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ORIGINAL ARTICLE

Environmental DNA reveals spatial patterns of fish and plankton diversity at a floating offshore wind farm

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Abstract

In this study, we collected water eDNA from sampling stations at the first full scale floating offshore wind farm (OWF), the Hywind Pilot Park, east of Peterhead, UK, and a nearby reference area. We combined targeted droplet digital PCR (ddPCR) quantification of two commercially important species, Atlantic mackerel (*Scombrus scombrus*) and Atlantic herring (*Clupea harengus*), with metabarcoding of fish and plankton communities. The goal of this study was to assess the performance of eDNA data to characterize pelagic communities and its use for environmental monitoring. The metabarcoding recovered 26 fish species including both pelagic and demersal taxa. The plankton data were dominated by dinoflagellates (*Karenia*) and calanoid copepods. We found that both specific quantification of eDNA from mackerel and herring and eDNA metabarcoding of fish communities were able to reveal spatial patterns: Mackerel was most abundant in the surface across both OWF and reference sites; herring was present at a wider depth range. While ddPCR and metabarcoding data for these two species were broadly congruent, we observed detection/non-detection mismatches for both methods, highlighting the need for robust sampling design. There was no consistent OWF versus reference area pattern in the demersal fraction of fish assemblages. We interpret our findings as representing a snapshot of fish school location around the time of sampling, and do not consider the single timepoint data from this pilot study to be sufficient to attribute any effects to the OWF itself. As a non-invasive tool, we conclude that eDNA has a high potential in future environmental monitoring of OWFs. We recommend further ground-truthing and biomass correlation of eDNA data with catch data and establishing eDNA time series as next steps towards implementation of eDNA in OWF environmental monitoring.

KEYWORDS
ddPCR, environmental DNA, environmental monitoring, marine fish, metabarcoding, MiFish, offshore wind farm, quantitative assay

Jon Thomassen Hestetun and Jessica Louise Ray contributed equally to the manuscript.

1 | INTRODUCTION

Construction and operation of offshore wind farms (OWFs) can have a variety of effects on local marine life. Effects during the operational phase may be negative or positive (Bergström et al., 2014) and potential impact sources range from noise (Wahlberg & Westerberg, 2005), changes in current and sediment dynamics (Vanhellemont & Ruddick, 2014), or magnetic fields (Cresci, Durif, et al., 2022; Cresci, Perrichon, et al., 2022) to the settling of hard-substrate organisms on artificial structures, nursery and shelter (the artificial reef effect; Krone et al., 2017; Langhamer & Wilhelmsson, 2009), the tendency of fish species to aggregate around artificial structures (the fish aggregating device, or FAD, effect; Bergström et al., 2013; Methratta & Dardick, 2019; Wilhelmsson et al., 2006), refuge effects due to the reduction of other human activities such as bottom trawling in the OWF area (Ashley et al., 2014; Stenberg et al., 2015), and effects on the pelagic ecosystem through increased upper ocean mixing that could increase primary production and lead to changes in community structure and trophic interactions (Broström, 2008; Buyse et al.,2023; Floeter et al., 2022).

While a growing body of literature exists on potential OWF environmental impact, differences in local conditions and the change over time toward larger turbine size and higher number of turbines per installation means that it is difficult to assess the impact of any particular OWF (Bergström et al., 2014). The cumulative impact of potential effects is complex, and it can be challenging to foresee how they interact in terms of changes in marine organism community dynamics in an area where an OWF has been established (Bat et al., 2013). The relative size of many of the hypothesized effects are also difficult to assess, with some effects likely small or otherwise difficult to measure in a dynamic ocean, depending on local conditions (Broström et al., 2008).

Impacts on fish stocks are of particular concern as they represent a potential conflict with the commercial interests of fisheries in the area. Indeed, there has been considerable resistance to the establishment of OWF sites from fishers, who perceive such sites as an encroachment on, and potential threat to, their interests (Gray et al., 2005; Haggett et al., 2020). The current uncertainty of OWF effects on local and regional fish abundances and spatial patterns thus highlights the need for baseline and monitoring studies of individual OWF sites to better understand effects on local fish stocks to fuel knowledge-based management decisions.

Fish stocks are typically assessed using a combination of acoustic and capture data. OWF studies employing such methods include studies of sand eel and dab at the North Sea Horns Rev I OWF site (Stenberg et al., 2015; van Deurs et al., 2012) and on the Dutch coast (Lindeboom et al., 2011), but studies on pelagic species are fewer, with less conclusive results (Methratta & Dardick, 2019). Metabarcoding of environmental DNA (eDNA) using high-throughput sequencing (HTS) is a recent alternative or complement to traditional methods. By detecting DNA shed by fish in the water column, eDNA metabarcoding is a cost-effective and non-invasive method to detect local species richness and monitor the spatiotemporal dynamics of fish communities (Miya, 2022; Shelton et al., 2022). Furthermore, it does not require trawling, which makes it feasible to use in OWF exclusion zones. A handful of different metabarcoding genetic markers are available that target fish communities, including the MiFish mitochondrial 12S rDNA markers (Miya et al., 2015).

While it is difficult to determine exact rates of DNA shedding, degradation, and transport in a particular marine setting (Harrison et al., 2019), studies suggest that eDNA rapidly disperses and degrades in the water column, allowing discrimination of local/recent differences in community composition, for example, at 30–60 m (Murakami et al., 2019) or 20-m intervals (Andruszkiewicz et al., 2017). Thus, eDNA metabarcoding could potentially be used to detect differences in fish communities in an OWF compared to baseline fish stocks in the area and assess impact on local fish species. The number of studies using metabarcoding in fish stock assessment has grown in recent years (Miya, 2022), but this methodology is still largely untested in an OWF setting (Lodge, 2022), with no current published studies.

As with other methods, eDNA metabarcoding has both advantages and limitations. In the case of metabarcoding, choice of genetic marker, bioinformatic pipeline (and filtering options), and the completeness of databases used to assign taxonomic identity all influence study findings (Zinger et al., 2019). Closely related species may not be detected as separate due to insufficient resolution in the marker used (Tang et al., 2012). Finally, PCR amplification introduces bias in metabarcoding abundance (number of sequences "reads"): Certain taxa may be over- or underrepresented in the data, or entirely lacking, that is, false negatives (Zhang et al., 2020), meaning that metabarcoding does not produce true quantitative data (Deagle et al., 2014).

More rigorous quantitative estimates are possible by employing quantitative PCR assays. Several such methods are available, such as quantitative PCR (qPCR), droplet digital PCR (ddPCR), or digital PCR (dPCR; Doi et al., 2015). In ecology, such methods are typically only used for single-species assays, limiting their use in whole community assessments, but they are still highly relevant for particular species of interest such as commercially important fish species (Allan et al., 2021; Fossøy et al., 2020). Thus, both quantitative assays and community metabarcoding have their use in eDNA environmental monitoring, depending on the particular aims of the study (NOAA, 2020).

The Hywind Scotland floating Pilot Park is an OWF consisting of five floating turbine units approximately 25 km East off the coast of Peterhead, UK. In this study, we evaluated the performance of (i) metabarcoding of the fish mitochondrial barcoding gene 12S rDNA (MiFish) and the nuclear 18S rDNA V1-V2 gene to distinguish spatial patterns in the local fish and plankton communities, and (ii) two ddPCR assays to provide quantitative estimates of the commercially important fish species Atlantic mackerel (*Scombrus scombrus*) and Atlantic herring (*Clupea harengus*).

Pelagic fish species are notoriously difficult to monitor in OWFs, and impact data are scarce (Methratta & Dardick, 2019). Floating OWF turbine structure and associated moorings are

physically different than seafloor jacket or pile foundations (Kramer et al., 2015). A previous video survey at the Hywind Pilot Park (Karlsson et al., 2022) revealed colonization of submerged structures consistent with earlier studies of fixed turbines, yet little additional information is available on floating OWFs in the literature (Farr et al., 2021).

The main objective of our study was to assess the performance of species-specific quantitative eDNA detection and metabarcoding community data in future OWF impact monitoring through the following research questions: (1) Does fish eDNA in the water column have sufficient resolution to discern differences in fish abundance (ddPCR) and community composition (metabarcoding) at different depths and between the designated study areas? (2) Are ddPCR and metabarcoding results congruent for species present in both datasets? (3) To what extent do the MiFish and 18S metabarcoding data give an insight into the targeted North Sea pelagic marine communities?

2 | MATERIALS AND METHODS

2.1 | Sample area and sampling design

The Hywind Pilot Park is an OWF with floating turbine design in operation since 2017. It is located 25 km east of Peterhead, Scotland, UK at the Buchan Deep. The OWF consists of five 6 MW turbine units with a hub height of 82–101 m and rotor diameter of 154 m, spaced 800–1600 m apart, extending to a depth of 80 m, anchored to the seafloor with three moorings each with a 600–1200 m mooring radius, and connected by 33 kV inter-array cables to the Peterhead Grange Substation. Water depth in the OWF area is 100–120 m. Currents are dominated by tides moving in a north–south direction, at times at significant speed. Bottom conditions are a blend of gravel and sand with scattered boulders (defined as "circalittoral fine sand"), with no significant sediment contamination levels (Statoil, 2015).

The study design included 10 water sampling stations. Five stations ("Impact Area," thus referred to as stations IA1–IA5) were placed in the OWF, and five stations ("Reference Area," thus referred to as stations RA1–RA5) were placed in a reference area designated in this study 10 km east of the OWF (Figure 1) with similar oceanographic conditions. We noted the degree of drift during sampling (Figure S1). To identify any stratification in the water column, we made CTD profiles at all sampling sites in both the impact and reference areas during sampling (Figure S2). We observed a thermocline around 20 m depth (from around 15 to 10°C) and decided to collect water samples at both 10 and 50 m depth at all 10 sampling stations (Table S1).

Samples were collected on board the OWF service vessel *MCS Swath 1* on August 10, 2021. Equipment and working surfaces were decontaminated with 5% (v/v) sodium hypochlorite, sodium hydroxide solution (household bleach) prior to sampling and between sampling stations. Three samples replicates were taken at 10 m, and three replicates at 50 m for each station. Water was collected using a weighted 5 L-Niskin bottle and dispensed into three 1 L volume capacity brown polypropylene bottles that had been thrice rinsed with sample water prior to filling. These triplicate water subsamples were filtered in parallel through 0.45 µm Sterivex PES filters using peristaltic pumps equipped with four-channel pump heads (Masterflex) and running at a speed of 300 rpm. Subsamples from 10 and 50 m depth were filtered simultaneously on two separate pumps to maximize throughput at each sampling station. Pump tubing was decontaminated between water samples and flushed with 100 mL of the next water sample prior to filter attachment and sample filtration. Excess water was expelled from filters using a 60 mL syringe filled with 0.22 µm sterile-filtered air. Air and water blank samples were collected at each station to control for ambient and carry-over contamination. Air blanks consisted of pressing non-sterile-filtered air from a 60-mL syringe into a 0.45-µm Sterivex PES filter. Water blanks were prepared by filtering 1 L of distilled water (the same water used for rinsing pump tubing) through a 0.45-µm Sterivex PES filter. Finally, all filters were filled with 2 mL ATL Buffer (QIAGEN) as preservative (Majaneva et al., 2018), capped using sterile Luer locks, placed individually inside sterile 50 mL polypropylene tubes, stored cool and dark until the next day, and subsequently stored at −20°C until eDNA extraction.

2.2 | DNA extraction

DNA extraction was done at clean NORCE lab facilities in Bergen (Norway), with lab surfaces and equipment pre-cleaned using 5% bleach solution. Lysis of filtered particles was done inside the Sterivex filters to minimize contamination and maximize lysis efficiency. Sixty µL of 20 mg mL^{-1} Proteinase K (QIAGEN) were added to each thawed filter, which were then incubated at 56°C with gentle rotation overnight. Lysate was aspirated from Sterivex filters using sterile 5 mL syringes. One mL of each lysate was taken for DNA purification, while the remaining lysate was archived at −80°C. DNA purification was conducted using the DNeasy Blood & Tissue kit (QIAGEN) according to the manufacturer's protocol, with two modifications: (1) Added volumes of RNAse A (100 mg mL^{-1}) and Buffer AL were adjusted to compensate for increased starting volume of lysate; (2) Buffer AL-treated lysates were applied to silica spin columns in multiple centrifugation rounds to allow binding of the entire lysate volume. Purified DNA was eluted in 200 µL Buffer EB (QIAGEN) and divided into one archive aliquot (−80°C storage) and one working aliquot (−20°C storage).

2.3 | Metabarcoding

Metabarcoding comprised the MiFish universal fish mt12S rDNA gene primer pair MiFish-U-F (5′-GTCGGTAAAACTCGTGCCAGC-3′) and MiFish-U-R (5′-CATAGTGGGGTATCTAATCCCAGTTTG-3′;

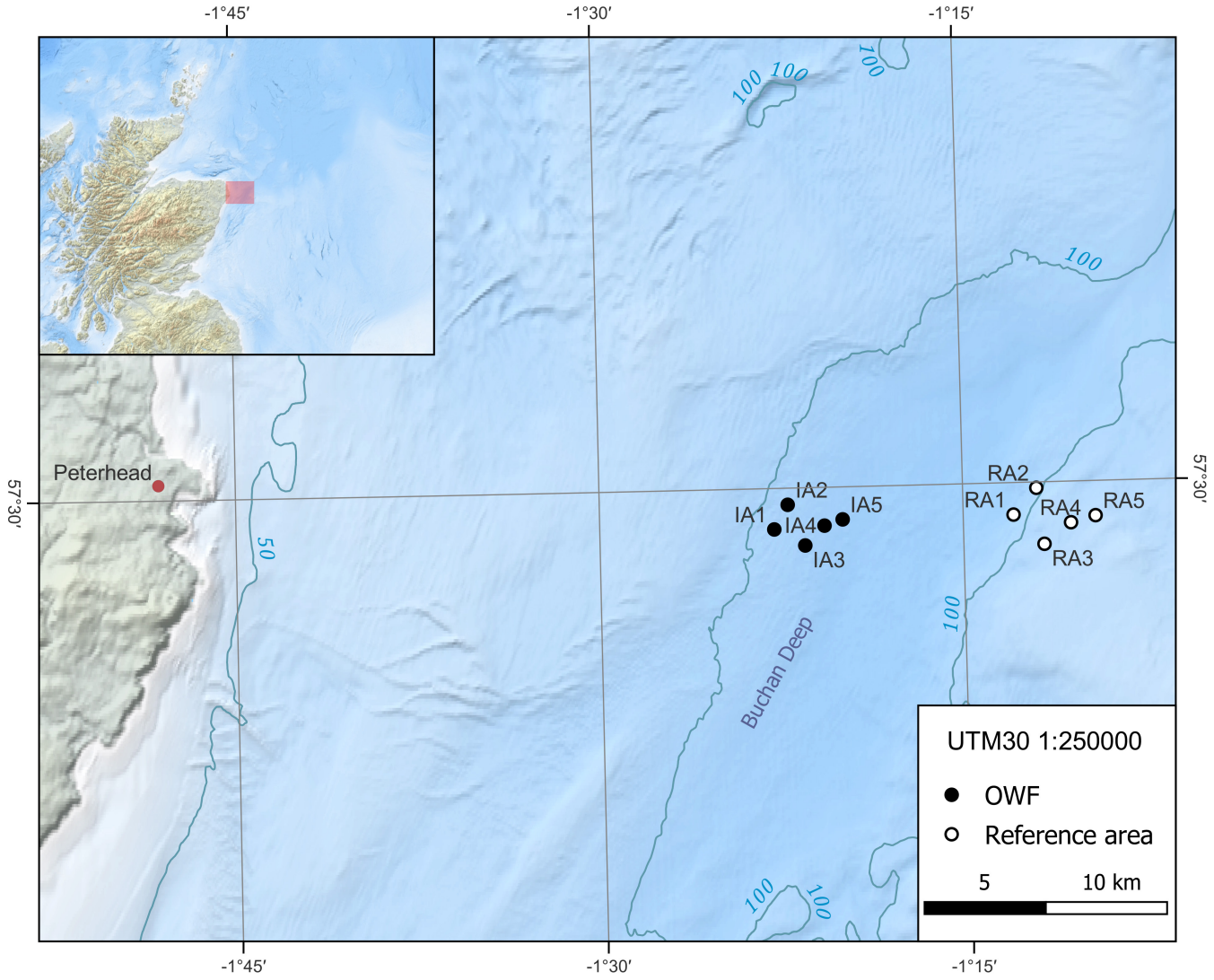

FIGURE 1 The location of the Hywind Pilot Park OWF with stations used for 10 and 50 m depth water sampling in this study. Black circles denote OWF water sampling stations (IA1–IA5), while white stations denote stations in the reference area (RA1–RA5).

Miya et al., 2015), and the 18S rDNA V1-V2 universal eukaryote primer pair SSU_F04mod (5′-GCTTGWCTCAAAGATTAAGCC-3′; Hestetun et al., 2021) and SSU_R22 (5′-CCTGCTGCCTTCCTTRGA-3′; Sinniger et al., 2016) to capture a broad range of eukaryote single-celled and metazoan diversity. PCR amplification was done in a clean-room UV cabinet with adapter-linked primers using the KAPA3G Plant PCR kit (KAPA Biosystems) with 35 cycles of 65°C for MiFish, and 30 cycles of 57°C for 18S primers respectively. Three (18S) and eight (MiFish) PCR replicates were made for each sample and subsequently pooled prior to second PCR (library prep). Purification and quantification after each PCR were done using Magbio beads (MAGBIO Genomics) and a Qubit 4 fluorometer (Thermo Fisher Scientific), respectively. Library preparation was done using equimolar pooled PCR product with Illumina dual index TruSeq i5/i7 barcodes. Contamination was monitored using field sampling, extraction, and PCR negative controls. Sequencing was performed on an Illumina MiSeq instrument using v3 with 300 bp chemistry at the Norwegian Sequencing Centre (University of Oslo, Norway). The raw data were deposited at the NCBI Sequence Read Archive (SRA) (18S, SUB13560331; MiFish 12S, SUB13563458) with BioProject number PRJNA985779.

Initial quality check of sequence fastq files was done using FastQC v0.11.8 (Andrews, 2010). Cutadapt v1.18 (Martin, 2011) and VSEARCH v2.111.1 (Rognes et al., 2016) were used for pairwise merging and filtering, then SWARM v2.2.1 ($d = 1$; Mahé et al., 2015) was used to derive OTUs from dataset sequences, with subsequent post-clustering curation using LULU with default (0.97) parameters (Frøslev et al., 2017). Finally, to reduce cross-sample mis-tagging, we removed OTU occurrences at very low (<1%) abundance compared to all-sample average, analogous to the UNCROSS algorithm (Edgar, 2016). Taxonomy was assigned using CREST4 with the SilvaMod v1.38 database for 18S data (Lanzén et al., 2012), and Sintax assignment using VSEARCH with the MitoFish database (Iwasaki et al., 2013) for the MiFish mt12S data. A few non-target (non-fish) sequences that could not be assigned using MitoFish were identified separately with GenBank blastn.

2.4 | Droplet digital PCR analysis

Quantitative eDNA detection was conducted using a DX200 droplet digital PCR (ddPCR) system (Bio-Rad) with two published assays targeting the mitochondrial cytochrome B gene (*cytB*) of Atlantic mackerel (*Scomber scombrus*) and Atlantic herring (*Clupea harengus*) (Knudsen et al., 2019). For Atlantic mackerel, triplicate 20 μL ddPCR assays consisted of 400 nM forward primer Scosco_CYBF14517 (5′-TTCCCTGC TTGGTCTCTGTT-3′), 800 nM reverse primer Scosco_CYBR14597 (5′-GGCGACTGAGTTGAATGCTG-3′), 200 nM probe Scosco_CYBP14541 (5′-[FAM]TTCCCAAATCCTCACAGGACTATTC[BHQ1]-3′). For Atlantic herring, triplicate 20 μL ddPCR assays per sample consisted of (final concentration) 200 nM forward primer Cluhar_CYBF14928 (5′-CCCATTTGTGATTGCAGGGG-3′), 1000 nM reverse primer Cluhar_CYBR15013 (5′-CTGAGTTAAGTCCTGCCGGG-3′), 200 nM probe Cluhar_CYBP14949 (5′-[FAM]TACTATTCTCCACCTTCTGT TCCTC[BHQ1]-3′). To both assays 1X ddPCR Supermix for probes (Bio-Rad) and 5 μL undiluted template were added. The PCR amplification protocol for mackerel consisted of an initial denaturation at 95°C for 10 min, 45 cycles of 94°C for 30 s and 54°C for 60 s, and a final denaturation at 98°C for 10 min. For herring the protocol was identical except we used 40 cycles with annealing temperature 59°C. After a brief equilibration to room temperature, droplet fluorescence was read using a droplet reader (Bio-Rad) with default settings for FAM detection. Absolute target gene copies per μL in ddPCR reactions were normalized to target gene copies L^{-1} seawater.

2.5 | Statistical analysis

Statistical analysis was done using R (R Core Team, 2020). GPS coordinates were converted using the parzer package (Chamberlain & Sagouis, 2022). Data visualization of ddPCR and metabarcoding results was done using the base (R Core Team, 2020) and ggplot2 (Wickham, 2016) packages. Single-factor (area or depth) explanatory power on ddPCR results (copies L^{-1}) was tested with the stats package (R Core Team, 2020) using the Kruskal–Wallis rank sum test with default parameters. Multivariate analysis on metabarcoding data, including Hellinger transformation, Bray–Curtis dissimilarity, non-metric multidimensional scaling (NMDS) and cluster plots, PERMANOVA and SIMPER analyses were done using the vegan package v 2.5-7 (Oksanen et al., 2019). Bray–Curtis pairwise dissimilarities were calculated on the Hellinger-transformed MiFish (species level) and 18S (OTU level) datasets and visualized using NMDS plots (sample level) and average linkage cluster analyses (station level).

3 | RESULTS

3.1 | MiFish metabarcoding results

The total number of raw sequences from the MiFish dataset was 4,176,470 reads from 60 eDNA samples (five OWF and five reference stations, each at 10 and 50 m depth, each with three sample replicates). The number of sequences in the negative controls were 46,097, of which traces of goldsinny wrasse at RA2 and RA3 (21,874 reads) water negative controls following detection of this species at station RA1, and an unexplained salmon signal in the water negative control at station IA2 (20,539 reads, no salmon signal in other samples except station RA5, sampled much later in the day) accounted for the vast majority. After bioinformatic processing and filtering, 3,206,410 sequences remained, with 29,027–267,001 reads per sample. SWARM clustering identified 2312 OTUs in the dataset, of which only 55 OTUs remained after chimera filtering and LULU curation. Taxonomic assignment of these OTUs using the MitoFish database yielded 26 fish species and five non-target species (harbor porpoise, cattle, sheep, human, and polychaete; Table S2). The non-target OTUs were removed from subsequent analysis of the MiFish dataset.

Atlantic mackerel (*Scomber scombrus*) was the most abundant species in the metabarcoding dataset, followed by sprat (*Sprattus sprattus*), Atlantic herring (*Clupea harengus*), haddock (*Melanogrammus aeglefinus*), pouting (*Trisopterus luscus*), and lemon sole (*Microstomus kitt*) (Figure 2a,b; Table S3).

Reads from the pelagic species mackerel, sprat, and herring (and at lower total abundance, garfish) exhibited higher relative abundance in the 10 m samples than in the 50 m samples (Figure 2a). This higher abundance at 10 m depth was particularly pronounced for mackerel (Table S3). Compared to the reference area, the OWF had higher relative abundances of sprat and herring at 50 m depth, suggesting either larger schools or prolonged periods of activity by these species near or within the OWF (Figure 2b). Mackerel was detected with lower relative abundance at 10 m in the OWF compared with the same depth in the reference area (Figure 2a), although it was only slightly less abundant in the OWF in terms of absolute read abundance at this depth (Figure 2b; Table S4). This suggests that schools of mackerel may have occurred with approximately similar frequency and/or duration in both OWF and reference areas.

The 50 m samples yielded a substantial number of reads from a more diverse assemblage of demersal or bentho-pelagic species, demonstrating a more equitable abundance distribution at this sampling depth (Figure 2b). Fish diversity at 50 m included gadids (cod, haddock, pouting, whiting, and blue ling), golden redfish, salmon (at one station), flatfish (lemon sole, thickback sole, plaice, and witch), and smaller demersal fish (lesser sand eel, goldsinny wrasse, argentine, sand goby, crystal goby, gray gurnard, lumpsucker, and spotted dragonet). Excepting plaice and lumpsucker, these species were detected with noteworthy abundance at both OWF and reference sites (Table S2).

Beta-diversity analysis at sample replicate level showed partial clustering of MiFish metabarcoding results based on sampling depth (10 m vs. 50 m), but no clear pattern based on sampling location (OWF vs. reference area; Figure 3a). Average linkage clustering analysis of Bray–Curtis pairwise dissimilarity (showed at station level here to aid readability) demonstrated local clustering, but no consistent overall pattern of separation based on sampling location or depth (Figure 3b).

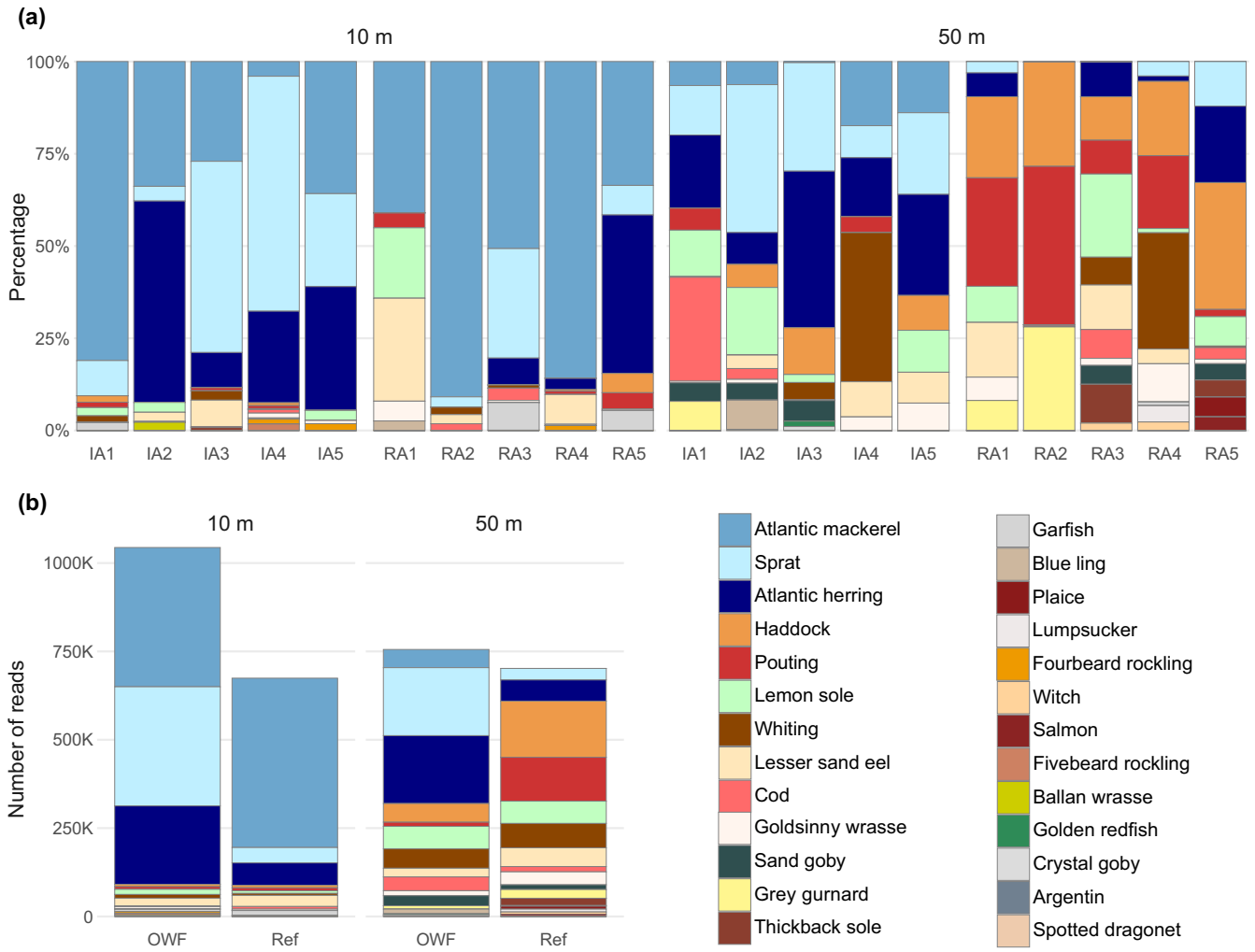

FIGURE 2 (a) Relative abundance of the species in the MiFish dataset at sample level for 10 and 50 m (IA are OWF stations; RA are reference stations). (b) Absolute number of reads for the OWF and reference area, sorted by depth.

PERMANOVA analysis of the MiFish dataset at the sample replicate level showed moderate differences between depths ($F = 15.843$; $p = 0.001$, $R^2 = 0.196$), and only slight differences between OWF and reference area ($F = 7.395$; $p = 0.001$, $R^2 = 0.091$). SIMPER analysis between depths showed that the abundant pelagic species identified in the MiFish sequence results had the highest discriminatory power to explain differences between samples: Atlantic mackerel explained close to 21% of the observed differences between samples, followed by Atlantic herring and sprat at 11% each. Top demersal species included haddock explaining 10.5% of the difference, pouting 7.5%, lemon sole 7%, and whiting and lesser sand eel each 5%. The final 18 species explained the remaining 22% of observed differences.

3.2 | 18S rDNA metabarcoding results

The 18S rDNA V1-V2 universal eukaryote dataset comprised 22,337,085 raw sequence reads from 60 samples (five OWF and five reference stations, each at 10 and 50 m depth, each with three replicates), with 45,828–827,730 sequences for individual samples (average: 337,268). After bioinformatic processing and filtering, 18,308,093 sequences remained. After SWARM clustering, chimera filtering and LULU curation 3591 OTUs were retained (Table S5). Taxonomic assignment of these OTUs after abundance filtering, using CREST4 with the SilvaMod 1.38 database, yielded 339 taxonomic groups at various levels of resolution (Table S6).

The most abundant taxon at kingdom level was the protist group Alveolata, which includes, among other taxa, dinoflagellates, and ciliates. Taxonomic analysis of 18S metabarcoding reads revealed that the dominance by Alveolates was due to the bloom-forming dinoflagellate *Karenia*, which accounted for 67% of total reads in the 18S dataset (Table S6). The second most abundant kingdom was Metazoa, constituting all multicellular animals, followed by unassigned sequences, Haptophyta algae and the protist group Stramenopiles (Figure 4).

Closer inspection of metazoan reads showed that the most abundant phylum was Arthropoda, with over 80%–95% relative abundance between stations. Within the Arthropoda, the copepod order Calanoida (1,716,376 reads) constituted 93.5% of all metazoan reads in the 18S rDNA dataset (Table S6). Following calanoid copepods in

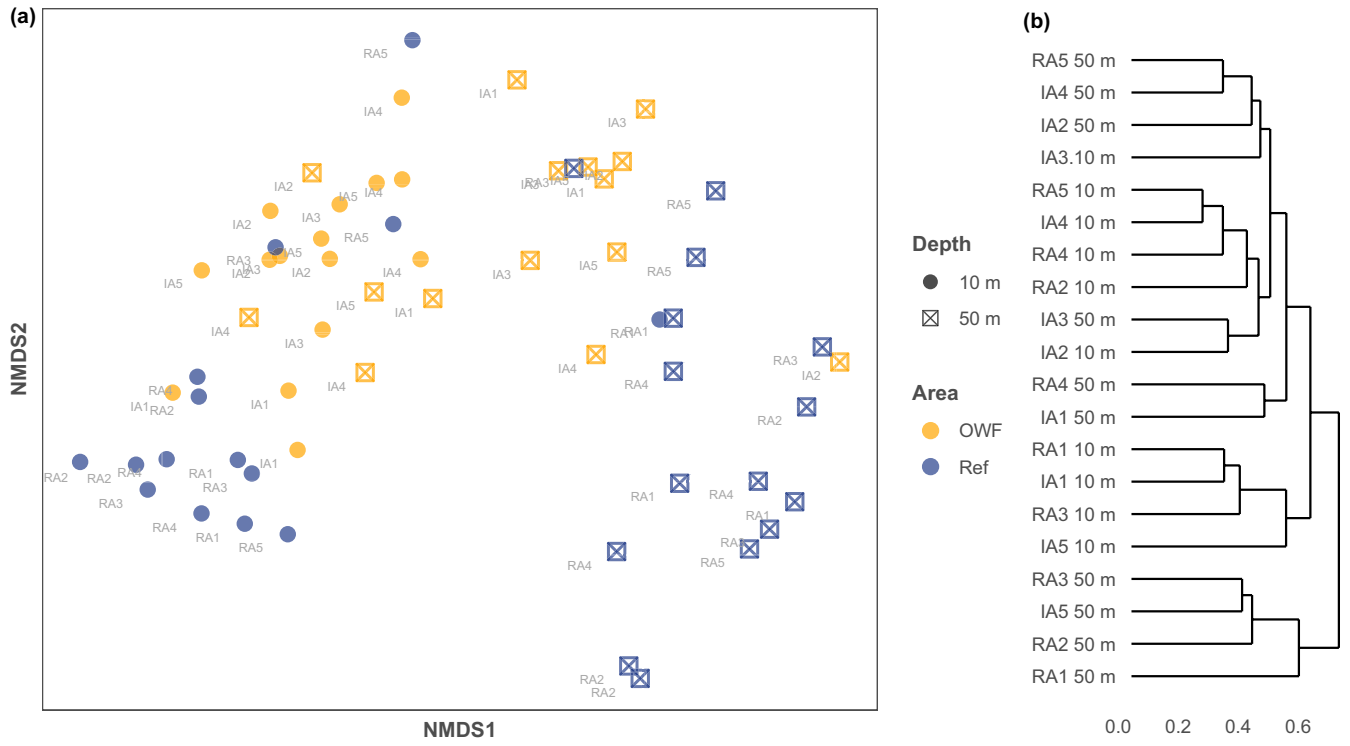

FIGURE 3 Beta-diversity of MiFish community profiles. (a) NMDS plot at sample replicate level, and (b) average linkage clustering at station level of Hellinger-transformed Bray–Curtis pairwise dissimilarities. Sampling depth is indicated as solid circles (10 m) or open squares with crosses (50 m). Samples are shown in yellow for OWF or blue for reference area.

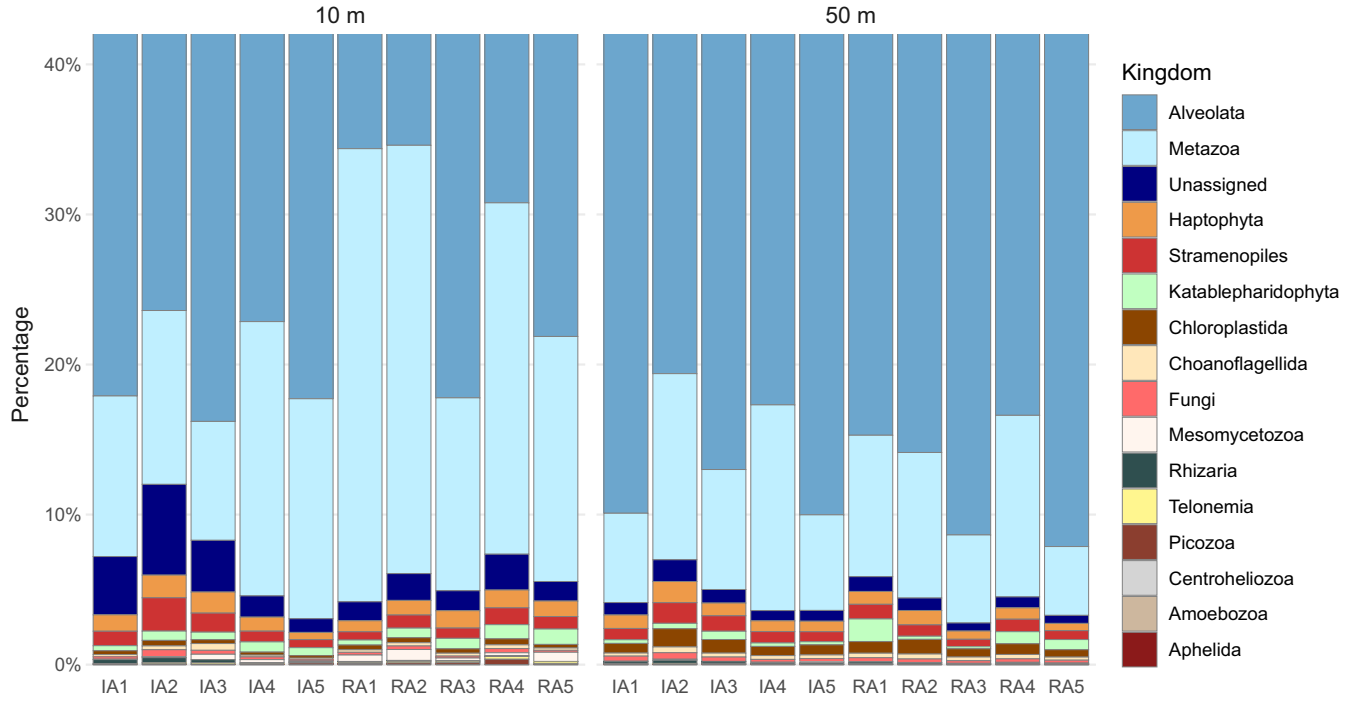

FIGURE 4 Relative abundance of the 16 taxa at kingdom level recovered in the 18S dataset. Due to the high abundance of reads belonging to the dinoflagellate *Karenia* (Alveolata), the *y* axis is scaled to the lower 40% to show less abundant taxa.

relative metazoan abundance were cnidarians (jellyfish, anemones, and hydrozoans), unidentified metazoans and annelids (segmented worms), and Ctenophora (comb jellies; Figure 5).

The NMDS (replicate level) and cluster (station level) analyses of the 18S metabarcoding results showed clear clustering based on depth (Figure 6). Three 10 m stations at the OWF (IA1–3) formed

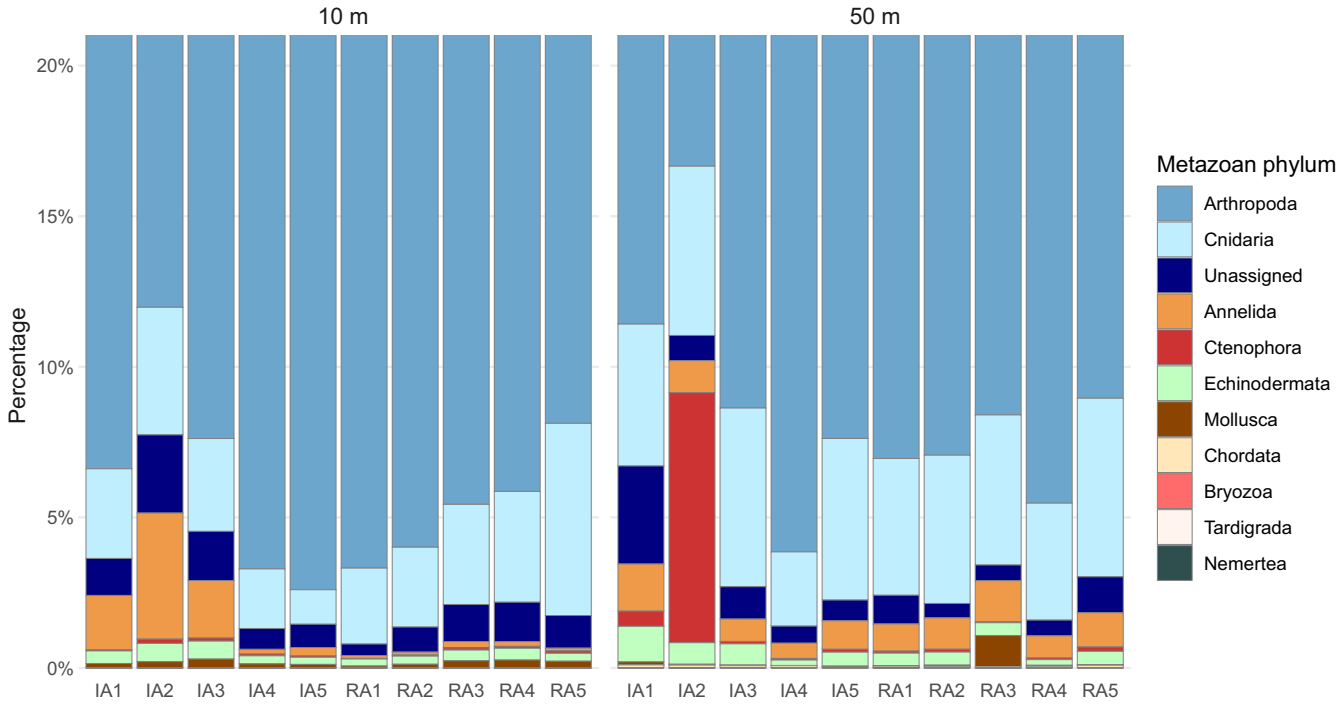

FIGURE 5 Metazoan relative abundance in the 18S rRNA dataset at phylum level. Due to the large abundance of Arthropoda (Calanoida) sequences, the y axis is scaled to the lower 20% to show less abundant taxa.

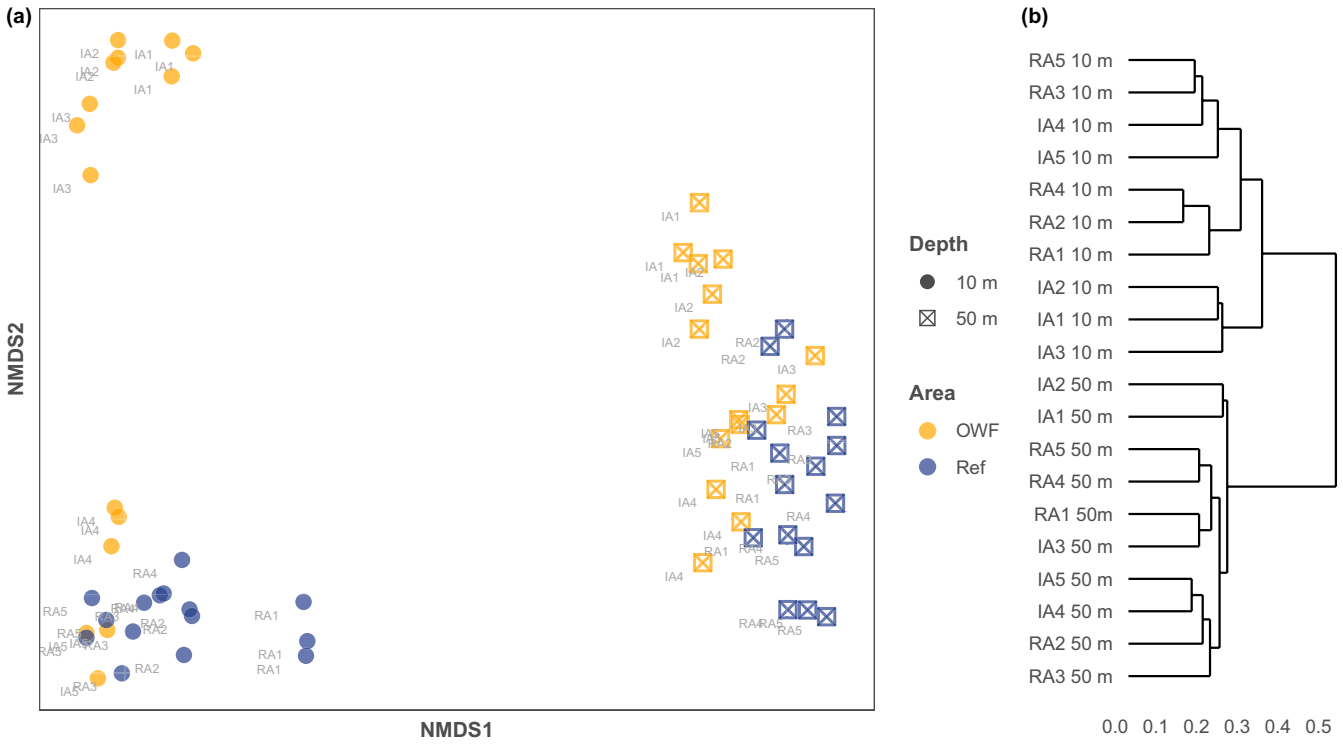

FIGURE 6 Beta-diversity of 18S community profiles. (a) NMDS plot at sample replicate level, color coded by depth, and with symbols indicating OWF or reference area, and (b) average linkage clustering of Bray–Curtis pairwise similarities at station level.

a separate cluster from the other 10 m stations, but no other clear pattern based on OWF versus reference area was evident.

PERMANOVA analysis of the 18S rDNA OTUs at sample replicate level showed strong and significant difference due to depth ($F = 101.047$; $p = 0.001$, $R^2 = 0.593$), and slight differences between OWF and reference area ($F = 7.261$; $p = 0.003$, $R^2 = 0.043$). SIMPER analysis between depths showed that dinoflagellate groups (mostly *Karenia*) accounted for over 52% of the observed differences

between samples, with higher relative abundance in the reference area and at 10 m (Figure 4). Arthropoda (almost exclusively calanoids) comprised just over 20% of reads, unassigned sequences a further 8.5% of reads, and all remaining taxa a collective 20% of reads.

3.3 | Droplet digital PCR results

We performed in total 486 ddPCR reactions to quantify eDNA of Atlantic mackerel (*Scomber scombrus*) and Atlantic herring (*Clupea harengus*) ($N = 243$ reactions each), including sampling, extraction, and PCR negative controls. The eDNA signal for mackerel ranged from 0 (non-detected; $N = 109$ samples) to 1016.4 copies L^{-1}, which occurred in a 10-m sample from station IA1 inside the OWF. For herring, ddPCR results ranged from 0 (non-detected; $N = 92$) to 1026.6 copies L^{-1} in a 50-m sample from station IA3. Detection rates for filter eDNA samples were 39% for mackerel (71 positive detections from 180 samples analyzed) and 49% (88 positive detections from 180 samples analyzed) for herring (Figure S3).

The ddPCR results revealed that the mackerel eDNA signal inside the OWF was not significantly different from the reference area (Figure 7a). We did, however, observe a significant difference in mackerel eDNA detection between 10 and 50 m depth (Figure 7b). Non-parametric rank sum tests using mackerel eDNA copies L^{-1} as response variable and either area (Kruskal–Wallis chi-squared = 0.0022121, df = 1, p-value = 0.9625) or depth (Kruskal–Wallis chi-squared = 66.232, df = 1, p-value = 4.009e-16) as explanatory variable confirmed these observations that mackerel eDNA was more abundant at 10 m depth than at 50 m depth regardless of sampling area.

Inter-station variability within both sampling areas was observed for mackerel eDNA detection at 10 m (Figure 8a,b; OWF: Kruskal–Wallis chi-squared = 16.574, df = 4, p-value = 0.002338; Ref: Kruskal–Wallis chi-squared = 18.001, df = 4, p-value = 0.001234) and individual non-significant detections at 50 m (Figure 8c,d; OWF: Kruskal–Wallis chi-squared = 3.0707, df = 4, p-value = 0.5461; Ref: Kruskal–Wallis chi-squared = 0.70753, df = 4, p-value = 0.9504).

For herring, ddPCR analysis revealed higher herring eDNA detection inside the OWF relative to the reference area (Figure 9a). We also observed higher detection of herring eDNA at 50 m sampling depth inside the OWF compared to the same depth in the reference area (Figure 9b), in contrast to the depth-dependent detection of mackerel shown above (Figure 7b). Non-parametric rank sum tests confirmed the significance of both sampling area (Kruskal–Wallis chi-squared = 31.548, df = 1, p-value = 1.946e-08) and depth (Kruskal–Wallis chi-squared = 6.7528, df = 1, p-value = 0.00936) for detection of herring eDNA.

Similar to mackerel, differences in herring eDNA detection were observed between sampling stations reflecting local relative concentrations (Figure 10a–d). Rank sum tests identified significant inter-station differences at both 10 m (Kruskal–Wallis chi-squared = 14.27, df = 4, p-value = 0.00648) and 50 m (Kruskal–Wallis chi-squared = 13.996, df = 4, p-value = 0.007308) within the OWF. In the reference area, however, significant inter-station differences were observed for 10 m samples (Kruskal–Wallis chi-squared = 10.061, df = 4, p-value = 0.03941) but not 50 m samples (Kruskal–Wallis chi-squared = 6.9158, df = 4, p-value = 0.1404).

In addition to eDNA filter samples, 63 control samples from triplicate analysis of 10 air blanks, 10 water blanks, 1 lysis buffer blank, as well as ddPCR negative controls, were analyzed

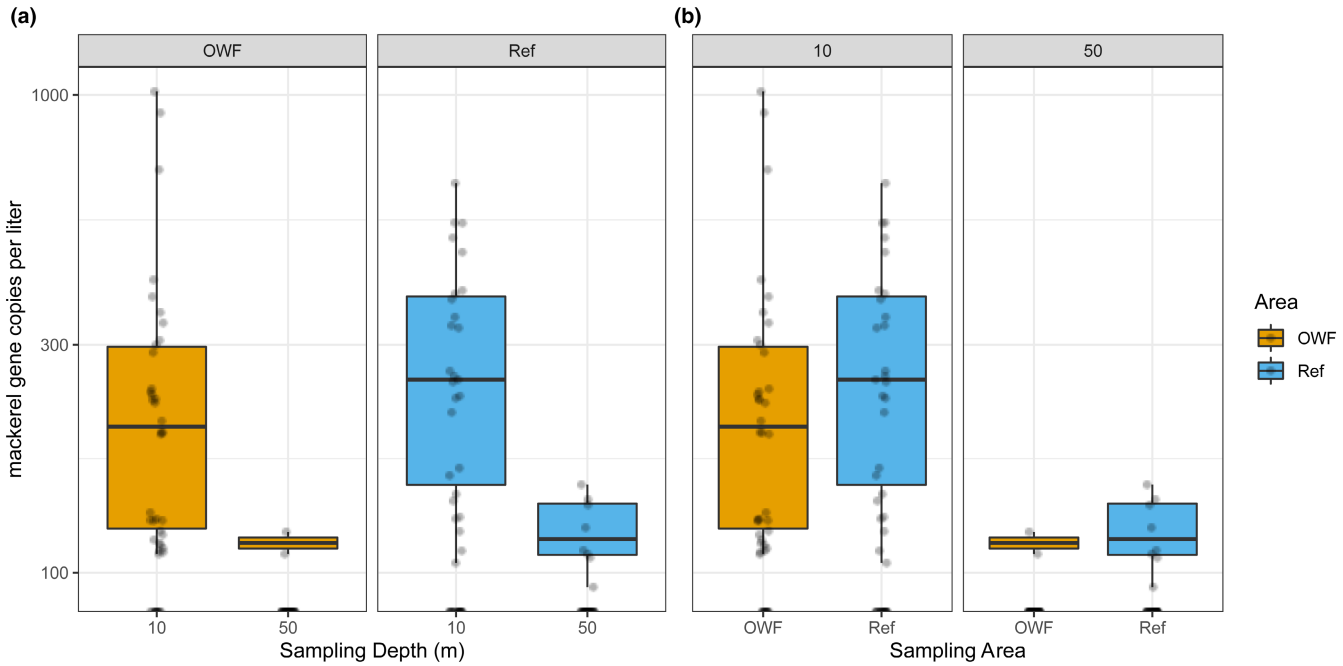

FIGURE 7 Box-and-whisker plots summarizing ddPCR results for Atlantic mackerel (a) between the OWF (yellow bars) and reference area (blue bars), and (b) by sampling depth (10 or 50 m). The logarithmic y-axis shows target gene copies per liter of seawater.

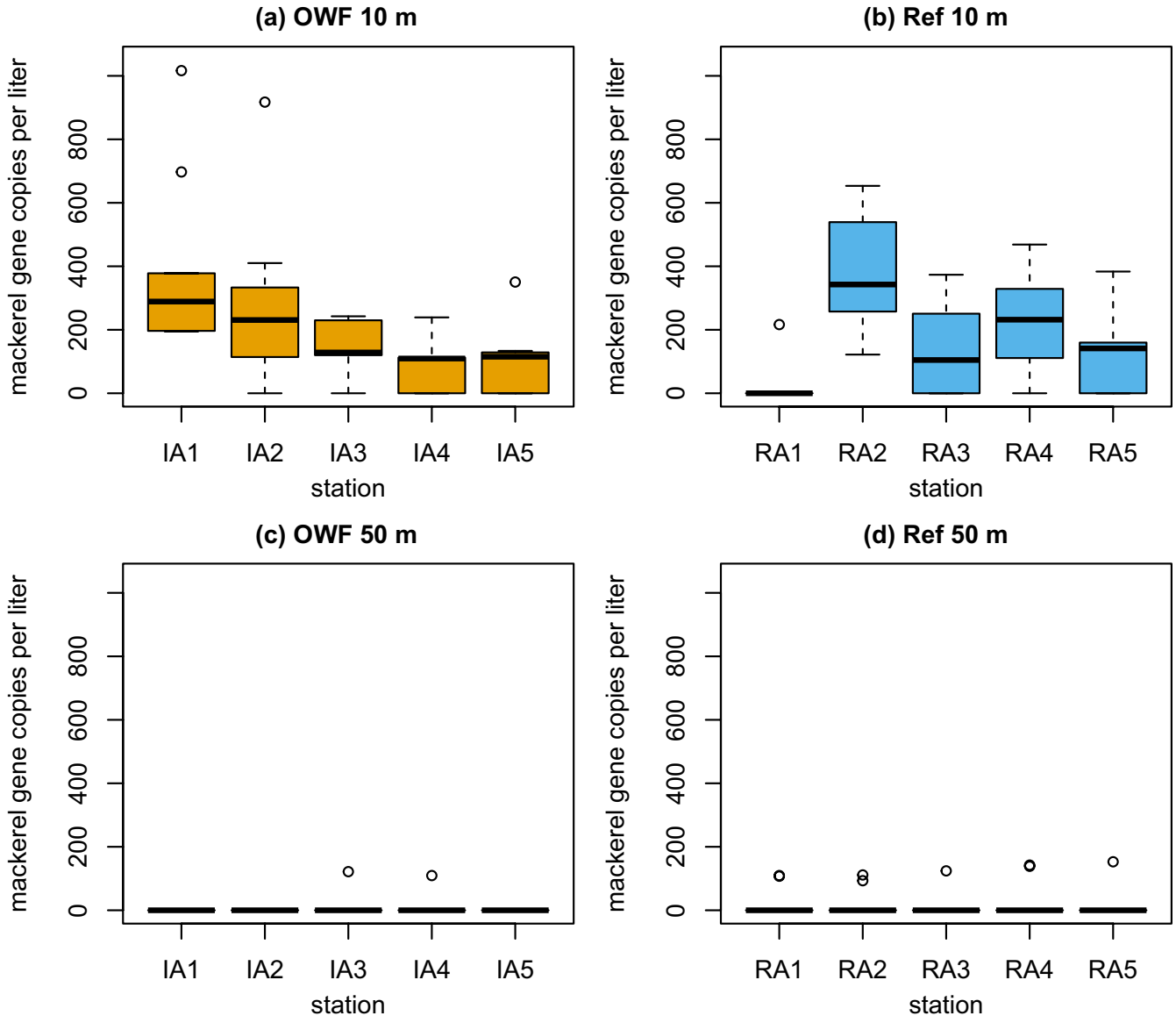

FIGURE 8 Box-and-whisker plots summarizing ddPCR results for Atlantic mackerel (*Scomber scombrus*) at sampling station level (IA1–IA5—OWF; RA1–RA5—reference area) by sampling depth (a, b—10 m or c, d—50 m) and sampling area (a, c—OWF or b, d—reference area), and by sampling station. Non-detections were arbitrarily set to 0 for visualization purposes.

in parallel to eDNA filters samples to control for background eDNA signal. Atlantic mackerel eDNA was not detected in any of the 63 controls samples or ddPCR negative controls. Atlantic herring eDNA, was detected in seven technical replicates from control samples: four air blanks ("AB"), two water blanks ("WB") and one Buffer ATL blank ("ATL"), with values ranging from 4.8 copies L^{-1} in the air blank from station IA2 to 255 copies L^{-1} in the water blank from station IA5. Positive detections in negative control samples were in general anecdotal and appeared in only one of three technical replicates, except for station IA5 where two of three replicates for the water blank gave positive detections (Figure S4).

To check for congruence between assay results and metabarcoding read abundances, ddPCR gene copies per liter of seawater and relative read abundance of metabarcoding reads were compared for Atlantic mackerel (Figure 11a) and Atlantic herring (Figure 11b). The metabarcoding abundance threshold for a positive detection was set to 0.25% of relative sample abundance for the purposes of this comparison. The results show correlation in total abundance between the two methods, but with variation and some false negatives/positives between the two methods for individual replicates. Specifically, for mackerel there were 61 of 180 (34%) samples that gave positive detection for both ddPCR and metabarcoding analysis, 68 (38%) that were negative for both methods, 10 samples were positive for ddPCR only (6%), and finally 41 samples were positive for metabarcoding only (23%). For herring, results from the two methods were both positive in 62 (34%) of the samples, both negative in 64 (36%) of the samples, positive

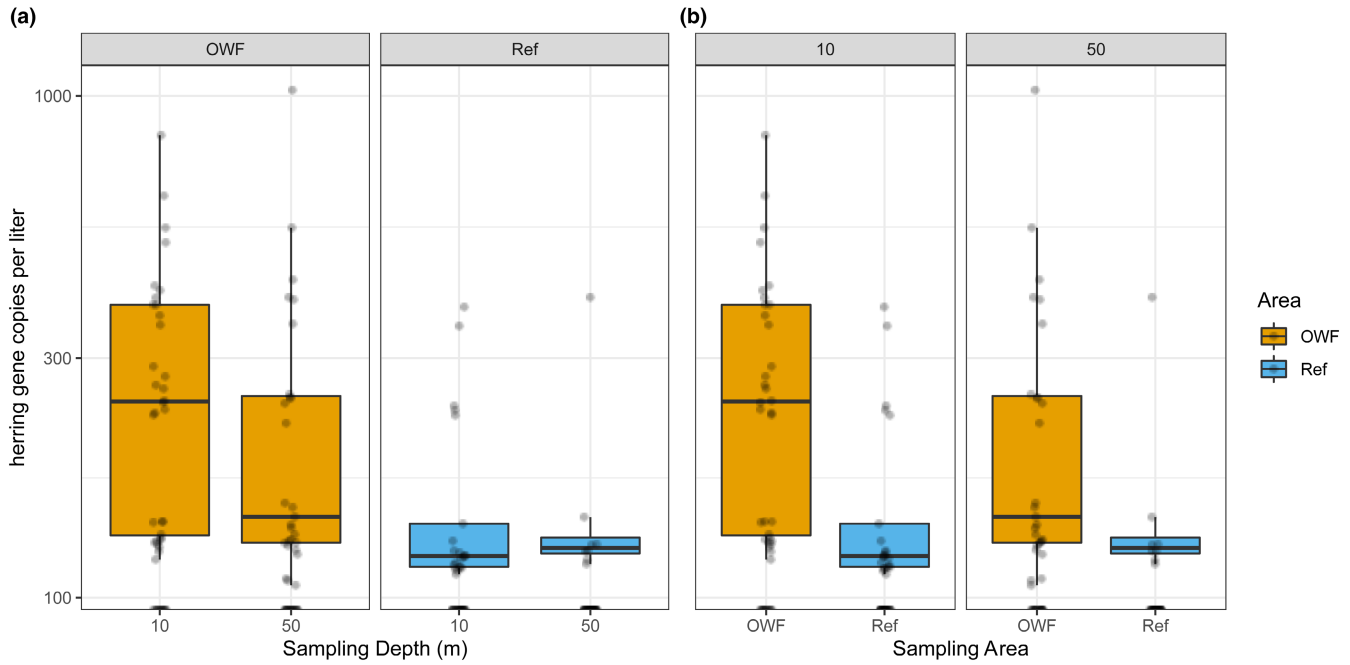

FIGURE 9 Box-and-whisker plots summarizing ddPCR results for Atlantic herring (a) between the OWF (yellow bars) and reference area (Ref, blue bars), and (b) by sampling depth (10 or 50 m). The logarithmic *y*-axis shows target gene copies per liter of seawater.

for ddPCR only in 26 (14%) of cases, and positive for metabarcoding only in 28 (16%) of cases. In all this represented a slightly higher sensitivity for metabarcoding data compared to the ddPCR data.

4 | DISCUSSION

4.1 | eDNA data from both ddPCR and metabarcoding were able to detect spatial patterns at station and 40 m depth resolution

The ability of eDNA to capture spatio-temporal patterns of marine fish distribution is dependent on the presence of DNA copies in quantities that reflect local biomass in the immediate vicinity. This is a challenge since the water is moving in relation to the installations requiring extra care and consideration for the sampling strategy (e.g., Doray et al., 2018). While precise estimates of degradation and dispersal of eDNA in water are a complex issue (e.g., Andruszkiewicz Allan et al., 2021), studies have shown that eDNA is quickly diluted in the water column and has a high site fidelity (Harrison et al., 2019). Empirical studies combining eDNA with ground-truthing from catch-data (Salter et al., 2019; Stoeckle et al., 2021) further indicate significant correlation between trawl data and eDNA detection (although some studies show weaker correlation; Knudsen et al., 2019).

Here, the quantitative ddPCR molecular analyses of two keystone pelagic fish species in the North Sea, Atlantic mackerel (*Scomber scombrus*) and Atlantic herring (*Clupea harengus*), demonstrated clear species-specific patterns of eDNA detection in the Hywind OWF and adjacent reference areas. In the case of Atlantic mackerel, there was a highly significant depth difference in gene copy distribution across both OWF and reference areas, with higher eDNA detection at the shallower depth, while Atlantic herring was abundant at both 10 and 50 m but with a significantly higher number of gene copies in the OWF compared to the reference area. These results mirror the MiFish metabarcoding data, where we found similar distribution patterns for these two species. A third abundant pelagic species in the MiFish metabarcoding dataset, sprat, was also more abundant in the OWF, at both depths, than in the reference area. The assemblage of demersal fish species showed no significant OWF vs. reference area pattern but had a higher MiFish metabarcoding richness and read abundance in the 50 m samples compared to the 10 m samples and showed station-specific detection of individual species. In sum, these results demonstrate that both the ddPCR and metabarcoding eDNA approaches used here were able to clearly discriminate between local fish richness and abundance at station and 40 m depth resolution. Our observation of non-homogeneous vertical eDNA distribution in the water column suggests that the optimal depth for water sampling in eDNA-based pelagic fish surveys may vary depending on the vertical stratification of water layers in the area at the given time (Closek et al., 2019; Jeunen et al., 2020).

As corroborated by MiFish SIMPER results, the observed differences in fish composition were mainly due to pelagic, schooling fishes. The presence of manufactured structures like OWFs in the water column create a fish aggregation device (FAD) effect (Bergström et al., 2013). Offshore wind farms have previously been shown to have a positive effect on fish abundances (Methratta & Dardick, 2019), yet this effect is highest for complex and soft bottom fishes, and no significant effect on pelagic fishes was reported (Methratta & Dardick, 2019). Pelagic fish are highly dynamic in time and space

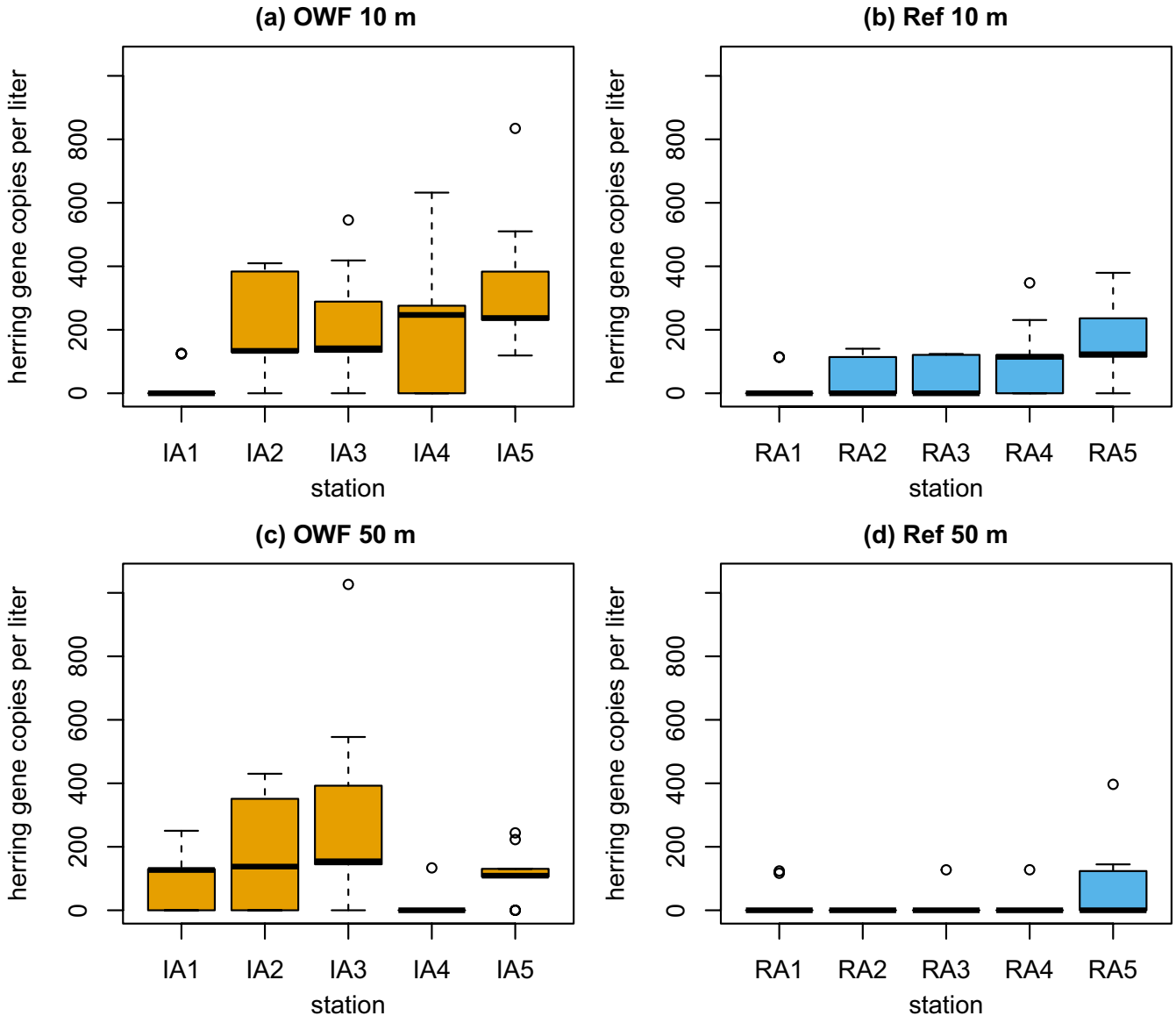

FIGURE 10 Box-and-whisker plots summarizing ddPCR results for Atlantic herring at sampling station level (IA1–IA5—OWF; RA1–RA5—reference area) by sampling depth (a, b—10 m or c, d—50 m) and sampling area (a, c—OWF or b, d—reference area), and by sampling station. Non-detections were arbitrarily set to 0 for visualization purposes.

(Lindeboom et al., 2011), and while we did see increased herring and sprat abundance in the OWF area at the time of sampling, we interpret this result as a snapshot of the placement of pelagic schools at the time of sampling; we cannot attribute this difference to the OWF, which would require resampling over several time points. The observation of higher mackerel eDNA detection at the shallower sampling depth corroborates previous observations that schools of feeding mackerel are more likely to occur at depths between 0 and 40 m (Godø et al., 2004). The potential considerable height and horizontal spread of mackerel schools in the water column (Walsh, 1995), however, supports the snapshot hypothesis of anecdotal dominance of mackerel at 10 m at the time of sampling for the present study.

Supplementing the MiFish metabarcoding results, the 18S rDNA V1-V2 metabarcoding dataset provided a complementary view into the wider diversity of multicellular and single-cell non-bacterial organisms in the two sampling areas. The 18S dataset beta diversity results showed a clear separation between 10 and 50 m depth. This confirmed the ability of the 18S metabarcoding data to discriminate between organism communities at different layers and validates the ability to pick up changes in eukaryote communities based on changes in environmental conditions. No absolute pattern emerged for differences between OWF and reference areas, though three 10 m samples from the OWF (IA1–IA3) were dissimilar to other stations both from OWF and reference areas. In the 18S V1-V2 dataset, the major abundant groups, especially the dinoflagellate *Karenia* and calanoids, explained most of the dissimilarity between samples. Although the metabarcoding results may represent real differences in organismal relative abundance, it is prudent to consider the large disparity in 18S

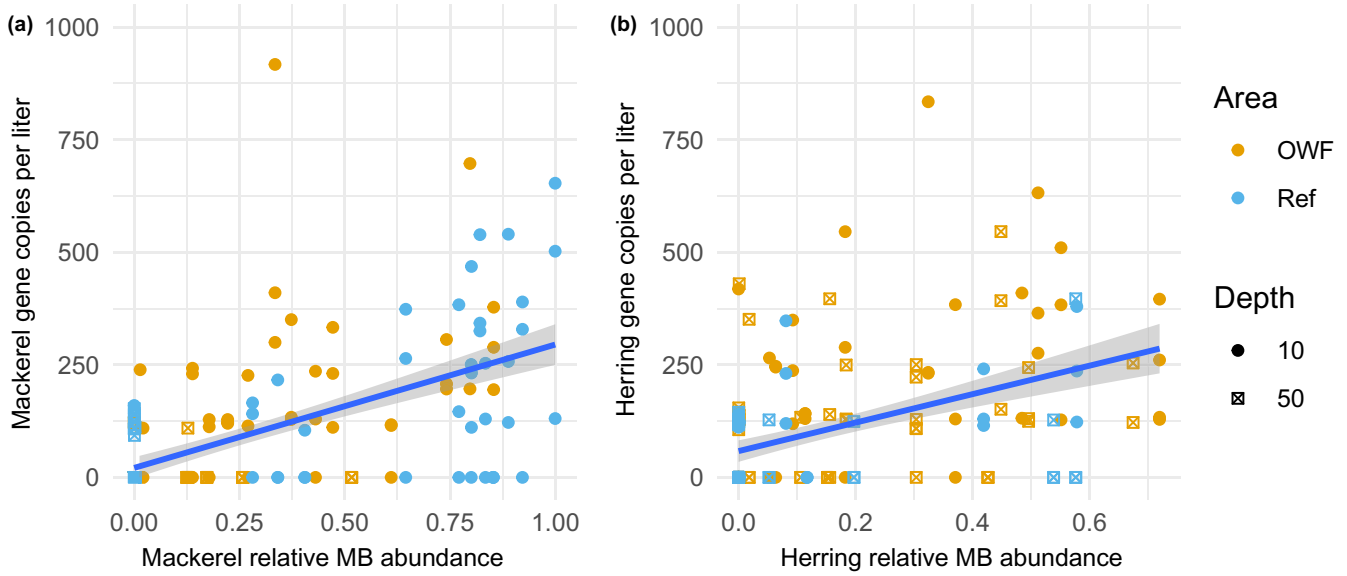

FIGURE 11 Comparison of metabarcoding and ddPCR results for (a) Atlantic mackerel and (b) Atlantic herring. Relative abundance of mackerel or herring reads in metabarcoding libraries is shown on the *x*-axis, while ddPCR target gene copies per liter of seawater is shown on the *y*-axis. Non-detections for each method were arbitrarily set to 0 for visualization purposes.

gene copy number between different groups of eukaryotic organisms, and in particular for dinoflagellates, which may possess up to hundreds or even thousands of 18S gene copies per cell (Godhe et al., 2008). Such disparities cannot be excluded as one potential explanation for the dominating abundance of, for example, *Karenia* in the metabarcoding data. While not pursued in detail in this study, our results hints at the potential of 18S V1-V2 metabarcoding data in tracking spatial patterns of predator–prey interactions between the planktivorous fish species here and local prey species congregations.

4.2 | Metabarcoding and ddPCR assays largely congruent, with some caveats

One persistent challenge with the development of eDNA-based studies for application within a regulatory framework for fisheries management is the unclear but critically important relationship between eDNA signal and biomass (Rourke et al., 2022). For metabarcoding in particular, given the range of relative biases in primer performance between organisms inherent in standard PCR amplification techniques, extrapolating relative abundance information is a complex issue and should be done with care (Kelly et al., 2019). Even so, strong correlation between metabarcoding read numbers and biomass have been reported in several studies (Afzali et al., 2021; Maiello et al., 2022; Russo et al., 2021).

Given the lack of ground-truthing from catch data in this pilot study, we were not in a position to do a direct comparison of eDNA quantities and biomass. However, the study design did allow a comparison of the absolute detected quantities of mackerel and herring eDNA (ddPCR results) with the relative abundance of both species in the MiFish metabarcoding results. Both mackerel and herring were detected using ddPCR and MiFish metabarcoding, and linear regression for each species indicates significant, albeit weak positive correlations between the two methods. The low strength of these correlations is likely due, in part, to non-detections (visualized as 0 values in Figure 11), of which the majority were ddPCR non-detections, but with some metabarcoding non-detections also present. Another reason for these discrepancies is that different markers with different performance for the target species were used in the metabarcoding and ddPCR analyses.

Shelton et al. (2022) indicate that small-scale inaccuracies in eDNA datasets underscore the need for sample replicates in obtaining robust data for ecological interpretation. In the present study both biological (filters) and technical (PCR) replicates were included for quantitative ddPCR detection of mackerel and herring, however ddPCR results were in general characterized by a sizable number of non-detections for both target species (Figure S3). Metabarcoding analysis (MiFish) of the same samples, however, yielded non-detection for only 5–6 (approx. 3%) of 180 samples. Using mean or median ddPCR results for each station did not improve metabarcoding correlation strength, meaning that non-detections could not be traced to variation between same-station replicates.

Our sampling method using triplicate one-liter water samples is aligned with multiple contemporary eDNA-based fish studies that aimed to find the best compromise between sample concentration and acquisition feasibility (Capo et al., 2020 and references therein). Despite this, the ddPCR results generated in this study were often near the limit of detection for the ddPCR instrument, raising questions about true versus false positive detections (Hunter et al., 2019). As the ddPCR assays utilized were obtained from a published study which reported rigorous optimization and specificity testing (Knudsen et al., 2019), there is no reason to suspect poor performance of the assay itself. Rather, the discrepancy suggests a considerable difference in detection sensitivity between the two methods,

with MiFish metabarcoding having superior detection sensitivity in comparison to ddPCR. On the other hand, higher metabarcoding sensitivity can be a double-edged sword, as PCR amplification may enhance weak sequence signal to give an over-estimate of the true sample abundance. The relationship between sample and metabarcoding abundances greatly depend on the overall community composition of the sample and their interactions during PCR amplification (Kelly et al., 2019), which likely explains a significant part of the abundance discrepancies in the correlation to ddPCR data.

Full validation of the assays applied and calculation of their reliable limits of detection on the ddPCR platform (Klymus et al., 2020) and with relevant levels of potential PCR inhibitors (Hunter et al., 2019) fall outside the scope of this pilot study. Possible focal points for follow-up investigation might include increasing sampling volume (i.e., of seawater per filter), measuring any differential effect of PCR inhibitors on the two different PCR chemistries (ddPCR vs. metabarcoding), and/or pooling of eDNA samples (biological replicates) prior to ddPCR analysis. From a broader perspective, these results also caution against the use of one-size-fits-all approaches for eDNA-based ecological investigations.

4.3 | The MiFish metabarcoding marker detects a wide assemblage of North Sea fish species

Although the focus of this pilot study was the eDNA based detection of pelagic fish, the MiFish metabarcoding approach also detected a number of demersal fish species (Figure 2 and Table S3). In a recent meta-analysis of bottom fast OWF impact on fish distribution patterns, Methratta and Dardick (2019) found an overall significant effect indicating greater demersal fish abundances inside OWF areas. Here, the MiFish metabarcoding data picked up 26 fish species, with the majority of detections belonging to demersal or bentho-pelagic fish species. Most of these species were detected with greater relative abundances at 50 m depth relative to the 10 m samples, with no discernable patterns of difference between the OWF and reference sites. Thus, we could not detect any demersal species FAD or artificial reef effect from the OWF. The low total number of turbines and moorings in the Hywind Scotland OWF could mean that such effects are too small to be easily detected. Additionally, Bergström et al. (2013, 2014) found that observations of increased demersal fish abundances in OWF were made at small spatial scale around, for example, turbine foundations, meaning any effect might not be picked up at the granularity of the current study.

Taxonomic assignment of MiFish metabarcoding data revealed fish species consistent with what would be expected from a North Sea near-shore environment. A possible exception is blue ling, which is generally found at greater (>350 m) depth. In this case, it is possible that the relevant OTUs have been misattributed from a closely related lotid species. As we could not do a direct comparison with data from morphological surveys in this pilot study, these assignments, as well as any resident species missing from the metabarcoding data, remain untested for the time being. In addition to fish species described above, the MiFish dataset also included non-target sequences identified as human, cattle (*Bos taurus*) and sheep (*Ovis aries*). These sequences were found throughout the dataset. While no rigorous study on their origin was done here, a possible explanation for these sequences could include human impact on the marine environment through for instance sewage effluents or ship activity.

An interesting detail was the presence (though at very low abundance) of harbor porpoise in the reference area MiFish dataset. While harbor porpoises tend to avoid OWFs during construction (Dähne et al., 2013), impact during the OWF operational phase is ambiguous with both positive and negative reported effects (Lindeboom et al., 2011; Scheidat et al., 2011; Teilmann & Carstensen, 2012). As a non-target species for the MiFish primers, presumably the actual abundance of harbor porpoise in the area could be higher than indicated in the MiFish data. Species-specific primers for harbor porpoise are available (Foote et al., 2012), and a design that included such primers could provide additional environmental monitoring utility of eDNA studies of environmental impact at OWF sites with modest additional effort (Suarez-Bregua et al., 2022).

4.4 | Environmental DNA has a high potential as a monitoring tool in OWF settings

With the ddPCR and metabarcoding data in this study, we were able to discriminate clearly between stations and depths, showing that eDNA data in this environment has the necessary spatial resolution for effective monitoring of impact. Abundance estimates between ddPCR and metabarcoding datasets were all-around in agreement with regards to detection and rough abundance estimates, but at the single replicate level, there were incongruences between ddPCR and metabarcoding detection highlighting the need for further refinement of the methodological approach, such as increasing the volume of filtered water. Metabarcoding of MiFish and 18S markers provided insights into the spatial patterns of the fish and plankton communities in the two areas. Catch data would also prove useful here to be able to estimate non-detection of additional fish species in the MiFish metabarcoding data.

The two ddPCR assays employed, for Atlantic mackerel and Atlantic herring, worked well in providing quantitative estimates of relative abundance throughout the OWF and reference areas. This shows the power of the approach for particular cases of keystone or commercially important species, where more rigorous quantification than what is obtainable by metabarcoding is needed to establish accurate spatial abundance patterns of such species. For future monitoring purposes, the utility of the assay data could be further enhanced through a combined ddPCR and catch data study to better correlate the ratio of biomass and gene copy number for these two species.

In conclusion, we found that the combination of ddPCR assays of keystone species, together with a community-based metabarcoding approach, has a large potential for generation of non-invasive fisheries management-relevant data for evaluating impact on the pelagic

ecosystem in OWF monitoring studies. Establishing time-series data of eDNA data would allow monitoring trends in community composition and abundance necessary for detecting OWF impact. Conducting a simultaneous catch-data and eDNA study would further increase reliability of the method by both working toward a reliable ddPCR versus biomass conversion and detecting any species not detected by metabarcoding.

AUTHOR CONTRIBUTIONS

Jon Thomassen Hestetun contributed to study sampling and metabarcode data design, metabarcoding data bioinformatics and ecological analysis, and writing the manuscript. Jessica Louise Ray contributed to study sampling, metabarcoding and ddPCR design, conducted the field work, performed ddPCR assay analysis and writing the manuscript. Kari Mette Murvoll and Ane Kjølhamar contributed to study design, facilitated, and organized field work, and writing the manuscript. Thomas G. Dahlgren conceived the study and contributed to study sampling and data design, ecological data interpretation, and writing the manuscript.

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CONFLICT OF INTEREST STATEMENT

Equinor funding was not in any way contingent on any study outcomes, and did not, directly or indirectly, limit study design, analysis, results, and interpretation of study data.

DATA AVAILABILITY STATEMENT

The raw data were deposited at the Sequence Read Archive (SRA) under project number PRJNA985779.

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SUPPORTING INFORMATION

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