveals bycatch diversity (P. Urban et al., 2024). Furthermore, monthly coastal eDNA sampling shows strong similarities with established, fish migration patterns and life history events (Lolivier et al., 2025), and can also improve the detection of rare and pelagic species that are usually under sampled with trawl surveys (He et al., 2023; Veron et al., 2023). Recent North Sea monitoring

efforts have demonstrated that eDNA results align with species community spatial patterns both horizontally and vertically (Dukan et al., 2024), can detect community shifts across spatial habitat gradients. Examples are, changes related to reef density, salinity and temperature gradients (Adams et al., 2023; Dukan et al., 2024; Lolivier et al., 2025; Ohnesorge et al., 2024) and detect non-indigenous species (Jansen et al., 2025). While eDNA application has transformed the study of North Sea biodiversity, challenges still need to be tackled, such as limited species-level resolution, uncertainty in eDNA transport and persistence, and quantification of biomass presence per species (Chapter 3 and 4).

Chapters 3 and 4 explored two innovative technological advancements. A long-read eDNA metabarcoding approach was developed and tested, and an eRNA-based metabarcoding approach was evaluated. The rationale for using alternative fragments (long-read DNA and short-read eRNA) is twofold. First, longer amplicon fragments often contain more phylogenetically informative sites, thereby enhancing species-level resolution, especially for closely related taxa (Zhang et al., 2020). Second, longer fragments and eRNA may reflect more recent biological material, since these fragments are generally less persistent in the water column than fragmented eDNA (Brandão-Dias et al., 2025; Jo, 2023), potentially providing a more recent snapshot of the community and allowing for finer spatial and temporal resolution.

Chapter 3 clearly showed that long-read metabarcoding significantly improves species-level resolution across various taxonomic groups, such as Gadidae, Clupeidae, and Ammodytidae. Other studies employing longer reads have also demonstrated enhanced taxonomic resolution, as seen in Ulva microbiomes using full-length 16S metabarcoding (van der Loos et al., 2023), and in full-length 18S protist community analyses, which reported better recovery of genera and species for groups like diatoms and dinoflagellates (Chwalińska et al., 2025). A different community composition observed between long and short read eDNA was initially hypothesised as longer reads reflecting a finer temporal and spatial scale due to more rapid degradation (Brandão-Dias et al., 2025; Jo, 2023). However, repeated comparisons of long and short read eDNA metabarcoding with eRNA metabarcoding (Chapter 4), indicate that eRNA more closely resembles the community composition observed with short-read eDNA. This contradicts the earlier hypothesis that mitochondrial eRNA degrades fastest and should therefore be more short-lived (Brandão-Dias et al., 2025). Several factors can explain this discrepancy: i) molecule size

and protection, short eRNA might be more abundant and/or spread more easily than it is degraded compared to full-length mtDNA (Jo, 2023); ii) low abundance of longer reads can lead to reduced amplification of rare species, causing biases (Shaffer et al., 2025; Zhao et al., 2021). Therefore, although different nucleic acid fragments can enhance spatial and/or temporal resolution, initial biases persist.

This thesis, along with recent studies, shows that over the last 5 years, there has been significant progress in molecular methods for biodiversity assessment (Figure 1). Nonetheless, significant knowledge gaps remain regarding factors such as shedding, transport, degradation, and absorption rates, which likely influence the detected community compositions. It is therefore essential to employ complementary methods, especially given eDNA metabarcoding's limited ability to provide biomass and life-stage information.

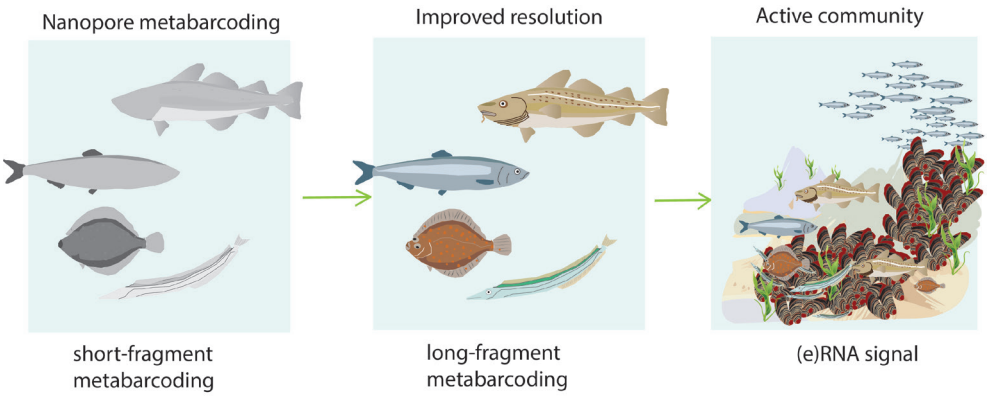


Figure 1: advances in novel methods for metabarcoding-based biodiversity assessments offer opportunities for enhanced marine monitoring. Short fragment metabarcoding (first illustration) detects the full range of fish and vertebrate taxonomies but is limited in identifying species specifically. In contrast, long-read metabarcoding can enhance observations by enabling species-level identification (second illustration). Complementing this with the eRNA signal allows for the detection of the active community, as eRNA degrades faster in the water column (third illustration).

7.4 eDNA metabarcoding shows distinct community compositions on natural and artificial North Sea ecosystems

Biogenic bivalve reefs, as well as artificial reefs from the North Sea, are biodiversity hotspots for benthic and mobile faunal communities (Coral et al., 2025). In Chapter 6, we described, using eDNA, that both

bivalve reefs in Scotland and the Netherlands hosted a high diversity of reef-associated fish species, including rocklings (*Lotidae*), clingfish (*Gobiesocidae*), and gunnels (*Pholis gunellus*). Their presence decreased with increasing distance from the reef. Additionally, benthic predators like sculpins (*Cottidae*) and scorpionfish (*Scorpaenidae*), which are known to primarily feed on crustaceans, molluscs, or other small fish (Heessen et al., 2023; Ivanova et al., 2018), were also present on the reef. Many commercially important species, such as cod (*Gadus morhua*), pollack (*Pollachius pollachius*), and poor cod (*Trispoterus sp.*), have been shown to benefit from the reefs by feeding on benthic prey (Almqvist et al., 2010; Fjøsne & Gjøsæter, 1996; Kristensen et al., 2015; Wennhage & Pihl, 2002), or as nursery areas for juveniles, such as cod and ling (Dunlop et al., 2022; Heessen et al., 2023; Wieland et al., 2009). These findings were accompanied by significantly more invertivore species observations within the *M. modiolus* reef than outside the reef, linked to food availability within reefs (Nunn et al., 2012). Furthermore, the lower number of planktivorous fish was associated with reduced plankton dnsity caused by increased bivalve filtration (Kristensen et al., 2015; Petersen et al., 2013; Tan et al., 2024).

Although direct comparisons between datasets and locations are never simple due to differing sampling strategies and sizes, some broader community patterns can be described when considering the reefs in the Southern North Sea (e.g., Voordelta) and the Northern North Sea (e.g., Noss Head). Generally, long-term large-scale surveys have revealed a gradient in community compositions between northern and southern habitats, primarily structured by depth, habitat type, and temperature (Heip & Craeymeersch, 1995). For example, Noss Head contained more Northern North Sea- and North Atlantic-associated species, such as flatfishes (*Hippoglossoides sp.*), snailfishes (*Liparis sp.*), and sculpins *(Micrenophrys sp*;Heessen et al., 2023). Interestingly, the majority of marine vertebrates were uniquely associated with the northern reef, such as Porbeagle shark (Lamna nasus), and blue skate (*Dipturus batis*), likely due to richer foraging grounds in the area (Sguotti et al., 2016). In addition, the southern community had species like mullets (*Chelon sp*.), sand smelts (*Atherina sp.*), and garfish (*Belone belone*) associated with estuaries and coastal zones (Breine et al., 2011; Edwards et al., 2025), but also species linked to the sandy characteristics of the southern North Sea, such as *Buglossidium luteum* and Ammodytidae (Heessen et al., 2023). The community across different associated reefs, whether artificial (e.g., shipwrecks or OWFs) or natural (e.g., Borkum reef, Noss Head), has a clear species

signature including *Micrenophrys lilljeborgii*, along with other structure-associated species (Chapter 3,6). eDNA-based metabarcoding can reveal community patterns that go beyond characterising biodiversity; by comparing community compositions, it can predict shifts in interactions, identify non-indigenous species and reveal hidden trophic links, providing valuable insights into the functioning of North Sea ecosystems (Boyse et al., 2024; Dukan et al., 2024; Jansen et al., 2025). Thus, molecular methods have become increasingly usable for North Sea biodiversity assessments (Cornelis et al., 2024; Derycke et al., 2021), but adopting eDNA as a standard monitoring technique still requires standardisation in laboratory workflows, reference database curation and use and (meta) data collection (Klymus et al., 2024; Shea et al., 2023). As discussed in Chapter 5, several initiatives are presented that aim to harmonise data sharing (e.g., FAANG, GEANS, OBON, ELIXIR, and eDNAquaplan; (Samuel et al., 2021; Waterhouse et al., 2022) and emphasise the importance of standardised protocols and metadata management practices for routine use are emphasized. This will improve the comparability of eDNA datasets between countries and projects, with the ultimate aim of facilitating the integration of metabarcoding as a standardised approach within marine biodiversity frameworks, such as MSDF and OSPAR.

7.5 The molecular toolbox: its possibilities and limitations help shape our perception of marine life

While eDNA metabarcoding is increasingly being integrated into standard and applied monitoring methods, the extent to which biodiversity can be accurately interpreted depends on several methodological factors and the availability of reference and environmental data. First, the hydrodynamic context of the sampling location is crucial, as nucleic acid fragments can be transported in particles by currents and moved up to 12 km (Harrison et al., 2019; Sunday et al., 2014). Especially in the turbulent North Sea, where currents are significant, eDNA signals can be detected over a broader spatial scale. Additionally, degradation rates fluctuate with factors such as pH, microbial activity, seasonal changes, and sediment absorption rates (Cristescu, 2019; Harrison et al., 2019). Nevertheless, eDNA sampling in a nearshore environment with varying tides showed that community patterns remained relatively stable over time and during the tide cycle, suggesting that eDNA signals are persistent for over 24 hours and can be accurately interpreted when sampling is both frequent and spatially structured (Collins et al., 2018; Hallam et al., 2023; Kelly et al., 2018). Consequently, environmental factors do influence eDNA results, but carefully planned sampling strategies can aid in their inter-

pretation, even when additional data are not available. Other limitations arise, such as incomplete reference databases; even for relatively well-represented groups, such as fish, reference databases lack references for COI and 12S sequences (Weigand et al., 2019). Laboratory workflows can also introduce biases; however, these limitations can largely be mitigated by using standardised, harmonised laboratory procedures across different labs (Van den Bulcke et al., 2021; Van den Bulcke et al., 2023). Finally, the financial resources available to a project, largely determine how surveys are designed and can therefore restrict replication, coverage, and frequency of sampling, reducing statistical power (Deiner et al., 2017; Goldberg et al., 2016; He et al., 2023; Takahashi et al., 2023)). Thus, several physicochemical, biological, and experimental design factors are critical to consider when interpreting eDNA metabarcoding results.

Complementing eDNA monitoring with visual surveys (AUVs and BRUVs) and acoustic methods (sonar and hydrophones) can substantially improve the coverage of North Sea biomonitoring surveys. In Chapter 6, the combination of BRUVS and eDNA demonstrated its complementary value by confirming the presence of juvenile Ling individuals identified in the eDNA results, while eDNA also detected more species. This is in accordance with other studies that combine eDNA with visual or acoustic surveys, which all report improved taxonomic recovery (Clark et al., 2024; Easson et al., 2020; Rey et al., 2023; Stat et al., 2019). Additional complementary signals of visual surveys can be used to obtain biomass estimates (Rey et al., 2023) and juvenile presence (Clark et al., 2024). Thus, while eDNA can detect a broad community and cryptic taxa, complementing with visual and acoustic surveys can confirm presence, size and numbers and improves the ecological information that single method approaches would otherwise miss.

Advancements beyond eDNA metabarcoding have considerably increased during the course of this thesis. As discussed and reviewed in Chapter 5, techniques as species-specific assays (Guri et al., 2024a; van Bleijswijk et al., 2020), long-read metabarcoding, metagenomics, epigenetics and population genomics can further improve ecological and taxonomic information from a monitoring survey and expand the molecular toolbox for marine monitoring. In addition, the innovative methods used in Chapters 3 and 4 and reviewed in Chapter 5 demonstrate that using longer and eRNA fragments significantly enhances species and community resolution. Furthermore, metagenomics methods, as described in Chapters 2 and 5, can potentially provide improved data on abundance and species,

especially as reference databases are extended with whole-genome sequences (Blasiak et al., 2020; Leray et al., 2022; Weigand et al., 2019). Multi-omics approaches can go beyond simple presence/absence studies and provide insights into functional potential and active gene expression, enabling an understanding of stress responses in an ecosystem (He et al., 2025; Hechler et al., 2025; Miyata et al., 2025). Additionally, omics data can reveal population genetic diversity, offering insights into connectivity, demographic structure, and adaptive capacity of populations (Crowley et al., 2025; Serivichyaswat et al., 2025). Moreover, epigenetic methods can detect modifications on the DNA in response to regulatory processes and changes enabling detection of environmental change, sex ratio, life-history characteristics, and can also uncover the age and sex ratios of individuals (Gerber et al., 2025; Silliman et al., 2023; Valk et al., 2025; Viña-Feás et al., 2024). This further expands the capabilities of studying the North Sea ecosystem using molecular methods.

During the period the work for this thesis was performed, molecular tools have diversified and have changed our view of the marine ecosystem (Figure 2).

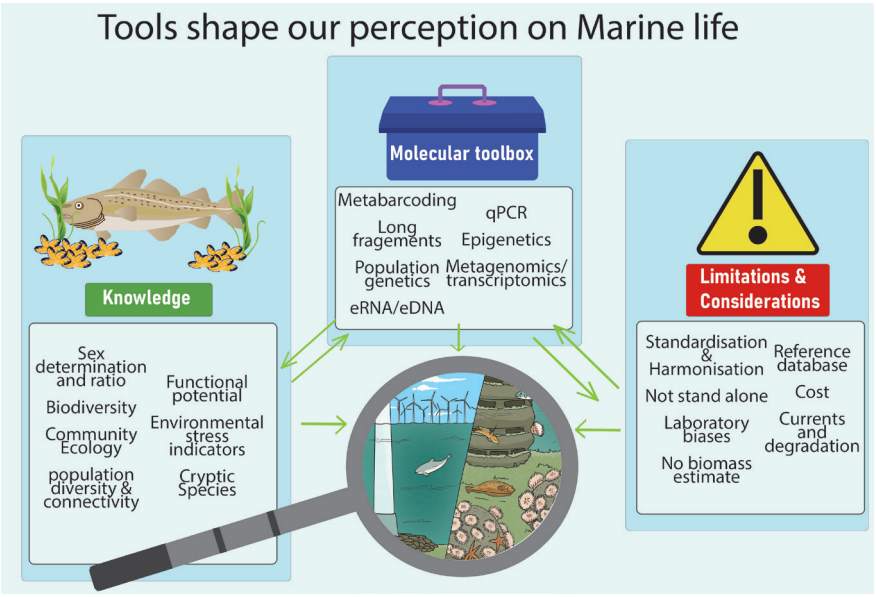


Figure 2: Representation of the interactions between developments of the molecular toolbox, its limitations and the knowledge that is generated through molecular techniques and beyond shape our perception of marine life

Conventional monitoring methods mostly rely on the 'visible' characteristics of an ecosystem, whereas molecular techniques such as metabarcoding and multi-

omics sequencing provide new layers of knowledge on stress response, species dynamics, and population connectivity that were previously unavailable. Thus, novel molecular tools provide additional knowledge about food webs, ecosystem dynamics and biodiversity. Simultaneously, methodological limitations and technical choices further shape how we view the North Sea ecosystem. In biodiversity monitoring contexts, molecular advances change the perception from collecting species lists to a more interconnected understanding that is structured by species interactions, functional processes and responses and become central to ecology. Technological development shapes new ecological research questions, thereby changing what we recognise as ecologically relevant and fundamentally reshaping our perception of marine life and redefining how we need to preserve it.

7.6 Future monitoring and research directions

Future molecular research should focus on developing and deploying integrated, multidimensional monitoring platforms that combine molecular tools, such as eDNA metabarcoding and metagenomics, with automated sampling and real-time sequencing. These systems (either in combination with robotics or animal tags) should improve cost efficiency and scalability for large-scale monitoring programs, enabling remote sampling over large areas and longer timeframes. In addition, automated molecular monitoring should be complemented with video and acoustic methods to improve estimates of life stage and biomass.

Simultaneously, molecular methods should be expanded from targeting single taxonomic groups (e.g., fish) to multi-trophic and community-wide biodiversity assessments, also incorporating microbial and planktonic taxa. In addition, advances in studying the functional community through multi-omics techniques should be integrated, as this type of data can help further understand responses to human pressures such as trawling, pollution, and warming. These developments will not be possible without improving reference databases that support the identification of omics data; therefore, consortia involved in North Sea molecular monitoring should focus on expanding these databases to include whole genomes and transcriptomes.

Harmonised and standardised molecular data should be used for ecological models. In addition, applying and developing machine learning models should be prioritised to connect patterns, thereby improving monitoring capabilities and scalability, and to better understand habitat connectivity, community dynamics, and functional responses to changing conditions and disturbance.

Finally, as anthropogenic marine infrastructure is likely to intensify, collaboration between offshore industry and conservation scientists will become increasingly important. These collaborations should focus on integrating automated, non-invasive monitoring devices into existing and future structures, such as wind turbines and artificial reef installations, enabling long-term and continuous data collection that can evaluate both ecological benefits and risks. By combining data generated from different locations and habitats using an automated tool, it is possible to scale up from local sampling to a seascape ecological network. In doing so, current biodiversity monitoring will evolve towards a more in-depth understanding of marine ecosystem health, dynamic and resilience.

Conclusions

This thesis has demonstrated how novel molecular methods can substantially improve biodiversity monitoring of the North Sea. Throughout the Chapters, sequencing platforms as Oxford Nanopore have been validated. The potential of molecular methods for harmonized and standardized monitoring are highlighted, and showed to deliver reliable information that aligns with conventional methods or known compositions. Innovative metabarcoding methods, such as long read metabarcoding, improve species-level identification, and eRNA metabarcoding reflects the active and more recent community. Application of the methods to natural reefs further illustrates the ecological value of eDNA metabarcoding, as distinct reef-associated communities show distinct functional trait compositions. Molecular data were further linked to a non-invasive video survey, demonstrating the importance of using complementary methods for in-depth biodiversity assessments.

These findings demonstrate that molecular monitoring tools are powerful and suitable for routine use, capturing part of the diversity that conventional methods might miss. Further development and integration into long-term monitoring programmes could enhance ecological assessment and support evidence-based management. By adopting scalable, faster, and more comprehensive approaches that are complementary and standardised, this thesis supports a fundamentally improved way of observing North Sea ecosystems, thereby guiding better sustainable industrial development and conservation management strategies.

REFERENCES

Aarts, G., Brasseur, S., Poos, J. J., Schop, J., Kirkwood, R., van Kooten, T., Mul, E., Reijnders, P., Rijnsdorp, A. D., & Tulp, I. (2019). Top-down pressure on a coastal ecosystem by harbor seals. Ecosphere, 10(1), e02538. https://doi.org/10.1002/ecs2.2538

Abarenkov, K., Nilsson, R. H., Larsson, K.-H., Taylor, A. F. S., May, T. W., Frøslev, T. G., Pawlowska, J., Lindahl, B., Põldmaa, K., Truong, C., Vu, D., Hosoya, T., Niskanen, T., Piirmann, T., Ivanov, F., Zirk, A., Peterson, M., Cheeke, T. E., Ishigami, Y., … Kõljalg, U. (2024). The UNITE database for molecular identification and taxonomic communication of fungi and other eukaryotes: Sequences, taxa and classifications reconsidered. Nucleic Acids Research, 52(D1), D791–D797. https://doi.org/10.1093/nar/gkad1039

Adams, C. I. M., Jeunen, G.-J., Cross, H., Taylor, H. R., Bagnaro, A., Currie, K., Hepburn, C., Gemmell, N. J., Urban, L., Baltar, F., Stat, M., Bunce, M., & Knapp, M. (2023). Environmental DNA metabarcoding describes biodiversity across marine gradients. ICES Journal of Marine Science, 80(4), 953–971. https://doi.org/10.1093/icesjms/fsad017

Akaike, H. (1973). Information Theory and an Extension of the Maximum Likelihood Principle. Trees - Structure and Function, 29(6), 655–662. https://doi.org/10.1007/978-1-4612-0919-5_38

Alberdi, A., Aizpurua, O., Bohmann, K., Gopalakrishnan, S., Lynggaard, C., Nielsen, M., & Gilbert, M. T. P. (2019). Promises and pitfalls of using high-throughput sequencing for diet analysis. Molecular Ecology Resources, 19(2), 327–348. https://doi.org/10.1111/1755-0998.12960

Alberdi, A., Aizpurua, O., Gilbert, M. T. P., & Bohmann, K. (2018). Scrutinizing key steps for reliable metabarcoding of environmental samples. Methods in Ecology and Evolution, 9(1), 134–147. https://doi.org/10.1111/2041-210X.12849

Almqvist, G., Strandmark, A. K., & Appelberg, M. (2010). Has the invasive round goby caused new links in Baltic food webs? Environmental Biology of Fishes, 89(1), 79–93. https://doi.org/10.1007/s10641-010-9692-z

Ammon, U. von, Wood, S. A., Laroche, O., Zaiko, A., Tait, L., Lavery, S., Inglis, G. J., & Pochon, X. (2018). Combining morpho-taxonomy and metabarcoding enhances the detection of non-indigenous marine pests in biofouling communities. Scientific Reports, 8(1), 16290. https://doi.org/10.1038/s41598-018-34541-1

An, H.-E., Mun, M.-H., & Kim, C.-B. (2023). Metabarcoding by Combining Environmental DNA with Environmental RNA to Monitor Fish Species in the Han River, Korea. Fishes, 8(11), Article 11. https://doi.org/10.3390/fishes8110550

Anastasiadi, D., & Piferrer, F. (2020). A clockwork fish: Age prediction using DNA methylation-based biomarkers in the European seabass. Molecular Ecology Resources, 20(2), 387–397. https://doi.org/10.1111/1755-0998.13111

Andersen, J. H., Al-Hamdani, Z., Harvey, E. T., Kallenbach, E., Murray, C., & Stock, A. (2020). Relative impacts of multiple human stressors in estuaries and coastal waters in the North Sea–Baltic Sea transition zone. Science of the Total Environment, 704, 135316–135316. https://doi.org/10.1016/j.scitotenv.2019.135316

Anderson, J. T., Van Holliday, D., Kloser, R., Reid, D. G., & Simard, Y. (2008). Acoustic seabed classification: Current practice and future directions. ICES Journal of Marine Science, 65(6), 1004–1011. https://doi.org/10.1093/icesjms/fsn061

Antich, A., Palacin, C., Wangensteen, O. S., & Turon, X. (2021). To denoise or to cluster, that is not the question: Optimizing pipelines for COI metabarcoding and metaphylogeography. BMC Bioinformatics, 22(1), 177. https://doi.org/10.1186/s12859-021-04115-6

Arkema, K. K., Abramson, S. C., & Dewsbury, B. M. (2006). Marine ecosystem-based management: From characterization to implementation. Frontiers in Ecology and the Environment, 4(10), 525–532. https://doi.org/10.1890/1540-9295(2006)4[525:MEMFCT]2.0.CO;2

Arranz, V., Pearman, W. S., Aguirre, J. D., & Liggins, L. (2020). MARES, a replicable pipeline and curated reference database for marine eukaryote metabarcoding. Scientific Data, 7(1), 209. https://doi.org/10.1038/s41597-020-0549-9

Aylagas, E., Borja, A., Pochon, X., Zaiko, A., Keeley, N., Bruce, K., Hong, P., Ruiz, G. M., Stein, E. D., Theroux, S., Geraldi, N., Ortega, A., Gajdzik, L., Coker, D. J., Katan, Y., Hikmawan, T., Saleem, A., Alamer, S., Jones, B. H., … Carvalho, S. (2020). Translational Molecular Ecology in practice: Linking DNA-based methods to actionable marine environmental management. Science of The Total Environment, 744, 140780. https://doi.org/10.1016/j.scitotenv.2020.140780

Aylagas, E., Mendibil, I., Borja, Á., & Rodríguez-Ezpeleta, N. (2016). Marine Sediment Sample Pre-processing for Macroinvertebrates Metabarcoding: Mechanical Enrichment and Homogenization. Frontiers in Marine Science, 3. https://doi.org/10.3389/fmars.2016.00203

Ayre, D. J. (1985). Localized adaptation of clones of the sea anemone Actinia tenebrosa. Evolution, 39(6), 1250–1260.

Badsha, K. S., & Sainsbury, M. (1978). Aspects of the biology and heavy metal accumulation of Ciliata mustela. Journal of Fish Biology, 12(3), 213–220. https://doi.org/10.1111/J.1095-8649.1978.TB04167.X

Baird, D. J., & Hajibabaei, M. (2012). Biomonitoring 2.0: A new paradigm in ecosystem assessment made possible by next-generation DNA sequencing. Molecular Ecology, 21(8), 2039–2044. https://doi.org/10.1111/j.1365-294X.2012.05519.x

Balard, A., Baltazar-Soares, M., Eizaguirre, C., & Heckwolf, M. J. (2024). An epigenetic toolbox for conservation biologists. Evolutionary Applications, 17(6), e13699. https://doi.org/10.1111/eva.13699

Balasingham, K. D., Walter, R. P., & Heath, D. D. (2017). Residual eDNA detection sensitivity assessed by quantitative real-time PCR in a river ecosystem. Molecular Ecology Resources, 17(3), 523–532. https://doi.org/10.1111/1755-0998.12598

Balasingham, K. D., Walter, R. P., Mandrak, N. E., & Heath, D. D. (2018). Environmental DNA detection of rare and invasive fish species in two Great Lakes tributaries. Molecular Ecology, 27(1), 112–127. https://doi.org/10.1111/mec.14395

Baloğlu, B., Chen, Z., Elbrecht, V., Braukmann, T., MacDonald, S., & Steinke, D. (2021). A workflow for accurate metabarcoding using nanopore MinION sequencing. Methods in Ecology and Evolution, 12(5), 794–804. https://doi.org/10.1111/2041-210X.13561

Barco, A., Kullmann, B., Knebelsberger, T., Sarrazin, V., Kuhs, V., Kreutle, A., Pusch, C., & Thiel, R. (2022). Detection of fish species from marine protected areas of the North Sea using environmental DNA. Journal of Fish Biology, 101(3), 722–727. https://doi.org/10.1111/jfb.15111

Bass, D., Christison, K. W., Stentiford, G. D., Cook, L. S. J., & Hartikainen, H. (2023). Environmental DNA/RNA for pathogen and parasite detection, surveillance, and ecology. Trends in Parasitology, 39(4), 285–304. https://doi.org/10.1016/j.pt.2022.12.010

Batavia, C., & Nelson, M. P. (2017). For goodness sake! What is intrinsic value and why should we care? Biological Conservation, 209, 366–376. https://doi.org/10.1016/j.biocon.2017.03.003

Beaugrand, G., & Kirby, R. R. (2010). Climate, plankton and cod. Global Change Biology, 16(4), 1268–1280. https://doi.org/10.1111/j.1365-2486.2009.02063.x

Beck, M. W., Heck, K. L., Able, K. W., Childers, D. L., Eggleston, D. B., Gillanders, B. M., Halpern, B., Hays, C. G., Hoshino, K., Minello, T. J., Orth, R. J., Sheridan, P. F., & Weinstein, M. P. (2001). The identification, conservation, and management of estuarine and marine nurseries for fish and invertebrates. BioScience, 51(8), 633–641. https://doi.org/10.1641/0006-3568(2001)051[0633:TICAMO]2.0.CO;2

Beentjes, K. K., Speksnijder, A. G. C. L., Schilthuizen, M., Hoogeveen, M., & Van Der Hoorn, B. B. (2019). The effects of spatial and temporal replicate sampling on eDNA metabarcoding. PeerJ, 2019(7), e7335–e7335. https://doi.org/10.7717/peerj.7335

Beermann, J., Gutow, L., Wührdemann, S., Konijnenberg, R., Heinicke, K., Bildstein, T., Jaklin, S., Gusky, M., Zettler, M. L., Dannheim, J., & Pesch, R. (2023). Characterization and differentiation of sublittoral sandbanks in the southeastern North Sea. Biodiversity and Conservation, 32(8), 2747–2768. https://doi.org/10.1007/s10531-023-02629-4

Belenguer, Á., Naya-Català, F., Calduch-Giner, J. À., & Pérez-Sánchez, J. (2024). Exploring Multifunctional Markers of Biological Age in Farmed Gilthead Sea Bream (Sparus aurata): A Transcriptomic and Epigenetic Interplay for an Improved Fish Welfare Assessment Approach. International Journal of Molecular Sciences, 25(18), 9836. https://doi.org/10.3390/ijms25189836

Beng, K. C., & Corlett, R. T. (2020). Applications of environmental DNA (eDNA) in ecology and conservation: Opportunities, challenges and prospects. Biodiversity and Conservation, 29(7), 2089–2121. https://doi.org/10.1007/s10531-020-01980-0

Bergstad, O. A., & Gordon, J. D. M. (1994). Deep-water ichthyoplankton of the Skagerrak with special reference to Coryphaenoides rupestris Gunnerus, 1765 (Pisces, Macrouridae) and Argentina silus (Ascanius, 1775) (Pisces, Argentinidae). Sarsia, 79(1), 33–43. https://doi.org/10.1080/00364827.1994.10413545

Bernatchez, L., Ferchaud, A.-L., Berger, C. S., Venney, C. J., & Xuereb, A. (2024). Genomics for monitoring and understanding species responses to global climate change. Nature Reviews Genetics, 25(3), 165–183. https://doi.org/10.1038/s41576-023-00657-y

Bessey, C., Jarman, S. N., Berry, O., Olsen, Y. S., Bunce, M., Simpson, T., Power, M., McLaughlin, J., Edgar, G. J., & Keesing, J. (2020). Maximizing fish detection with eDNA metabarcoding. Environmental DNA, edn3.74-edn3.74. https://doi.org/10.1002/edn3.74

Biodiversity strategy for 2030—European Commission. (2025, November 6). European Commission. https://environment.ec.europa.eu/strategy/biodiversity-strategy-2030_en

Bista, I., Carvalho, G. R., Tang, M., Walsh, K., Zhou, X., Hajibabaei, M., Shokralla, S., Seymour, M., Bradley, D., Liu, S., Christmas, M., & Creer, S. (2018). Performance of amplicon and shotgun sequencing for accurate biomass estimation in invertebrate community samples. Molecular Ecology Resources, 18(5), 1020–1034. https://doi.org/10.1111/1755-0998.12888

Bista, I., Carvalho, G. R., Walsh, K., Seymour, M., Hajibabaei, M., Lallias, D., Christmas, M., & Creer, S. (2017). Annual time-series analysis of aqueous eDNA reveals ecologically relevant dynamics of lake ecosystem biodiversity. Nature Communications, 8(1), 14087. https://doi.org/10.1038/ncomms14087

Blackman, R., Couton, M., Keck, F., Kirschner, D., Carraro, L., Cereghetti, E., Perrelet, K., Bossart, R., Brantschen, J., Zhang, Y., & Altermatt, F. (2024). Environmental DNA: The next chapter. Molecular Ecology, 33(11). https://doi.org/10.1111/mec.17355

Blasiak, R., Wynberg, R., Grorud-Colvert, K., Thambisetty, S., Bandarra, N. M., Canário, A. V. M., Da Silva, J., Duarte, C. M., Jaspars, M., Rogers, A., Sink, K., & Wabnitz, C. C. C. (2020). The ocean genome and future prospects for conservation and equity. Nature Sustainability, 3(8), 588–596. https://doi.org/10.1038/s41893-020-0522-9

Bleidorn, C. (2016). Third generation sequencing: Technology and its potential impact on evolutionary biodiversity research. Systematics and Biodiversity, 14(1), 1–8. https://doi.org/10.1080/14772000.2015.1099575

Bleijswijk, J. D. L., Engelmann, J. C., Klunder, L., Witte, H. J., Witte, J. IJ., & Veer, H. W. (2020). Analysis of a coastal North Sea fish community: Comparison of aquatic environmental DNA concentrations to fish catches. Environmental DNA, edn3.67-edn3.67. https://doi.org/10.1002/edn3.67

Bludau, D., Sieber, G., Shah, M., Deep, A., Boenigk, J., & Beisser, D. (2025). Breaking the Standard: Can Oxford Nanopore Technologies Sequencing Compete With Illumina in Protistan Amplicon Studies? Environmental DNA, 7(2), e70084. https://doi.org/10.1002/edn3.70084

Bock, S. L., Smaga, C. R., McCoy, J. A., & Parrott, B. B. (2022). Genome-wide DNA methylation patterns harbour signatures of hatchling sex and past incubation temperature in a species with environmental sex determination. Molecular Ecology, 31(21), 5487–5505. https://doi.org/10.1111/mec.16670

Bogan, S. N., & Yi, S. V. (2024). Potential Role of DNA Methylation as a Driver of Plastic Responses to the Environment Across Cells, Organisms, and Populations. Genome Biology and Evolution, 16(2), evae022. https://doi.org/10.1093/gbe/evae022

BOLD. (n.d.). Retrieved March 30, 2025, from https://www.ibol.org/phase1/bold/

Bolger, A. M., Lohse, M., & Usadel, B. (2014). Trimmomatic: A flexible trimmer for Illumina sequence data. Bioinformatics, 30(15), 2114–2120. https://doi.org/10.1093/bioinformatics/btu170

Bonasio, R. (2015). The expanding epigenetic landscape of non-model organisms. Journal of Experimental Biology, 218(1), 114–122. https://doi.org/10.1242/jeb.110809

Borchardt, M. A., Boehm, A. B., Salit, M., Spencer, S. K., Wigginton, K. R., & Noble, R. T. (2021). The Environmental Microbiology Minimum Information (EMMI) Guidelines: qPCR and dPCR Quality and Reporting for Environmental Microbiology. Environmental Science & Technology, 55(15), 10210–10223. https://doi.org/10.1021/acs.est.1c01767

Bordoux, V., Parcerisas, C., Debusschere, E., Jälmby, M., Murk, A. J., & van der Ven, R. M. (2025). Rapid Fish Sound Detection Using Human-in-The-Loop Active Learning (SSRN Scholarly Paper No. 5403106). Social Science Research Network. https://doi.org/10.2139/ssrn.5403106

Borja, Á., Elliott, M., Carstensen, J., Heiskanen, A.-S., & van de Bund, W. (2010). Marine management – Towards an integrated implementation of the European Marine Strategy Framework and the Water Framework Directives. Marine Pollution Bulletin, 60(12), 2175–2186. https://doi.org/10.1016/j.marpolbul.2010.09.026

Bourdonnais, E., Le Bris, C., Brauge, T., & Midelet, G. (2024). Tracking antimicrobial resistance indicator genes in wild flatfish from the English Channel and the North Sea area: A one health concern. Environmental Pollution, 343, 123274. https://doi.org/10.1016/j.envpol.2023.123274

Bowers, H., Pochon, X., Von Ammon, U., Gemmell, N., Stanton, J.-A., Jeunen, G.-J., Sherman, C., & Zaiko, A. (2021). Towards the Optimization of eDNA/eRNA Sampling Technologies for Marine Biosecurity Surveillance. Water, 13(8), 1113. https://doi.org/10.3390/w13081113

Boyse, E., Robinson, K. P., Beger, M., Carr, I. M., Taylor, M., Valsecchi, E., & Goodman, S. J. (2024). Environmental DNA reveals fine-scale spatial and temporal variation of marine mammals and their prey species in a Scottish marine protected area. Environmental DNA, 6(4), e587. https://doi.org/10.1002/edn3.587

Boyse, E., Robinson, K. P., Carr, I. M., Valsecchi, E., Beger, M., & Goodman, S. J. (2025). Inferring Species Interactions From Co-occurrence Networks With Environmental DNA Metabarcoding Data in a Coastal Marine Food Web. Molecular Ecology, 34(7), e17701. https://doi.org/10.1111/mec.17701

Brandt, M. I., Trouche, B., Quintric, L., Günther, B., Wincker, P., Poulain, J., & Arnaud-Haond, S. (2021). Bioinformatic pipelines combining denoising and clustering tools allow for more comprehensive prokaryotic and eukaryotic metabarcoding. Molecular Ecology Resources, 21(6), 1904–1921. https://doi.org/10.1111/1755-0998.13398

Brandão-Dias, P. F., Shaffer, M., Guri, G., Parsons, K. M., Kelly, R. P., & Allan, E. A. (2025). Differential decay of multiple environmental nucleic acid components. Scientific Reports, 15(1), 26791. https://doi.org/10.1038/s41598-025-12916-5

Breine, J., Maes, J., Ollevier, F., & Stevens, M. (2011). Fish assemblages across a salinity gradient in the Zeeschelde estuary (Belgium). Belgian Journal of Zoology, 141, 21–44. https://doi.org/10.26496/bjz.2011.148

Breine, N. T., De Backer, A., Van Colen, C., Moens, T., Hostens, K., & Van Hoey, G. (2018). Structural and functional diversity of soft-bottom macrobenthic communities in the Southern North Sea. Estuarine, Coastal and Shelf Science, 214, 173–184. https://doi.org/10.1016/j.ecss.2018.09.012

Brian, A., Venables, B., Bates, D. M., Firth, D., & Ripley, M. B. (2024). Package ‘ MASS .’

Brink, K., Thomas, C. L., Jones, A., Chan, T. W., & Mallon, E. B. (2024). Exploring the ageing methylome in the model insect, Nasonia vitripennis. BMC Genomics, 25(1), 305. https://doi.org/10.1186/s12864-024-10211-7

Brooks, S. M., Spencer, T., & Christie, E. K. (2017). Storm impacts and shoreline recovery: Mechanisms and controls in the southern North Sea. Geomorphology, 283, 48–60. https://doi.org/10.1016/j.geomorph.2017.01.007

Brown, C. J., Broadley, A., Adame, M. F., Branch, T. A., Turschwell, M. P., & Connolly, R. M. (2019). The assessment of fishery status depends on fish habitats. Fish and Fisheries, 20(1), 1–14. https://doi.org/10.1111/faf.12318

Bucklin, A., Lindeque, P. K., Rodriguez-Ezpeleta, N., Albaina, A., & Lehtiniemi, M. (2016). Metabarcoding of marine zooplankton: Prospects, progress and pitfalls. Journal of Plankton Research, 38(3), 393–400. https://doi.org/10.1093/plankt/fbw023

Bucklin, A., Steinke, D., & Blanco-Bercial, L. (2011). DNA Barcoding of Marine Metazoa. Annual Review of Marine Science, 3(1), 471–508. https://doi.org/10.1146/annurev-marine-120308-080950

Bulcke, L. V. den, Backer, A. D., Ampe, B., Maes, S., Wittoeck, J., Waegeman, W., Hostens, K., & Derycke, S. (2021). Towards harmonization of DNA metabarcoding for monitoring marine macrobenthos: The effect of technical replicates and pooled DNA extractions on species detection. Metabarcoding and Metagenomics, 5, e71107. https://doi.org/10.3897/mbmg.5.71107

Bunholi, I. V., Foster, N. R., & Casey, J. M. (2023). Environmental DNA and RNA in aquatic community ecology: Toward methodological standardization. Environmental DNA, 5(6), 1133–1147. https://doi.org/10.1002/edn3.476

Burgers, L., Meijer, E., Nowak, E., & Noordzee (Amsterdam), A. van de. (2020). De stem van de Noordzee: Een pleidooi voor vloeibaar denken. Boom.

Bustin, S. A. (2024). Improving the quality of quantitative polymerase chain reaction experiments: 15 years of MIQE. Molecular Aspects of Medicine, 96, 101249. https://doi.org/10.1016/j.mam.2024.101249

Bustin, S. A., Benes, V., Garson, J. A., Hellemans, J., Huggett, J., Kubista, M., Mueller, R., Nolan, T., Pfaffl, M. W., Shipley, G. L., Vandesompele, J., & Wittwer, C. T. (2009). The MIQE Guidelines: Minimum Information for Publication of Quantitative Real-Time PCR Experiments. Clinical Chemistry, 55(4), 611–622. https://doi.org/10.1373/clinchem.2008.112797

Bustin, S., & Nolan, T. (2017). Talking the talk, but not walking the walk: RT-qPCR as a paradigm for the lack of reproducibility in molecular research. European Journal of Clinical Investigation, 47(10), 756–774. https://doi.org/10.1111/eci.12801

Cahill, A. E., Pearman, J. K., Borja, A., Carugati, L., Carvalho, S., Danovaro, R., Dashfield, S., David, R., Féral, J.-P., Olenin, S., Šiaulys, A., Somerfield, P. J., Trayanova, A., Uyarra, M. C., & Chenuil, A. (2018). A comparative analysis of metabarcoding and morphology-based identification of benthic communities across different regional seas. Ecology and Evolution, 8(17), 8908–8920. https://doi.org/10.1002/ece3.4283

Cai, W., Harper, L. R., Neave, E. F., Shum, P., Craggs, J., Arias, M. B., Riesgo, A., & Mariani, S. (2022). Environmental DNA persistence and fish detection in captive sponges. Molecular Ecology Resources, 22(8), 2956–2966. https://doi.org/10.1111/1755-0998.13677

Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., & Holmes, S. P. (2016). DADA2: High-resolution sample inference from Illumina amplicon data. Nature Methods, 13(7), 581–583. https://doi.org/10.1038/nmeth.3869

Callens, M., Le Berre, G., Van Den Bulcke, L., Lolivier, M., & Derycke, S. (2025). An Accessible Metagenomic Strategy Allows for Better Characterisation of Invertebrate Bulk Samples. Molecular Ecology Resources, e14126. https://doi.org/10.1111/1755-0998.14126

Camphuysen, C. J., & Henderson, P. A. (2017). North Sea fish and their remains. In Royal Netherlands Institute for Sea Research & Pisces Conservation Limited. Royal Netherlands Institute for Sea Research & Pisces Conservation Limited.

Caporaso, J. G., Lauber, C. L., Costello, E. K., Berg-Lyons, D., Gonzalez, A., Stombaugh, J., Knights, D., Gajer, P., Ravel, J., Fierer, N., Gordon, J. I., & Knight, R. (2011). Moving pictures of the human microbiome. Genome Biology, 12(5). https://doi.org/10.1186/gb-2011-12-5-r50

Cappo, M., Speare, P., & De'ath, G. (2004). Comparison of baited remote underwater video stations (BRUVS) and prawn (shrimp) trawls for assessments of fish biodiversity in inter-reefal areas of the Great Barrier Reef Marine Park. Journal of Experimental Marine Biology and Ecology, 302(2), 123–152. https://doi.org/10.1016/j.jembe.2003.10.006

Capuzzo, E., Lynam, C. P., Barry, J., Stephens, D., Forster, R. M., Greenwood, N., McQuatters-Gollop, A., Silva, T., van Leeuwen, S. M., & Engelhard, G. H. (2018). A decline in primary production in the North Sea over 25 years, associated with reductions in zooplankton abundance and fish stock recruitment. Global Change Biology, 24(1), e352–e364. https://doi.org/10.1111/gcb.13916

Carr, C. M., Hardy, S. M., Brown, T. M., Macdonald, T. A., & Hebert, P. D. N. (2011). A Tri-Oceanic Perspective: DNA Barcoding Reveals Geographic Structure and Cryptic Diversity in Canadian Polychaetes. PLOS ONE, 6(7), e22232. https://doi.org/10.1371/journal.pone.0022232

Carradec, Q., Pelletier, E., Da Silva, C., Alberti, A., Seeleuthner, Y., Blanc-Mathieu, R., Lima-Mendez, G., Rocha, F., Tirichine, L., Labadie, K., Kirilovsky, A., Bertrand, A., Engelen, S., Madoui, M., Méheust, R., Poulain, J., Romac, S., Richter, D., Yoshikawa, G., … Wincker, P. (2018). A global ocean atlas of eukaryotic genes. NATURE COMMUNICATIONS, 9. https://doi.org/10.1038/s41467-017-02342-1

Carvalho, L., Mackay, E. B., Cardoso, A. C., Baattrup-Pedersen, A., Birk, S., Blackstock, K. L., Borics, G., Borja, A., Feld, C. K., Ferreira, M. T., Globevnik, L., Grizzetti, B., Hendry, S., Hering, D., Kelly, M., Langaas, S., Meissner, K., Panagopoulos, Y., Penning, E., … Solheim, A. L. (2019). Protecting and restoring Europe's waters: An analysis of the future development needs of the Water Framework Directive. Science of The Total Environment, 658, 1228–1238. https://doi.org/10.1016/j.scitotenv.2018.12.255

Castro-Wallace, S. L., Chiu, C. Y., John, K. K., Stahl, S. E., Rubins, K. H., McIntyre, A. B. R., Dworkin, J. P., Lupisella, M. L., Smith, D. J., Botkin, D. J., Stephenson, T. A., Juul, S., Turner, D. J., Izquierdo, F., Federman, S., Stryke, D., Somasekar, S., Alexander, N., Yu, G., … Burton, A. S. (2017). Nanopore DNA Sequencing and Genome Assembly on the International Space Station. Scientific Reports, 7, 18022. https://doi.org/10.1038/s41598-017-18364-0

Chamberlain, S. A., & Szöcs, E. (2013). Taxize: Taxonomic search and retrieval in R. F1000Research, 2. https://doi.org/10.12688/f1000research.2-191.v2

Chang, J. J. M., Ip, Y. C. A., Neo, W. L., Mowe, M. A. D., Jaafar, Z., & Huang, D. (2024). Primed and ready: Nanopore metabarcoding can now recover highly accurate consensus barcodes that are generally indel-free. BMC Genomics, 25(1), 842. https://doi.org/10.1186/s12864-024-10767-4

Chen, P., Sun, Z., Wang, J., Liu, X., Bai, Y., Chen, J., Liu, A., Qiao, F., Chen, Y., Yuan, C., Sha, J., Zhang, J., Xu, L.-Q., & Li, J. (2023). Portable nanopore-sequencing technology: Trends in development and applications. Frontiers in Microbiology, 14. https://doi.org/10.3389/fmicb.2023.1043967

Chevene, F., Doleadec, S., & Chessel, D. (1994). A fuzzy coding approach for the analysis of long-term ecological data. Freshwater Biology, 31(3), 295–309. https://doi.org/10.1111/J.1365-2427.1994.TB01742.X

Christianen, M. J. A., Lengkeek, W., Bergsma, J. H., Coolen, J. W. P., Didderen, K., Dorenbosch, M., Driessen, F. M. F., Kamermans, P., Reuchlin-Hugenholtz, E., Sas, H., Smaal, A., van den Wijngaard, K. A., & van der Have, T. M. (2018). Return of the native facilitated by the invasive? Population composition, substrate preferences and epibenthic species richness of a recently discovered shellfish reef with native European flat oysters (Ostrea edulis) in the North Sea. Marine Biology Research, 14(6), 590–597. https://doi.org/10.1080/17451000.2018.1498520

Christodoulou, M., Derycke, S., Beentjes, K. K., Hillewaert, H., Laakmann, S., Lundin, K., Kamyab, E., Khodami, S., Maes, S., Reiss, H., Uhlir, C., Van Den Bulcke, L., Van Der Hoorn, B., De Backer, A., & Arbizu, P. M. (2025). A taxonomically reliable DNA barcode reference library for North Sea macrobenthos. Scientific Data, 12(1), 1198. https://doi.org/10.1038/s41597-025-05500-z

Christodoulou, M., van der Hoorn, B., Van den Bulcke, L., Derycke, S., De Backer, A., Kröncke, I., & Arbizu, P. M. (2021). A reliable DNA barcode reference library for the identifica-

tion of benthic invertebrates: Essential for biomonitoring of the North Sea. ARPHA Conference Abstracts, 4, e65063. https://aca.pensoft.net/article/65063/download/pdf/

Chwalińska, M., Karlicki, M., Romac, S., Not, F., & Karnkowska, A. (2025). From short to long reads: Enhanced protist diversity profiling via Nanopore metabarcoding. Metabarcoding and Metagenomics, 9, e163750. https://doi.org/10.3897/mbmg.9.163750

Claire Rosemond, R., Paxton, A. B., Lemoine, H. R., Fegley, S. R., & Peterson, C. H. (2018). Fish use of reef structures and adjacent sand flats: Implications for selecting minimum buffer zones between new artificial reefs and existing reefs. Marine Ecology Progress Series, 587, 187–199. https://doi.org/10.3354/MEPS12428

Claisse, J. T., Pondella, D. J., Love, M., Zahn, L. A., Williams, C. M., Williams, J. P., & Bull, A. S. (2014). Oil platforms off California are among the most productive marine fish habitats globally. Proceedings of the National Academy of Sciences of the United States of America, 111(43), 15462–15467. https://doi.org/10.1073/pnas.1411477111

Clare, D. S., Robinson, L. A., & Frid, C. L. J. (2015). Community variability and ecological functioning: 40 years of change in the North Sea benthos. Marine Environmental Research, 107, 24–34. https://doi.org/10.1016/j.marenvres.2015.03.012

Clark, A. J., Atkinson, S. R., Scarponi, V., Cane, T., Geraldi, N. R., Hendy, I. W., Shipway, J. R., & Peck, M. (2024). Cost-effort analysis of Baited Remote Underwater Video (BRUV) and environmental DNA (eDNA) in monitoring marine ecological communities. PeerJ, 12, e17091. https://doi.org/10.7717/peerj.17091

Claudet, J. (2018). Six conditions under which MPAs might not appear effective (when they are). ICES Journal of Marine Science, 75(3), 1172–1174. https://doi.org/10.1093/icesjms/fsx074

Clover, C. (2022). Rewilding the Sea: How to Save our Oceans. Random House.

Coates, D. A., Van Hoey, G., Colson, L., Vincx, M., & Vanaverbeke, J. (2015). Rapid macrobenthic recovery after dredging activities in an offshore wind farm in the Belgian part of the North Sea. Hydrobiologia, 756(1), 3–18. https://doi.org/10.1007/s10750-014-2103-2

Coen, L. D., Luckenbach, M. W., & Breitburg, D. L. (1999). The Role of Oyster Reefs as Essential Fish Habitat: A Review of Current Knowledge and Some New Perspectives. American Fisheries Society Symposium, 22, 438–454.

Cole, J. R., Chai, B., Marsh, T. L., Farris, R. J., Wang, Q., Kulam, S. A., Chandra, S., McGarrell, D. M., Schmidt, T. M., Garrity, G. M., & Tiedje, J. M. (2003). The Ribosomal Database Project (RDP-II): Previewing a new autoaligner that allows regular updates and the new prokaryotic taxonomy. Nucleic Acids Research, 31(1), 442–443. https://doi.org/10.1093/NAR/GKG039

Collins, R. A., Wangensteen, O. S., O'Gorman, E. J., Mariani, S., Sims, D. W., & Genner, M. J. (2018). Persistence of environmental DNA in marine systems. Communications Biology, 1(1), 185. https://doi.org/10.1038/s42003-018-0192-6

Connolly, R. M., Jinks, K. I., Shand, A., Taylor, M. D., Gaston, T. F., Becker, A., & Jinks, E. L. (2023). Out of the shadows: Automatic fish detection from acoustic cameras. Aquatic Ecology, 57(4), 833–844. https://doi.org/10.1007/s10452-022-09967-5

Cook, L. S. J., Briscoe, A. G., Fonseca, V. G., Boenigk, J., Woodward, G., & Bass, D. (2025). Microbial, holobiont, and Tree of Life eDNA/eRNA for enhanced ecological assessment. Trends in Microbiology, 33(1), 48–65. https://doi.org/10.1016/j.tim.2024.07.003

Coolen JWP, Bittner O, Driessen FMF, van Dongen U, Siahaya MS, de Groot W, Mavraki N, Bolam SG, van der Weide B (2020) Ecological implications of removing a concrete gas platform in the North Sea. J Sea Res 166:101968.

Coolen JWP, Bos OG, Glorius S, Lengkeek W, Cuperus J, van der Weide B, Agüera A (2015) Reefs, sand and reef-like sand: A comparison of the benthic biodiversity of habitats in the Dutch Borkum Reef Grounds. J Sea Res 103:84–92.

Coolen JWP, Coral C, Witbaard R, Doorenspleet K, Hovenkamp P, Schaars LK, Colona F, Yeuw TT, Heidemans Z, Studies T (2023) Cruise report ReViFES 64PE524 Noss Head Modiolus modiolus reef. 1–101.

Coolen, J. W. P., Bittner, O., Driessen, F. M. F., van Dongen, U., Siahaya, M. S., de Groot, W., Mavraki, N., Bolam, S. G., & van der Weide, B. (2020). Ecological implications of removing a concrete gas platform in the North Sea. Journal of Sea Research, 166(September), 101968. https://doi.org/10.1016/j.seares.2020.101968

Coolen, J. W. P., Bos, O. G., Glorius, S., Lengkeek, W., Cuperus, J., van der Weide, B., & Agüera, A. (2015). Reefs, sand and reef-like sand: A comparison of the benthic biodiversity of habitats in the Dutch Borkum Reef Grounds. Journal of Sea Research, 103, 84–92. https://doi.org/10.1016/j.seares.2015.06.010

Coolen, J. W. P., Coral, C., Witbaard, R., Doorenspleet, K., Hovenkamp, P., Schaars, L. K., Colona, F., Yeuw, T. T., Heidemans, Z., & Studies, T. (2023). Cruise report ReViFES 64PE524 Noss Head Modiolus modiolus reef. (October), 1–101.

Coolen, J. W. P., Vanaverbeke, J., Dannheim, J., Garcia, C., Birchenough, S. N. R., Krone, R., & Beermann, J. (2022). Generalized changes of benthic communities after construction of wind farms in the southern North Sea. Journal of Environmental Management, 315, 115173. https://doi.org/10.1016/j.jenvman.2022.115173

Coral, C., Kornau, L. M., van der Heide, T., Coolen, J. W. P., Witbaard, R., Bouma, T. J., & Christianen, M. J. A. (2025). *Subtidal temperate reefs in marginal seas enhance biodiversity, food web complexity, and ecosystem stability.* Marine Ecology Progress Series, 764, 15–32. https://doi.org/10.3354/meps14883

Cordier, T., Esling, P., Lejzerowicz, F., Visco, J., Ouadahi, A., Martins, C., Cedhagen, T., & Pawlowski, J. (2017). Predicting the Ecological Quality Status of Marine Environments from eDNA Metabarcoding Data Using Supervised Machine Learning. Environmental Science & Technology, 51(16), 9118–9126. https://doi.org/10.1021/acs.est.7b01518

Cordier, T., Frontalini, F., Cermakova, K., Apothéloz-Perret-Gentil, L., Treglia, M., Scantamburlo, E., Bonamin, V., & Pawlowski, J. (2019a). Multi-marker eDNA metabarcoding survey to assess the environmental impact of three offshore gas platforms in the North Adriatic Sea (Italy). Marine Environmental Research, 146, 24–34. https://doi.org/10.1016/J.MARENVRES.2018.12.009

Cordier, T., Lanzén, A., Apothéloz-Perret-Gentil, L., Stoeck, T., & Pawlowski, J. (2019b). Embracing Environmental Genomics and Machine Learning for Routine Biomonitoring. Trends in Microbiology, 27(5), 387–397. https://doi.org/10.1016/j.tim.2018.10.012

Cornelis, I., De Backer, A., Maes, S., Vanhollebeke, J., Brys, R., Ruttink, T., Hostens, K., & Derycke, S. (2024). Environmental DNA for monitoring the impact of offshore wind farms on fish and invertebrate community structures. Environmental DNA, 6(4), e575. https://doi.org/10.1002/edn3.575

Couce, E., Schratzberger, M., & Engelhard, G. H. (2020). Reconstructing three decades of total international trawling effort in the North Sea. Earth System Science Data, 12(1), 373–386. https://doi.org/10.5194/essd-12-373-2020

Courtaillac, K.-L., Landschoff, J., Hull, K., & von der Heyden, S. (2024). The Effect of Spatio-Temporal Sampling and Biological Replication on the Detection of Kelp Forest Fish Communities Using eDNA Metabarcoding. Environmental DNA, 6(5), e70023. https://doi.org/10.1002/edn3.70023

Cousson, A., Mahé, F., Guyet, U., Razafimahafaly, D., & Bernard, L. (2025). NanoASV: A snakemake workflow for reproducible field-based Nanopore full-length 16S metabarcoding amplicon data analysis. Bioinformatics, 41(3), btaf089. https://doi.org/10.1093/bioinformatics/btaf089

Crampton-Platt, A., Yu, D. W., Zhou, X., & Vogler, A. P. (2016). Mitochondrial metagenomics: Letting the genes out of the bottle. GigaScience, 5(1), 15. https://doi.org/10.1186/s13742-016-0120-y

Creer, S., Deiner, K., Frey, S., Porazinska, D., Taberlet, P., Thomas, W. K., Potter, C., & Bik, H. M. (2016). The ecologist's field guide to sequence-based identification of biodiversity. Methods in Ecology and Evolution, 7(9), 1008–1018. https://doi.org/10.1111/2041-210X.12574

Cristescu, M. E. (2019). Can Environmental RNA Revolutionize Biodiversity Science? Trends in Ecology & Evolution, 34(8), 694–697. https://doi.org/10.1016/j.tree.2019.05.003

Crowley, S. E., Kess, T., Bentzen, P., Neville, V. K., Bloom, C., Smith, N., Fahner, N., Berghuis, L., Hobrecker, K., Hajibabaei, M., Duffy, S. J., & Bradbury, I. R. (2025). eDNA metabarcoding and whole genome sequencing detect European–American eel hybrids in northeastern Canada. Canadian Journal of Fisheries and Aquatic Sciences, 82, 1–11. https://doi.org/10.1139/cjfas-2024-0371

Cruz, C. D. L., & Manuel, P. (2021). The Knowledge Status of Coastal and Marine Ecosystem Services—Challenges, Limitations and Lessons Learned From the Application of the Ecosystem Services Approach in Management. Frontiers in Marine Science, 8. https://doi.org/10.3389/fmars.2021.684770

Daan, N., Bromley, P. J., Hislop, J. R. G., & Nielsen, N. A. (1990). Ecology of North Sea fish. Netherlands Journal of Sea Research, 26(2), 343–386. https://doi.org/10.1016/0077-7579(90)90096-Y

Daan, N., Gislason, H., G. Pope, J., & C. Rice, J. (2005a). Changes in the North Sea fish community: Evidence of indirect effects of fishing? ICES Journal of Marine Science, 62(2), 177–188. https://doi.org/10.1016/j.icesjms.2004.08.020

Daan, N., Heessen, H., & ter Hofstede, R. (2005b). North Sea Elasmobranchs: Distribution, abundance and biodiversity. ICES CM, 2005, 06.

Dahlgren, T. G., Hestetun, J. T., & Ray, J. (2023). The Use of eDNA to Monitor Pelagic Fish in Offshore Floating Wind Farms. Oceanography, 36(1), 94–95. https://doi.org/10.5670/oceanog.2023.s1.30

Dang, X., Lim, Y.-K., Li, Y., Roberts, S. B., Li, L., & Thiyagarajan, V. (2023). Epigenetic-associated phenotypic plasticity of the ocean acidification-acclimated edible oyster in the mariculture environment. Molecular Ecology, 32(2), 412–427. https://doi.org/10.1111/mec.16751

Danovaro, R., Carugati, L., Berzano, M., Cahill, A. E., Carvalho, S., Chenuil, A., Corinaldesi, C., Cristina, S., David, R., Dell'Anno, A., Dzhembekova, N., Garcés, E., Gasol, J. M., Goela, P., Féral, J.-P., Ferrera, I., Forster, R. M., Kurekin, A. A., Rastelli, E., … Borja, A. (2016). Implementing and Innovating Marine Monitoring Approaches for Assessing Marine Environmental Status. Frontiers in Marine Science, 3. https://doi.org/10.3389/fmars.2016.00213

Darling, J. A., & Frederick, R. M. (2018). Nucleic acids-based tools for ballast water surveillance, monitoring, and research. Journal of Sea Research, 133, 43–52. https://doi.org/10.1016/j.seares.2017.02.005

de Mendonça, S. N., & Metaxas, A. (2021). Comparing the Performance of a Remotely Operated Vehicle, a Drop Camera, and a Trawl in Capturing Deep-Sea Epifaunal Abundance and Diversity. Frontiers in Marine Science, 8. https://doi.org/10.3389/fmars.2021.631354

Degraer, S., Carey, D. A., Coolen, J. W. P., Hutchison, Z. L., Rumes, B., & Vanaverbeke, J. (2020). Offshore Wind Farm Artificial. 33(4).

Deiner, K., Bik, H. M., Mächler, E., Seymour, M., Lacoursière-Roussel, A., Altermatt, F., Creer, S., Bista, I., Lodge, D. M., de Vere, N., Pfrender, M. E., & Bernatchez, L. (2017). Environmental DNA metabarcoding: Transforming how we survey animal and plant communities. Molecular Ecology, 26(21), 5872–5895. https://doi.org/10.1111/mec.14350

Derycke, S., Maes, S., Van Den Bulcke, L., Vanhollebeke, J., Wittoeck, J., Hillewaert, H., Ampe, B., Haegeman, A., Hostens, K., & De Backer, A. (2021). Detection of Macrobenthos Species With Metabarcoding Is Consistent in Bulk DNA but Dependent on Body Size and Sclerotization in eDNA From the Ethanol Preservative. Frontiers in Marine Science, 8, 637858. https://doi.org/10.3389/fmars.2021.637858

DeSalle, R., & Goldstein, P. (2019). Review and Interpretation of Trends in DNA Barcoding. Frontiers in Ecology and Evolution, 7. https://doi.org/10.3389/fevo.2019.00302

Devloo-Delva, F., Huerlimann, R., Chua, G., Matley, J. K., Heupel, M. R., Simpfendorfer, C. A., & Maes, G. E. (2018). How does marker choice affect your diet analysis: Comparing genetic markers and digestion levels for diet metabarcoding of tropical-reef piscivores. Marine and Freshwater Research, 70(1), 8–18. https://doi.org/10.1071/MF17209

Di Muri, C., Lawson Handley, L., Bean, C. W., Li, J., Peirson, G., Sellers, G. S., Walsh, K., Watson, H. V., Winfield, I. J., & Hänfling, B. (2020). Read counts from environmental DNA (eDNA) metabarcoding reflect fish abundance and biomass in drained ponds. Metabarcoding and Metagenomics, 4, e56959. https://doi.org/10.3897/mbmg.4.56959

DiBattista, J. D., Coker, D. J., Sinclair-Taylor, T. H., Stat, M., Berumen, M. L., & Bunce, M. (2017). Assessing the utility of eDNA as a tool to survey reef-fish communities in the Red Sea. Coral Reefs, 36(4), 1245–1252. https://doi.org/10.1007/s00338-017-1618-1

Dickey-Collas, M., Nash, R. D. M., Brunel, T., van Damme, C. J. G., Marshall, C. T., Payne, M. R., Corten, A., Geffen, A. J., Peck, M. A., Hatfield, E. M. C., Hintzen, N. T., Enberg, K., Kell, L. T., & Simmonds, E. J. (2010). Lessons learned from stock collapse and recovery of North Sea herring: A review. ICES Journal of Marine Science, 67(9), 1875–1886. https://doi.org/10.1093/icesjms/fsq033

Dickson, J., Franken, O., Watson, M. S., Monnich, B., Holthuijsen, S., Eriksson, B. K., Govers, L. L., van der Heide, T., & Bouma, T. J. (2023). Who lives in a pear tree under the sea? A first look at tree reefs as a complex natural biodegradable structure to enhance biodiversity in marine systems. Frontiers in Marine Science, 10. https://doi.org/10.3389/fmars.2023.1213790

Didderen, K., Lengkeek, W., Bergsma, J. H., & Dongen, U. V. (2019). WWF & ARK Borkum Reef Ground oyster pilot. September.

Didderen, K., Lengkeek, W., Kamermans, P., Deden, B., Reuchlin-Hugenholtz, E., Bergsma, J. H., & van Gool, A. (2019b). Comprehensive report to share lessons learned in 2018.

Ditria, E. M., Buelow, C. A., Gonzalez-Rivero, M., & Connolly, R. M. (2022). Artificial intelligence and automated monitoring for assisting conservation of marine ecosystems: A perspective. Frontiers in Marine Science, 9. https://doi.org/10.3389/fmars.2022.918104

Divoky G, Tremblay Y (2013) Shifting prey in a melting ocean: seabirds reveal annual and seasonal changes in Arctic nearshore fish.

Doi, H., Fukaya, K., Oka, S. ichiro, Sato, K., Kondoh, M., & Miya, M. (2019). Evaluation of detection probabilities at the water-filtering and initial PCR steps in environmental DNA metabarcoding using a multispecies site occupancy model. Scientific Reports, 9(1), 1–8. https://doi.org/10.1038/s41598-019-40233-1

Doorenspleet, K., Jansen, L., Oosterbroek, S., Kamermans, P., Bos, O., Wurz, E., Murk, A., & Nijland, R. (2023). The long and the short of it: Nanopore based eDNA metabarcoding of marine vertebrates works; sensitivity and specificity depend on amplicon lengths (p. 2021.11.26.470087). bioRxiv. https://doi.org/10.1101/2021.11.26.470087

Doorenspleet, K., Jansen, L., Oosterbroek, S., Kamermans, P., Bos, O., Wurz, E., Murk, A., & Nijland, R. (2025). The Long and the Short of It: Nanopore-Based eDNA Metabarcoding of Marine Vertebrates Works; Sensitivity and Species-Level Assignment Depend on Amplicon Lengths. Molecular Ecology Resources, e14079. https://doi.org/10.1111/1755-0998.14079

dos Santos, L. N., Brotto, D. S., & Zalmon, I. R. (2010). Fish responses to increasing distance from artificial reefs on the Southeastern Brazilian Coast. Journal of Experimental Marine Biology and Ecology, 386(1–2), 54–60. https://doi.org/10.1016/J.JEMBE.2010.01.018

Duarte, C. M., Agusti, S., Barbier, E., Britten, G. L., Castilla, J. C., Gattuso, J.-P., Fulweiler, R. W., Hughes, T. P., Knowlton, N., Lovelock, C. E., Lotze, H. K., Predragovic, M., Poloczanska, E., Roberts, C., & Worm, B. (2020a). Rebuilding marine life. Nature, 580(7801), 39–51. https://doi.org/10.1038/s41586-020-2146-7

Duarte, S., Vieira, P. E., & Costa, F. O. (2020b). Assessment of species gaps in DNA barcode libraries of non-indigenous species (NIS) occurring in European coastal regions. Metabarcoding and Metagenomics, 4, e55162. https://doi.org/10.3897/mbmg.4.55162

Duarte, S., Vieira, P. E., Lavrador, A. S., & Costa, F. O. (2021). Status and prospects of marine NIS detection and monitoring through (e)DNA metabarcoding. Science of The Total Environment, 751, 141729. https://doi.org/10.1016/j.scitotenv.2020.141729

Dubois, B., Delitte, M., Lengrand, S., Bragard, C., Legrève, A., & Debode, F. (2024). PRONAME: A user-friendly pipeline to process long-read nanopore metabarcoding data by generating high-quality consensus sequences. Frontiers in Bioinformatics, 4. https://doi.org/10.3389/fbinf.2024.1483255

Dukan, N., Cornelis, I., Maes, S., Hostens, K., De Backer, A., & Derycke, S. (2024). Vertical and horizontal environmental DNA (eDNA) patterns of fish in a shallow and well-mixed North Duncan, E. J., Gluckman, P. D., & Dearden, P. K. (2014). Epigenetics, plasticity, and evolution: How do we link epigenetic change to phenotype? Journal of Experimental Zoology Part B: Molecular and Developmental Evolution, 322(4), 208–220. https://doi.org/10.1002/jez.b.22571

Dunlop, K., Staby, A., van der Meeren, T., Keeley, N., Olsen, E. M., Bannister, R., & Skjæraasen, J. E. (2022). Habitat associations of juvenile Atlantic cod (Gadus morhua L.) and sympatric demersal fish communities within shallow inshore nursery grounds. Estuarine, Coastal and Shelf Science, 279(July), 108111. https://doi.org/10.1016/j.ecss.2022.108111

Dunn, N., Curnick, D. J., Carbone, C., Carlisle, A. B., Chapple, T. K., Dowell, R., Ferretti, F., Jacoby, D. M. P., Schallert, R. J., Steyaert, M., Tickler, D. M., Williamson, M. J., Block, B. A., & Savolainen, V. (2023). Environmental DNA helps reveal reef shark distribution across a remote archipelago. Ecological Indicators, 154, 110718. https://doi.org/10.1016/j.ecolind.2023.110718

Dye, B., Tulp, I., van Leeuwen, A., Blom, E., & Schram, E. (2024). A rockling's choice: The trade-off between thermal preference and physical structure in the five bearded rockling, Ciliata mustela. Journal of Experimental Marine Biology and Ecology, 570(September 2023), 151959. https://doi.org/10.1016/j.jembe.2023.151959

Dziedzic, E., Sidlauskas, B., Cronn, R., Anthony, J., Cornwell, T., Friesen, T. A., Konstantinidis, P., Penaluna, B. E., Stein, S., & Levi, T. (2023). Creating, curating and evaluating a mitogenomic reference database to improve regional species identification using environmental DNA. Molecular Ecology Resources, 23(8), 1880–1904. https://doi.org/10.1111/1755-0998.13855

Easson, C. G., Boswell, K. M., Tucker, N., Warren, J. D., & Lopez, J. V. (2020). Combined eDNA and Acoustic Analysis Reflects Diel Vertical Migration of Mixed Consortia in the Gulf of Mexico. Frontiers in Marine Science, 7. https://doi.org/10.3389/fmars.2020.00552

Edgar, R. C. (2004). MUSCLE: A multiple sequence alignment method with reduced time and space complexity. BMC Bioinformatics, 5. https://doi.org/10.1186/1471-2105-5-113

eDNA Approaches for Ecosystem Health Monitoring: Focus on Pathogens, Vectors, and Microbial Assessment. (2025). In B. Mandal & B. K. Das, Laboratory Techniques for Fish Disease Diagnosis (pp. 573–622). Springer Nature Singapore. https://doi.org/10.1007/978-981-96-4620-3_25

Edwards, J. E., Buijse, A. D., Winter, H. V., & Bijleveld, A. I. (2025). Seasonal coastal residency and large-scale migration of two grey mullet species in temperate European waters. Movement Ecology, 13, 2. https://doi.org/10.1186/s40462-024-00528-z

Egeter, B., Veríssimo, J., Lopes-Lima, M., Chaves, C., Pinto, J., Riccardi, N., Beja, P., & Fonseca, N. A. (2020). Speeding up the detection of invasive aquatic species using environmental DNA and nanopore sequencing (p. 2020.06.09.142521). bioRxiv. https://doi.org/10.1101/2020.06.09.142521

Egeter, B., Veríssimo, J., Lopes-Lima, M., Chaves, C., Pinto, J., Riccardi, N., Beja, P., & Fonseca, N. A. (2022). Speeding up the detection of invasive bivalve species using environmental DNA: A Nanopore and Illumina sequencing comparison. Molecular Ecology Resources, 22(6), 2232–2247. https://doi.org/10.1111/1755-0998.13610

Eggleton, J. D., Depestele, J., Kenny, A. J., Bolam, S. G., & Garcia, C. (2018). How benthic habitats and bottom trawling affect trait composition in the diet of seven demersal and benthivorous fish species in the North Sea. Journal of Sea Research, 142, 132–146. https://doi.org/10.1016/j.seares.2018.09.013

Eirin-Lopez, J. M., & Putnam, H. M. (2019). Marine Environmental Epigenetics. Annual Review of Marine Science, 11(1), 335–368. https://doi.org/10.1146/annurev-marine-010318-095114

Eisma, D., Johnston, R., & Cadogan, J. I. G. (1997). The North Sea: An overview. Philosophical Transactions of the Royal Society of London. B, Biological Sciences, 316(1181), 461–485. https://doi.org/10.1098/rstb.1987.0032

Elbrecht, V., & Leese, F. (2015). Can DNA-Based Ecosystem Assessments Quantify Species Abundance? Testing Primer Bias and Biomass—Sequence Relationships with an Innovative Metabarcoding Protocol. PLOS ONE, 10(7), e0130324. https://doi.org/10.1371/journal.pone.0130324

Elbrecht, V., Vamos, E. E., Meissner, K., Aroviita, J., & Leese, F. (2017). Assessing strengths and weaknesses of DNA metabarcoding-based macroinvertebrate identification for routine stream monitoring. Methods in Ecology and Evolution, 8(10), 1265–1275. https://doi.org/10.1111/2041-210X.12789

Elbrecht, V., Vamos, E. E., Steinke, D., & Leese, F. (2018). Estimating intraspecific genetic diversity from community DNA metabarcoding data. PeerJ, 6, e4644. https://doi.org/10.7717/peerj.4644

Emeis, K.-C., Van Beusekom, J., Callies, U., Ebinghaus, R., Kannen, A., Kraus, G., Kröncke, I., Lenhart, H., Lorkowski, I., Matthias, V., Möllmann, C., Pätsch, J., Scharfe, M., Thomas, H., Weisse, R., & Zorita, E. (2015). The North Sea—A shelf sea in the Anthropocene. Journal of Marine Systems, 141, 18–33. https://doi.org/10.1016/j.jmarsys.2014.03.012

ENA Browser. (2025). Retrieved March 30, 2025, from https://www.ebi.ac.uk/ena/browser/home

Engelhard, G. H. (2008). One Hundred and Twenty Years of Change in Fishing Power of English North Sea Trawlers. In Advances in Fisheries Science (pp. 1–25). John Wiley & Sons, Ltd. https://doi.org/10.1002/9781444302653.ch1

Engelhard, G. H., Righton, D. A., & Pinnegar, J. K. (2014). Climate change and fishing: A century of shifting distribution in North Sea cod. Global Change Biology, 20(8), 2473–2483. https://doi.org/10.1111/gcb.12513

Engesmo, A., Strand, D., Gran-Stadniczeñko, S., Edvardsen, B., Medlin, L. K., & Eikrem, W. (2018). Development of a qPCR assay to detect and quantify ichthyotoxic flagellates along the Norwegian coast, and the first Norwegian record of Fibrocapsa japonica (Raphidophyceae). Harmful Algae, 75, 105–117. https://doi.org/10.1016/j.hal.2018.04.007

Ershova, E. A., Wangensteen, O. S., Descoteaux, R. ;, Barth-Jensen, C. ;, & Præbel, K. (2021). Metabarcoding as a quantitative tool for estimating biodiversity and relative biomass of marine zooplankton. ICES Journal of Marine Science. https://doi.org/10.1093/icesjms/fsab171

ESTABLISHMENT OF BLUE MUSSEL BEDS TO ENHANCE FISH HABITATS. (2015). Applied Ecology and Environmental Research, 13(3). https://doi.org/10.15666/aeer/1303_783798

Evans, N. T., Olds, B. P., Renshaw, M. A., Turner, C. R., Li, Y., Jerde, C. L., Mahon, A. R., Pfrender, M. E., Lamberti, G. A., & Lodge, D. M. (2016). Quantification of mesocosm fish and amphibian species diversity via environmental DNA metabarcoding. Molecular Ecology Resources, 16(1), 29–41. https://doi.org/10.1111/1755-0998.12433

Ewels, P., Magnusson, M., Lundin, S., & Käller, M. (2016). MultiQC: Summarize analysis results for multiple tools and samples in a single report. Bioinformatics, 32(19), 3047–3048. https://doi.org/10.1093/bioinformatics/btw354

FAANG data portal. (n.d.). Retrieved May 20, 2025, from https://data.faang.org/home

Farnsworth, K. D., Lyashevska, O., & Fung, T. (2012). Functional complexity: The source of value in biodiversity. Ecological Complexity, 11, 46–52. https://doi.org/10.1016/j.ecocom.2012.02.001

Fediajevaite, J., Priestley, V., Arnold, R., & Savolainen, V. (2021). Meta-analysis shows that environmental DNA outperforms traditional surveys, but warrants better reporting standards. Ecology and Evolution, 11(9), 4803–4815. https://doi.org/10.1002/ece3.7382

Feist, S. M., & Lance, R. F. (2021). Advanced molecular-based surveillance of quagga and zebra mussels: A review of environmental DNA/RNA (eDNA/eRNA) studies and considerations for future directions. NeoBiota, 66, 117–159.

Fernandes, P. G., & Cook, R. M. (2013). Reversal of fish stock decline in the northeast atlantic. Current Biology, 23(15), 1432–1437. https://doi.org/10.1016/j.cub.2013.06.016

Ferreira, A. O., Azevedo, O. M., Barroso, C., Duarte, S., Egas, C., Fontes, J. T., Ré, P., Santos, A. M. P., & Costa, F. O. (2024). Multi-marker DNA metabarcoding for precise species

identification in ichthyoplankton samples. Scientific Reports, 14(1), 19772. https://doi.org/10.1038/s41598-024-69963-7

Ficetola, G. F., & Taberlet, P. (2023). Towards exhaustive community ecology via DNA metabarcoding. Molecular Ecology, 32(23), 6320–6329. https://doi.org/10.1111/mec.16881

Ficetola, G. F., Miaud, C., Pompanon, F., & Taberlet, P. (2008). Species detection using environmental DNA from water samples. Biology Letters, 4(4), 423–425. https://doi.org/10.1098/rsbl.2008.0118

Fjøsne, K., & Gjøsæter, J. (1996). Dietary composition and the potential of food competition between 0-group cod (Gadus morhua L.) and some other fish species in the littoral zone. ICES Journal of Marine Science, 53(5), 757–770. https://doi.org/10.1006/jmsc.1996.0097

Fluharty D (2000) Habitat protection, ecological issues, and implementation of the Sustainable Fisheries Act. Ecol Appl 10:325–337.

Fluharty, D. (2000). Habitat protection, ecological issues, and implementation of the Sustainable Fisheries Act. Ecological Applications, 10(2), 325–337. https://doi.org/10.1890/1051-0761(2000)010[0325:HPEIAI]2.0.CO;2

Flávio, H., Seitz, R., Eggleston, D., Svendsen, J. C., & Støttrup, J. (2023). Hard-bottom habitats support commercially important fish species: A systematic review for the North Atlantic Ocean and Baltic Sea. In PeerJ (Vol. 11, p. e14681). PeerJ Inc. https://doi.org/10.7717/peerj.14681

Fonseca, V. G., Nichols, B., Lallias, D., Quince, C., Carvalho, G. R., Power, D. M., & Creer, S. (2012). Sample richness and genetic diversity as drivers of chimera formation in nSSU metagenetic analyses. Nucleic Acids Research, 40(9), e66. https://doi.org/10.1093/nar/gks002

Fontes, J. T., Ferreira, A. O., Lavrador, A. S., Machado, C., Soares, P., Costa, F. O., & Canchaya, C. A. (2024). Exploring artificial neural networks for the curation of DNA barcode reference libraries: A pilot study with marine fish species of the Eastern Atlantic. Preprints. https://doi.org/10.22541/au.172374899.92498971/v1

Fowler, A. M., & Booth, D. J. (2012). How well do sunken vessels approximate fish assemblages on coral reefs? Conservation implications of vessel-reef deployments. Marine Biology, 159(12), 2787–2796. https://doi.org/10.1007/s00227-012-2039-x

Fowler, A. M., Jørgensen, A.-M., Coolen, J. W. P., Jones, D. O. B., Svendsen, J. C., Brabant, R., Rumes, B., & Degraer, S. (2020). The ecology of infrastructure decommissioning in the North Sea: What we need to know and how to achieve it. ICES Journal of Marine Science, 77(3), 1109–1126. https://doi.org/10.1093/icesjms/fsz143

Framst, I., Wolking, R. M., Schonfeld, J., Ricker, N., Beeler-Marfisi, J., Chalmers, G., Kamath, P. L., & Maboni, G. (2024). High-throughput rapid amplicon sequencing for multilocus sequence typing of Mycoplasma ovipneumoniae from archived clinical DNA samples. Frontiers in Veterinary Science, 11, 1443855.

Frøslev, T. G., Kjøller, R., Bruun, H. H., Ejrnæs, R., Brunbjerg, A. K., Pietroni, C., & Hansen, A. J. (2017). Algorithm for post-clustering curation of DNA amplicon data yields reliable biodiversity estimates. Nature Communications, 8(1), 1188. https://doi.org/10.1038/s41467-017-01312-x

Gaither, M. R., Szabó, Z., Crepeau, M. W., Bird, C. E., & Toonen, R. J. (2011). Preservation of corals in salt-saturated DMSO buffer is superior to ethanol for PCR experiments. Coral Reefs, 30(2), 329–333. https://doi.org/10.1007/s00338-010-0687-1

Garin-Fernandez, A., Pereira-Flores, E., Glöckner, F. O., & Wichels, A. (2018). The North Sea goes viral: Occurrence and distribution of North Sea bacteriophages. Marine Genomics, 41, 31–41. https://doi.org/10.1016/j.margen.2018.05.004

Garlapati, D., Charankumar, B., Ramu, K., Madeswaran, P., & Ramana Murthy, M. V. (2019). A review on the applications and recent advances in environmental DNA (eDNA) metagenomics. Reviews in Environmental Science and Bio/Technology, 18(3), 389–411. https://doi.org/10.1007/s11157-019-09501-4

Garofalo, G., Fortibuoni, T., Gristina, M., Sinopoli, M., & Fiorentino, F. (2011). Persistence and co-occurrence of demersal nurseries in the Strait of Sicily (central Mediterranean): Implications for fishery management. Journal of Sea Research, 66(1), 29–38. https://doi.org/10.1016/J.SEARES.2011.04.008

GBIF Secretariat. (2024). Metabarcoding Data Toolkit—User guide. Copenhagen: Global Biodiversity Information Facility. https://doi.org/10.35035/doc-wkpc-m352

GBIF. (n.d.). Retrieved March 30, 2025, from https://www.gbif.org/

GEANS. (n.d.). Retrieved May 20, 2025, from https://www.geans.eu/

Gell, F. R., & Roberts, C. M. (2003). Benefits beyond boundaries: The fishery effects of marine reserves. Trends in Ecology & Evolution, 18(9), 448–455. https://doi.org/10.1016/S0169-5347(03)00189-7

Gerber, L., Peters, K. J., King, S. L., Allen, S. J., Connor, R. C., Forbes, O., Holmes, K. G., Kearns, A. M., Willems, E. P., Krützen, M., & Rollins, L. A. (2025). Social bonds decrease epigenetic age in male bottlenose dolphins. Communications Biology. https://doi.org/10.1038/s42003-025-09227-w

Gielings, R., Fais, M., Fontaneto, D., Creer, S., Costa, F. O., Renema, W., & Macher, J.-N. (2021). DNA Metabarcoding Methods for the Study of Marine Benthic Meiofauna: A Review. Frontiers in Marine Science, 8. https://doi.org/10.3389/fmars.2021.730063

Gilbert, K. J., Andrew, R. L., Bock, D. G., Franklin, M. T., Kane, N. C., Moore, J., Moyers, B. T., Renaut, S., Rennison, D. J., Veen, T., & Vines, T. H. (2012). Recommendations for utilizing and reporting population genetic analyses: The reproducibility of genetic clustering using the program structure. Molecular Ecology, 21(20), 4925–4930. https://doi.org/10.1111/j.1365-294X.2012.05754.x

Gilby, B. L., Olds, A. D., Chapman, S., Goodridge Gaines, L. A., Henderson, C. J., Ortodossi, N. L., Didderen, K., Lengkeek, W., van der Heide, T., & Schlacher, T. A. (2021). Attraction versus production in restoration: Spatial and habitat effects of shellfish reefs for fish in coastal seascapes. Restoration Ecology, 29(7), 1–12. https://doi.org/10.1111/rec.13413

Gilby, B. L., Olds, A. D., Henderson, C. J., Ortodossi, N. L., Connolly, R. M., & Schlacher, T. A. (2019). Seascape context modifies how fish respond to restored oyster reef structures. ICES Journal of Marine Science, 76(4), 1131–1139. https://doi.org/10.1093/icesjms/fsz019

Giroux, M. S., Reichman, J. R., Langknecht, T., Burgess, R. M., & Ho, K. T. (2022). Environmental RNA as a Tool for Marine Community Biodiversity Assessments. Scientific Reports, 12(1), 17782. https://doi.org/10.1038/s41598-022-22198-w

Gleason, J. E., Hanner, R. H., & Cottenie, K. (2023). Hidden diversity: DNA metabarcoding reveals hyper-diverse benthic invertebrate communities. BMC Ecology and Evolution, 23(1), 19. https://doi.org/10.1186/s12862-023-02118-w

Gold, Z., Koch, M. Q., Schooler, N. K., Emery, K. A., Dugan, J. E., Miller, R. J., Page, H. M., Schroeder, D. M., Hubbard, D. M., Madden, J. R., Whitaker, S. G., & Barber, P. H. (2023a). A comparison of biomonitoring methodologies for surf zone fish communities. PLoS ONE, 18(6 June), 1–26. https://doi.org/10.1371/journal.pone.0260903

Gold, Z., Shelton, A. O., Casendino, H. R., Duprey, J., Gallego, R., Van Cise, A., Fisher, M., Jensen, A. J., D'Agnese, E., Andruszkiewicz Allan, E., Ramón-Laca, A., Garber-Yonts, M., Labare, M., Parsons, K. M., & Kelly, R. P. (2023b). Signal and noise in metabarcoding data. PLOS ONE, 18(5), e0285674. https://doi.org/10.1371/journal.pone.0285674

Goldberg, C. S., Turner, C. R., Deiner, K., Klymus, K. E., Thomsen, P. F., Murphy, M. A., Spear, S. F., McKee, A., Oyler-McCance, S. J., Cornman, R. S., Laramie, M. B., Mahon, A. R., Lance, R. F., Pilliod, D. S., Strickler, K. M., Waits, L. P., Fremier, A. K., Takahara, T., Herder, J. E., & Taberlet, P. (2016). Critical considerations for the application of environmental DNA methods to detect aquatic species. Methods in Ecology and Evolution, 7(11), 1299–1307. https://doi.org/10.1111/2041-210X.12595

Gombeau, K., Pereira, S., Ravanat, J.-L., Camilleri, V., Cavalie, I., Bourdineaud, J.-P., & Adam-Guillermin, C. (2016). Depleted uranium induces sex- and tissue-specific methylation patterns in adult zebrafish. Journal of Environmental Radioactivity, 154, 25–33. https://doi.org/10.1016/j.jenvrad.2016.01.004

Gonçalves, E. J., Henriques, M., & Almada, V. C. (2002). Use of a Temperate Reef-Fish Community To Identify Priorities in the Establishment of a Marine Protected Area. Aquatic Protected Areas: What Works Best And How Do We Know? Proceedings of the World Congress on Aquatic Protected Areas, (August), 261–272.

Goodwin, K. D., Thompson, L. R., Duarte, B., Kahlke, T., Thompson, A. R., Marques, J. C., & Caçador, I. (2017). DNA Sequencing as a Tool to Monitor Marine Ecological Status. Frontiers in Marine Science, 4. https://doi.org/10.3389/fmars.2017.00107

Gostel, M. R., & Kress, W. J. (2022). The Expanding Role of DNA Barcodes: Indispensable Tools for Ecology, Evolution, and Conservation. Diversity, 14(3), Article 3. https://doi.org/10.3390/d14030213

Govers, L. L., Heusinkveld, J. H. T., Gräfnings, M. L. E., Smeele, Q., & Heide, T. van der. (2022). Adaptive intertidal seed-based seagrass restoration in the Dutch Wadden Sea. PLOS ONE, 17(2), e0262845. https://doi.org/10.1371/journal.pone.0262845

Greco, M., Lejzerowicz, F., Reo, E., Caruso, A., Maccotta, A., Coccioni, R., Pawlowski, J., & Frontalini, F. (2022). Environmental RNA outperforms eDNA metabarcoding in assessing impact of marine pollution: A chromium-spiked mesocosm test. Chemosphere, 298, 134239. https://doi.org/10.1016/j.chemosphere.2022.134239

Grip, K., & Blomqvist, S. (2020). Marine nature conservation and conflicts with fisheries. Ambio, 49(7), 1328–1340. https://doi.org/10.1007/s13280-019-01279-7

Groot, R. de, Fisher, B., Christie, M., Aronson, J., Braat, L., Gowdy, J., Haines-Young, R., Maltby, E., Neuville, A., & Polasky, S. (2011). Integrating the Ecological and Economic Dimensions in Biodiversity and Ecosystem Service Valuation. In The Economics of Ecosystems and Biodiversity: Ecological and Economic Foundations. Routledge.

Guidelines for developing national biodiversity monitoring systems—UNECE. (2023, January 1). UNECE. https://unece.org/environment-policy/publications/guidelines-developing-national-biodiversity-monitoring-systems

Guri, G., Ray, J. L., Shelton, A. O., Kelly, R. P., Præbel, K., Andruszkiewicz Allan, E., Yoccoz, N., Johansen, T., Wangensteen, O. S., Hanebrekke, T., & Westgaard, J. (2024a). Quantifying the Detection Sensitivity and Precision of qPCR and ddPCR Mechanisms for eDNA Samples. Ecology and Evolution, 14(12). https://doi.org/10.1002/ece3.70678

Guri, G., Shelton, A. O., Kelly, R. P., Yoccoz, N., Johansen, T., Præbel, K., Hanebrekke, T., Ray, J. L., Fall, J., & Westgaard, J.-I. (2024b). Predicting trawl catches using environmental DNA. ICES Journal of Marine Science, 81(8), 1536–1548. https://doi.org/10.1093/icesjms/fsae097

Gygax, D., Ramirez, S., Chibesa, M., Simpamba, T., Riffel, M., Riffel, T., Srivathsan, A., Nijland, R., & Urban, L. (2025). Evaluation of nanopore sequencing for increasing accessibility of eDNA studies in biodiverse countries. PLOS ONE, 20(10), e0333994. https://doi.org/10.1371/journal.pone.0333994

Günther, B., Knebelsberger, T., Neumann, H., Laakmann, S., & Martínez Arbizu, P. (2018). Metabarcoding of marine environmental DNA based on mitochondrial and nuclear genes. Scientific Reports, 8(1), 14822. https://doi.org/10.1038/s41598-018-32917-x

Hadley Wickham (2016) Ggplot2: Elegant Graphics for Data Analysis. J R Stat Soc Ser A Stat Soc 174:245–246.

Hadley Wickham. (2016). ggplot2: Elegant Graphics for Data Analysis. Journal of the Royal Statistical Society Series A: Statistics in Society, 174(1), 245–246.

Hadziavdic, K., Lekang, K., Lanzen, A., Jonassen, I., Thompson, E. M., & Troedsson, C. (2014). Characterization of the 18s rRNA gene for designing universal eukaryote specific primers. PLoS ONE, 9(2). https://doi.org/10.1371/journal.pone.0087624

Haelters, J., & Kerckhof, F. (2010). The first historic record of a humpback whale (Megaptera novaeangliae) from the Low Countries (Southern Bight of the North Sea).

Hakimzadeh, A., Asbun, A. A., Albanese, D., Bernard, M., Buchner, D., Callahan, B., Caporaso, J. G., Curd, E., Djemiel, C., Durling, M. B., Elbrecht, V., Gold, Z., Gweon, H. S., Hajibabaei, M., Hildebrand, F., Mikryukov, V., Normandeau, E., Özkurt, E., Palmer, J. M., … Anslan, S. (2024). A pile of pipelines: An overview of the bioinformatics software for metabarcoding data analyses. Molecular Ecology Resources, 24(5), e13847. https://doi.org/10.1111/1755-0998.13847

Hallam, J., Clare, E. L., Jones, J. I., & Day, J. J. (2023). High frequency environmental DNA metabarcoding provides rapid and effective monitoring of fish community dynamics. Environmental DNA, 5(6), 1623–1640. https://doi.org/10.1002/edn3.486

Hanel, R., & Rubner, M. (n.d.). AquaGene. Retrieved November 12, 2025, from https://www.aquagene.org/

Harrison, J. B., Sunday, J. M., & Rogers, S. M. (2019). Predicting the fate of eDNA in the environment and implications for studying biodiversity. Proceedings of the Royal Society B: Biological Sciences, 286(1915), 20191409. https://doi.org/10.1098/rspb.2019.1409

Hashemipetroudi, S. H., Nematzadeh, G., Ahmadian, G., Yamchi, A., & Kuhlmann, M. (2018). Assessment of DNA Contamination in RNA Samples Based on Ribosomal DNA. Journal of Visualized Experiments : JoVE, 131, 55451. https://doi.org/10.3791/55451

He, X., Jeffery, N. W., Stanley, R. R. E., Hamilton, L. C., Rubidge, E. M., & Abbott, C. L. (2023). eDNA metabarcoding enriches traditional trawl survey data for monitoring biodiversity in the marine environment. ICES Journal of Marine Science, 80(5), 1529–1538. https://doi.org/10.1093/icesjms/fsad083

He, X., Maruki, T., Morgado-Gamero, W. B., Barrett, R. D. H., Fugère, V., Fussmann, G. F., Gonzalez, A., Shapiro, B. J., & Cristescu, M. E. (2025). Environmental RNA-Based Metatranscriptomics as a Novel Biomonitoring Tool: A Case Study of Glyphosate-Based Herbicide Effects on Freshwater Eukaryotic Communities. Molecular Ecology, 34(22), e70164. https://doi.org/10.1111/mec.70164

Heberling, J. M., Miller, J. T., Noesgaard, D., Weingart, S. B., & Schigel, D. (2021). Data integration enables global biodiversity synthesis. Proceedings of the National Academy of Sciences, 118(6). https://doi.org/10.1073/pnas.2018093118

Hebert, P. D. N., Cywinska, A., Ball, S. L., & deWaard, J. R. (2003). Biological identifications through DNA barcodes. Proceedings. Biological Sciences, 270(1512), 313–321. https://doi.org/10.1098/rspb.2002.2218

Hechler, R. M., Yates, M. C., Chain, F. J. J., & Cristescu, M. E. (2025). Environmental transcriptomics under heat stress: Can environmental RNA reveal changes in gene expression of aquatic organisms? Molecular Ecology, 34(13), e17152. https://doi.org/10.1111/mec.17152

Heessen HJL, Daan N, Ellis JR (2015) Fish atlas of the Celtic Sea, North Sea, and Baltic Sea. 572.

Heessen, H. J. L., Daan, N., & Ellis, J. R. (2015). Fish atlas of the Celtic Sea, North Sea, and Baltic Sea (p. 572). Wageningen Academic Publishers. https://doi.org/doi:10.3920/978-90-8686-878-0

Heikema, A. P., Horst-Kreft, D., Boers, S. A., Jansen, R., Hiltemann, S. D., de Koning, W., Kraaij, R., de Ridder, M. A. J., van Houten, C. B., Bont, L. J., Stubbs, A. P., & Hays, J. P. (2020). Comparison of Illumina versus Nanopore 16S rRNA Gene Sequencing of the Human Nasal Microbiota. Genes, 11(9), Article 9. https://doi.org/10.3390/genes11091105

Heip, C., & Craeymeersch, J. A. (1995). Benthic community structures in the North Sea. Helgoländer Meeresuntersuchungen, 49(1), 313–328. https://doi.org/10.1007/BF02368359

Hering, D., Borja, A., Jones, J. I., Pont, D., Boets, P., Bouchez, A., Bruce, K., Drakare, S., Hänfling, B., Kahlert, M., Leese, F., Meissner, K., Mergen, P., Reyjol, Y., Segurado, P., Vogler, A., & Kelly, M. (2018). Implementation options for DNA-based identification into ecological status assessment under the European Water Framework Directive. Water Research, 138, 192–205. https://doi.org/10.1016/j.watres.2018.03.003

Hermans, A., Bos, O. G., & Prusina, I. (2020). Nature-Inclusive Design: A catalogue for offshore wind infrastructure: Technical report. https://research.wur.nl/en/publications/nature-inclusive-design-a-catalogue-for-offshore-wind-infrastruct/

Hernandez-Santin, C., Amati, M., Bekessy, S., & Desha, C. (2022). A Review of Existing Ecological Design Frameworks Enabling Biodiversity Inclusive Design. Urban Science, 6(4), 95. https://doi.org/10.3390/urbansci6040095

Hestetun, J. T., Bye-Ingebrigtsen, E., Nilsson, R. H., Glover, A. G., Johansen, P. O., & Dahlgren, T. G. (2020). Significant taxon sampling gaps in DNA databases limit the operational use of marine macrofauna metabarcoding. Marine Biodiversity, 50(5), 1–9. https://doi.org/10.1007/s12526-020-01093-5

Hestetun, J. T., Lanzén, A., Skaar, K. S., & Dahlgren, T. G. (2021). The impact of DNA extract homogenization and replication on marine sediment metabarcoding diversity and heterogeneity. Environmental DNA, 3(5), 997–1006. https://doi.org/10.1002/edn3.223

Hiddink, J. G., Johnson, A. F., Kingham, R., & Hinz, H. (2011). Could our fisheries be more productive? Indirect negative effects of bottom trawl fisheries on fish condition. Journal of Applied Ecology, 48(6), 1441–1449. https://doi.org/10.1111/j.1365-2664.2011.02036.x

Hinz, S., Coston-Guarini, J., Marnane, M., & Guarini, J.-M. (2022). Evaluating eDNA for Use within Marine Environmental Impact Assessments. Journal of Marine Science and Engineering, 10(3), Article 3. https://doi.org/10.3390/jmse10030375

Hirst, N., Clark, L., & Sanderson, W. G. (2012). The distribution of selected MPA search features and Priority Marine Features off the NE coast of Scotland. Scottish Natural Heritage Commissioned Report No.500.132pp., (5).

Hoffmann, R. C. (2005). A brief history of aquatic resource use in medieval Europe. Helgoland Marine Research, 59(1), 22–30. https://doi.org/10.1007/s10152-004-0203-5

Hofstede, R. T., Hiddink, J. G., & Rijnsdorp, A. D. (2010). Regional warming changes fish species richness in the eastern North Atlantic Ocean. Marine Ecology Progress Series, 414(August), 1–9. https://doi.org/10.3354/meps08753

Holligan, P. M., Aarup, T., & Groom, S. B. (1989). The North Sea: Satellite colour atlas. Continental Shelf Research, The North Sea Satellite Colour Atlas, 9(8), 667–765. https://doi.org/10.1016/0278-4343(89)90096-4

Holm, P., Ludlow, F., Scherer, C., Travis, C., Allaire, B., Brito, C., Hayes, P. W., Matthews, J. A., Rankin, K. J., Breen, R. J., Legg, R., Lougheed, K., & Nicholls, J. (2022). The North Atlantic Fish Revolution (ca. AD 1500). Quaternary Research, 108, 92–106. https://doi.org/10.1017/qua.2018.153

Holman, L. E., Hollenbeck, C. M., Ashton, T. J., & Johnston, I. A. (2019). Demonstration of the Use of Environmental DNA for the Non-Invasive Genotyping of a Bivalve Mollusk, the European Flat Oyster (Ostrea edulis). Frontiers in Genetics, 10(November), 1–7. https://doi.org/10.3389/fgene.2019.01159

Holman, L. E., Zampirolo, G., Gyllencreutz, R., Scourse, J., Frøslev, T., Carøe, C., Gopalakrishnan, S., Pedersen, M. W., & Bohmann, K. (2025). Navigating Past Oceans: Comparing Metabarcoding and Metagenomics of Marine Ancient Sediment Environmental DNA. Molecular Ecology Resources, e14086. https://doi.org/10.1111/1755-0998.14086

Holmes, J. A., Cronkite, G. M. W., Enzenhofer, H. J., & Mulligan, T. J. (2006). Accuracy and precision of fish-count data from a "dual-frequency identification sonar" (DIDSON) imaging system. ICES Journal of Marine Science, 63(3), 543–555. https://doi.org/10.1016/j.icesjms.2005.08.015

Holt, T. J. ., Rees, E. I., Hawkins, S. J., & Seed, R. (1998). BIOGENIC REEFS An overview of dynamic and sensitivity characteristics for conservation management of marine SACs. UK Marine SACs Project, Task Manager, A.M.W. Wilson, SAMS, IX, 170. https://doi.org/10.1007/s12518-019-00263-w

Hu, T., Chitnis, N., Monos, D., & Dinh, A. (2021). Next-generation sequencing technologies: An overview. Human Immunology, Next Generation Sequencing and Its Application to Medical Laboratory Immunology, 82(11), 801–811. https://doi.org/10.1016/j.humimm.2021.02.012

Huang, Y., Niu, B., Gao, Y., Fu, L., & Li, W. (2010). CD-HIT Suite: A web server for clustering and comparing biological sequences. Bioinformatics, 26(5), 680–682. https://doi.org/10.1093/bioinformatics/btq003

Hubert, J., Neo, Y. Y., Winter, H. V., & Slabbekoorn, H. (2020). The role of ambient sound levels, signal-to-noise ratio, and stimulus pulse rate on behavioural disturbance of seabass in a net pen. Behavioural Processes, 170, 103992. https://doi.org/10.1016/j.beproc.2019.103992

Hunter, E., Buckley, A. A., Stewart, C., & Metcalfe, J. D. (2005). Migratory behaviour of the thornback ray, Raja clavata, in the southern North Sea. Journal of the Marine Biological Association of the United Kingdom, 85(5), 1095–1105. https://doi.org/10.1017/S0025315405012142

Hunter, M. E., Dorazio, R. M., Butterfield, J. S. S., Meigs-Friend, G., Nico, L. G., & Ferrante, J. A. (2017). Detection limits of quantitative and digital PCR assays and their influence in presence–absence surveys of environmental DNA. Molecular Ecology Resources, 17(2), 221–229. https://doi.org/10.1111/1755-0998.12619

Hunter, W. R., & Sayer, M. D. J. (2009). The comparative effects of habitat complexity on faunal assemblages of northern temperate artificial and natural reefs. ICES Journal of Marine Science, 66(4), 691–698. https://doi.org/10.1093/icesjms/fsp058

Huserbråten, M. B. O., Moland, E., & Albretsen, J. (2018). Cod at drift in the North Sea. Progress in Oceanography, 167, 116–124. https://doi.org/10.1016/j.pocean.2018.07.005

Hylkema, A., Hakkaart, Q. C. A., Reid, C. B., Osinga, R., Murk, A. J., & Debrot, A. O. (2021). Artificial reefs in the Caribbean: A need for comprehensive monitoring and integration into marine management plans. Ocean & Coastal Management, 209, 105672. https://doi.org/10.1016/J.OCECOAMAN.2021.105672

Ibanez-Erquiaga B, Baktoft H, Mildenberger TK, Teilmann J, Kleivane L, Kornau LM, Agersted MD, Hüllert SM, Svendsen JC (2025) Increased fish abundance, biodiversity, and body size near a North Sea oil and gas platform. Mar Environ Res 204.

Ibanez-Erquiaga B, Baktoft H, Wilms T, Mildenberger TK, Svendsen JC (2024) Fish aggregations at oil and gas platform foundations in the North Sea. Fish Manag Ecol 31:e12693.

Ibanez-Erquiaga, B., Baktoft, H., Mildenberger, T. K., Teilmann, J., Kleivane, L., Kornau, L. M., Agersted, M. D., Hüllert, S. M., & Svendsen, J. C. (2025). Increased fish abundance, biodiversity, and body size near a North Sea oil and gas platform. Marine Environmental Research, 204, 106959. https://doi.org/10.1016/j.marenvres.2025.106959

Ibanez-Erquiaga, B., Baktoft, H., Wilms, T., Mildenberger, T. K., & Svendsen, J. C. (2024). Fish aggregations at oil and gas platform foundations in the North Sea. Fisheries Management and Ecology, 31(4), e12693. https://doi.org/10.1111/FME.12693

IJsseldijk, L. L., Van Den Broek, J., Kik, M. J. L., Leopold, M. F., Bravo Rebolledo, E., Gröne, A., & Heesterbeek, H. (2024). Using marine mammal necropsy data in animal health surveillance: The case of the harbor porpoise in the Southern North Sea. Frontiers in Marine Science, 10, 1306294. https://doi.org/10.3389/fmars.2023.1306294

Interpretation Manual of EU Union Habitats—European Commission. (2023, March 23). European Commission. https://circabc.europa.eu/ui/group/3f466d71-92a7-49eb-9c63-6cb0fadf29dc/library/37d9e6d9-b7de-42ce-b789-622e9741b68f/details

Ivanova, S. V., Kessel, S. T., Landry, J., O'Neill, C., McLean, M. F., Espinoza, M., Vagle, S., Hussey, N. E., & Fisk, A. T. (2018). Impact of vessel traffic on the home ranges and movement of shorthorn sculpin (Myoxocephalus scorpius) in the nearshore environment of the high Arctic. Canadian Journal of Fisheries and Aquatic Sciences, 75(12), 2390–2400. https://doi.org/10.1139/cjfas-2017-0418

Jackson, J. B. C., Kirby, M. X., Berger, W. H., Bjorndal, K. A., Botsford, L. W., Bourque, B. J., Bradbury, R. H., Cooke, R., Erlandson, J., Estes, J. A., Hughes, T. P., Kidwell, S., Lange, C. B., Lenihan, H. S., Pandolfi, J. M., Peterson, C. H., Steneck, R. S., Tegner, M. J., & Warner, R. R. (2001). Historical Overfishing and the Recent Collapse of Coastal Ecosystems. Science, 293(5530), 629–637. https://doi.org/10.1126/science.1059199

Jahnke, M., Moknes, P.-O., Le Moan, A., Martens, G. A., & Jonsson, P. R. (2022). Seascape genomics identify adaptive barriers correlated to tidal amplitude in the shore crab Carcinus maenas. Molecular Ecology, 31(7), 1980–1994. https://doi.org/10.1111/mec.16371

Jain, M., Fiddes, I. T., Miga, K. H., Olsen, H. E., Paten, B., & Akeson, M. (2015). Improved data analysis for the MinION nanopore sequencer. Nature Methods, 12(4), 351–356. https://doi.org/10.1038/nmeth.3290

Jansen, L., Loos, E., Murk, A. J., Priesmann, B., Valk, S., Walles, B., Kamermans, P., & Nijland, R. (2025). Coming of age: 13 years after construction an artificial oyster reef in a North Sea estuary hosts a mature benthic community. Marine Environmental Research, 212, 107551. https://doi.org/10.1016/j.marenvres.2025.107551

Janssens, L., Asselman, J., & De Troch, M. (2024). Effects of ocean warming on the fatty acid and epigenetic profile of Acartia tonsa: A multigenerational approach. Marine Pollution Bulletin, 201, 116265. https://doi.org/10.1016/j.marpolbul.2024.116265

Jenkins, C., Eggleton, J., Barry, J., & O'Connor, J. (2018). Advances in assessing Sabellaria spinulosa reefs for ongoing monitoring. Ecology and Evolution, 8(15), 7673–7687. https://doi.org/10.1002/ece3.4292

Jennings, S., Dinmore, T. A., Duplisea, D. E., Warr, K. J., & Lancaster, J. E. (2001). Trawling disturbance can modify benthic production processes. Journal of Animal Ecology, 70(3), 459–475. https://doi.org/10.1046/j.1365-2656.2001.00504.x

Jeunen, G.-J., Urban, L., Lewis, R., Knapp, M., Lamare, M., Rayment, W., Dawson, S., & Gemmell, N. (2020). Marine environmental DNA (eDNA) for biodiversity assessments: A one-to-one comparison between eDNA and baited remote underwater video (BRUV) surveys. https://doi.org/10.22541/au.160278512.26241559/v1

Jin, J., Chang, M., Cheong, Y., Ip, A., Bauman, A. G., & Huang, D. (2020). MinION-in-ARMS: Nanopore Sequencing To Expedite Barcoding Of Specimen-Rich Macrofaunal Samples From Autonomous Reef 2 Monitoring Structures 3 4. bioRxiv, 2020.03.30.009654-2020.03.30.009654. https://doi.org/10.1101/2020.03.30.009654

Jo, T. S. (2023). Utilizing the state of environmental DNA (eDNA) to incorporate time-scale information into eDNA analysis. Proceedings of the Royal Society B: Biological Sciences, 290(1999), 20230979. https://doi.org/10.1098/rspb.2023.0979

Jo, T., & Minamoto, T. (2021). Complex interactions between environmental DNA (eDNA) state and water chemistries on eDNA persistence suggested by meta-analyses. Molecular Ecology Resources, 21(5), 1490–1503. https://doi.org/10.1111/1755-0998.13354

Jo, T., Murakami, H., Masuda, R., & Minamoto, T. (2020). Selective collection of long fragments of environmental DNA using larger pore size filter. Science of The Total Environment, 735, 139462. https://doi.org/10.1016/j.scitotenv.2020.139462

Jo, T., Murakami, H., Masuda, R., Sakata, M. K., Yamamoto, S., & Minamoto, T. (2017). Rapid degradation of longer DNA fragments enables the improved estimation of distribution and biomass using environmental DNA. Molecular Ecology Resources, 17(6), e25–e33. https://doi.org/10.1111/1755-0998.12685

Johnson, A. F., Jenkins, S. R., Hiddink, J. G., & Hinz, H. (2013). Linking temperate demersal fish species to habitat: Scales, patterns and future directions. Fish and Fisheries, 14(3), 256–280. https://doi.org/10.1111/J.1467-2979.2012.00466.X

Johnson, J. S., Spakowicz, D. J., Hong, B. Y., Petersen, L. M., Demkowicz, P., Chen, L., Leopold, S. R., Hanson, B. M., Agresta, H. O., Gerstein, M., Sodergren, E., & Weinstock, G. M. (2019). Evaluation of 16S rRNA gene sequencing for species and strain-level microbiome analysis. Nature Communications, 10(1). https://doi.org/10.1038/s41467-019-13036-1

Johnson, L. E. (1993). A Morally Deep World: An Essay on Moral Significance and Environmental Ethics. Cambridge University Press.

Johnson, S. S., Zaikova, E., Goerlitz, D. S., Bai, Y., & Tighe, S. W. (2017). Real-Time DNA Sequencing in the Antarctic Dry Valleys Using the Oxford Nanopore Sequencer. Journal of Biomolecular Techniques : JBT, 28(1), 2–7. https://doi.org/10.7171/jbt.17-2801-009

Jones, P. A. (2012). Functions of DNA methylation: Islands, start sites, gene bodies and beyond. Nature Reviews Genetics, 13(7), 484–492. https://doi.org/10.1038/nrg3230

Joseph, C., Faiq, M. E., Li, Z., & Chen, G. (2022). Persistence and degradation dynamics of eDNA affected by environmental factors in aquatic ecosystems. Hydrobiologia, 849(19), 4119–4133. https://doi.org/10.1007/S10750-022-04959-W/METRICS

Kaandorp, M. L. A., Ypma, S. L., Boonstra, M., Dijkstra, H. A., & Van Sebille, E. (2022). Using machine learning and beach cleanup data to explain litter quantities along the Dutch North Sea coast. Ocean Science, 18(1), 269–293. https://doi.org/10.5194/os-18-269-2022

Kachelriess, D., Wegmann, M., Gollock, M., & Pettorelli, N. (2014). The application of remote sensing for marine protected area management. Ecological Indicators, 36, 169–177. https://doi.org/10.1016/j.ecolind.2013.07.003

Kagzi, K., Hechler, R. M., Fussmann, G. F., & Cristescu, M. E. (2022). Environmental RNA degrades more rapidly than environmental DNA across a broad range of pH conditions. Molecular Ecology Resources, 22(7), 2640–2650. https://doi.org/10.1111/1755-0998.13655

Kagzi, K., Millette, K. L., Littlefair, J. E., Pochon, X., Wood, S. A., Fussmann, G. F., & Cristescu, M. E. (2023). Assessing the degradation of environmental DNA and RNA based on genomic origin in a metabarcoding context. Environmental DNA, 5(5), 1016–1031. https://doi.org/10.1002/edn3.437

Kaiser, M. J., Clarke, K. R., Hinz, H., Austen, M. C. V., Somerfield, P. J., & Karakassis, I. (2006). Global analysis of response and recovery of benthic biota to fishing. Marine Ecology Progress Series, 311, 1–14. https://doi.org/10.3354/meps311001

Kamermans P, Bos O, Tonk L, Bakker R, Didderen K (2023) Monitoring platte-oesterbank Voordelta 2023 Monitoring platte-oesterbank Voordelta.

Kamermans, P., Bos, O., Tonk, L., Bakker, R., & Didderen, K. (2023). Monitoring platte-oesterbank Voordelta 2023

Kamermans, P., van Duren, L., & Kleissen, F. (2018a). European flat oysters on offshore wind farms: Additional locations; Opportunities for the development of European flat oyster (Ostrea edulis) populations on planned wind farms and additional locations in the Dutch section of the North Sea Wageningen, Wage. Wageningen Marine Research, C053/18, 33–33.

Kamermans, P., Walles, B., Kraan, M., Van Duren, L. A., Kleissen, F., Van der Have, T. M., Smaal, A. C., & Poelman, M. (2018b). Offshore wind farms as potential locations for flat oyster (Ostrea edulis) restoration in the Dutch North Sea. Sustainability (Switzerland), 10, 308.

Kappel, K., Haase, I., Käppel, C., Sotelo, C. G., & Schröder, U. (2017). Species identification in mixed tuna samples with next-generation sequencing targeting two short cytochrome b gene fragments. Food Chemistry, 234, 212–219. https://doi.org/10.1016/j.foodchem.2017.04.178

Karin, B. R., Arellano, S., Wang, L., Walzer, K., Pomerantz, A., Vasquez, J. M., Chatla, K., Sudmant, P. H., Bach, B. H., Smith, L. L., & McGuire, J. A. (2023). Highly-multiplexed and efficient long-amplicon PacBio and Nanopore sequencing of hundreds of full mitochondrial genomes. BMC Genomics, 24, 229. https://doi.org/10.1186/s12864-023-09277-6

Kasmi, Y., Eschbach, E., & Hanel, R. (2023). Mare-MAGE curated reference database of fish mitochondrial genes. BMC Genomic Data, 24(1), 18. https://doi.org/10.1186/s12863-023-01119-4

Katoh, K., & Standley, D. M. (2013). MAFFT Multiple Sequence Alignment Software Version 7: Improvements in Performance and Usability. Molecular Biology and Evolution, 30(4), 772–780. https://doi.org/10.1093/molbev/mst010

Katz, K., Shutov, O., Lapoint, R., Kimelman, M., Brister, J. R., & O'Sullivan, C. (2022). The Sequence Read Archive: A decade more of explosive growth. Nucleic Acids Research, 50(D1), D387–D390. https://doi.org/10.1093/nar/gkab1053

Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C., Thierer, T., Ashton, B., Meintjes, P., & Drummond, A. (2012). Geneious Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics, 28(12), 1647–1649. https://doi.org/10.1093/bioinformatics/bts199

Keller, A. G., Grason, E. W., McDonald, P. S., Ramón-Laca, A., & Kelly, R. P. (2022). Tracking an invasion front with environmental DNA. Ecological Applications, 32(4), e2561. https://doi.org/10.1002/eap.2561

Kelly, R. P., Closek, C. J., O'Donnell, J. L., Kralj, J. E., Shelton, A. O., & Samhouri, J. F. (2017). Genetic and Manual Survey Methods Yield Different and Complementary Views of an Ecosystem. Frontiers in Marine Science, 3. https://doi.org/10.3389/fmars.2016.00283

Kelly, R. P., Gallego, R., & Jacobs-Palmer, E. (2018). The effect of tides on nearshore environmental DNA. PeerJ, 6, e4521. https://doi.org/10.7717/peerj.4521

Kelly, R. P., Port, J. A., Yamahara, K. M., & Crowder, L. B. (2014). Using environmental DNA to census marine fishes in a large mesocosm. PLoS ONE, 9(1), 86175–86175. https://doi.org/10.1371/journal.pone.0086175

Kelly, R. P., Shelton, A. O., & Gallego, R. (2019). Understanding PCR Processes to Draw Meaningful Conclusions from Environmental DNA Studies. Scientific Reports, 9(1). https://doi.org/10.1038/S41598-019-48546-X

Kent, F. E. A., Gray, M. J., Last, K. S., & Sanderson, W. G. (2016). Horse mussel reef ecosystem services: Evidence for a whelk nursery habitat supporting a shellfishery. International Journal of Biodiversity Science, Ecosystem Services and Management, 12(3), 172–180. https://doi.org/10.1080/21513732.2016.1188330

Kent, F. E. A., Last, K. S., Harries, D. B., & Sanderson, W. G. (2017a). In situ biodeposition measurements on a Modiolus modiolus (horse mussel) reef provide insights into ecosystem services. Estuarine, Coastal and Shelf Science, 184, 151–157. https://doi.org/10.1016/j.ecss.2016.11.014

Kent, F. E. A., Mair, J. M., Newton, J., Lindenbaum, C., Porter, J. S., & Sanderson, W. G. (2017b). Commercially important species associated with horse mussel (Modiolus modiolus) biogenic reefs: A priority habitat for nature conservation and fisheries benefits. Marine Pollution Bulletin, 118(1), 71–78. https://doi.org/10.1016/j.marpolbul.2017.02.051

Kent, F. E. A., Mair, J. M., Newton, J., Lindenbaum, C., Porter, J. S., Sanderson, W. G., Vallina, S. M., Le Quéré, C., Khripounoff, A., Caprais, J. C., Decker, C., Le Bruchec, J., Noel, P., Husson, B., Post, D. M., Avelar, S., van der Voort, T. S., Eglinton, T. I., van Oevelen, D., … Barot, S. (2017c). Integrating ecosystem engineering and food web ecology: Testing the effect of biogenic reefs on the food web of a soft-bottom intertidal area. PLoS ONE, 10(3), 703–718. https://doi.org/10.1371/journal.pone.0140857

Kingma, E. M., ter Hofstede, R., Kardinaal, E., Bakker, R., Bittner, O., van der Weide, B., & Coolen, J. W. P. (2024). Guardians of the seabed: Nature-inclusive design of scour protection in offshore wind farms enhances benthic diversity. Journal of Sea Research, 199, 102502. https://doi.org/10.1016/j.seares.2024.102502

Klemetsen, T., Raknes, I. A., Fu, J., Agafonov, A., Balasundaram, S. V., Tartari, G., Robertsen, E., & Willassen, N. P. (2018). The MAR databases: Development and implementa-

tion of databases specific for marine metagenomics. Nucleic Acids Research, 46(D1), D692–D699. https://doi.org/10.1093/nar/gkx1036

Klunder, L., Lavaleye, M. S. S., Filippidi, A., Van Bleijswijk, J. D. L., Reichart, G.-J., Van Der Veer, H. W., Duineveld, G. C. A., & Mienis, F. (2020). Impact of an artificial structure on the benthic community composition in the southern North Sea: Assessed by a morphological and molecular approach. ICES Journal of Marine Science, 77(3), 1167–1177. https://doi.org/10.1093/icesjms/fsy114

Klunder, L., van Bleijswijk, J. D. L., Kleine Schaars, L., van der Veer, H. W., Luttikhuizen, P. C., & Bijleveld, A. I. (2022). Quantification of marine benthic communities with metabarcoding. Molecular Ecology Resources, 22(3), 1043–1054. https://doi.org/10.1111/1755-0998.13536

Klymus, K. E., Baker, J. D., Abbott, C. L., Brown, R. J., Craine, J. M., Gold, Z., Hunter, M. E., Johnson, M. D., Jones, D. N., Jungbluth, M. J., Jungbluth, S. P., Lor, Y., Maloy, A. P., Merkes, C. M., Noble, R., Patin, N. V., Sepulveda, A. J., Spear, S. F., Steele, J. A., … Theroux, S. (2024). The MIEM guidelines: Minimum information for reporting of environmental metabarcoding data. Metabarcoding and Metagenomics, 8. https://doi.org/10.3897/mbmg.8.128689

Knoester, E. G., Rienstra, J. J., Schürmann, Q. J. F., Wolma, A. E., Murk, A. J., & Osinga, R. (2023). Community-managed coral reef restoration in southern Kenya initiates reef recovery using various artificial reef designs. Frontiers in Marine Science, 10, 1152106. https://doi.org/10.3389/FMARS.2023.1152106/BIBTEX

Kopp, D., Faillettaz, R., Le Joncour, A., Simon, J., Morandeau, F., Le Bourdonnec, P., Bouché, L., & Méhault, S. (2023). Assessing without harvesting: Pros and cons of environmental DNA sampling and image analysis for marine biodiversity evaluation. Marine Environmental Research, 188, 106004. https://doi.org/10.1016/j.marenvres.2023.106004

Korpinen, S., Kahlert, M., Kuosa, H., Mack, L., Meissner, K., Pitkänen, H., Pyhälahti, T., & Uusitalo, L. (2022). Marine monitoring in transition: On the verge of technological revolution? Frontiers in Marine Science, 9, 1066769. https://doi.org/10.3389/fmars.2022.1066769

Kraan, C., Haslob, H., Probst, W. N., Stelzenmüller, V., Rehren, J., & Neumann, H. (2024). Thresholds of seascape fauna composition along gradients of human pressures and natural conditions to inform marine spatial planning. Science of The Total Environment, 914, 169940. https://doi.org/10.1016/j.scitotenv.2024.169940

Krabberød, A. K., Stokke, E., Thoen, E., Skrede, I., & Kauserud, H. (2025). The Ribosomal Operon Database: A Full-Length rDNA Operon Database Derived From Genome Assemblies. Molecular Ecology Resources, 25(1), e14031. https://doi.org/10.1111/1755-0998.14031

Krehenwinkel, H., Pomerantz, A., & Prost, S. (2019). Genetic biomonitoring and biodiversity assessment using portable sequencing technologies: Current uses and future directions. Genes, 10(11). https://doi.org/10.3390/genes10110858

Krehenwinkel, H., Wolf, M., Lim, J. Y., Rominger, A. J., Simison, W. B., & Gillespie, R. G. (2017). Estimating and mitigating amplification bias in qualitative and quantitative arthropod metabarcoding. Scientific Reports, 7(1), 17668. https://doi.org/10.1038/s41598-017-17333-x

Kristensen, L. D., Stenberg, C., Støttrup, J. G., Poulsen, L. K., Christensen, H. T., Dolmer, P., Landes, A., Røjbek, M., Thorsen, S. W., Holmer, M., Deurs, M. V., & Grønkjær, P. (2015). Establishment of blue mussel beds to enhance fish habitats. Applied Ecology and Environmental Research, 13(3), 783–798. https://doi.org/10.15666/aeer/1303_783798

Krolicka, A., Mæland Nilsen, M., Klitgaard Hansen, B., Wulf Jacobsen, M., Provan, F., & Baussant, T. (2022). Sea lice (Lepeophtherius salmonis) detection and quantification around aquaculture installations using environmental DNA. PLOS ONE, 17(9), e0274736. https://doi.org/10.1371/journal.pone.0274736

Krone, R., & Schröder, A. (2011). Wrecks as artificial lobster habitats in the German Bight. Helgoland Marine Research, 65(1), 11–16. https://doi.org/10.1007/S10152-010-0195-2/FIGURES/2

Krone, R., Dederer, G., Kanstinger, P., Krämer, P., Schneider, C., & Schmalenbach, I. (2017). Mobile demersal megafauna at common offshore wind turbine foundations in the German Bight (North Sea) two years after deployment—Increased production rate of Cancer pagurus. Marine Environmental Research, 123, 53–61. https://doi.org/10.1016/j.marenvres.2016.11.011

Krone, R., Gutow, L., Brey, T., Dannheim, J., & Schröder, A. (2013). Mobile demersal megafauna at artificial structures in the German Bight e Likely effects of offshore wind farm development. Estuarine, Coastal and Shelf Science, 125, 1–9. https://doi.org/10.1016/j.ecss.2013.03.012

Kusters, J. E. H., Van Kann, F. M. G., & Zuidema, C. (2025). Spatial conflict resolution in marine spatial plans and permitting procedures for offshore wind energy: An analysis of measures adopted in Denmark, England and the Netherlands. Frontiers in Marine Science, 12, 1468734. https://doi.org/10.3389/fmars.2025.1468734

Käse, L., Metfies, K., Kraberg, A. C., Neuhaus, S., Meunier, C. L., Wiltshire, K. H., & Boersma, M. (2021). Metabarcoding analysis suggests that flexible food web interactions in the eukaryotic plankton community are more common than specific predator–prey relationships at Helgoland Roads, North Sea. ICES Journal of Marine Science, 78(9), 3372–3386. https://doi.org/10.1093/icesjms/fsab058

Kühn, B., Taylor, M., & Kempf, A. (2021). Using machine learning to link spatiotemporal information to biological processes in the ocean: A case study for North Sea cod recruitment. Marine Ecology Progress Series, 664, 1–22. https://doi.org/10.3354/meps13689

Kühne, S., & Rachor, E. (1996). The macrofauna of a stony sand area in the German Bight (North Sea). Helgoländer Meeresuntersuchungen, 50(4), 433–452. https://doi.org/10.1007/BF02367159

Laakmann, S., Boos, K., Knebelsberger, T., Raupach, M. J., & Neumann, H. (2017). Species identification of echinoderms from the North Sea by combining morphology and molecular data. Helgoland Marine Research, 70(1), 18. https://doi.org/10.1186/s10152-016-0468-5

Lacoursière-Roussel, A., Côté, G., Leclerc, V., & Bernatchez, L. (2016). Quantifying relative fish abundance with eDNA: a promising tool for fisheries management. Journal of Applied Ecology, 53(4), 1148–1157. https://doi.org/10.1111/1365-2664.12598

Lamb, P. D., Fonseca, V. G., Maxwell, D. L., & Nnanatu, C. C. (2022). Systematic review and meta-analysis: Water type and temperature affect environmental DNA decay. Molecular Ecology Resources, 22(7), 2494–2505. https://doi.org/10.1111/1755-0998.13627

Lance, R. F., & Guan, X. (2020). Variation in inhibitor effects on qPCR assays and implications for eDNA surveys. Canadian Journal of Fisheries and Aquatic Sciences, 77(1), 23–33. https://doi.org/10.1139/cjfas-2018-0263

Langlois, T., Goetze, J., Bond, T., Monk, J., Abesamis, R. A., Asher, J., Barrett, N., Bernard, A. T. F., Bouchet, P. J., Birt, M. J., Cappo, M., Currey-Randall, L. M., Driessen, D., Fairclough, D. V., Fullwood, L. A. F., Gibbons, B. A., Harasti, D., Heupel, M. R., Hicks, J., … Harvey, E. S. (2020). A field and video annotation guide for baited remote un-

derwater stereo-video surveys of demersal fish assemblages. Methods in Ecology and Evolution, 11(11), 1401–1409. https://doi.org/10.1111/2041-210X.13470

Langlois, T., Williams, J., Monk, J., Bouchet, P., Currey, L., Goetze, J., & Whitmarsh, S. (2018). Marine Sampling Field Manual for Benthic Stereo BRUVS (Baited Remote Underwater Videos). Field Manuals for Marine Sampling to Monitor Australian Waters, (June), 82–104.

Langlois, V. S., Allison, M. J., Bergman, L. C., To, T. A., & Helbing, C. C. (2021). The need for robust qPCR-based eDNA detection assays in environmental monitoring and species inventories. Environmental DNA, 3(3), 519–527. https://doi.org/10.1002/edn3.164

Lanzén, A., Lekang, K., Jonassen, I., Thompson, E. M., & Troedsson, C. (2017). DNA extraction replicates improve diversity and compositional dissimilarity in metabarcoding of eukaryotes in marine sediments. PLOS ONE, 12(6), e0179443. https://doi.org/10.1371/journal.pone.0179443

Lavrador, A. S., Fontes, J. T., Vieira, P. E., Costa, F. O., & Duarte, S. (2023). Compilation, Revision, and Annotation of DNA Barcodes of Marine Invertebrate Non-Indigenous Species (NIS) Occurring in European Coastal Regions. Diversity, 15(2), Article 2. https://doi.org/10.3390/d15020174

Lee, Y. H., Kim, M.-S., Jeong, H., Hagiwara, A., & Lee, J.-S. (2020). Genome-wide identification and transcriptional modulation of histone variants and modification related genes in the low pH-exposed marine rotifer Brachionus koreanus. Comparative Biochemistry and Physiology Part D: Genomics and Proteomics, 36, 100748. https://doi.org/10.1016/j.cbd.2020.100748

Lenth R V. (2025) Emmeans: Estimated Marginal Means, aka Least-Squares Means. R package version 1.10.7-100001. https://rvlenth.github.io/emmeans/authors.html#citation (accessed 21 February 2025)

Leray, M., & Knowlton, N. (2015). DNA barcoding and metabarcoding of standardized samples reveal patterns of marine benthic diversity. Proceedings of the National Academy of Sciences, 112(7), 2076–2081. https://doi.org/10.1073/pnas.1424997112

Leray, M., & Knowlton, N. (2016). Censusing marine eukaryotic diversity in the twenty-first century. Philosophical Transactions of the Royal Society B: Biological Sciences, 371(1702), 20150331. https://doi.org/10.1098/rstb.2015.0331

Leray, M., Ho, S.-L., Lin, I.-J., & Machida, R. J. (2018). MIDORI server: A webserver for taxonomic assignment of unknown metazoan mitochondrial-encoded sequences using a curated database. Bioinformatics, 34(21), 3753–3754. https://doi.org/10.1093/bioinformatics/bty454

Leray, M., Knowlton, N., & Machida, R. J. (2022). MIDORI2: A collection of quality controlled, preformatted, and regularly updated reference databases for taxonomic assignment of eukaryotic mitochondrial sequences. Environmental DNA, 4(4), 894–907. https://doi.org/10.1002/edn3.303

Leray, M., Yang, J. Y., Meyer, C. P., Mills, S. C., Agudelo, N., Ranwez, V., Boehm, J. T., & Machida, R. J. (2013). A new versatile primer set targeting a short fragment of the mitochondrial COI region for metabarcoding metazoan diversity: Application for characterizing coral reef fish gut contents. Frontiers in Zoology, 10(1), 1–14. https://doi.org/10.1186/1742-9994-10-34

Levin, P. S., & Stunz, G. W. (2005). Habitat triage for exploited fishes: Can we identify essential "Essential Fish Habitat?" Estuarine, Coastal and Shelf Science, 64(1), 70–78. https://doi.org/10.1016/J.ECSS.2005.02.007

Lewis, J. P., Ryves, D. B., Rasmussen, P., Olsen, J., Knudsen, K.-L., Andersen, S. H., Weckström, K., Clarke, A. L., Andrén, E., & Juggins, S. (2016). The shellfish enigma across the Mesolithic-Neolithic transition in southern Scandinavia. Quaternary Science Reviews, 151, 315–320. https://doi.org/10.1016/j.quascirev.2016.09.004

Li, H. (2018). Minimap2: Pairwise alignment for nucleotide sequences. Bioinformatics, 34(18), 3094–3100. https://doi.org/10.1093/bioinformatics/bty191

Li, S., & Tollefsbol, T. O. (2021). DNA methylation methods: Global DNA methylation and methylomic analyses. Methods, 187, 28–43. https://doi.org/10.1016/j.ymeth.2020.10.002

Li, W., Jaroszewski, L., & Godzik, A. (2002). Tolerating some redundancy significantly speeds up clustering of large protein databases. Bioinformatics, 18(1), 77–82. https://doi.org/10.1093/bioinformatics/18.1.77

Li, W., Jia, H., & Zhang, H. (2024). Evaluating the effectiveness of the eRNA technique in monitoring fish biodiversity – A case study in the Qingdao offshore, China. Global Ecology and Conservation, 51, e02888. https://doi.org/10.1016/j.gecco.2024.e02888

Li, Z., Zhao, W., Jiang, Y., Wen, Y., Li, M., Liu, L., & Zou, K. (2024). New insights into biologic interpretation of bioinformatic pipelines for fish eDNA metabarcoding: A case study in Pearl River estuary. Journal of Environmental Management, 368, 122136. https://doi.org/10.1016/j.jenvman.2024.122136

Lindeboom, H. J. (1995). Protected areas in the North Sea: An absolute need for future marine research. Helgoländer Meeresuntersuchungen, 49(1), 591–602. https://doi.org/10.1007/BF02368384

Lindholm, J. B., Auster, P. J., Ruth, M., & Kaufman, L. (2001). Modeling the effects of fishing and implications for the design of marine protected areas: Juvenile fish responses to variations in seafloor habitat. Conservation Biology, 15(2), 424–437. https://doi.org/10.1046/J.1523-1739.2001.015002424.X

Littlefair, J. E., Rennie, M. D., & Cristescu, M. E. (2022). Environmental nucleic acids: A field-based comparison for monitoring freshwater habitats using eDNA and eRNA. Molecular Ecology Resources, 22(8), 2928–2940. https://doi.org/10.1111/1755-0998.13671

Liu, C., Xia, F., Hirayama, I., Yagi, G., & Ushio, M. (2025). Epi-eDNA: From Methylation Signal Detection to Functional Ecological Monitoring. Life Sciences. https://doi.org/10.32942/X2R340

Liu, F., Zhang, P., Liang, Z., Yuan, Y., Liu, Y., & Wu, Y. (2023). The global dynamic of DNA methylation in response to heat stress revealed epigenetic mechanism of heat acclimation in Saccharina japonica. Journal of Phycology, 59(1), 249–263. https://doi.org/10.1111/jpy.13305

Liu, Z., Zhou, T., & Gao, D. (2022). Genetic and epigenetic regulation of growth, reproduction, disease resistance and stress responses in aquaculture. Frontiers in Genetics, 13, 994471. https://doi.org/10.3389/fgene.2022.994471

Lobo, J., Shokralla, S., Costa, M. H., Hajibabaei, M., & Costa, F. O. (2017). DNA metabarcoding for high-throughput monitoring of estuarine macrobenthic communities. Scientific Reports, 7(1), 15618. https://doi.org/10.1038/s41598-017-15823-6

Loeza-Quintana, T., Abbott, C. L., Heath, D. D., Bernatchez, L., & Hanner, R. H. (2020). Pathway to Increase Standards and Competency of eDNA Surveys (PISCeS)—Advancing collaboration and standardization efforts in the field of eDNA. Environmental DNA, 2(3), 255–260. https://doi.org/10.1002/edn3.112

Lolivier, M., Cornelis, I., Maes, S., Mortelmans, J., Polet, H., & Derycke, S. (2025). Monthly eDNA Metabarcoding Data From the Coastal North Sea Area Align With Fish Species-

Specific Life Cycle Events. Environmental DNA, 7(4), e70175. https://doi.org/10.1002/edn3.70175

Lopez, J., Moreno, G., Boyra, G., & Dagorn, L. (2016). A model based on data from echosounder buoys to estimate biomass of fish species associated with fish aggregating devices. Fishery Bulletin, 114(2), 166. https://doi.org/10.7755/fb.114.2.4

Lopez, M. L. D., Lin, Y., Sato, M., Hsieh, C., Shiah, F.-K., & Machida, R. J. (2022). Using metatranscriptomics to estimate the diversity and composition of zooplankton communities. Molecular Ecology Resources, 22(2), 638–652. https://doi.org/10.1111/1755-0998.13506

Luisetti, T., & Schratzberger, M. (2023). Including biological diversity in natural capital accounts for marine biodiversity conservation and human well-being. Biodiversity and Conservation, 32(1), 405–413. https://doi.org/10.1007/s10531-021-02342-0

Lynch, J. (1990). PCR Technology: Principles and Applications for DNA Amplification. Journal of Medical Genetics, 27(8), 536.

Ma, J., Ma, R., Pan, Q., Liang, X., Wang, J., & Ni, X. (2023). A Global Review of Progress in Remote Sensing and Monitoring of Marine Pollution. Water, 15(19), 3491. https://doi.org/10.3390/w15193491

Macher, T.-H., Arle, J., Beermann, A. J., Frank, L., Hupało, K., Koschorreck, J., Schütz, R., & Leese, F. (2024). Is it worth the extra mile? Comparing environmental DNA and RNA metabarcoding for vertebrate and invertebrate biodiversity surveys in a lowland stream. PeerJ, 12, e18016. https://doi.org/10.7717/peerj.18016

Macher, T.-H., Schütz, R., Arle, J., Beermann, A. J., Koschorreck, J., & Leese, F. (2021). Beyond fish eDNA metabarcoding: Field replicates disproportionately improve the detection of stream associated vertebrate species. Metabarcoding and Metagenomics, 5, e66557. https://doi.org/10.3897/mbmg.5.66557

Mackelworth, P. C., Teff Seker, Y., Vega Fernández, T., Marques, M., Alves, F. L., D'Anna, G., Fa, D. A., Goldborough, D., Kyriazi, Z., Pita, C., Portman, M. E., Rumes, B., Warr, S. J., & Holcer, D. (2019). Geopolitics and Marine Conservation: Synergies and Conflicts. Frontiers in Marine Science, 6, 759. https://doi.org/10.3389/fmars.2019.00759

Maes, J., Egoh, B., Willemen, L., Liquete, C., Vihervaara, P., Schägner, J. P., Grizzetti, B., Drakou, E. G., Notte, A. L., Zulian, G., Bouraoui, F., Luisa Paracchini, M., Braat, L., & Bidoglio, G. (2012). Mapping ecosystem services for policy support and decision making in the European Union. Ecosystem Services, 1(1), 31–39. https://doi.org/10.1016/j.ecoser.2012.06.004

Maggini, S., Jacobsen, M. W., Urban, P., Hansen, B. K., Kielgast, J., Bekkevold, D., Jardim, E., Martinsohn, J. T., Carvalho, G. R., Nielsen, E. E., & Papadopulos, A. S. T. (2024). Nanopore environmental DNA sequencing of catch water for estimating species composition in demersal bottom trawl fisheries. Environmental DNA, 6(3), e555. https://doi.org/10.1002/edn3.555

Maitland PS, Herdson D, Coates S (2009) Key to the marine and freshwater fishes of Britain and Ireland: A guide to the identification of more than 370 species.

Majaneva, M., Diserud, O. H., Eagle, S. H. C., Boström, E., Hajibabaei, M., & Ekrem, T. (2018). Environmental DNA filtration techniques affect recovered biodiversity. Scientific Reports, 8(1), 4682. https://doi.org/10.1038/s41598-018-23052-8

Mallet, D., & Pelletier, D. (2014). Underwater video techniques for observing coastal marine biodiversity: A review of sixty years of publications (1952–2012). Fisheries Research, 154, 44–62. https://doi.org/10.1016/j.fishres.2014.01.019

Mangel, M. (2000). Trade-offs between fish habitat and fishing mortality and the role of reserves. Bulletin of Marine Science, 66(3), 663–674.

Manu, S., & Umapathy, G. (2023). Deep sequencing of extracellular eDNA enables total biodiversity assessment of ecosystems. Ecological Indicators, 156, 111171. https://doi.org/10.1016/j.ecolind.2023.111171

MARCO-BOLO. (2025, November 5). Marco-Bolo. https://marcobolo-project.eu/

Mariani, S., Baillie, C., Colosimo, G., & Riesgo, A. (2019). Sponges as natural environmental DNA samplers. Current Biology, 29(11), R401–R402. https://doi.org/10.1016/j.cub.2019.04.031

Marine environment—Environment—European Commission. (2025, November 7). European Commission. https://environment.ec.europa.eu/topics/marine-environment_en

Marshall, N. T., Vanderploeg, H. A., & Chaganti, S. R. (2021). Environmental (e)RNA advances the reliability of eDNA by predicting its age. Scientific Reports, 11(1), 2769. https://doi.org/10.1038/s41598-021-82205-4

Martens, N., Russnak, V., Woodhouse, J., Grossart, H.-P., & Schaum, C.-E. (2024). Metabarcoding reveals potentially mixotrophic flagellates and picophytoplankton as key groups of phytoplankton in the Elbe estuary. Environmental Research, 252, 119126. https://doi.org/10.1016/j.envres.2024.119126

Martin, M. (2011). Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnet.Journal, 17(1), 10–10. https://doi.org/10.14806/EJ.17.1.200

Mateos-Rivera, A., Skern-Mauritzen, R., Dahle, G., Sundby, S., Mozfar, B., Thorsen, A., Wehde, H., & Krafft, B. A. (2020). Comparison of visual and molecular taxonomic methods to identify ichthyoplankton in the North Sea. Limnology and Oceanography: Methods. https://doi.org/10.1002/lom3.10387

Matsuo, Y., Komiya, S., Yasumizu, Y., Yasuoka, Y., Mizushima, K., Takagi, T., Kryukov, K., Fukuda, A., Morimoto, Y., Naito, Y., Okada, H., Bono, H., Nakagawa, S., & Hirota, K. (2021). Full-length 16S rRNA gene amplicon analysis of human gut microbiota using MinIONTM nanopore sequencing confers species-level resolution. BMC Microbiology, 21(1). https://doi.org/10.1186/s12866-021-02094-5

Mauffrey, F., Cordier, T., Apothéloz-Perret-Gentil, L., Cermakova, K., Merzi, T., Delefosse, M., Blanc, P., & Pawlowski, J. (2021). Benthic monitoring of oil and gas offshore platforms in the North Sea using environmental DNA metabarcoding. Molecular Ecology, 30(13), 3007–3022. https://doi.org/10.1111/mec.15698

Mauger, S., Fouqueau, L., Avia, K., Reynes, L., Serrao, E. A., Neiva, J., & Valero, M. (2021). Development of tools to rapidly identify cryptic species and characterize their genetic diversity in different European kelp species. Journal of Applied Phycology, 33(6), 4169–4186. https://doi.org/10.1007/s10811-021-02613-x

McCartin, L. J., Vohsen, S. A., Ambrose, S. W., Layden, M., McFadden, C. S., Cordes, E. E., McDermott, J. M., & Herrera, S. (2022). Temperature Controls eDNA Persistence across Physicochemical Conditions in Seawater. Environmental Science and Technology, 56(12), 8629–8639. https://doi.org/10.1021/ACS.EST.2C01672/ASSET/IMAGES/LARGE/ES2C01672_0005.JPEG

McGeady, R., Runya, R. M., Dooley, J. S. G., Howe, J. A., Fox, C. J., Wheeler, A. J., Summers, G., Callaway, A., Beck, S., Brown, L. S., Dooly, G., & McGonigle, C. (2023). A review of new and existing non-extractive techniques for monitoring marine protected areas. Frontiers in Marine Science, 10, 1126301. https://doi.org/10.3389/fmars.2023.1126301

McGee, K. M., Robinson, C. V., & Hajibabaei, M. (2019). Gaps in DNA-Based Biomonitoring Across the Globe. Frontiers in Ecology and Evolution, 7, 337–337. https://doi.org/10.3389/fevo.2019.00337

McKnight, D. T., Huerlimann, R., Bower, D. S., Schwarzkopf, L., Alford, R. A., & Zenger, K. R. (2019). microDecon: A highly accurate read-subtraction tool for the post-sequencing removal of contamination in metabarcoding studies. Environmental DNA, 1(1), 14–25. https://doi.org/10.1002/edn3.11

Melvin, G. D., & Cochrane, N. A. (2015). Multibeam Acoustic Detection of Fish and Water Column Targets at High-Flow Sites. Estuaries and Coasts, 38(1), 227–240. https://doi.org/10.1007/s12237-014-9828-z

Meyer, A., Boyer, F., Valentini, A., Bonin, A., Ficetola, G. F., Beisel, J.-N., Bouquerel, J., Wagner, P., Gaboriaud, C., Leese, F., Dejean, T., Taberlet, P., & Usseglio-Polatera, P. (2021). Morphological vs. DNA metabarcoding approaches for the evaluation of stream ecological status with benthic invertebrates: Testing different combinations of markers and strategies of data filtering. Molecular Ecology, 30(13), 3203–3220. https://doi.org/10.1111/mec.15723

Meyer, M., & Kircher, M. (2010). Illumina Sequencing Library Preparation for Highly Multiplexed Target Capture and Sequencing. Cold Spring Harbor Protocols, 2010(6), pdb.prot5448. https://doi.org/10.1101/pdb.prot5448

Mirimin, L., Desmet, S., Romero, D. L., Fernandez, S. F., Miller, D. L., Mynott, S., Brincau, A. G., Stefanni, S., Berry, A., Gaughan, P., & Aguzzi, J. (2021). Don't catch me if you can – Using cabled observatories as multidisciplinary platforms for marine fish community monitoring: An in situ case study combining Underwater Video and environmental DNA data. Science of The Total Environment, 773, 145351. https://doi.org/10.1016/J.SCITOTENV.2021.145351

Mittelstrass, J., Heinzelmann, R., Eschen, R., Hartmann, M., Kupper, Q., Schneider, S., Prospero, S., & Franić, I. (2025). Metabarcoding with Illumina and Oxford Nanopore Technologies provides complementary insights into tree seed mycobiota. Environmental Microbiome, 20(1), 53. https://doi.org/10.1186/s40793-025-00712-7

Miya, M. (2022). Environmental DNA Metabarcoding: A Novel Method for Biodiversity Monitoring of Marine Fish Communities. Annual Review of Marine Science, 14(1), 161–185. https://doi.org/10.1146/annurev-marine-041421-082251

Miya, M., Sato, Y., Fukunaga, T., Sado, T., Poulsen, J. Y., Sato, K., Minamoto, T., Yamamoto, S., Yamanaka, H., Araki, H., Kondoh, M., & Iwasaki, W. (2015). MiFish, a set of universal PCR primers for metabarcoding environmental DNA from fishes: Detection of more than 230 subtropical marine species. Royal Society Open Science, 2(7), 150088. https://doi.org/10.1098/rsos.150088

Miyata, K., Inoue, Y., Amano, Y., Nishioka, T., Nagaike, T., Kawaguchi, T., Morita, O., Yamane, M., & Honda, H. (2022). Comparative environmental RNA and DNA metabarcoding analysis of river algae and arthropods for ecological surveys and water quality assessment. Scientific Reports, 12(1), 19828. https://doi.org/10.1038/s41598-022-23888-1

Miyata, K., Inoue, Y., Yamane, M., & Honda, H. (2025). Fish environmental RNA sequencing sensitively captures accumulative stress responses through short-term aquarium sampling. Science of The Total Environment, 959, 178182. https://doi.org/10.1016/j.scitotenv.2024.178182

Mohrbeck, I., Raupach, M. J., Martínez Arbizu, P., Knebelsberger, T., & Laakmann, S. (2015). High-Throughput Sequencing—The Key to Rapid Biodiversity Assessment of Marine Metazoa? PLOS ONE, 10(10), e0140342. https://doi.org/10.1371/journal.pone.0140342

Monchamp, M.-È., Walsh, D. A., Garner, R. E., Kraemer, S. A., Beisner, B. E., Cristescu, M. E., & Gregory-Eaves, I. (2022). Comparative analysis of zooplankton diversity in freshwaters: What can we gain from metagenomic analysis? Environmental DNA, 4(6), 1250–1264. https://doi.org/10.1002/edn3.335

Moore C (2019) SNH Research Report 1085: Biological analyses of underwater video from monitoring and research cruises in Lochs Ailort and Fyne, the Sounds of Barra and Mull, inner Moray Firth, off Wester Ross, Noss Head and Rattray Head, and around the Southern Trench.

Moore, C. (2019). SNH Research Report 1085: Biological analyses of underwater video from monitoring and research cruises in Lochs Ailort and Fyne, the Sounds of Barra and Mull, inner Moray Firth, off Wester Ross, Noss Head and Rattray Head, and around the Southern Trench. (1085).

Moore, C. G. and Roberts, J. M. (2011). An assessment of the conservation importance of species and habitats identified during a series of recent research cruises around Scotland. Scottish Natural Heritage Commissioned Report No. 446., 507.

Morey, K. C., Bartley, T. J., & Hanner, R. H. (2020). Validating environmental DNA metabarcoding for marine fishes in diverse ecosystems using a public aquarium. Environmental DNA, 2(3), 330–342. https://doi.org/10.1002/edn3.76

Morin, F., Panova, M. A. Z., Schweizer, M., Wiechmann, M., Eliassen, N., Sundberg, P., Cluzel-Burgalat, L., & Polovodova Asteman, I. (2023). Hidden aliens: Application of digital PCR to track an exotic foraminifer across the Skagerrak (North Sea) correlates well with traditional morphospecies analysis. Environmental Microbiology, 25(11), 2321–2337. https://doi.org/10.1111/1462-2920.16458

Moutinho, J., Costa, F. O., & Duarte, S. (2024). Advancements in DNA Metabarcoding Protocols for Monitoring Zooplankton in Marine and Brackish Environments. Journal of Marine Science and Engineering, 12(11), 2093. https://doi.org/10.3390/jmse12112093

Mugnai, F., Meglécz, E., Abbiati, M., Bavestrello, G., Bertasi, F., Bo, M., Capa, M., Chenuil, A., Colangelo, M. A., De Clerck, O., Gutiérrez, J. M., Lattanzi, L., Leduc, M., Martin, D., Matterson, K. O., Mikac, B., Plaisance, L., Ponti, M., Riesgo, A., … Costantini, F. (2021). Are well-studied marine biodiversity hotspots still blackspots for animal barcoding? Global Ecology and Conservation, 32, e01909. https://doi.org/10.1016/j.gecco.2021.e01909

Munday, P. L. (2004). Habitat loss, resource specialization, and extinction on coral reefs. Global Change Biology, 10(10), 1642–1647. https://doi.org/10.1111/J.1365-2486.2004.00839.X

Murgier, J., McLean, M., Maire, A., Mouillot, D., Loiseau, N., Munoz, F., Violle, C., & Auber, A. (2021). Rebound in functional distinctiveness following warming and reduced fishing in the North Sea. Proceedings of the Royal Society B: Biological Sciences, 288(1942), 20201600. https://doi.org/10.1098/rspb.2020.1600

Murphy, H. M., & Jenkins, G. P. (2010). Observational methods used in marine spatial monitoring of fishes and associated habitats: A review. Marine and Freshwater Research, 61(2), 236–252. https://doi.org/10.1071/MF09068

Mérou, N., Lecadet, C., Pouvreau, S., & Arzul, I. (2020). An eDNA/eRNA-based approach to investigate the life cycle of non-cultivable shellfish micro-parasites: The case of

Bonamia ostreae , a parasite of the European flat oyster Ostrea edulis. Microbial Biotechnology, 13(6), 1807–1818. https://doi.org/10.1111/1751-7915.13617
Nagler, M., Podmirseg, S. M., Ascher-Jenull, J., Sint, D., & Traugott, M. (2022). Why eDNA fractions need consideration in biomonitoring. Molecular Ecology Resources, 22(7), 2458–2470. https://doi.org/10.1111/1755-0998.13658
National Center for Biotechnology Information. (n.d.). Retrieved March 30, 2025, from https://www.ncbi.nlm.nih.gov/
NatureScot. (2024). Conservation Objectives and Advice to Support Management: Noss Head MPA. (June), 1–25.
Newell, R. I. E. (2004). SpringerLink—Estuaries and Coasts, Volume 27, Number 5. Estuaries and Coasts, 27(5), 793–806.
Nielsen, T. G., & Maar, M. (2007). Effects of a blue mussel Mytilus edulis bed on vertical distribution and composition of the pelagic food web. Marine Ecology Progress Series, 339, 185–198. https://doi.org/10.3354/meps339185
Niku, J., Brooks, W., Herliansyah, R., Hui, F. K. C., Korhonen, P., Taskinen, S., Veen, B. van der, & Warton, D. I. (2024). Gllvm: Generalized Linear Latent Variable Models. R package version 2.0. Cran. https://doi.org/10.18637/jss.v070.i05
Nolan, J. M., Skujina, I., Hurpy, G., Tighe, A. J., Whelan, C., & Teeling, E. C. (2025). Evaluation of Oxford Nanopore Technologies MinION Sequencer as a Novel Short Amplicon Metabarcoding Tool Using Arthropod Mock Sample and Irish Bat Diet Characterisation. Ecology and Evolution, 15(5), e71333. https://doi.org/10.1002/ece3.71333
Nunn, A. D., Tewson, L. H., & Cowx, I. G. (2012). The foraging ecology of larval and juvenile fishes. Reviews in Fish Biology and Fisheries, 22(2), 377–408. https://doi.org/10.1007/s11160-011-9240-8
Ocean Biodiversity Information System. (n.d.). Retrieved May 20, 2025, from https://obis.org/
Ohnesorge, A., John, U., Kuczynski, L., Neuhaus, S., Beng, K. C., Krock, B., & Laakmann, S. (2024). Roaming the Seas—Assessing Marine Invertebrate Biodiversity Along Salinity Gradients With Zooplankton and eDNA Metabarcoding. Environmental DNA, 6(6), e70037. https://doi.org/10.1002/edn3.70037
Oksanen, J., Simpson, G. L., Blanchet, F. G., Kindt, R., Legendre, P., Minchin, P. R., O'Hara, R. B., Solymos, P., Stevens, M. H. H., Szoecs, E., Wagner, H., Barbour, M., Bedward, M., Bolker, B., Borcard, D., Carvalho, G., Chirico, M., De Caceres, M., Durand, S., … Weedon, J. (2022). Package "Vegan" Title Community Ecology Package. Cran, 1–297.
Olsen, O. (1883). The piscatorial atlas of the North Sea. Taylor Fr London.
Ortega-Recalde, O., Goikoetxea, A., Hore, T. A., Todd, E. V., & Gemmell, N. J. (2020). The Genetics and Epigenetics of Sex Change in Fish. Annual Review of Animal Biosciences, 8(1), 47–69. https://doi.org/10.1146/annurev-animal-021419-083634
Otto, L., Zimmerman, J. T. F., Furnes, G. K., Mork, M., Saetre, R., & Becker, G. (1990). Review of the physical oceanography of the North Sea. Netherlands Journal of Sea Research, 26(2), 161–238. https://doi.org/10.1016/0077-7579(90)90091-T
O'Brien, A. L., Dafforn, K. A., Chariton, A. A., Johnston, E. L., & Mayer-Pinto, M. (2019). After decades of stressor research in urban estuarine ecosystems the focus is still on single stressors: A systematic literature review and meta-analysis. Science of the Total Environment, 684, 753–764. https://doi.org/10.1016/j.scitotenv.2019.02.131
O'Cathail, C., Ahamed, A., Burgin, J., Cummins, C., Devaraj, R., Gueye, K., Gupta, D., Gupta, V., Haseeb, M., Ihsan, M., Ivanov, E., Jayathilaka, S., Kadhirvelu, V., Kumar, M., Lathi, A., Leinonen, R., McKinnon, J., Meszaros, L., Pauperio, J., … Cochrane, G. (2025).

The European Nucleotide Archive in 2024. Nucleic Acids Research, 53(D1), D49–D55. https://doi.org/10.1093/nar/gkae975

O'Leary, B. C., Fonseca, C., Cornet, C. C., de Vries, M. B., Degia, A. K., Failler, P., Furlan, E., Garrabou, J., Gil, A., Hawkins, J. P., Krause-Jensen, D., Le Roux, X., Peck, M. A., Pérez, G., Queirós, A. M., Różyński, G., Sanchez-Arcilla, A., Simide, R., Sousa Pinto, I., … Roberts, C. M. (2023). Embracing Nature-based Solutions to promote resilient marine and coastal ecosystems. Nature-Based Solutions, 3, 100044. https://doi.org/10.1016/j.nbsj.2022.100044

Parsley, M. B., & Goldberg, C. S. (2024). Environmental RNA can distinguish life stages in amphibian populations. Molecular Ecology Resources, 24(4), e13857. https://doi.org/10.1111/1755-0998.13857

Paska, C., Barta, I., Drozdovszky, O., & Antus, B. (2019). Elimination of bacterial DNA during RNA isolation from sputum: Bashing bead vortexing is preferable over prolonged DNase treatment. PLoS ONE, 14(3), e0214609. https://doi.org/10.1371/journal.pone.0214609

Paula, D. P., Barros, S. K. A., Pitta, R. M., Barreto, M. R., Togawa, R. C., & Andow, D. A. (2022). Metabarcoding versus mapping unassembled shotgun reads for identification of prey consumed by arthropod epigeal predators. GigaScience, 11, giac020. https://doi.org/10.1093/gigascience/giac020

Pawlowski, J., Kelly-Quinn, M., Altermatt, F., Apothéloz-Perret-Gentil, L., Beja, P., Boggero, A., Borja, A., Bouchez, A., Cordier, T., Domaizon, I., Feio, M. J., Filipe, A. F., Fornaroli, R., Graf, W., Herder, J., van der Hoorn, B., Iwan Jones, J., Sagova-Mareckova, M., Moritz, C., … Kahlert, M. (2018). The future of biotic indices in the ecogenomic era: Integrating (e)DNA metabarcoding in biological assessment of aquatic ecosystems. Science of The Total Environment, 637–638, 1295–1310. https://doi.org/10.1016/j.scitotenv.2018.05.002

Paxton, A. B., Shertzer, K. W., Bacheler, N. M., Kellison, G. T., Riley, K. L., & Taylor, J. C. (2020). Meta-analysis reveals artificial reefs can be effective tools for fish community enhancement but are not one-size-fits-all. Frontiers in Marine Science, 7, 534479. https://doi.org/10.3389/FMARS.2020.00282/BIBTEX

Pearce, B., Hill, J. M., Wilson, C., Griffin, R., Earnshaw, S., & Pitts, J. (2013). Sabellaria spinulosa Reef Ecology and Ecosystem Services. In The Crown Estate 120 pages ISBN 978-1-906410-27-8 (Issue January). https://doi.org/10.13140/2.1.4856.0644

Petersen, J. K., Maar, M., Ysebaert, T., & Herman, P. M. J. (2013). Near-bed gradients in particles and nutrients above a mussel bed in the limfjorden: Influence of physical mixing and mussel filtration. Marine Ecology Progress Series, 490, 137–146. https://doi.org/10.3354/meps10444

Peterson, C. H., Grabowski, J. H., & Powers, S. P. (2003). Estimated enhancement of fish production resulting from restoring oyster reef habitat: Quantitative valuation. Marine Ecology Progress Series, 264, 249–264. https://doi.org/10.3354/meps264249

Pierce, G. J., Santos, M. B., Smeenk, C., Saveliev, A., & Zuur, A. F. (2007). Historical trends in the incidence of strandings of sperm whales (Physeter macrocephalus) on North Sea coasts: An association with positive temperature anomalies. Fisheries Research, History of Marine Animal Populations and Their Exploitation in Northern Europe, 87(2), 219–228. https://doi.org/10.1016/j.fishres.2007.06.001

Pikesley, S. K., Solandt, J.-L., Trundle, C., & Witt, M. J. (2021). Benefits beyond "features": Cooperative monitoring highlights MPA value for enhanced seabed integrity. Marine Policy, 134, 104801. https://doi.org/10.1016/j.marpol.2021.104801

Piñol, J., Senar, M. A., & Symondson, W. O. C. (2019). The choice of universal primers and the characteristics of the species mixture determine when DNA metabarcoding can be quantitative. Molecular Ecology, 28(2), 407–419. https://doi.org/10.1111/mec.14776

Plonus, R., & Floeter, J. (2024). Identification of plankton habitats in the North Sea. Ecology and Evolution, 14(10), e70342. https://doi.org/10.1002/ece3.70342

Pochon, X., Zaiko, A., Fletcher, L. M., Laroche, O., & Wood, S. A. (2017). Wanted dead or alive? Using metabarcoding of environmental DNA and RNA to distinguish living assemblages for biosecurity applications. PLOS ONE, 12(11), e0187636. https://doi.org/10.1371/journal.pone.0187636

Polanco F., A., Richards, E., Flück, B., Valentini, A., Altermatt, F., Brosse, S., Walser, J.-C., Eme, D., Marques, V., Manel, S., Albouy, C., Dejean, T., & Pellissier, L. (2021). Comparing the performance of 12S mitochondrial primers for fish environmental DNA across ecosystems. Environmental DNA, 3(6), 1113–1127. https://doi.org/10.1002/edn3.232

Ponti, M., Linares, C., Cerrano, C., Rodolfo-Metalpa, R., & Hoeksema, B. W. (2021). Editorial: Biogenic Reefs at Risk: Facing Globally Widespread Local Threats and Their Interaction With Climate Change Editorial on the Research Topic Biogenic Reefs at Risk: Facing Globally Widespread Local Threats and Their Interaction With Climate Change. Frontiers in Marine Science | Www.Frontiersin.Org, 8, 793038. https://doi.org/10.3389/fmars.2021.793038

Pope, L. C., Liggins, L., Keyse, J., Carvalho, S. B., & Riginos, C. (2015). Not the time or the place: The missing spatio-temporal link in publicly available genetic data. Molecular Ecology, 24(15), 3802–3809. https://doi.org/10.1111/mec.13254

Porz, L., Zhang, W., Christiansen, N., Kossack, J., Daewel, U., & Schrum, C. (2024). Quantification and mitigation of bottom-trawling impacts on sedimentary organic carbon stocks in the North Sea. Biogeosciences, 21(10), 2547–2570. https://doi.org/10.5194/bg-21-2547-2024

Pruitt, K. D. (2004). NCBI Reference Sequence (RefSeq): A curated non-redundant sequence database of genomes, transcripts and proteins. Nucleic Acids Research, 33(Database issue), D501–D504. https://doi.org/10.1093/nar/gki025

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., & Glöckner, F. O. (2012). The SILVA ribosomal RNA gene database project: Improved data processing and web-based tools. Nucleic Acids Research, 41(D1), D590–D596. https://doi.org/10.1093/nar/gks1219

Quince, C., Walker, A. W., Simpson, J. T., Loman, N. J., & Segata, N. (2017). Shotgun metagenomics, from sampling to analysis. Nature Biotechnology, 35(9), 833–844. https://doi.org/10.1038/nbt.3935

Quintana, C. O., Méléder, V., Sousa, A. I., Drakou, E. G., van der Wal, D., Krause-Jensen, D., & Andreu-Boussut, V. (2025). Editorial: Coastal rewilding as a nature-based solution. Frontiers in Marine Science, 11. https://doi.org/10.3389/fmars.2024.1547177

Radulovici, A. E., Vieira, P. E., Duarte, S., Teixeira, M. A. L., Borges, L. M. S., Deagle, B. E., Majaneva, S., Redmond, N., Schultz, J. A., & Costa, F. O. (2021). Revision and annotation of DNA barcode records for marine invertebrates: Report of the 8th iBOL conference hackathon. Metabarcoding and Metagenomics, 5, e67862. https://doi.org/10.3897/mbmg.5.67862

Rahman, S. R., & Lozier, J. D. (2023). Genome-wide DNA methylation patterns in bumble bee (Bombus vosnesenskii) populations from spatial-environmental range extremes. Scientific Reports, 13(1), 14901. https://doi.org/10.1038/s41598-023-41896-7

Ramón-Laca, A., Nichols, K. M., Parsons, K. M., Gallego, R., Vieites, D. R., Doadrio, I., & Riesgo Gil, A. (2025). Prospects of environmental nucleic acids (eDNA and eRNA) as multipurpose biomonitoring tools for fisheries management. https://digital.csic.es/handle/10261/389419

Rang, F. J., Kloosterman, W. P., & de Ridder, J. (2018). From squiggle to basepair: Computational approaches for improving nanopore sequencing read accuracy. Genome Biology, 19(1), 1–11. https://doi.org/10.1186/s13059-018-1462-9

Ransome, E., Geller, J. B., Timmers, M., Leray, M., Mahardini, A., Sembiring, A., Collins, A. G., & Meyer, C. P. (2017). The importance of standardization for biodiversity comparisons: A case study using autonomous reef monitoring structures (ARMS) and metabarcoding to measure cryptic diversity on Mo'orea coral reefs, French Polynesia. PLOS ONE, 12(4), e0175066. https://doi.org/10.1371/journal.pone.0175066

Ratnasingham, S., & Hebert, P. D. N. (2007). BOLD: The Barcode of Life Data System: Barcoding. Molecular Ecology Notes, 7(3), 355–364. https://doi.org/10.1111/j.1471-8286.2007.01678.x

Rees, E. I. S., Sanderson, W. G., Mackie, A. S. Y., & Holt, R. H. F. (2008). Small-scale variation within a Modiolus modiolus (Mollusca: Bivalvia) reef in the Irish Sea. III. Crevice, sediment infauna and epifauna from targeted cores. Journal of the Marine Biological Association of the United Kingdom, 88(1), 151–156. https://doi.org/10.1017/S0025315408000052

Rees, H. C., Maddison, B. C., Middleditch, D. J., Patmore, J. R. M., & Gough, K. C. (2014). REVIEW: The detection of aquatic animal species using environmental DNA – a review of eDNA as a survey tool in ecology. Journal of Applied Ecology, 51(5), 1450–1459. https://doi.org/10.1111/1365-2664.12306

Reijnders, P. J. H., & Lankester, K. (1990). Status of marine mammals in the North sea. Netherlands Journal of Sea Research, 26(2), 427–435. https://doi.org/10.1016/0077-7579(90)90098-2

Reiss, H., Degraer, S., Duineveld, G. C. A., Kröncke, I., Aldridge, J., Craeymeersch, J. A., Eggleton, J. D., Hillewaert, H., Lavaleye, M. S. S., Moll, A., Pohlmann, T., Rachor, E., Robertson, M., Vanden Berghe, E., Van Hoey, G., & Rees, H. L. (2010). Spatial patterns of infauna, epifauna, and demersal fish communities in the North Sea. ICES Journal of Marine Science, 67(2), 278–293. https://doi.org/10.1093/icesjms/fsp253

Reubens, J. T., Braeckman, U., Vanaverbeke, J., Van Colen, C., Degraer, S., & Vincx, M. (2013). Aggregation at windmill artificial reefs: CPUE of Atlantic cod (Gadus morhua) and pouting (Trisopterus luscus) at different habitats in the Belgian part of the North Sea. Fisheries Research, 139, 28–34. https://doi.org/10.1016/j.fishres.2012.10.011

Reubens, J. T., Degraer, S., & Vincx, M. (2011). Aggregation and feeding behaviour of pouting (Trisopterus luscus) at wind turbines in the Belgian part of the North Sea. Fisheries Research, 108(1), 223–227. https://doi.org/10.1016/J.FISHRES.2010.11.025

Reubens, J. T., Degraer, S., & Vincx, M. (2014). The ecology of benthopelagic fishes at offshore wind farms: A synthesis of 4 years of research. Hydrobiologia, 727(1), 121–136. https://doi.org/10.1007/s10750-013-1793-1

Rey, A., Viard, F., Lizé, A., Corre, E., Valentini, A., & Thiriet, P. (2023). Coastal rocky reef fish monitoring in the context of the Marine Strategy Framework Directive: Environmental DNA metabarcoding complements underwater visual census. Ocean & Coastal Management, 241, 106625. https://doi.org/10.1016/j.ocecoaman.2023.106625

Riaz, T., Shehzad, W., Viari, A., Pompanon, F., Taberlet, P., & Coissac, E. (2011). EcoPrimers: Inference of new DNA barcode markers from whole genome sequence analysis. Nucleic Acids Research, 39(21), e145–e145. https://doi.org/10.1093/nar/gkr732

Riemann, B., Nielsen, T., Horsted, S., Bjomsen, P., & Pock-Steen, J. (1988). Regulation of phytoplankton biomass in estuarine enclosures. Marine Ecology Progress Series, 48, 205–215. https://doi.org/10.3354/meps048205

Riginos, C., Crandall, E. D., Liggins, L., Gaither, M. R., Ewing, R. B., Meyer, C., Andrews, K. R., Euclide, P. T., Titus, B. M., Therkildsen, N. O., Salces-Castellano, A., Stewart, L. C., Toonen, R. J., & Deck, J. (2020). Building a global genomics observatory: Using GEOME (the Genomic Observatories Metadatabase) to expedite and improve deposition and retrieval of genetic data and metadata for biodiversity research. Molecular Ecology Resources, 20(6), 1458–1469. https://doi.org/10.1111/1755-0998.13269

Rijnsdorp, A. (1996). Changes in growth of North Sea plaice since 1950 in relation to density, eutrophication, beam-trawl effort, and temperature. ICES Journal of Marine Science, 53(6), 1199–1213. https://doi.org/10.1006/jmsc.1996.0145

Rijnsdorp, A. D., Bastardie, F., Bolam, S. G., Buhl-Mortensen, L., Eigaard, O. R., Hamon, K. G., Hiddink, J. G., Hintzen, N. T., Ivanović, A., Kenny, A., Laffargue, P., Nielsen, J. R., O'Neill, F. G., Piet, G. J., Polet, H., Sala, A., Smith, C., van Denderen, P. D., van Kooten, T., & Zengin, M. (2016). Towards a framework for the quantitative assessment of trawling impact on the seabed and benthic ecosystem. ICES Journal of Marine Science, 73(suppl_1), i127–i138. https://doi.org/10.1093/icesjms/fsv207

Rijnsdorp, A. D., Bolam, S. G., Garcia, C., Hiddink, J. G., Hintzen, N. T., van Denderen, P. D., & van Kooten, T. (2018). Estimating sensitivity of seabed habitats to disturbance by bottom trawling based on the longevity of benthic fauna. Ecological Applications, 28(5), 1302–1312. https://doi.org/10.1002/eap.1731

Ritari, J., Salojärvi, J., Lahti, L., & de Vos, W. M. (2015). Improved taxonomic assignment of human intestinal 16S rRNA sequences by a dedicated reference database. BMC Genomics, 16(1), 1056. https://doi.org/10.1186/s12864-015-2265-y

Roberts, L., Pérez-Domínguez, R., & Elliott, M. (2016). Use of baited remote underwater video (BRUV) and motion analysis for studying the impacts of underwater noise upon free ranging fish and implications for marine energy management. Marine Pollution Bulletin, 112(1), 75–85. https://doi.org/10.1016/j.marpolbul.2016.08.039

Roberts, S. B., & Gavery, M. R. (2012). Is There a Relationship between DNA Methylation and Phenotypic Plasticity in Invertebrates? Frontiers in Physiology, 2. https://doi.org/10.3389/fphys.2011.00116

Robinson, C. V., Porter, T. M., Maitland, V. C., Wright, M. T. G., & Hajibabaei, M. (2022). Multi-marker metabarcoding resolves subtle variations in freshwater condition: Bioindicators, ecological traits, and trophic interactions. Ecological Indicators, 145, 109603. https://doi.org/10.1016/j.ecolind.2022.109603

Rodríguez-Pérez, H., Ciuffreda, L., & Flores, C. (2020). NanoCLUST: a species-level analysis of 16S rRNA nanopore sequencing data. Bioinformatics. https://doi.org/10.1093/bioinformatics/btaa900

Rodríguez-Ezpeleta, N., Zinger, L., Kinziger, A., Bik, H. M., Bonin, A., Coissac, E., Emerson, B. C., Lopes, C. M., Pelletier, T. A., Taberlet, P., & Narum, S. (2021). Biodiversity monitoring using environmental DNA. Molecular Ecology Resources, 21(5), 1405–1409. https://doi.org/10.1111/1755-0998.13399

Rourke, M. L., Fowler, A. M., Hughes, J. M., Broadhurst, M. K., DiBattista, J. D., Fielder, S., Wilkes Walburn, J., & Furlan, E. M. (2022). Environmental DNA (eDNA) as a tool for

assessing fish biomass: A review of approaches and future considerations for resource surveys. Environmental DNA, 4(1), 9–33. https://doi.org/10.1002/edn3.185

Rubbens, P., Brodie, S., Cordier, T., Destro Barcellos, D., Devos, P., Fernandes-Salvador, J. A., Fincham, J. I., Gomes, A., Handegard, N. O., Howell, K., Jamet, C., Kartveit, K. H., Moustahfid, H., Parcerisas, C., Politikos, D., Sauzède, R., Sokolova, M., Uusitalo, L., Van den Bulcke, L., … Irisson, J.-O. (2023). Machine learning in marine ecology: An overview of techniques and applications. ICES Journal of Marine Science, 80(7), 1829–1853. https://doi.org/10.1093/icesjms/fsad100

Ruiz, E., Leprieur, F., Sposito, G., Lüthi, M., Schmidlin, M., Panfili, J., Pellissier, L., & Albouy, C. (2025). Environmental DNA Epigenetics Accurately Predicts the Age of Cultured Fish Larvae. Ecology and Evolution, 15(2), e70645. https://doi.org/10.1002/ece3.70645

Ruppert, K. M., Kline, R. J., & Rahman, M. S. (2019). Past, present, and future perspectives of environmental DNA (eDNA) metabarcoding: A systematic review in methods, monitoring, and applications of global eDNA. Global Ecology and Conservation, 17, e00547–e00547. https://doi.org/10.1016/j.gecco.2019.e00547

Sahlin, K., Lim, M. C. W., & Prost, S. (2021). NGSpeciesID: DNA barcode and amplicon consensus generation from long-read sequencing data. Ecology and Evolution, 11(3), 1392–1398. https://doi.org/10.1002/ece3.7146

Saito, T., Espe, M., Mommens, M., Bock, C., Fernandes, J. M. O., & Skjærven, K. H. (2025). Altered spawning seasons of Atlantic salmon broodstock transcriptionally and epigenetically influence cell cycle and lipid-mediated regulations in their offspring. PLOS ONE, 20(2), e0317770. https://doi.org/10.1371/journal.pone.0317770

Samuel, R. M., Meyer, R., Buttigieg, P. L., Davies, N., Jeffery, N. W., Meyer, C., Pavloudi, C., Pitz, K. J., Sweetlove, M., Theroux, S., Van De Kamp, J., & Watts, A. (2021). Toward a Global Public Repository of Community Protocols to Encourage Best Practices in Biomolecular Ocean Observing and Research. Frontiers in Marine Science, 8, 758694. https://doi.org/10.3389/fmars.2021.758694

Santos, A., van Aerle, R., Barrientos, L., & Martinez-Urtaza, J. (2020). Computational methods for 16S metabarcoding studies using Nanopore sequencing data. Computational and Structural Biotechnology Journal, 18, 296–305. https://doi.org/10.1016/j.csbj.2020.01.005

Sard, N. M., Herbst, S. J., Nathan, L., Uhrig, G., Kanefsky, J., Robinson, J. D., & Scribner, K. T. (2019). Comparison of fish detections, community diversity, and relative abundance using environmental DNA metabarcoding and traditional gears. Environmental DNA, 1(4), 368–384. https://doi.org/10.1002/edn3.38

Sassoubre, L. M., Yamahara, K. M., Gardner, L. D., Block, B. A., & Boehm, A. B. (2016). Quantification of Environmental DNA (eDNA) Shedding and Decay Rates for Three Marine Fish. Environmental Science & Technology, 50(19), 10456–10464. https://doi.org/10.1021/acs.est.6b03114

Sayers, E. W., Cavanaugh, M., Frisse, L., Pruitt, K. D., Schneider, V. A., Underwood, B. A., Yankie, L., & Karsch-Mizrachi, I. (2025). GenBank 2025 update. Nucleic Acids Research, 53(D1), D56–D61. https://doi.org/10.1093/nar/gkae1114

Scarano, C., Veneruso, I., De Simone, R. R., Di Bonito, G., Secondino, A., & D'Argenio, V. (2024). The Third-Generation Sequencing Challenge: Novel Insights for the Omic Sciences. Biomolecules, 14(5), 568. https://doi.org/10.3390/biom14050568

Scheidat, M., Tougaard, J., Brasseur, S., Carstensen, J., Van Polanen Petel, T., Teilmann, J., & Reijnders, P. (2011). Harbour porpoises (Phocoena phocoena) and wind farms: A case

study in the Dutch North Sea. Environmental Research Letters, 6(2), 025102. https://doi.org/10.1088/1748-9326/6/2/025102

Schenk, J., Geisen, S., Kleinbölting, N., & Traunspurger, W. (2019). Metabarcoding data allow for reliable biomass estimates in the most abundant animals on earth. Metabarcoding and Metagenomics, 3, 117–126. https://doi.org/10.3897/mbmg.3.46704

Schon, E. A. (2000). Mitochondrial genetics and disease. Trends in Biochemical Sciences, 25(11), 555–560. https://doi.org/10.1016/S0968-0004(00)01688-1

Schramm, K. D., Harvey, E. S., Goetze, J. S., Travers, M. J., Warnock, B., & Saunders, B. J. (2020). A comparison of stereo-BRUV, diver operated and remote stereo-video transects for assessing reef fish assemblages. Journal of Experimental Marine Biology and Ecology, 524, 151273. https://doi.org/10.1016/j.jembe.2019.151273

Schwartzbach A, Behrens J, Svendsen J (2020) Atlantic cod Gadus morhua save energy on stone reefs: implications for the attraction versus production debate in relation to reefs. Mar Ecol Prog Ser 635:81–87.

Schwartzbach, A., Behrens, J., & Svendsen, J. (2020). Atlantic cod Gadus morhua save energy on stone reefs: Implications for the attraction versus production debate in relation to reefs. Marine Ecology Progress Series, 635, 81–87. https://doi.org/10.3354/meps13192

Scriver, M., Zaiko, A., Pochon, X., & von Ammon, U. (2023). Harnessing decay rates for coastal marine biosecurity applications: A review of environmental DNA and RNA fate. Environmental DNA, 5(5), 960–972. https://doi.org/10.1002/edn3.405

Seitz, R. D., Wennhage, H., Bergström, U., Lipcius, R. N., & Ysebaert, T. (2014). Ecological value of coastal habitats for commercially and ecologically important species. ICES Journal of Marine Science, 71(3), 648–665. https://doi.org/10.1093/icesjms/fst152

Sell, A. F., & Kröncke, I. (2013). Correlations between benthic habitats and demersal fish assemblages—A case study on the Dogger Bank (North Sea). Journal of Sea Research, 80, 12–24. https://doi.org/10.1016/J.SEARES.2013.01.007

Semmouri, I., De Schamphelaere, K. A. C., Willemse, S., Vandegehuchte, M. B., Janssen, C. R., & Asselman, J. (2021). Metabarcoding reveals hidden species and improves identification of marine zooplankton communities in the North Sea. ICES Journal of Marine Science, 78(9), 3411–3427. https://doi.org/10.1093/icesjms/fsaa256

Serite, C. P., Emami-Khoyi, A., Ntshudisane, O. K., James, N. C., Jansen Van Vuuren, B., Bodill, T., Cowley, P. D., Whitfield, A. K., & Teske, P. R. (2023). eDNA metabarcoding vs metagenomics: An assessment of dietary competition in two estuarine pipefishes. Frontiers in Marine Science, 10, 1116741. https://doi.org/10.3389/fmars.2023.1116741

Serivichyaswat, P. T., Scholte, T., Wilms, T., Stranddorf, L., & van der Valk, T. (2025). Metagenomic biodiversity assessment within an offshore wind farm. Scientific Reports, 15(1), 16786. https://doi.org/10.1038/s41598-025-01541-x

Seymour, M., Durance, I., Cosby, B. J., Ransom-Jones, E., Deiner, K., Ormerod, S. J., Colbourne, J. K., Wilgar, G., Carvalho, G. R., de Bruyn, M., Edwards, F., Emmett, B. A., Bik, H. M., & Creer, S. (2018). Acidity promotes degradation of multi-species environmental DNA in lotic mesocosms. Communications Biology, 1(1). https://doi.org/10.1038/s42003-017-0005-3

Seymour, M., Edwards, F. K., Cosby, B. J., Bista, I., Scarlett, P. M., Brailsford, F. L., Glanville, H. C., de Bruyn, M., Carvalho, G. R., & Creer, S. (2021). Environmental DNA provides higher resolution assessment of riverine biodiversity and ecosystem function via spatio-temporal nestedness and turnover partitioning. Communications Biology, 4(1), 512. https://doi.org/10.1038/s42003-021-02031-2

Sguotti, C., Blöcker, A. M., Färber, L., Blanz, B., Cormier, R., Diekmann, R., Letschert, J., Rambo, H., Stollberg, N., Stelzenmüller, V., Stier, A. C., & Möllmann, C. (2022). Irreversibility of regime shifts in the North Sea. Frontiers in Marine Science, 9. https://doi.org/10.3389/fmars.2022.945204

Sguotti, C., Lynam, C. P., García-Carreras, B., Ellis, J. R., & Engelhard, G. H. (2016). Distribution of skates and sharks in the North Sea: 112 years of change. Global Change Biology, 22(8), 2729–2743. https://doi.org/10.1111/gcb.13316

Shabardina, V., Kischka, T., Manske, F., Grundmann, N., Frith, M. C., Suzuki, Y., & Makałowski, W. (2019). NanoPipe—A web server for nanopore MinION sequencing data analysis. GigaScience, 8(2), giy169. https://doi.org/10.1093/gigascience/giy169

Shaffer, M. R., Andruszkiewicz Allan, E., Van Cise, A. M., Parsons, K. M., Shelton, A. O., & Kelly, R. P. (2025). Observation Bias in Metabarcoding. Molecular Ecology Resources, 25(7), e14119. https://doi.org/10.1111/1755-0998.14119

Shao, C., Li, Q., Chen, S., Zhang, P., Lian, J., Hu, Q., Sun, B., Jin, L., Liu, S., Wang, Z., Zhao, H., Jin, Z., Liang, Z., Li, Y., Zheng, Q., Zhang, Y., Wang, J., & Zhang, G. (2014). Epigenetic modification and inheritance in sexual reversal of fish. Genome Research, 24(4), 604–615. https://doi.org/10.1101/gr.162172.113

Shea, M. M., Kuppermann, J., Rogers, M. P., Smith, D. S., Edwards, P., & Boehm, A. B. (2023). Systematic review of marine environmental DNA metabarcoding studies: Toward best practices for data usability and accessibility. PeerJ, 11, e14993. https://doi.org/10.7717/peerj.14993

Shelton, A. O., Gold, Z. J., Jensen, A. J., D'Agnese, E., Andruszkiewicz Allan, E., Van Cise, A., Gallego, R., Ramón-Laca, A., Garber-Yonts, M., Parsons, K., & Kelly, R. P. (2023). Toward quantitative metabarcoding. Ecology, 104(2), e3906. https://doi.org/10.1002/ecy.3906

Shelton, A. O., Ramón-Laca, A., Wells, A., Clemons, J., Chu, D., Feist, B. E., Kelly, R. P., Parker-Stetter, S. L., Thomas, R., Nichols, K. M., & Park, L. (2022). Environmental DNA provides quantitative estimates of Pacific hake abundance and distribution in the open ocean. Proceedings of the Royal Society B: Biological Sciences, 289(1971), 20212613. https://doi.org/10.1098/rspb.2021.2613

Sherman, C. S., Heupel, M. R., Johnson, M., Kaimuddin, M., Qamar, L. M. S., Chin, A., & Simpfendorfer, C. A. (2020). Repeatability of baited remote underwater video station (BRUVS) results within and between seasons. PLoS ONE, 15(12), e0244154. https://doi.org/10.1371/journal.pone.0244154

Shields, T. G., & Lee, W. B. (1998). The incidence of decompression sickness arising from commercial offshore air-diving operations in the UK sector of the North Sea during 1982/83. https://www.osti.gov/etdeweb/biblio/595332

Shin, J., Lee, S., Go, M. J., Lee, S. Y., Kim, S. C., Lee, C. H., & Cho, B. K. (2016). Analysis of the mouse gut microbiome using full-length 16S rRNA amplicon sequencing. Scientific Reports, 6. https://doi.org/10.1038/srep29681

Shu, L., Ludwig, A., & Peng, Z. (2020). Standards for methods utilizing environmental dna for detection of fish species. Genes, 11(3), 296–296. https://doi.org/10.3390/genes11030296

Shum, P., Wäge-Recchioni, J., Sellers, G. S., Johnson, M. L., & Joyce, D. A. (2023). DNA metabarcoding reveals the dietary profiles of a benthic marine crustacean, Nephrops norvegicus. PLOS ONE, 18(11), e0289221. https://doi.org/10.1371/journal.pone.0289221

Sigsgaard, E. E., Jensen, M. R., Winkelmann, I. E., Møller, P. R., Hansen, M. M., & Thomsen, P. F. (2020). Population-level inferences from environmental DNA—Current status

and future perspectives. Evolutionary Applications, 13(2), 245–262. https://doi.org/10.1111/eva.12882

Sigurpalsdottir, B. D., Stefansson, O. A., Holley, G., Beyter, D., Zink, F., Hardarson, M. Þ., Sverrisson, S. Þ., Kristinsdottir, N., Magnusdottir, D. N., Magnusson, O. Þ., Gudbjartsson, D. F., Halldorsson, B. V., & Stefansson, K. (2024). A comparison of methods for detecting DNA methylation from long-read sequencing of human genomes. Genome Biology, 25(1), 69. https://doi.org/10.1186/s13059-024-03207-9

Silliman, K., Spencer, L. H., White, S. J., & Roberts, S. B. (2023). Epigenetic and Genetic Population Structure is Coupled in a Marine Invertebrate. Genome Biology and Evolution, 15(2), evad013. https://doi.org/10.1093/gbe/evad013

Singer, G. A. C., Fahner, N. A., Barnes, J. G., McCarthy, A., & Hajibabaei, M. (2019). Comprehensive biodiversity analysis via ultra-deep patterned flow cell technology: A case study of eDNA metabarcoding seawater. Scientific Reports, 9(1), 5991. https://doi.org/10.1038/s41598-019-42455-9

Simon-Ekeland, A., & Delsett, L. L. (2024). Whales as a Nordic speciality: Collecting cetaceans for Natural History Museums (1860s–1910s). Journal Nordic Museology / Nordisk Museologi, 36(1). https://www.researchgate.net/profile/Lene-Delsett/publication/382149492_Whales_as_a_Nordic_speciality_Collecting_cetaceans_for_Natural_History_Museums_1860s-1910s/links/668fa8d9af9e615a15de1cfd/Whales-as-a-Nordic-speciality-Collecting-cetaceans-for-Natural-History-Museums-1860s-1910s.pdf

Sinniger, F., Pawlowski, J., Harii, S., Gooday, A. J., Yamamoto, H., Chevaldonné, P., Cedhagen, T., Carvalho, G., & Creer, S. (2016). Worldwide Analysis of Sedimentary DNA Reveals Major Gaps in Taxonomic Knowledge of Deep-Sea Benthos. Frontiers in Marine Science, 3. https://doi.org/10.3389/fmars.2016.00092

Skelton, J., Cauvin, A., & Hunter, M. E. (2023). Environmental DNA metabarcoding read numbers and their variability predict species abundance, but weakly in non-dominant species. Environmental DNA, 5(5), 1092–1104. https://doi.org/10.1002/EDN3.355

Smaal, A. C., Ferreira, J. G., Grant, J., Petersen, J. K., & Strand, Ø. (2018). Goods and services of marine bivalves. In Goods and Services of Marine Bivalves. https://doi.org/10.1007/978-3-319-96776-9

Smeenk, C. (1997). Strandings of sperm whales Physeter macrocephalus in the North Sea: History and patterns. Bull Inst R Sci Nat Belg Biol, 67, 15–28.

Sobha, T. R., Vibija, C. P., & Fahima, P. (2023). Coral Reef: A Hot Spot of Marine Biodiversity. 171–194. https://doi.org/10.1007/978-981-19-5841-0_8

Sokolova, M., Thompson, F., Mariani, P., & Krag, L. A. (2021). Towards sustainable demersal fisheries: NepCon image acquisition system for automatic Nephrops norvegicus detection. PLOS ONE, 16(6), e0252824. https://doi.org/10.1371/journal.pone.0252824

Sommer, C., Seuret, M., Gourmelon, N., Bahrami, M., Christlein, V., & Braun, M. (2025, March 15). AI-based animal monitoring for marine biodiversity conservation along the North Sea and Baltic Sea coasts. https://doi.org/10.5194/egusphere-egu25-16694

Sonnewald, M., & Türkay, M. (2012). Environmental influence on the bottom and near-bottom megafauna communities of the Dogger Bank: A long-term survey. Helgoland Marine Research, 66(4), 503–511. https://doi.org/10.1007/s10152-011-0286-8

Spalding, M. D., Ravilious, C., & Green, P. E. (2002). World atlas of coral reefs. In Choice Reviews Online (Vol. 39, Issue 05). https://doi.org/10.5860/choice.39-2540

Śpibida, M., Krawczyk, B., Olszewski, M., & Kur, J. (2017). Modified DNA polymerases for PCR troubleshooting. Journal of Applied Genetics, 58(1), 133–142. https://doi.org/10.1007/s13353-016-0371-4

Srivathsan, A., Baloğlu, B., Wang, W., Tan, W. X., Bertrand, D., Ng, A. H. Q., Boey, E. J. H., Koh, J. J. Y., Nagarajan, N., & Meier, R. (2018). A MinIONTM-based pipeline for fast and cost-effective DNA barcoding. Molecular Ecology Resources, 18(5), 1035–1049. https://doi.org/10.1111/1755-0998.12890

Srivathsan, A., Hartop, E., Puniamoorthy, J., Lee, W. T., Kutty, S. N., Kurina, O., & Meier, R. (2019). Rapid, large-scale species discovery in hyperdiverse taxa using 1D MinION sequencing. BMC Biology, 17(1), 96. https://doi.org/10.1186/s12915-019-0706-9

Srivathsan, A., Lee, L., Katoh, K., Hartop, E., Kutty, S. N., Wong, J., Yeo, D., & Meier, R. (2021). ONTbarcoder and MinION barcodes aid biodiversity discovery and identification by everyone, for everyone. BMC Biology, 19(1), 217. https://doi.org/10.1186/s12915-021-01141-x

Staehr, P. A. U., Dahl, K., Buur, H., Göke, C., Sapkota, R., Winding, A., Panova, M., Obst, M., & Sundberg, P. (2022). Environmental DNA Monitoring of Biodiversity Hotspots in Danish Marine Waters. Frontiers in Marine Science, 8, 800474. https://doi.org/10.3389/fmars.2021.800474

Stat, M., Huggett, M. J., Bernasconi, R., DiBattista, J. D., Berry, T. E., Newman, S. J., Harvey, E. S., & Bunce, M. (2017). Ecosystem biomonitoring with eDNA: Metabarcoding across the tree of life in a tropical marine environment. Scientific Reports, 7(1), 12240. https://doi.org/10.1038/s41598-017-12501-5

Stat, M., John, J., DiBattista, J. D., Newman, S. J., Bunce, M., & Harvey, E. S. (2019). Combined use of eDNA metabarcoding and video surveillance for the assessment of fish biodiversity. Conservation Biology, 33(1), 196–205. https://doi.org/10.1111/cobi.13183

Stefanni, S., Mirimin, L., Stanković, D., Chatzievangelou, D., Bongiorni, L., Marini, S., Modica, M. V., Manea, E., Bonofiglio, F., Del Rio Fernandez, J., Cukrov, N., Gavrilović, A., De Leo, F. C., & Aguzzi, J. (2022a). Framing Cutting-Edge Integrative Deep-Sea Biodiversity Monitoring via Environmental DNA and Optoacoustic Augmented Infrastructures. Frontiers in Marine Science, 8, 797140. https://doi.org/10.3389/fmars.2021.797140

Stevens, B. M., Creed, T. B., Reardon, C. L., & Manter, D. K. (2023a). Comparison of Oxford Nanopore Technologies and Illumina MiSeq sequencing with mock communities and agricultural soil. Scientific Reports, 13(1), 9323. https://doi.org/10.1038/s41598-023-36101-8

Stevens, J. D., & Parsley, M. B. (2023b). Environmental RNA applications and their associated gene targets for management and conservation. Environmental DNA, 5(2), 227–239. https://doi.org/10.1002/edn3.386

Steyaert, M., Priestley, V., Osborne, O., Herraiz, A., Arnold, R., & Savolainen, V. (2020). Advances in metabarcoding techniques bring us closer to reliable monitoring of the marine benthos. Journal of Applied Ecology, 57(11), 2234–2245. https://doi.org/10.1111/1365-2664.13729

Stoeck, T., Bass, D., Nebel, M., Christen, R., & Meredith, D. (2010). Multiple marker parallel tag environmental DNA sequencing reveals a highly complex eukaryotic community in marine anoxic water. Molecular Ecology, 19(s1), 21–31. https://doi.org/10.1111/j.1365-294X.2009.04480.x

Støttrup, J. G., Stenberg, C., Dahl, K., Kristensen, L. D., & Richardson, K. (2014). Restoration of a Temperate Reef: Effects on the Fish Community. Open Journal of Ecology, 4(16), 1045–1059. https://doi.org/10.4236/oje.2014.416086

Suarez-Bregua, P., Álvarez-González, M., Parsons, K. M., Rotllant, J., Pierce, G. J., & Saavedra, C. (2022). Environmental DNA (eDNA) for monitoring marine mammals: Challenges and opportunities. Frontiers in Marine Science, 9, 987774. https://doi.org/10.3389/fmars.2022.987774

Sun, D., Yu, H., & Li, Q. (2023). Starvation-induced changes in sex ratio involve alterations in sex-related gene expression and methylation in Pacific oyster Crassostrea gigas. Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology, 267, 110863. https://doi.org/10.1016/j.cbpb.2023.110863

Sunday, J. M., Popovic, I., Palen, W. J., Foreman, M. G. G., & Hart, M. W. (2014). Ocean circulation model predicts high genetic structure observed in a long-lived pelagic developer. Molecular Ecology, 23(20), 5036–5047. https://doi.org/10.1111/mec.12924

Sutherland, B. J. G., Finke, J. F., Saunders, R., Warne, S., Schulze, A. D., Strohm, J. H. T., Chan, A. M., Suttle, C. A., & Miller, K. M. (2023). Metatranscriptomics reveals a shift in microbial community composition and function during summer months in a coastal marine environment. Environmental DNA, 5(5), 920–933. https://doi.org/10.1002/edn3.353

Svetovidov, A. N. (1986). Gadidae. Fishes of the North-Eastern Atlantic and the Mediterranean, 2, 680–710.

Swartz, S. L., Taylor, B. L., & Rugh, D. J. (2006). Gray whale Eschrichtius robustus population and stock identity. Mammal Review, 36(1), 66–84. https://doi.org/10.1111/j.1365-2907.2006.00082.x

Szoboszlay, M., Schramm, L., Pinzauti, D., Scerri, J., Sandionigi, A., & Biazzo, M. (2023). Nanopore Is Preferable over Illumina for 16S Amplicon Sequencing of the Gut Microbiota When Species-Level Taxonomic Classification, Accurate Estimation of Richness, or Focus on Rare Taxa Is Required. Microorganisms, 11(3), 804. https://doi.org/10.3390/microorganisms11030804

Taberlet, P., Coissac, E., Hajibabaei, M., & Rieseberg, L. H. (2012a). Environmental DNA. Molecular Ecology, 21(8), 1789–1793. https://doi.org/10.1111/j.1365-294X.2012.05542.x

Taberlet, P., Coissac, E., Pompanon, F., Brochmann, C., & Willerslev, E. (2012b). Towards next-generation biodiversity assessment using DNA metabarcoding. Molecular Ecology, 21(8), 2045–2050. https://doi.org/10.1111/j.1365-294X.2012.05470.x

Takahashi, M., Frøslev, T. G., Paupério, J., Thalinger, B., Klymus, K., Helbing, C. C., Villacorta-Rath, C., Silliman, K., Thompson, L. R., Jungbluth, S. P., Yong, S. Y., Formel, S., Jenkins, G., Laporte, M., Deagle, B., Rajbhandari, S., Jeppesen, T. S., Bissett, A., Jerde, C., … Berry, O. (2025). A Metadata Checklist and Data Formatting Guidelines to Make eDNA FAIR (Findable, Accessible, Interoperable, and Reusable). Environmental DNA, 7(3), e70100. https://doi.org/10.1002/edn3.70100

Takahashi, M., Frøslev, T., Jenkins, G., Laporte, M., Thalinger, B., Jerde, C., Rajbhandari, S., Jeppesen, T., Bissett, A., Deagle, B., Hahn, E., Pauperio, J., Newman, P., Klymus, K., & Berry, O. (2024). Best Practice for Publishing Environmental DNA (eDNA) Data According to FAIR Principles. Biodiversity Information Science and Standards, 8, e137742. https://doi.org/10.3897/biss.8.137742

Takahashi, M., Saccò, M., Kestel, J. H., Nester, G., Campbell, M. A., van der Heyde, M., Heydenrych, M. J., Juszkiewicz, D. J., Nevill, P., Dawkins, K. L., Bessey, C., Fernandes, K., Miller, H., Power, M., Mousavi-Derazmahalleh, M., Newton, J. P., White, N. E.,

Richards, Z. T., & Allentoft, M. E. (2023). Aquatic environmental DNA: A review of the macro-organismal biomonitoring revolution. Science of The Total Environment, 873, 162322. https://doi.org/10.1016/j.scitotenv.2023.162322

Tan, G., Opitz, L., Schlapbach, R., & Rehrauer, H. (2019). Long fragments achieve lower base quality in Illumina paired-end sequencing. Scientific Reports, 9(1), 1–7. https://doi.org/10.1038/s41598-019-39076-7

Tan, K., Xu, P., Huang, L., Luo, C., Huang, J., Fazhan, H., & Kwan, K. Y. (2024). Effects of bivalve aquaculture on plankton and benthic community. Science of The Total Environment, 914, 169892. https://doi.org/10.1016/J.SCITOTENV.2024.169892

Tateno, Y., & Gojobori, T. (1997). DNA Data Bank of Japan in the age of information biology. Nucleic Acids Research, 25(1), 14–17. https://doi.org/10.1093/nar/25.1.14

Tauber, H. (1981). 13C evidence for dietary habits of prehistoric man in Denmark. Nature, 292(5821), 332–333. https://doi.org/10.1038/292332a0

Teletchea, F. (2009). Molecular identification methods of fish species: Reassessment and possible applications. Reviews in Fish Biology and Fisheries, 19(3), 265–293. https://doi.org/10.1007/s11160-009-9107-4

Ter Hofstede, R., & Rijnsdorp, A. D. (2011). Comparing demersal fish assemblages between periods of contrasting climate and fishing pressure. ICES Journal of Marine Science, 68(6), 1189–1198. https://doi.org/10.1093/icesjms/fsr053

Thalinger, B., Wolf, E., Traugott, M., & Wanzenböck, J. (2019). Monitoring spawning migrations of potamodromous fish species via eDNA. Scientific Reports, 9(1), 15388. https://doi.org/10.1038/s41598-019-51398-0

Whale, A. S., De Spiegelaere, W., Trypsteen, W., Nour, A. A., Bae, Y.-K., Benes, V., Burke, D., Cleveland, M., Corbisier, P., Devonshire, A. S., Dong, L., Drandi, D., Foy, C. A., Garson, J. A., He, H.-J., Hellemans, J., Kubista, M., Lievens, A., … Huggett, J. F. (2020). The Digital MIQE Guidelines Update: Minimum Information for Publication of Quantitative Digital PCR Experiments for 2020. Clinical Chemistry, 66(8), 1012–1029. https://doi.org/10.1093/clinchem/hvaa125

Theissinger, K., Fernandes, C., Formenti, G., Bista, I., Berg, P. R., Bleidorn, C., Bombarely, A., Crottini, A., Gallo, G. R., Godoy, J. A., Jentoft, S., Malukiewicz, J., Mouton, A., Oomen, R. A., Paez, S., Palsbøll, P. J., Pampoulie, C., Ruiz-López, M. J., Secomandi, S., … Zammit, G. (2023). How genomics can help biodiversity conservation. Trends in Genetics, 39(7), 545–559. https://doi.org/10.1016/j.tig.2023.01.005

Thompson, L. R., & Thielen, P. (2023). Decoding dissolved information: Environmental DNA sequencing at global scale to monitor a changing ocean. Current Opinion in Biotechnology, 81, 102936. https://doi.org/10.1016/j.copbio.2023.102936

Thomsen, P. F., & Willerslev, E. (2015). Environmental DNA – An emerging tool in conservation for monitoring past and present biodiversity. Biological Conservation, 183, 4–18. https://doi.org/10.1016/j.biocon.2014.11.019

Thomsen, P. F., Kielgast, J., Iversen, L. L., Møller, P. R., Rasmussen, M., & Willerslev, E. (2012). Detection of a Diverse Marine Fish Fauna Using Environmental DNA from Seawater Samples. PLoS ONE, 7(8), e41732–e41732. https://doi.org/10.1371/journal.pone.0041732

Thorpe, R. B., Arroyo, N. L., Safi, G., Niquil, N., Preciado, I., Heath, M., Pace, M. C., & Lynam, C. P. (2022). The Response of North Sea Ecosystem Functional Groups to Warming and Changes in Fishing. Frontiers in Marine Science, 9. https://doi.org/10.3389/fmars.2022.841909

Thurstan, R. H., Brockington, S., & Roberts, C. M. (2010). The effects of 118 years of industrial fishing on UK bottom trawl fisheries. Nature Communications, 1(1), 15. https://doi.org/10.1038/ncomms1013

Thurstan, R. H., McCormick, H., Preston, J., Ashton, E. C., Bennema, F. P., Bratoš Cetinić, A., Brown, J. H., Cameron, T. C., da Costa, F., Donnan, D. W., Ewers, C., Fortibuoni, T., Galimany, E., Giovanardi, O., Grancher, R., Grech, D., Hayden-Hughes, M., Helmer, L., Jensen, K. T., … zu Ermgassen, P. S. E. (2024). Historical dataset details the distribution, extent and form of lost Ostrea edulis reef ecosystems. Scientific Data, 11(1), 1198. https://doi.org/10.1038/s41597-024-04048-8

Thurstan, R. H., McCormick, H., Preston, J., Ashton, E. C., Bennema, F. P., Cetinić, A. B., Brown, J. H., Cameron, T. C., da Costa, F., Donnan, D. W., Ewers, C., Fortibuoni, T., Galimany, E., Giovanardi, O., Grancher, R., Grech, D., Hayden-Hughes, M., Helmer, L., Jensen, K. T., … zu Ermgassen, P. S. E. (2024). Records reveal the vast historical extent of European oyster reef ecosystems. Nature Sustainability, 7(12), 1719–1729. https://doi.org/10.1038/s41893-024-01441-4

Tilman, D., Isbell, F., & Cowles, J. M. (2014). Biodiversity and Ecosystem Functioning. Annual Review of Ecology, Evolution, and Systematics, 45(1), 471–493. https://doi.org/10.1146/annurev-ecolsys-120213-091917

Toxqui Rodríguez, M. del S., Vanhollebeke, J., & Derycke, S. (2023). Evaluation of DNA metabarcoding using Oxford Nanopore sequencing for authentication of mixed seafood products. Food Control, 145, 109388. https://doi.org/10.1016/j.foodcont.2022.109388

Trigg, S. A., Venkataraman, Y. R., Gavery, M. R., Roberts, S. B., Bhattacharya, D., Downey-Wall, A., Eirin-Lopez, J. M., Johnson, K. M., Lotterhos, K. E., Puritz, J. B., & Putnam, H. M. (2022). Invertebrate methylomes provide insight into mechanisms of environmental tolerance and reveal methodological biases. Molecular Ecology Resources, 22(4), 1247–1261. https://doi.org/10.1111/1755-0998.13542

Truelove, N. K., Andruszkiewicz, E. A., & Block, B. A. (2019). A rapid environmental DNA method for detecting white sharks in the open ocean. Methods in Ecology and Evolution, 10(8), 1128–1135. https://doi.org/10.1111/2041-210X.13201

Tsuri, K., Ikeda, S., Hirohara, T., Shimada, Y., Minamoto, T., & Yamanaka, H. (2021). Messenger RNA typing of environmental RNA (eRNA): A case study on zebrafish tank water with perspectives for the future development of eRNA analysis on aquatic vertebrates. Environmental DNA, 3(1), 14–21. https://doi.org/10.1002/edn3.169

UNESCO World Heritage Centre. (n.d.). *Wadden Sea*. UNESCO. https://whc.unesco.org/en/list/1314/

Urban, L., Perlas, A., Francino, O., Martí-Carreras, J., Muga, B. A., Mwangi, J. W., Boykin Okalebo, L., Stanton, J. L., Black, A., Waipara, N., Fontsere, C., Eccles, D., Urel, H., Reska, T., Morales, H. E., Palmada-Flores, M., Marques-Bonet, T., Watsa, M., Libke, Z., … van Oosterhout, C. (2023). Real-time genomics for One Health. Molecular Systems Biology, 19(8), MSB202311686. https://doi.org/10.15252/msb.202311686

Urban, P., Bekkevold, D., Degel, H., Hansen, B. K., Jacobsen, M. W., Nielsen, A., & Nielsen, E. E. (2023). Scaling from eDNA to biomass: Controlling allometric relationships improves precision in bycatch estimation. ICES Journal of Marine Science, 80(4), 1066–1078. https://doi.org/10.1093/ICESJMS/FSAD027

Urban, P., Bekkevold, D., Hansen, B. K., Jacobsen, M. W., Nielsen, A., & Nielsen, E. E. (2024). Using eDNA to estimate biomass of bycatch in pelagic fisheries. Environmental DNA, 6(1), e377. https://doi.org/10.1002/edn3.377

Urban, P., Jacobsen, M. W., Bekkevold, D., Nielsen, A., Storr-Paulsen, M., Nijland, R., & Nielsen, E. E. (2024). eDNA based bycatch assessment in pelagic fish catches. Scientific Reports, 14(1), 2976. https://doi.org/10.1038/s41598-024-52543-0

Valk, S., Engelsma, M., Megens, H.-J., Kamermans, P., Murk, A. J., & Nijland, R. (2025). Oysters in transition: Hermaphrodite oysters display unique DNA methylation patterns in gill tissue. BMC Genomics, 26(1), 567. https://doi.org/10.1186/s12864-025-11736-1

van Bleijswijk, J. D. L., Engelmann, J. C., Klunder, L., Witte, H. J., Witte, J. IJ., & van der Veer, H. W. (2020). Analysis of a coastal North Sea fish community: Comparison of aquatic environmental DNA concentrations to fish catches. Environmental DNA, 2(4), 429–445. https://doi.org/10.1002/edn3.67

Van den Bulcke, L., Annelies, D. B., Hans, H., Sara, M., Stephie, S., Willem, W., Jan, W., Kris, H., & Sofie, D. (2024). Comparative study of traditional and DNA-based methods for environmental impact assessment: A case study of marine aggregate extraction in the North Sea. Science of The Total Environment, 946, 174106. https://doi.org/10.1016/j.scitotenv.2024.174106

Van den Bulcke, L., De Backer, A., Ampe, B., Maes, S., Wittoeck, J., Waegeman, W., Hostens, K., & Derycke, S. (2021). Towards harmonization of DNA metabarcoding for monitoring marine macrobenthos: The effect of technical replicates and pooled DNA extractions on species detection. Metabarcoding and Metagenomics, 5, e71107.

Van den Bulcke, L., De Backer, A., Wittoeck, J., Beentjes, K., Maes, S., Christodoulou, M., Martinez Arbizu, P., Sapkota, R., Van der Hoorn, B., Winding, A., Hostens, K., & Derycke, S. (2023). DNA metabarcoding on repeat: Sequencing data of marine macrobenthos are reproducible and robust across labs and protocols. Ecological Indicators, 150, 110207. https://doi.org/10.1016/j.ecolind.2023.110207

van der Loos, L. M., & Nijland, R. (2021). Biases in bulk: DNA metabarcoding of marine communities and the methodology involved. Molecular Ecology, 30(13), 3270–3288. https://doi.org/10.1111/mec.15592

van der Loos, L. M., D'hondt, S., Engelen, A. H., Pavia, H., Toth, G. B., Willems, A., Weinberger, F., De Clerck, O., & Steinhagen, S. (2023). Salinity and host drive Ulva-associated bacterial communities across the Atlantic–Baltic Sea gradient. Molecular Ecology, 32(23), 6260–6277. https://doi.org/10.1111/mec.16462

van der Molen, J., & Pätsch, J. (2022). An overview of Atlantic forcing of the North Sea with focus on oceanography and biogeochemistry. Journal of Sea Research, 189, 102281. https://doi.org/10.1016/j.seares.2022.102281

van der Reijden, K. J., Koop, L., O'Flynn, S., Garcia, S., Bos, O., van Sluis, C., Maaholm, D. J., Herman, P. M. J., Simons, D. G., Olff, H., Ysebaert, T., Snellen, M., Govers, L. L., Rijnsdorp, A. D., & Aguilar, R. (2019). Discovery of Sabellaria spinulosa reefs in an intensively fished area of the Dutch Continental Shelf, North Sea. Journal of Sea Research, 144, 85–94. https://doi.org/10.1016/j.seares.2018.11.008

van der Reis, A. L., Beckley, L. E., Olivar, M. P., & Jeffs, A. G. (2023). Nanopore short-read sequencing: A quick, cost-effective and accurate method for DNA metabarcoding. Environmental DNA, 5(2), 282–296. https://doi.org/10.1002/edn3.374

van Hal, R., Griffioen, A. B., & van Keeken, O. A. (2017). Changes in fish communities on a small spatial scale, an effect of increased habitat complexity by an offshore wind farm. Marine Environmental Research, 126, 26–36. https://doi.org/10.1016/J.MARENVRES.2017.01.009

Van Walraven, L., Hoekendijk, J., Jak, R., Keur, M., Peck, N., Polling, M., Van Dalen, J., & Van Oevelen, D. (2025). Development of integrated zooplankton monitoring for the

Dutch North Sea within the MONS project: ID14 MONS Monitoring Zooplankton Phase 1. Wageningen Marine Research. https://doi.org/10.18174/688432

Vaser, R., Sović, Ivan, Sović, Sović, Nagarajan, N., Šikić1, M., & Šikić1, Š. (2017). Fast and accurate de novo genome assembly from long uncorrected reads. Genome.Cshlp.Org. https://doi.org/10.1101/gr.214270.116

Veilleux, H. D., Misutka, M. D., & Glover, C. N. (2021). Environmental DNA and environmental RNA: Current and prospective applications for biological monitoring. Science of The Total Environment, 782, 146891. https://doi.org/10.1016/j.scitotenv.2021.146891

Verfuss, U. K., Gillespie, D., Gordon, J., Marques, T. A., Miller, B., Plunkett, R., Theriault, J. A., Tollit, D. J., Zitterbart, D. P., Hubert, P., & Thomas, L. (2018). Comparing methods suitable for monitoring marine mammals in low visibility conditions during seismic surveys. Marine Pollution Bulletin, 126, 1–18. https://doi.org/10.1016/j.marpolbul.2017.10.034

Veron, P., Rozanski, R., Marques, V., Joost, S., Deschez, M. E., Trenkel, V. M., Lorance, P., Valentini, A., Polanco F., A., Pellissier, L., Eme, D., & Albouy, C. (2023). Environmental DNA complements scientific trawling in surveys of marine fish biodiversity. ICES Journal of Marine Science, 80(8), 2150–2165. https://doi.org/10.1093/icesjms/fsad139

Viña-Feás, A., Temes-Rodríguez, J., Vidal-Capón, A., Novas, S., Rodríguez-Castro, J., Pequeño-Valtierra, A., Pasantes, J. J., Tubío, J. M. C., & Garcia-Souto, D. (2024). Unravelling epigenetic mechanisms in Cerastoderma edule genome: A comparison of healthy and neoplastic cockles. Molecular Genetics and Genomics, 299(1), 58. https://doi.org/10.1007/s00438-024-02148-z

Voorhuijzen-Harink, M. M., Hagelaar, R., van Dijk, J. P., Prins, T. W., Kok, E. J., & Staats, M. (2019). Toward on-site food authentication using nanopore sequencing. Food Chemistry: X, 2, 100035. https://doi.org/10.1016/j.fochx.2019.100035

Wang Q, Wang Z, Zheng K, Zhang P, Shen L, Chen W, Fan P, Zhang L (2022) Assessing the Diet of a Predator Using a DNA Metabarcoding Approach. Front Ecol Evol 10:902412.

Wang, F., Xiong, W., Liu, Y., Zhai, X., Zhou, J., Li, H., Huang, X., Chen, Y., Zhou, K., & Zhan, A. (2025). Exploring technical improvements for environmental nucleic acids-based biodiversity assessment and management in coastal ecosystems. Journal of Environmental Management, 377, 124724. https://doi.org/10.1016/j.jenvman.2025.124724

Wang, Q., Garrity, G. M., Tiedje, J. M., & Cole, J. R. (2007). Naïve Bayesian Classifier for Rapid Assignment of rRNA Sequences into the New Bacterial Taxonomy. Applied and Environmental Microbiology, 73(16), 5261–5267. https://doi.org/10.1128/AEM.00062-07

Wang, Q., Wang, Z., Zheng, K., Zhang, P., Shen, L., Chen, W., Fan, P., & Zhang, L. (2022). Assessing the Diet of a Predator Using a DNA Metabarcoding Approach. Frontiers in Ecology and Evolution, 10, 902412. https://doi.org/10.3389/FEVO.2022.902412/BIBTEX

Wang, S., Yan, Z., Hänfling, B., Zheng, X., Wang, P., Fan, J., & Li, J. (2021). Methodology of fish eDNA and its applications in ecology and environment. Science of the Total Environment, 755, 142622–142622. https://doi.org/10.1016/j.scitotenv.2020.142622

Wang, X., Li, A., Wang, W., Que, H., Zhang, G., & Li, L. (2021). DNA methylation mediates differentiation in thermal responses of Pacific oyster (Crassostrea gigas) derived from different tidal levels. Heredity, 126(1), 10–22. https://doi.org/10.1038/s41437-020-0351-7

Wang, Y., Zhao, Y., Bollas, A., Wang, Y., & Au, K. F. (2021). Nanopore sequencing technology, bioinformatics and applications. Nature Biotechnology, 39(11), 1348–1365. https://doi.org/10.1038/s41587-021-01108-x

Waterhouse, R. M., Adam-Blondon, A.-F., Agosti, D., Baldrian, P., Balech, B., Corre, E., Davey, R. P., Lantz, H., Pesole, G., Quast, C., Glöckner, F. O., Raes, N., Sandionigi, A., Santamaria, M., Addink, W., Vohradsky, J., Nunes-Jorge, A., Willassen, N. P., & Lanfear, J. (2022). Recommendations for connecting molecular sequence and biodiversity research infrastructures through ELIXIR. F1000Research, 10, 1238. https://doi.org/10.12688/f1000research.73825.2

Watson, D. L., Harvey, E. S., Anderson, M. J., & Kendrick, G. A. (2005). A comparison of temperate reef fish assemblages recorded by three underwater stereo-video techniques. Marine Biology, 148(2), 415–425. https://doi.org/10.1007/S00227-005-0090-6

Watson, M. S., Dickson, J., Franken, O., Govers, L. L., van der Heide, T., Witte, S., & Eriksson, B. K. (2025). Introduced substrates trigger colonization by reef-associated fish in a degraded coastal system. PLoS ONE, 20(1 January), 1–15. https://doi.org/10.1371/journal.pone.0317431

Weber, S., Junk, I., Brink, L., Wörner, M., Künzel, S., Veith, M., Teubner, D., Klein, R., Paulus, M., & Krehenwinkel, H. (2023). Molecular diet analysis in mussels and other metazoan filter feeders and an assessment of their utility as natural eDNA samplers. Molecular Ecology Resources, 23(2), 471–485. https://doi.org/10.1111/1755-0998.13710

Weigand, H., Beermann, A. J., Čiampor, F., Costa, F. O., Csabai, Z., Duarte, S., Geiger, M. F., Grabowski, M., Rimet, F., Rulik, B., Strand, M., Szucsich, N., Weigand, A. M., Willassen, E., Wyler, S. A., Bouchez, A., Borja, A., Čiamporová-Zaťovičová, Z., Ferreira, S., … Ekrem, T. (2019). DNA barcode reference libraries for the monitoring of aquatic biota in Europe: Gap-analysis and recommendations for future work. Science of The Total Environment, 678, 499–524. https://doi.org/10.1016/j.scitotenv.2019.04.247

Weinert, M., Kröncke, I., Meyer, J., Mathis, M., Pohlmann, T., & Reiss, H. (2022). Benthic ecosystem functioning under climate change: Modelling the bioturbation potential for benthic key species in the southern North Sea. PeerJ, 10, e14105. https://doi.org/10.7717/peerj.14105

Wennhage, H., & Pihl, L. (2002). Fish feeding guilds in shallow rocky and soft bottom areas on the Swedish west coast. Journal of Fish Biology, 0(61), 207–228. https://doi.org/10.1016/S0022-1112(02)92078-7

Wieking, G., & Kröncke, I. (2003). Macrofauna communities of the Dogger Bank (central North Sea) in the late 1990s: Spatial distribution, species composition and trophic structure. Helgoland Marine Research, 57(1), 34–46. https://doi.org/10.1007/s10152-002-0130-2

Wieland, K., Pedersen, E. M. F., Olesen, H. J., & Beyer, J. E. (2009). Effect of bottom type on catch rates of North Sea cod (Gadus morhua) in surveys with commercial fishing vessels. Fisheries Research, 96(2–3), 244–251. https://doi.org/10.1016/J.FISHRES.2008.11.010

Wilkinson, M. D., Dumontier, M., Aalbersberg, Ij. J., Appleton, G., Axton, M., Baak, A., Blomberg, N., Boiten, J.-W., Da Silva Santos, L. B., Bourne, P. E., Bouwman, J., Brookes, A. J., Clark, T., Crosas, M., Dillo, I., Dumon, O., Edmunds, S., Evelo, C. T., Finkers, R., … Mons, B. (2016). The FAIR Guiding Principles for scientific data management and stewardship. Scientific Data, 3(1), 160018. https://doi.org/10.1038/sdata.2016.18

Willassen, E., Westgaard, J.-I., Kongsrud, J. A., Hanebrekke, T., Buhl-Mortensen, P., & Holte, B. (2022). Benthic invertebrates in Svalbard fjords—When metabarcoding does

not outperform traditional biodiversity assessment. PeerJ, 10, e14321. https://doi.org/10.7717/peerj.14321

Wiltshire, K. H., Malzahn, A. M., Wirtz, K., Greve, W., Janisch, S., Mangelsdorf, P., Manly, B. F. J., & Boersma, M. (2008). Resilience of North Sea phytoplankton spring bloom dynamics: An analysis of long-term data at Helgoland Roads. Limnology and Oceanography, 53(4), 1294–1302. https://doi.org/10.4319/lo.2008.53.4.1294

Witbaard, R., Kamermans, P., & Bos, O. G. (2020). "Age" determination of Oysters from the Voordelta reef. NIOZ Report, 29.

Wood, C. (2018). The Diver's Guide to Marine Life of Britain and Ireland.

Wood, S. A., Biessy, L., Latchford, J. L., Zaiko, A., Von Ammon, U., Audrezet, F., Cristescu, M. E., & Pochon, X. (2020). Release and degradation of environmental DNA and RNA in a marine system. Science of The Total Environment, 704, 135314. https://doi.org/10.1016/j.scitotenv.2019.135314

WoRMS - World Register of Marine Species. (n.d.). Retrieved March 30, 2025, from https://www.marinespecies.org/

Yang, J., Li, C., Lo, L. S. H., Zhang, X., Chen, Z., Gao, J., U, C., Dai, Z., Nakaoka, M., Yang, H., & Cheng, J. (2024). Artificial Intelligence-Assisted Environmental DNA Metabarcoding and High-Resolution Underwater Optical Imaging for Noninvasive and Innovative Marine Environmental Monitoring. Journal of Marine Science and Engineering, 12(10), 1729. https://doi.org/10.3390/jmse12101729

Yates, M. C., Glaser, D. M., Post, J. R., Cristescu, M. E., Fraser, D. J., & Derry, A. M. (2021). The relationship between eDNA particle concentration and organism abundance in nature is strengthened by allometric scaling. Molecular Ecology, 30(13), 3068–3082. https://doi.org/10.1111/mec.15543

Zanni, M. Y., Trenkel, V. M., & Faillettaz, R. (2025). Decaying Uncertainties: Exploring the Role of Decay Rate Variability in Marine eDNA Dispersal Using Lagrangian Transport Modeling. Environmental DNA, 7(3), e70140. https://doi.org/10.1002/edn3.70140

Zhang, G. K., Chain, F. J. J., Abbott, C. L., & Cristescu, M. E. (2018). Metabarcoding using multiplexed markers increases species detection in complex zooplankton communities. Evolutionary Applications, 11(10), 1901–1914. https://doi.org/10.1111/eva.12694

Zhang, S., Zhao, J., & Yao, M. (2020). A comprehensive and comparative evaluation of primers for metabarcoding eDNA from fish. Methods in Ecology and Evolution, 11(12), 1609–1625. https://doi.org/10.1111/2041-210X.13485

Zhang, T., Li, H., Ma, S., Cao, J., Liao, H., Huang, Q., & Chen, W. (2023). The newest Oxford Nanopore R10.4.1 full-length 16S rRNA sequencing enables the accurate resolution of species-level microbial community profiling. Applied and Environmental Microbiology, 89(10), e00605-23. https://doi.org/10.1128/aem.00605-23

Zhang, Y., Pavlovska, M., Stoica, E., Prekrasna, I., Yang, J., Slobodnik, J., Zhang, X., & Dykyi, E. (2020). Holistic pelagic biodiversity monitoring of the Black Sea via eDNA metabarcoding approach: From bacteria to marine mammals. Environment International, 135, 105307. https://doi.org/10.1016/j.envint.2019.105307

Zhang, Y., Qiu, Y., Liu, K., Zhong, W., Yang, J., Altermatt, F., & Zhang, X. (2024). Evaluating eDNA and eRNA metabarcoding for aquatic biodiversity assessment: From bacteria to vertebrates. Environmental Science and Ecotechnology, 21, 100441. https://doi.org/10.1016/j.ese.2024.100441

Zhao, B., van Bodegom, P. M., & Trimbos, K. (2021). The particle size distribution of environmental DNA varies with species and degradation. Science of The Total Environment, 797, 149175. https://doi.org/10.1016/j.scitotenv.2021.149175

Zhao, B., van Bodegom, P. M., & Trimbos, K. B. (2023). Environmental DNA methylation of Lymnaea stagnalis varies with age and is hypermethylated compared to tissue DNA. Molecular Ecology Resources, 23(1), 81–91. https://doi.org/10.1111/1755-0998.13691

Zhou, X., Li, Y., Liu, S., Yang, Q., Su, X., Zhou, L., Tang, M., Fu, R., Li, J., & Huang, Q. (2013). Ultra-deep sequencing enables high-fidelity recovery of biodiversity for bulk arthropod samples without PCR amplification. GigaScience, 2(1), 2047-217X-2–4. https://doi.org/10.1186/2047-217X-2-4

zu Ermgassen, P. S. E., Grabowski, J. H., Gair, J. R., & Powers, S. P. (2016). Quantifying fish and mobile invertebrate production from a threatened nursery habitat. Journal of Applied Ecology, 53(2), 596–606. https://doi.org/10.1111/1365-2664.12576

zu Ermgassen, P. S. E., McCormick, H., Debney, A., Fariñas-Franco, J. M., Gamble, C., Gillies, C., Hancock, B., Laugen, A. T., Pouvreau, S., Preston, J., Sanderson, W. G., Strand, Å., & Thurstan, R. H. (2024). European Native Oyster Reef Ecosystems Are Universally Collapsed. Conservation Letters, 1–12. https://doi.org/10.1111/conl.13068

zu Ermgassen, P., Bos, O., Debney, A., Gamble, C., Glover, A., Pogoda, B., Pouvreau, S., Sanderson, W., Smyth, D., & Preston, J. (2021). European native oyster habitat restoration monitoring handbook. https://researchportal.port.ac.uk/files/49260381/European_Native_Oyster_Habitat_Monitoring_Handbook_WEB_Final.pdf

Zuur, A. F., & Ieno, E. N. (2025). Book: The World of Zero-Inflated Models. Volume 3: Using GLLVM. Highland Statistics. https://www.highstat.com/index.php/order-books-hika/product/56-coming-in-july-2024-e-book-the-world-of-zero-inflated-models-volume-3-using-multivariate-glmm-and-gllvm

SUMMARY

The North Sea is one of the most productive seas globally and contains several biogenic reefs that host a range of fish and invertebrate species. Yet the North Sea is heavily used, putting substantial pressure on its habitats and marine communities. In response, several conservation and restoration measures, as well as nature-inclusive designs in industry, are in place and need to be guided by robust, transparent monitoring methods. eDNA metabarcoding holds great promise for marine biodiversity assessments, and novel sequencing platforms, such as Oxford Nanopore sequencing, can facilitate long-read metabarcoding and potentially improve the resolution of species detection for monitoring the North Sea. In five chapters, new molecular platforms and methods were verified and applied to explore the biodiversity of natural and artificial habitats of the North Sea.

Chapter 2 compares short-read metabarcoding using Oxford Nanopore sequencing with metabarcoding using a conventional sequencing platform Illumina MiSeq and metagenomics, and assesses their performance for macrobenthos biodiversity assessments in offshore windfarms in the Belgian part of the North Sea. Using marine invertebrate specific markers, Nanopore and MiSeq yielded very similar alpha and beta diversity compositions and were consistent with observed location-specific community patterns. Novaseq metagenomics yielded slightly different community compositions compared to metabarcoding and correlated poorly with size-corrected morphological abundance data. All methods had mismatches with classical monitoring methods, based on morphological identification of species, and likely reflect primer biases and an incomplete reference database. Overall, this chapter emphasises that nanopore-based metabarcoding is a viable alternative to Illumina MiSeq for macrobenthos biodiversity monitoring.

Chapter 3 presents a long-read eDNA metabarcoding method using a 2kb mitochondrial primer targeting the 12S and 16S mitochondrial rRNA and the DECONA bioinformatics processing pipeline to handle error-prone nanopore reads. The results from the 2kb primer were compared with a widely used universal fish (MiFish) primer. Mock communities and aquarium eDNA showed improved species-level resolution with the 2kb fragment. Still, North Sea samples from shipwrecks, the Borkum reef grounds, and a wind park yielded more species detections with the MiFish primer. This is likely due to a lower presence of longer reads in the

environment, as they degrade to shorter fragments over time. This demonstrates the potential of long-read metabarcoding to provide a more accurate picture of species that were recently present.

Chapter 4 presents an eRNA-based metabarcoding method to assess the vertebrate biodiversity on a natural Horse mussel (*Modiolus modiolus)* reef in a marine protected area in the Scottish part of the North Sea. By comparing short-read eDNA, long-read eDNA and short-read eRNA fragments, this chapter evaluates how nucleic acid type and fragment length influence community resulting species community detected. Short-read metabarcoding detected the highest diversity and was the only method that distinguished communities between high and low reef density. eRNA was similar to short-read eDNA but missed many large vertebrates, such as sharks and rays, suggesting that eRNA reflects the active local benthic community. Long fragments from the 2kb primer showed the lowest detectability, contradicting earlier statements that long-read metabarcoding may provide a more temporally accurate signal.

Chapter 5 reviews current and future monitoring tools for assessing North Sea biodiversity and effective monitoring. The chapter outlines the strengths and limitations of existing molecular methods, including eDNA metabarcoding, and metagenomics, and describes improvements to their application. This chapter also explores emerging DNA and RNA-based methods, such as epigenetics and metatranscriptomics, that can potentially improve understanding of ecosystem functioning. The chapter also discusses the challenges of reference database curation and standardisation, and concludes with recommendations for further integrating novel molecular methods into harmonised, long-term biodiversity monitoring of the North Sea.

Chapter 6 applies metabarcoding methods to study marine vertebrate diversity in two North Sea shellfish reefs: a mixed shellfish reef in the Dutch Voordelta and a Horse mussel reef at Noss Head, Scotland, to evaluate their role as essential fish habitats. By comparing stations inside and outside the reef using eDNA and baited remote underwater video (BRUVs), this chapter shows that vertebrate diversity was consistently higher inside the reef and that richness declined with increasing distance from it. The reef community included reef-associated species such as gobies, gunnels, and clingfish, as well as commercially important species like cod and pollock. eDNA detected more species overall, but BRUVs

captured juvenile ling individuals, as verified by eDNA, demonstrating the value of using complementary methods for monitoring.

Chapter 7 discusses the thesis outcomes by examining the effectiveness of novel molecular methods for biodiversity assessment and monitoring in the North Sea. The findings indicate that i) Nanopore metabarcoding is comparable to Illumina metabarcoding for assessing macrobenthos, making it a viable option; ii) Long-read and eRNA metabarcoding enhance species identification and active community detection; and iii) eDNA metabarcoding reveals distinct biodiversity patterns linked to reef density and natural reefs. In summary, this thesis highlights the value of these methods for improved, standardised, non-invasive monitoring of the North Sea ecosystem.

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ABOUT THE AUTHOR



Karlijn Doorenspleet was born in Haarlem on a Wednesday, the 4th of March 1992. Her first inspirations for her career path came from her grandfather, a radiologist who showed her the medical books he kept around, and from summer holidays in La Costa Brava, Spain, where she had her first encounter with the region's colourful benthic life. What could be more inspiring for a curious child than feeling the sticky tentacles of an anemone?

After 6 beautiful years at a high school in Groningen, she decided to pursue a BSc in applied sciences in Medical Laboratory Research at Hanze Hogeschool Groningen, where she gained practical laboratory experience. After a course in environmental microbiology, she completed only her propaedeutic year and then further studied Ecology and Evolution and Marine Biology at the Rijksuniversiteit of Groningen (RUG). During her bachelor's, she took a 6-month Erasmus programme at Umea University and a research internship at the Swedish University of Agricultural Sciences (SLU) in Uppsala, Sweden. Here, she learned about the potential power of high-throughput sequencing technologies for Ecology.

She decided to pursue a Master's degree in Molecular Ecology at Wageningen University (WUR), where she further specialised in the Ecology and diversity of microorganisms and other marine life. For her master's thesis, she joined a research cruise to the Black Sea aboard the R/V Pelagia as part of a larger research program at the Royal Dutch Institute for Marine Sciences (NIOZ), where she conducted fieldwork to understand Anoxic microbial biodiversity associated with several organic carbon sources using next-generation sequencing techniques. As her interests extend beyond microbes alone, she joined the Marine Animal Ecology group at WUR for a PhD on innovative molecular tools to study the biodiversity of the North Sea. She focuses on techniques such as eDNA metabarcoding and Nanopore to detect the diversity of marine fish and invertebrates.

LIST OF PUBLICATIONS

Coral, C*., **Doorenspleet, K.***, C., Christianen, M., Nijland, R., Witbaard, R., Bouma, T. J., van der Heide, T., Murk, A. J., van den Brink, X., Vilijn, M., Oudelaar, R. R., & Coolen, J. W. P. (2026). *Shellfish reefs as Essential Fish Habitats: complementary insights from eDNA and BRUVs in the North Sea.* Marine Ecology Progress Series. https://doi.org/10.3354/meps15179

Doorenspleet, K*., Jansen, L.*, Oosterbroek, S.*, Kamermans, P., Bos, O., Wurz | Erik, Murk, A., & Nijland, R. (2025). *The Long and the Short of It: Nanopore-Based eDNA Metabarcoding of Marine Vertebrates Works; Sensitivity and Species-Level Assignment Depend on Amplicon Lengths.* Molecular Ecology Resources, e14079. https://doi.org/10.1111/1755-0998.14079

Doorenspleet, K., Mailli, A. A., van der Hoorn, B. B., Beentjes, K. K., De Backer, A., Derycke, S., Murk, A. J., Reiss, H., & Nijland, R. (2025). *Advancing molecular macrobenthos biodiversity monitoring: a comparison between Oxford Nanopore and Illumina based metabarcoding and metagenomics.* PeerJ, 13:e19158. https://doi.org/10.7717/peerj.19158

Suominen, S., **Doorenspleet, K.**, Sinninghe Damsté, J. S., & Villanueva, L. (2021). *Microbial community development on model particles in the deep sulfidic waters of the Black Sea.* Environmental Microbiology, 23(6), 2729–2746. https://doi.org/10.1111/1462-2920.15588

In preparation/review;

Valk S.**, Doorenspleet, K***., van Berkel, D., van den Brink, X., Jansen, L., Oosterbroek, S., Nijland, R., & Sumner-Hempel, A. (2025). *Current and future molecular tools for monitoring biodiversity in a changing North Sea.* BMC Marine Biology. In review.

Doorenspleet, K., van den Brink, X., Valk S., Coral, C, Coolen, J. W. P.[6], Murk, A. J., Nijland, R. (2026). *The potential of eRNA to assess the local biodiversity in North Sea bivalve reef.* BMC Marine Biology. In preparation.

AUTHORSHIP STATEMENT

PhD candidate's name: Karlijn Doorenspleet
First promotor: Albertinka Murk
Co-promotor: Reindert Nijland
Title of PhD thesis: Improving North Sea biodiversity monitoring using novel molecular approaches

Chapter 1: ***General introduction***
I structured the outline of Chapter 1 and wrote the chapter, the research questions, and the objectives. I generated all the figures, except for Figure 1. After one round of feedback from (co-)promoters, I drafted the final version of Chapter 1. I used AI as a source of inspiration for grammar and sentence structure suggestions.

Chapter 2: ***Advancing molecular macrobenthos biodiversity monitoring: a comparison between Nanopore and Illumina based metabarcoding and metagenomics***
I developed the research questions and conducted lab experiments on Nanopore metabarcoding data. While coauthors handled other experiments, I performed the combined data analysis with input from the shared first coauthor. I drafted the manuscript, created figures and tables, and revised it after feedback from all coauthors and peer review.

Chapter 3: ***The long and the short of it: Nanopore-based eDNA metabarcoding of marine vertebrates works; sensitivity and specificity depend on amplicon lengths***
I formulated the research questions and objectives. Coauthor Lara Jansen designed the primers, and I conducted the laboratory experiments. I advised Saskia Oosterbroek on the bioinformatics pipeline, analysed data independently, created the tables and figures, drafted and revised the chapter based on feedback from coauthors and peer review.

Chapter 4: ***The potential of eRNA to assess the local biodiversity in North Sea bivalve reefs***
I formulated the research questions and objectives, designed the study and with coauthor Xantia van den Brink conducted the experiments. I performed the analysis, produced the final tables and figures and drafted the text for this Chapter. After incorporating input and comments from all coauthors, I improved the figures and the chapter text.

Chapter 5: ***Integrating current and future molecular tools for standardised monitoring of North Sea biodiversity and ecosystem dynamics***
I conceived the idea for this Chapter and, together with coauthor Sophie Valk, formulated the research questions and objectives. I prepared the structure of the review Chapter, and, together with all coauthors, conducted the literature review. I conducted the analysis and drafted the discussion together with co-authors Sophie Valk and Auriel Sumner-Hempel. My coauthors and I revised and modified this Chapter.

Chapter 6: ***Shellfish reefs as Essential Fish Habitats: complementary insights from eDNA and BRUVs in the North Sea***
Together with coauthor Caterina Coral, I formulated the research questions and objectives and conducted fieldwork. I designed and supervised two thesis projects for conducting lab work and video analysis. I conducted and refined the data analysis after input from coauthors. Together with Caterina Coral, I wrote the chapter and created the figures, incorporating feedback from all coauthors.

Chapter 7**:** ***General Discussion.***
I structured the outline of Chapter 7 and wrote the chapter, the research questions, and the objectives. I generated all the figures. After one round of feedback from (co-) promoters, I drafted the final version of Chapter 7. I used AI as a source of inspiration for grammar and sentence structure suggestions.

TRAINING AND EDUCATION STATEMENT

Netherlands Research School for the
Socio-Economic and Natural Sciences of the Environment

D I P L O M A

for specialised PhD training

The Netherlands research school for the
Socio-Economic and Natural Sciences of the Environment
(SENSE) declares that

Karlijn Doorenspleet

born on 4th of March 1992 in Haarlem, The Netherlands

has successfully fulfilled all requirements of the
educational PhD programme of SENSE.

Wageningen, 25 June Month 2026

SENSE coordinator PhD education

Dr Ir Peter Vermeulen

The SENSE Director

Dr Jampel Dell'Angelo

The SENSE Research School declares that **Karlijn Doorenspleet** has successfully fulfilled all requirements of the educational PhD programme of SENSE with a workload of 42.0 EC, including the following activities:

SENSE PhD Courses

- A1: Environmental Research in Context (2020 (1))
- A2 Research in context activity: 'Theatrical performance MENS DNA ZEE, with the aim to communicate and to emotionally connect with the ecology of the North Sea and the molecular work that is needed to understand it.' (2022)

In depth PhD courses (> 2 ECTS)

- Mothur Course, Path sloss (2020)
- Bioinformatics with Python and Linux, WIMEK (2021)
- Advanced Molecular ecology, WUR (2022)
- Biotech 101, WUR (2023)

External training at a foreign research institute

- Learning in depth statistics, Ecology and Fieldwork management, NIOZ/WMR (2022-2024)

Teaching and supervision

- Supervising three MSc students with thesis (2020-2024)
- Supervising one BSc student with thesis (2023)

Oral Presentations

- Biomonitoring of North Sea vertebrates using accurate nanopore based long-read genetics and genomics. Nanopore community meeting NY, 2-3 December 2020, New York, USA
- Complete mitogenome sequencing of marine animals using Cas9 enrichment and Nanopore sequencing. Nanopore community meeting Utrecht, 27 November 2019, Utrecht, the Netherlands

Colophon

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