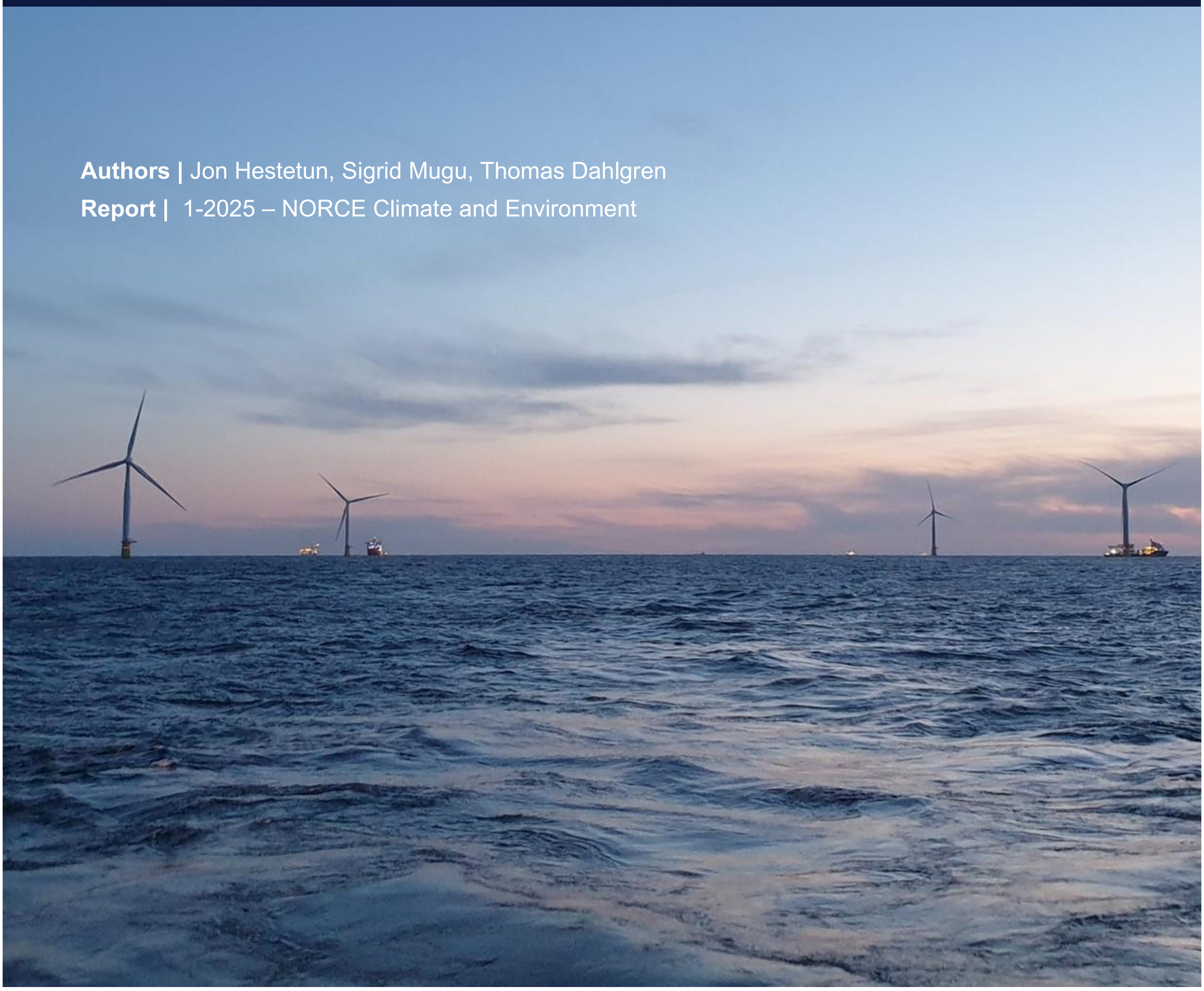
Supplementary eDNA analyses at the Hywind Tampen FOWF

Enhanced metabarcoding shark and skate detection and additional demersal fish analyses



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Summary

The Hywind Tampen Offshore Floating Wind Park is a floating OWF (FOWF) situated in deep-water on the Norwegian Shelf in the Northern North Sea. On behalf of Equinor, in 2023-2024, NORCE Climate and Environment conducted an environmental DNA survey of surface (20 m) and bottom water samples from sampling stations upstream, inside, downstream and some distance away from the FOWF to assess fish (MiFish-U) and plankton (18S V1-V2) communities for potential impact (Hestetun et al., 2024). Study results revealed differences in community composition over time and with depth but could not detect impact – negative or positive – from the construction and operation of the FOWF itself. The study used fish capture and ROV data from IMR-conducted surveys in the area to ground truth completeness of the data. While the MiFish-U primer set was able to detect the vast majority of fish species reported in the fish capture and ROV studies, and detect some additional species, elasmobranchs (sharks and skates) were missing from the eDNA data.

This technical note contains a re-sequencing of the Hestetun et al. (2024) samples using a combination of MiFish-U and MiFish-E primer sets, MiFish-E being a modification of the MiFish-U primer set specifically designed to detect elasmobranchs. The goal of this analysis was to assess the ability of this approach to get a more comprehensive overview of local fish communities also including elasmobranch species. In addition, new analyses of bottom water fish communities were made removing dominating and pelagic species to see if this revealed further information on differences in demersal fish composition between sampling stations.

Re-sequencing of Hywind Tampen samples using the MiFish-U/E mixed primer approach yielded a dataset that retained 32 of 35 species from the previous MiFish-U only dataset of Hestetun et al. (2024). The approach was also successful in detecting several elasmobranchs not part of the MiFish-U dataset but reported from the fish capture and ROV surveys in the area, including the thorny skate (*Amblyraja radiata*), common skate (*Dipturus* sp.), blackmouth catshark (*Galeus melastomus*), and spurdog (*Squalus* sp.). In addition, a couple of previously unreported elasmobranchs, including velvet belly lanternshark (*Etmopterus spinax*) and porbeagle (*Lamna nasus*), were detected. Most elasmobranchs were detected with relatively low abundance, however. The results also highlight some ambiguities in taxonomic assignment where several species were equally similar in sequence identity, suggesting the need for taxonomist validation of taxonomy results based on knowledge of regional fish communities.

In conclusion, the MiFish-U/E primer set approach was successfully able to recreate local fish communities with greater elasmobranch coverage with little reduction in non-elasmobranch coverage and represents a good alternative for maximum coverage in metabarcoding of fish communities.

Concerning the reanalysis of the bottom water fish community datasets, both the previous MiFish-U and the newly sequenced MiFish-U/E datasets were analyzed removing pelagic and dominating species. This reanalysis reaffirmed the conclusions from the full dataset analysis in the original report: While there was a statistically significant support for

differences between sites and time, the size of this effect was small. The main impression is that demersal fish communities are stable and comparable between sampling stations and time points, with no detectable impact due to the FOWF. Importantly, bottom depth is similar across the sampling station, situated along a slope, in the Hywind Tampen study here in contrast to e.g. (de Jong et al., 2022), who did a transect perpendicular to the slope itself.

Contents

Abbreviations and terms

18S – The ribosomal small subunit rRNA gene, parts of which is commonly used as marker in barcoding and metabarcoding, divided into regions from V1 to V9. Several markers exist, typically identified by the region of 18S they target.
ASV – Amplicon sequence variant. A unique read in a metabarcoding dataset, often associated with dada2 sequence data processing.
Barcoding – Sequencing one or several genes from a specific organism
Benthic – Pertaining to the seafloor.
CTD – Conductivity, temperature, depth – a sensor array, typically also including additional sensors such as oxygen, chlorophyll and/or turbidity etc. often lowered from a vessel down through the water column.
ddPCR – Droplet digital PCR, a method to subdivide a PCR reaction into a large number of reactions contained within individual nanodroplets, detection of positive or negative PCR amplification within each droplet allows quantitative assessment of gene copies in the template.
Demersal – Descriptor of fish living above the seafloor.
DNA extract – DNA extracted from an environmental sample or tissue suspended in a buffered solution, used as template in a PCR reaction.
eDNA – Environmental DNA, DNA from environmental samples such as water, soil or air
Elasmobranchs – Sharks and rays
FOWF – Floating offshore wind farm
HTS – High throughput sequencing, the simultaneous sequencing of a large number of DNA sequences using e.g. Illumina, PacBio SMRT, or Oxford Nanopore sequencers. (Sometimes NGS – next generation sequencing.)
Marker – A gene used in barcoding or metabarcoding applications.
Metabarcoding – Sequencing one or several genes from a large set of organisms in an environmental sample.
MiFish-E – A modification of the MiFish-U marker to enhance capture of elasmobranch fish species (skates and sharks).
MiFish-U – A genetic marker for eDNA amplification specific for fish species situated on the mitochondrial 12S rRNA gene.
OWF – Offshore wind farm
PCR – Polymerase chain reaction, exponential amplification of a target gene from a DNA extract, creating a PCR product, numerous copies of a single gene suspended in a buffered solution.
Pelagic – Pertaining to the water column.
Primer pair – A pair of complementary forward and reverse sequences that bind to a DNA template on each side to the gene marker to be amplified.
Sequencing – Reading DNA sequences present in e.g. a PCR product into electronic sequence files.

1. Introduction

Hywind Tampen is a floating offshore wind farm (FOWF) situated in deep-water (~250-300 m) on the Norwegian Shelf in the Northern North Sea (environmental monitoring region IV) along a NW-SE bottom slope gradient towards the Norwegian trench (Fig. 1). On behalf of Equinor, in 2023 NORCE Climate and Environment conducted an eDNA water sample environmental study to investigate fish and eukaryote organism communities in the area around the Hywind Tampen FOWF (Hestetun et al., 2024). This study was itself a follow-up study based on the methodology trialed in a 2021 eDNA pilot study at the Hywind Scotland FOWF off the coast of Peterhead (UK) (Ray et al., 2022), subsequently published (Dahlgren et al., 2023; Hestetun et al., 2023). The methodology included the use of two metabarcoding markers, MiFish-U (noted as MiFish in the report) and the V1-V2 region of the 18S rRNA gene; and two ddPCR assays, for Atlantic herring and mackerel, on water samples collected in and around the FOWFs.

Both studies showed that metabarcoding data from water samples taken at depth and close to the surface was able to provide a coherent and mostly comprehensive picture of local fish and plankton communities at time of sampling, and that the data could detect differences in local populations between stations and depth. The larger-scale 2023 Hywind Tampen study also included three time points (T0 = initial sampling, T1 = after one day, T2 = after one week) to assess the stability of the eDNA signal over time as well as any effects from the prevailing NW-SE current in the area, both questions left unexplored in the initial 2021 single-timepoint Hywind Scotland study. Finally, the Hywind Tampen FOWF eDNA study allowed ground-truthing of fish eDNA metabarcoding data comprehensiveness as the area is subject to separate baseline studies by the Institute of Marine Research (IMR) and Equinor, including capture surveys conducted by IMR (de Jong et al., 2022; Palm et al., 2023).

Results confirmed the utility of eDNA from water samples in recording differences in local community composition but did not detect any clear positive or negative effect on fish or plankton communities due to the Hywind Tampen FOWF, potentially due to the fact that the FOWF was still under construction at the time of sampling and features a limited number of turbines in total. Further, no significant eDNA current transport effect could be detected. As part of the ground-truthing effort, however, it was noticed that similar to previous reports (Miya et al., 2015), the MiFish-U marker used did not detect skate or shark (elasmobranch) species reported from the Hywind Tampen area. A variant of the MiFish-U primer, MiFish-E, has been developed to detect elasmobranch species (Miya et al., 2015), and was identified as an alternative or complement for future eDNA fish studies. As an additional item, Equinor expressed an interest in analyses of demersal fish communities from the Hywind Tampen data without the presence of dominating or clearly pelagic fish species in the data from bottom samples.

This technical note serves as supplement to the previous Hywind Tampen report, and includes two parts:

1. A re-amplification and sequencing of Hywind Tampen samples, using the same extracts as for the previous Hestetun et al. (2024) study, with a mixture of MiFish-U

and MiFish-E primer pairs for PCR amplification, to assess ability to detect shark and skate species using this approach.
2. A reanalysis of demersal fish communities removing dominating species to take a closer look at detailed community characteristics for both the previous 2024 and the newly sequenced datasets.

This technical note should be considered a companion to the 2024 Hywind Tampen report. For a full description of the Hywind Tampen study and previous discussions, it is advised to consult the main Hywind Tampen report (Hestetun et al., 2024).

2. Materials and methods

Materials and methods described here comprise the additional analyses done for this technical note. For a full treatment of samples and choice of sampling scheme, please consult the 2024 Hywind Tampen report (Hestetun et al., 2024).

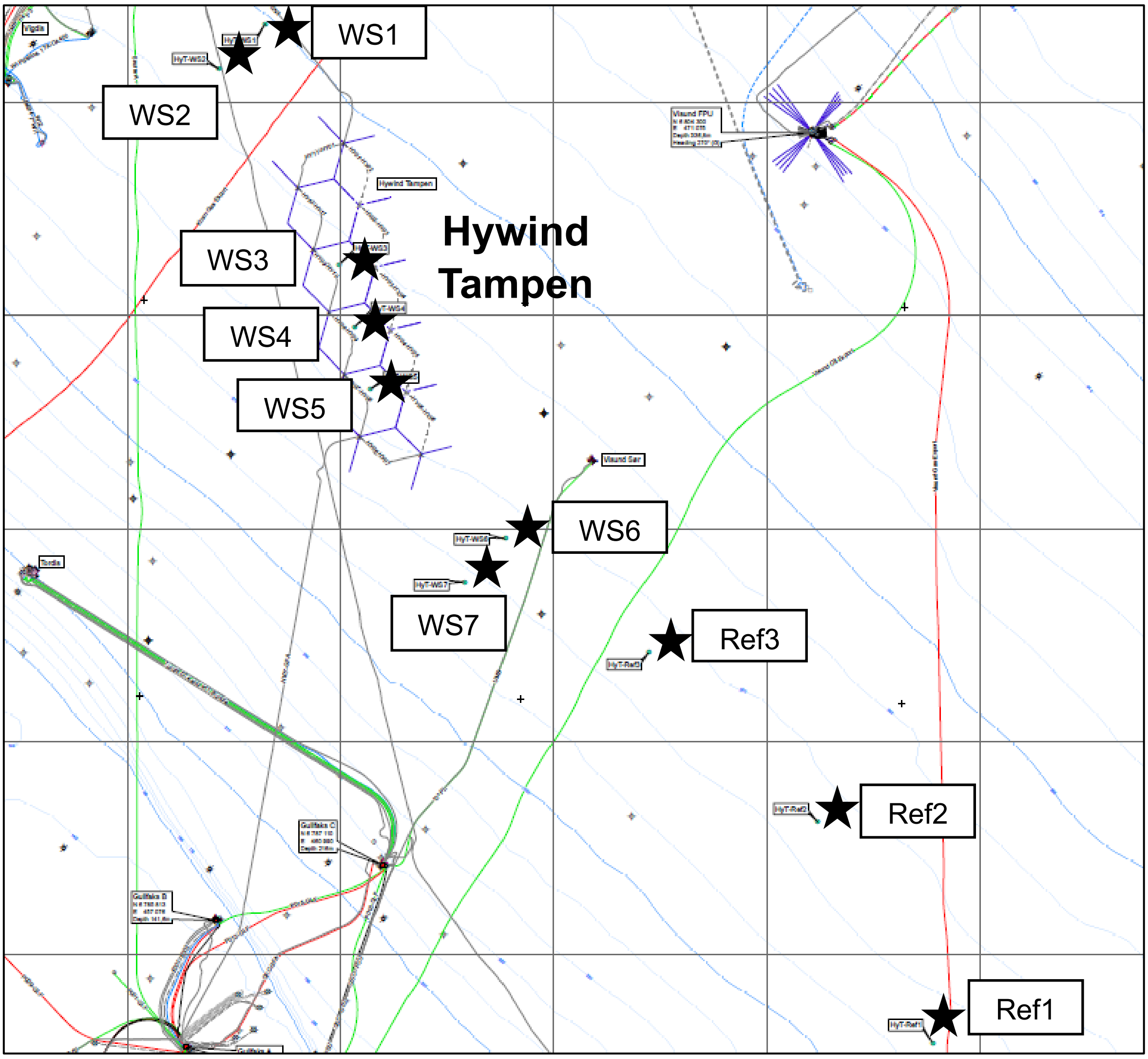

Figure 1. Placement of Hywind Tampen water sampling stations. Stations are divided into upstream (WS1-2), OWF (WS3-5), downstream (WS6-7) and reference

stations (Ref1-3) along the prevailing slope into the Norwegian trench in this area and following the dominating current direction. Figure from Hestetun et al., (2024).

Table 1. Positions of the Hywind Tampen sampling stations (UTM30). Table from Hestetun et al. (2024).

Name	Easting	Northing	Depth
HyT-WS1	458236	6806835	300
HyT-WS2	457154	6805790	292
HyT-WS3	459976	6801193	287
HyT-WS4	460340	6799734	285
HyT-WS5	460701	6798275	282
HyT-WS6	463875	6794764	275
HyT-WS7	462914	6793753	266
HyT-Ref1	473890	6782941	264
HyT-Ref2	471198	6788122	274
HyT-Ref3	467228	6792105	276

2.1. Elasmobranch detection

The scope of the elasmobranch detection work included resequencing the existing Hywind Tampen DNA extracts from the previous study with the inclusion of a molecular marker better able to detect elasmobranch fish species (MiFish-E). All Hywind Tampen samples part of the original study were reanalyzed here.

A literature review was conducted to assess the optimal approach to incorporate elasmobranch detection in the MiFish-U eDNA workflow established by previous studies at Hywind Scotland and Hywind Tampen. The MiFish-E primer set is a variant of the MiFish-U primer set specifically for elasmobranch detection (Miya et al., 2015), and was chosen for the reanalysis here. Within the scope of a single PCR run, two main options showed promising support in the literature: 1) a MiFish-E-only option, and 2) a primer mix containing both MiFish-E, and MiFish-U primer sets. Based on the reports of Dunn et al. (2022) and Sato et al., (2021), who both reported good results using the mixed approach, combining both primer sets (MiFish-E/MiFish-U) in the same amplification was chosen as the method here.

A full description of lab processing can be found in Hestetun et al. (2024). In brief, PCR amplification was done with adapter-linked primers using the KAPA3G Plant PCR kit (KAPA Biosystems) at an annealing temperature of 65 °C for a 50/50 equal concentration mix of MiFish-U and MiFish-E primer sets. Three PCR replicates were made for each sample and pooled before sequencing. Library preparation was done using equimolar pooled PCR product with Illumina dual index TruSeq i5/i7 barcodes. Field sampling, extraction and PCR negative controls were used to detect potential sampling and processing contamination. Sequencing was performed on an Illumina MiSeq instrument using v3 with 300 bp chemistry at the Norwegian Sequencing Centre (University of Oslo, Norway).

Multivariate analyses were done using Bray-Curtis pairwise dissimilarity data made with ASVs grouped into assigned taxon on Hellinger-transformed data.

2.2. New demersal fish analyses

A re-analysis of demersal fish dataset from the original 2024 Hywind Tampen report was requested in order to see if removal of dominating and/or pelagic fish species could improve detection of differences in patterns between bottom sampling stations. From the original MiFish-U demersal dataset, blue whiting (*Micromesistius poutassou*) was removed due to the large number of sequences belonging to this species in demersal datasets. Fish species with a closer pelagic affinity also removed for this analysis included Atlantic mackerel (*Scomber scombrus*), silvery cod (*Gadiculus argenteus*), Mueller's pearlside (*Maurolicus muelleri*), Atlantic herring (*Clupea harengus*), garfish (*Belone belone*), Atlantic salmon (*Salmo salar*), and lancet fish (*Notoscopelus kroyeri*). Original datasets can be found in Hestetun et al. (2024). For the MiFish-U/E mixed dataset, a similar approach was done in addition to the standard analysis of this dataset presented in this note. Here, porbeagle (*Lamna nasus*) was also removed from the dataset in addition to the species above.

3. Results

3.1. MiFish-U/MiFish-E mix metabarcoding results

Initial inspection of PCR product gels showed secondary bands for both MiFish U/E plates sent to the sequencing center representing 4 and 8% of main band strength respectively. Similar non-target amplification was reported by (Baidouri et al., 2024). While noted, sequencing proceeded without removal of these secondary bands, and sequences derived from them were removed using length filtering during dada2 processing.

The total number of raw sequences from the MiFish U/E dataset was 27,511,714 reads from 192 data points (seven stations with three timepoints, two depths, three replicates = 126) three reference stations (one time point, two depths, three replicates = 18), and 48 sampling (air, water), extraction and PCR controls. Bioinformatic filtering, denoising, merging and chimera detection reduced this to 23,732,322 sequences (also removing secondary band sequences); after uncross and the R package decontam additional filtering, 22,566,983 sequences remained distributed over 1038 ASVs in the 144 station samples. Taxonomic assignment using the MitoFish v396 database yielded 791 ASVs in 19,206,277 reads of genus rank and below, while 247 ASVs (3,648,509 reads) could not be assigned to at least genus level. The 791 genus and species rank ASVs represented 43 separate fish taxa (Appendix B) and were grouped by their assigned taxon for subsequent analyses.

The most abundant species in the entire MiFish dataset was blue whiting (*Micromesistius poutassou*), followed by Atlantic mackerel (*Scombrus scombrus*), Atlantic herring (*Clupea harengus*), saithe/pollack (*Pollachus* sp.), and pearlside (*Maurolicus* sp.) (Fig. 2; Table 2).

Elasmobranchs detected included the thorny skate (*Amblyraja radiata*), common skate (*Dipturus* sp.), velvet belly lanternshark (*Etmopterus spinax*), blackmouth catshark (*Galeus melastomus*), porbeagle (*Lamna nasus*), and spurdog (*Squalus* sp.).

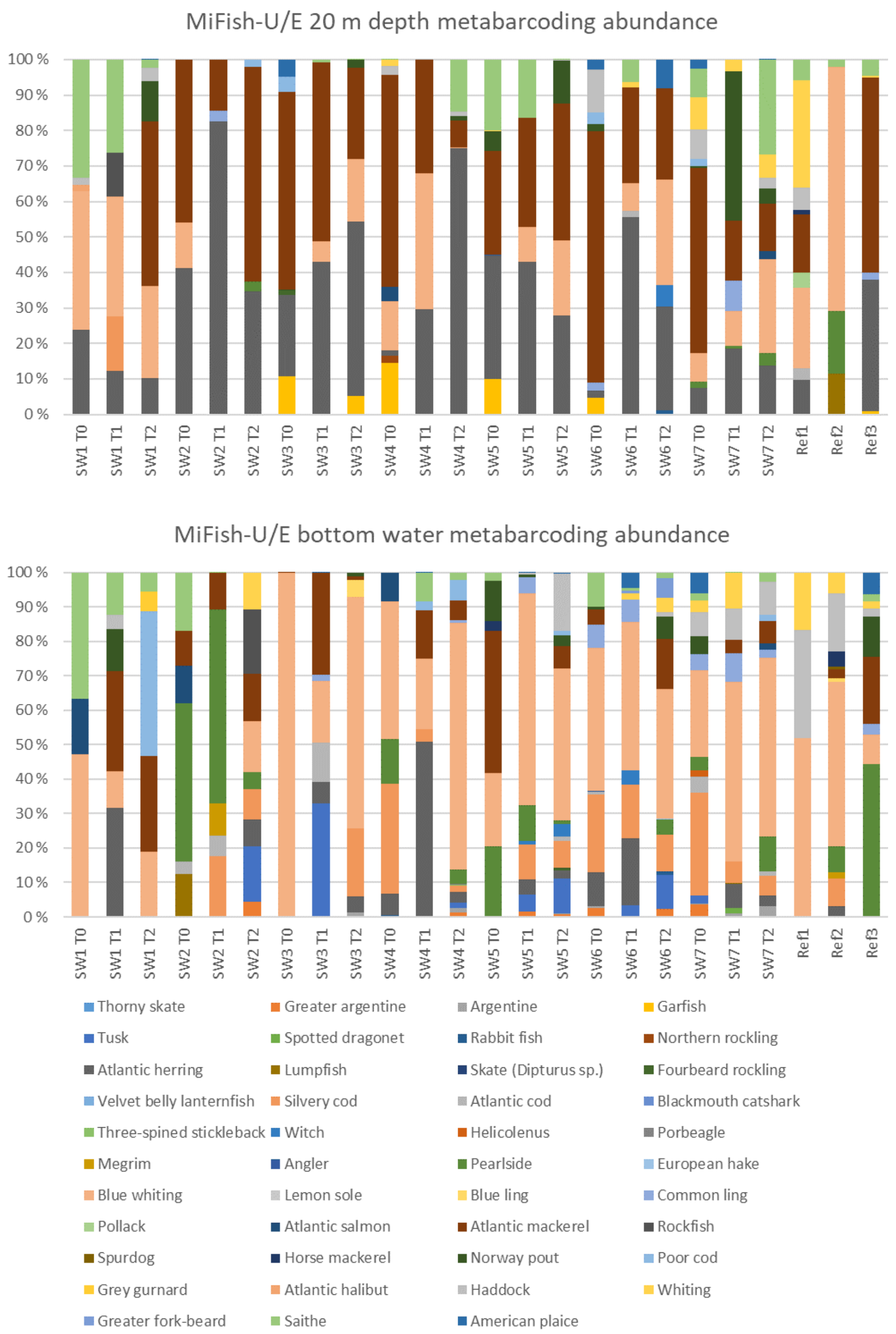

Figure 2. Relative abundance of all identified species in the MiFish-U/E dataset at sample level and sorted by depth.

Table 2. Absolute number of sequence reads for the 20 fish species with the highest number of sequences in the MiFish-U/E dataset, as identified by the MitoFish 3.96 database.

Name	Total	20 m	Bottom	Name	Total	20 m	Bottom
Blue whiting	5036956	1365152	3671804	**Poor cod**	247850	54904	192946
Atlantic mackerel	4087819	3171135	916684	**Common ling**	231420	60173	171247
Atlantic herring	3442289	2775232	667057	**Garfish**	214550	214550	0
Saithe	1118462	790904	327558	**Atlantic salmon**	152515	30262	122253
Pearlside	953027	84437	868590	**American plaice**	137285	65629	71656
Silvery cod	891599	60710	830889	**Atlantic cod**	124127	16087	108040
Haddock	514567	187456	327111	**Rockfish**	120254	49003	71251
Norway pout	502134	296817	205317	**Lumpfish**	85339	29314	56025
Whiting	368701	197750	170951	**Greater argentine**	81297	138	81159
Tusk	344607	36	344571	**Megrim**	52260	13585	38675

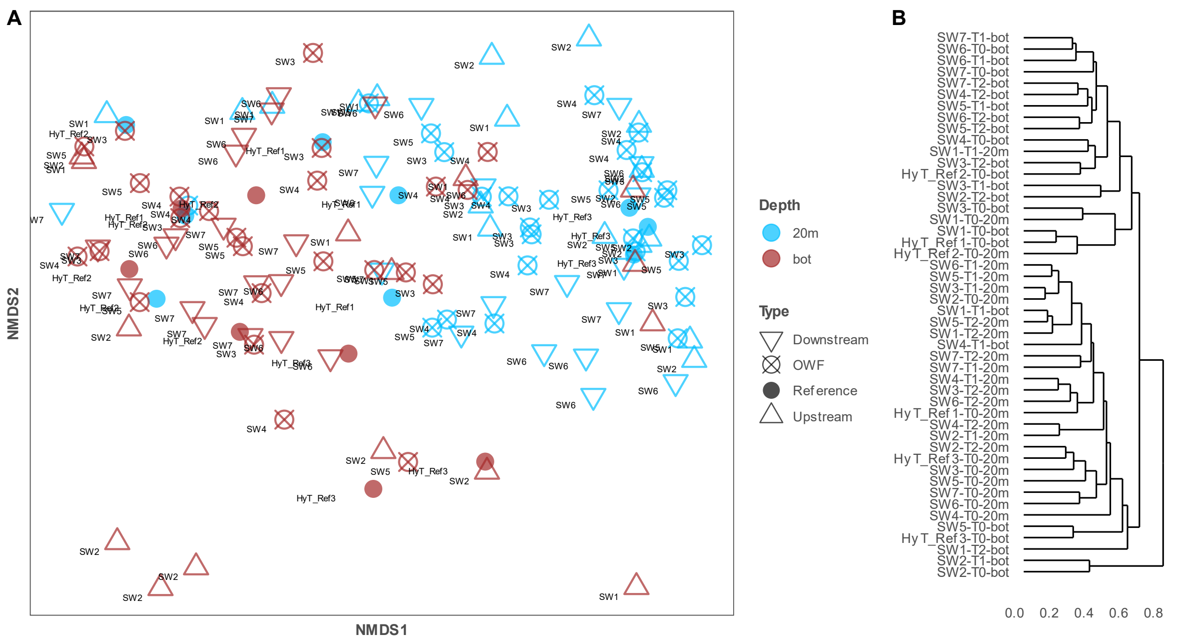

Figure 3. MiFish U/E analyses based on Bray-Curtis dissimilarities of Hellinger-transformed data. (A) NMDS analysis based of 20 m (light blue) and bottom water stations (brown) at sample level (three samples per station and depth), and (B) cluster analysis at station level, showing relative similarities in fish community composition. Stations are color-coded based on depth, and with symbols showing position relative to the wind farm (*cf.* Fig. 1).

The NMDS analysis of all stations at sample level (Fig. 3) indicated clustering based on depth, showing that recovered fish species communities were different at surface relative to bottom samples.

PERMANOVA analysis of the entire MiFish-U/E dataset showed significant differences for depth (F = 24.808; p = 0.001), and weaker but still significant differences based on location (F = 3.519; p = 0.001) and time point, indicating relatively stable conditions over time (F = 2.230; p = 0.023). SIMPER analysis of depth differences showed that Atlantic mackerel, Atlantic herring, and blue whiting explained 17% of the observed differences each, followed by pearlside at 8%, saithe at 6%, silvery cod at 6%, and all remaining species slightly over 29% in total. These patterns mirror the results of the Hestetun et al. (2024) MiFish-U only dataset.

3.2. MiFish U/E, U-only and fish capture checklist comparison

An overview of relative coverage based on reported taxa for the MiFish-U/E dataset was made compared to taxa reported in the Hestetun et al. (2024) MiFish-U only data and the fish capture data from the previous IMR-conducted surveys (de Jong et al., 2022; Palm et al., 2023). This data, available in Table form in Appendix B, is shown here as Euler diagrams both as a pairwise comparison and including all three datasets. Species detected in the 2024 MiFish-U-only survey not redetected here include *Echiodon drummondii* (Drummond's pearlfish), *Salmo trutta* (trout), and *Notoscopelus kroyeri* (lancet fish). The four fish from capture surveys not detected in the MiFish-U/E data also include *E. drummondii*, and not reported in the MiFish-U data, *Lophius budegassa* (blackbellied angler), *Leucoraja fullonica* (shagreen skate), and *L. naevus* (cuckoo ray) (Fig. 4).

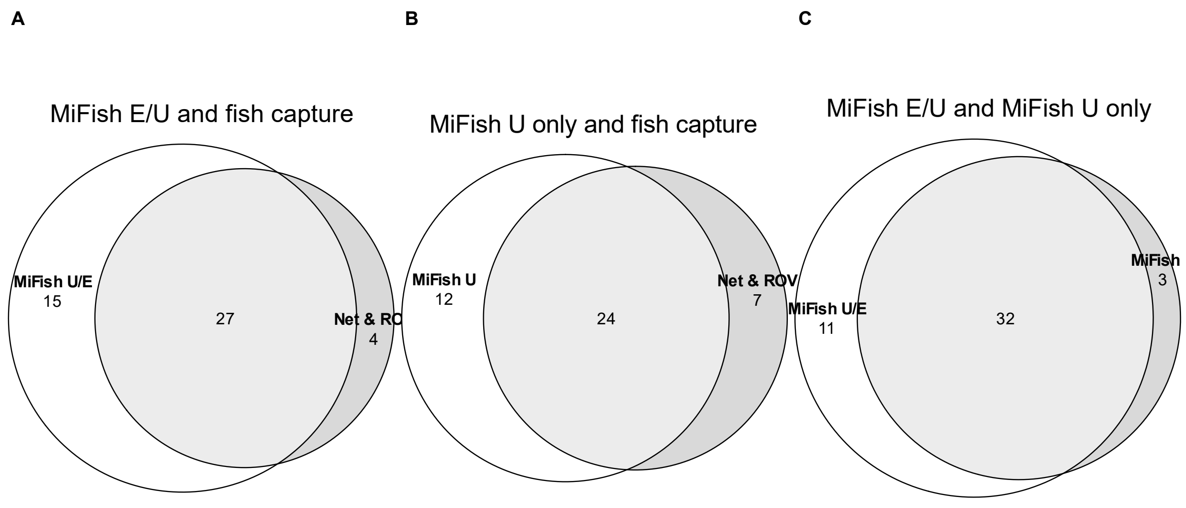

Figure 4. Euler diagrams showing overlap in reported species for (A) the newly sequenced MiFish-U/E dataset and species reported from fish capture surveys, (B) the previous MiFish-U only and capture data from Hestetun et al. (2024), and (C)

direct comparison of the MiFish-U/E and 2024 dataset. As in Hestetun et al. (2024), *Helicolenus* sp. And *Sebastes* sp. In the MiFish data have been synonymized with *H. dactylopterus* and *S. norvegicus* in the fish capture data. In addition, *Squalus* sp. In the MiFish-U/E data was synonymized with *S. acanthias* in the fish capture data.

3.3. Demersal fish analyses

New multivariate analyses with pelagic and dominating species removed were made for both the original MiFish-U dataset from Hestetun et al. (2024) and the MiFish U/E mix dataset sequenced for this note. In the original MiFish-U dataset, the demersal fish dataset with dominating and pelagic species removed contained 3,817,126 reads from 27 identified species (down from 13,580,659 reads and 39 species in the original demersal MiFish-U dataset). In the newly sequenced MiFish-U/E dataset, the demersal fish dataset with dominating and pelagic species removed contained 1,746,257 reads from 27 identified species (down from 8,826,451 reads and 34 species in the original demersal MiFish-U/E dataset).

NMDS plots and cluster analyses of data points from both datasets at sample level are given in Figures 5-6.

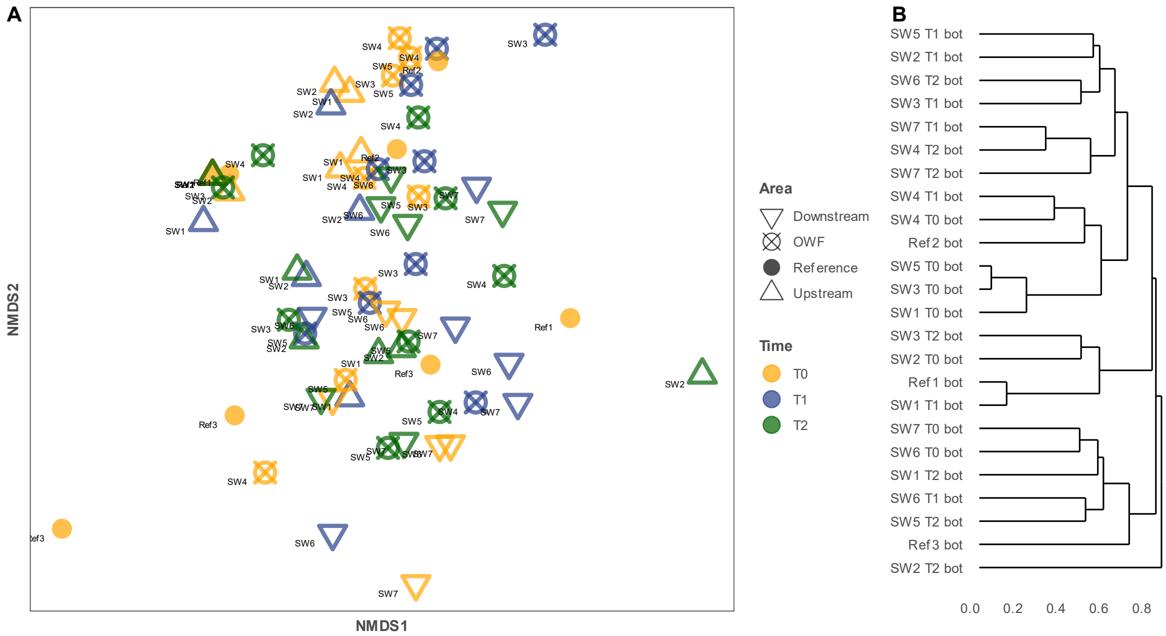

Figure 5. MiFish-U (A) NMDS and (B) cluster analysis of bottom water stations at station level with dominating and pelagic species removed, showing relative similarities in fish community composition across stations and time points.

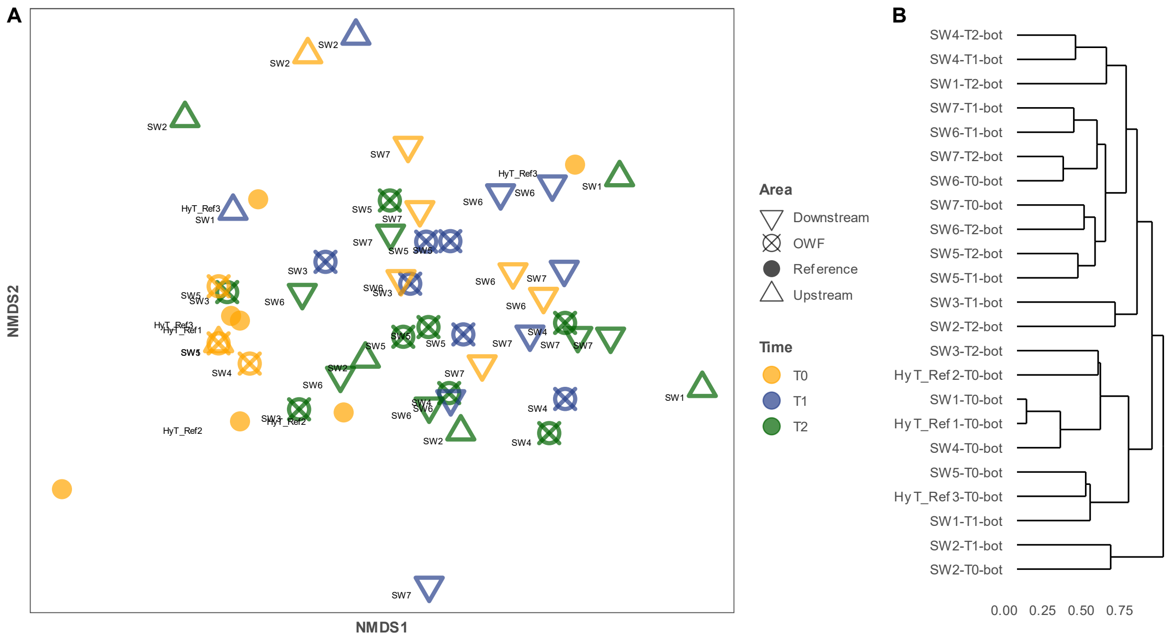

Figure 6. MiFish-U/E (A) NMDS and (B) cluster analysis of bottom water stations at sample level with dominating and pelagic species removed, showing relative similarities in fish community composition across stations and time points.

PERMANOVA results showed weak differences based on both area (MiFish-U: $F = 3.268$; $p = 0.001$; MiFish-U/E: $F = 1.938$; $p = 0.004$) and time (MiFish-U: $F = 1.664$; $p = 0.034$; MiFish-U/E: $F = 1.255$; $p = 0.224$). While only time for the MiFish-U/E dataset was found to be clearly not significant per se, time was also close to the limit of significance for the original dataset.

4. Discussion

4.1. Performance of the U/E mixed primer set

4.1.1. Comprehensiveness of elasmobranchs

In the original MiFish-U dataset of Hestetun et al., (2024), elasmobranchs reported from gillnet and ROV surveys in the area (de Jong et al., 2022; Palm et al., 2023), were not recovered. These species included the thorny skate (*Amblyraja radiata*), common skate (blue skate, flapper skate; *Dipturus intermedius*/*batis*), shagreen skate (*Leucoraja fullonica*), cuckoo ray (*L. naevus*), blackmouth catshark (*Galeus melastomus*) and spiny dogfish (*Squalus acanthias*).

For the mixed MiFish-U/E dataset, detected elasmobranchs from this list of species included *Amblyraja radiata*, *Dipturus* sp., *Galeus melastomus*, and *Squalus* sp. However, *Leucoraja fullonica* and *L. naevus* were not detected, and both skates (*Amblyraja*, *Dipturus*) as well as the blackmouth catshark (*Galeus melanostomus*) had comparatively few reads in the dataset (Appendix B). Two elasmobranchs were detected not reported previously: velvet belly lanternshark (*Etmopterus spinax*) and porbeagle (*Lamna nasus*). With the exception of the porbeagle, read numbers for elasmobranchs were from single digit up to ~100 reads. Thus, there is a possibility that elasmobranchs remain under-represented with regards of read abundance relative to non-molecular methods. This could be due to physiological reasons such as shedding rates (bony fishes typically have a thick mucous outer layer), ecology/behavior, or due to PCR amplification in the mixed U/E reaction. Trialing a MiFish-E-only sequencing run could answer the latter question, though a putative effect of a MiFish-E-only amplification experiment is beyond the scope of the work here. Given that metabarcoding abundances are not a precise quantitative measure, a MiFish-U/E run might be considered to give sufficient information for species inventory or monitoring purposes even with comparatively low read counts for elasmobranch species.

4.1.2. Comprehensiveness of non-elasmobranchs

The approach chosen here was designed to see if a combination of the MiFish-U and MiFish-E primers could serve as a way to get comprehensive coverage of both elasmobranchs and other fish species in a single amplification run. This is an approach that has been trialed in several previous studies (Baidouri et al., 2024; Dunn et al., 2022). In a detailed study of the relative efficacy of both primer sets in mixed conditions Dunn et al. (2022) reported that preference seemed to be given to the MiFish-E over the MiFish-U primer set in mixed conditions, yet as the primary aim here was elasmobranch detection, this did not serve as discouragement to try the mixed method in this study. Still, the MiFish-U data from the Hestetun et al. (2024) study allows an overall comparison of the comprehensiveness of MiFish-U/E data over a MiFish-U-only dataset.

In general, results between the previous MiFish-U dataset and the newly sequenced MiFish-U/E dataset agree. Pelagic fish species abundances are roughly similar for 20 m samples. A comparison of bottom water read abundances for species in the dataset is given in Appendix B: A few species from the previous dataset were not recovered for this analysis, including Drummond's pearlfish (*Echiodon drummondi*), lancet fish (*Notoscopelus kroyeri*), and brown trout (*Salmo trutta*). A couple of previously undetected non-elasmobranch species were reported for the U/E dataset, including three-spined stickleback (*Gasterosteus aculeatus*), lemon sole (*Microstomus kitt*), and blue ling (*Molva dypterygia*).

A combination of either the MiFish-U/E primer mix, or stochastic PCR effects (or both), could have the potential for non-detection, which is probable in the case of the pearlfish and lancet fish here: No similar sequence was found during a manual validation check of the ASVs in the dataset. However, some differences point to the taxonomic assignment protocol: For instance, lemon sole is very close to witch (*Glyptocephalus cynoglossus*) in sequence identity. Other instances include brown trout from the MiFish-U dataset, American plaice (*Hippoglossoides platessoides*) (with some ASVs erroneously assigned to *Limanda sakhalensis* and corrected here), and Pacific herring (*Clupea pallasii*) reported in the 2024 report. More puzzling was the assignment (with 100% identity) of three ASVs in both the original 2024 and present dataset to the freshwater species common bleak (*Alburnus alburnus*), goby (*Gobio gobio*), and catfish (*Rhamdia* sp.); while these were removed from the dataset, no obvious explanation for their presence could be found (either misattributed sequences in the database or an unknown contamination vector; they were not present in control samples, however), and these examples highlight the need for quality checking assignments against knowledge of local fish communities. In some cases, assignment ambiguity is the result of local genotype variation not included in the MitoFish database (thus resulting in several equal slightly lower-percentage scores for several species); alternatively certain ASVs could include sequencing artifacts not successfully removed during processing. Here, we used crest4, using a lowest common ancestor (LCA) assignment protocol (Lanzén et al., 2012) with some manual curation based on species known from the region. Still, these ambiguities highlight that metabarcoding datasets need to be subject to taxonomic scrutiny by experts in the field and suggests that the MiFish marker may struggle to distinguish between closely related species in some cases.

4.1.3. Evaluation of the MiFish-U/E mixed primer set approach

Overall, the MiFish-U/E protocol used here performed very well in terms of both detecting most elasmobranchs (all except both *Leucoraja* species) known from fish capture surveys in the area, and two additional unreported shark species. Only minor discrepancies in non-elasmobranch coverage were detected; 32 of 35 species from the 2024 MiFish-U dataset were recovered in the MiFish-U/E dataset here. A level of ambiguity was evident in species-rank assignments, highlighting the need for taxonomist validation of assignment results (not inherent to the MiFish-U/E approach but applicable to the MiFish marker in general). Thus, the MiFish-U/E approach used here can readily be recommended as a cost-effective method for future studies to increase coverage of elasmobranch taxa in MiFish studies without the need for separate MiFish-U and MiFish-E PCR amplifications.

4.2. Demersal fish analyses

As part of this note, new bottom water analyses were done on both the original MiFish-U dataset from the Hestetun et al. (2024) Hywind Tampen report, and the newly sequenced MiFish-U/E dataset. In the original report (Hestetun et al., 2024) (Fig. 8), only weak differences in fish community composition were evident between sites based on location (F = 2.624; p = 0.001) and time (F = 1.559; p = 0.05).

Removing a selection of pelagic species and the species with the highest number of reads (38% of total bottom water MiFish-U reads), blue whiting (*Micromesistius poutassou*), no further level of discrimination was evident in the MiFish-U dataset and differences based on location (F = 3.268; p = 0.001) and time (F = 1.664; p = 0.034) remained weak (Fig. 4).

Similarly, for the newly sequenced MiFish-U/E dataset, dissimilarities between stations (F = 1.789; p = 0.011) were weak and over time both weak and not significant (F = 1.645; p = 0.056) (Fig. 5). Removal of dominating/pelagic species thus did not give any improved discrimination ability for demersal fish communities here. Rather, these analyses reaffirm the main conclusion from the original Hywind Tampen report, namely that bottom fish community composition remains stable over time and with similar conditions across stations (Hestetun et al., 2024).

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Appendix A – Fish scientific names

Scientific name	English	Norwegian
Amblyraja radiata	Thorny skate	Kloskate
Argentina silus	Greater argentine	Vassild
Argentina sphyraena	Argentine	Strømsild
Belone belone	Garfish	Horngjel
Brosme brosme	Tusk	Brosme
Callionymus maculatus	Spotted dragonet	Flekket fløyfisk
Chimaera monstrosa	Rabbit fish	Havmus
Clupea harengus	Atlantic herring	Sild
Cyclopterus lumpus	Lumpfish	Rognkjeks
Dipturus batis	Common skate	Gulringskate
Dipturus intermedius	Flapper skate	Storskate
Echiodon drummondi	Drummond's pearlfish	Snyltefisk
Enchelyopus cimbrius	Fourbeard rockling	Firetrådet tangbrosme
Etmopterus spinax	Velvet belly lanternshark	Svarthå
Eutrigla gurnardus	Grey gurnard	Knurr
Gadiculus argenteus	Silvery cod	Sølvtorsk
Gadus morhua	Atlantic cod	Torsk
Galeus melastomus	Blackmouth catshark	Hågjel
Gasterosteus aculeatus	Three-spined stickleback	Trepigget stingsild
Glyptocephalus cynoglossus	Witch	Smørflyndre
Helicolenus dactylopterus	Blackbelly rosefish	Blåkjeft
Hippoglossoides platessoides	American plaice	Gapeflyndre
Hippoglossus hippoglossus	Atlantic halibut	Kveite
Lamna nasus	Porbeagle	Håbrann
Lepidorhombus whiffiagonis	Megrim	Glassvar
Leucoraja fullonica	Shagreen skate	Nebbskate
Leucoraja naevus	Cuckoo ray	Gjøkskate
Lophius piscatorius	European angler	Breiflabb
Lophius budegassa	Blackbellied angler	Svartflabb
Maurolicus muelleri	Mueller's pearlside	Laksesild
Melanogrammus aeglefinus	Atlantic haddock	Hyse
Merlangius merlangus	Whiting	Hvitting
Merluccius merluccius	European hake	Lysing
Micromesistius poutassou	Blue whiting	Kolmule
Microstomus kitt	Lemon sole	Lomre
Molva dypterygia	Blue ling	Blålange
Molva molva	Common ling	Lange
Notoscopelus kroyeri	Lancet fish	Stor lysprikkfisk
Phycis blennoides	Greater forkbeard	Skjellbrosme
Pollachius pollachius	Pollack	Lyr
Pollachius virens	Saithe	Sei
Salmo salar	Atlantic salmon	Laks
Salmo trutta	Brown trout	Ørret
Scomber scombrus	Atlantic mackerel	Makrell
Sebastes norvegicus	Atlantic redfish	Uer
Squalus acanthias	Spiny dogfish	Pigghå
Trachurus trachurus	Horse mackerel	Hestmakrell
Trisopterus esmarkii	Norway pout	Øyepål
Trisopterus minutus	Poor cod	Sypike

Appendix B – MiFish-U/E and capture study species composition

Checklist of fish species in the bottom samples from the MiFish-U/E data in this note against the MiFish-U data in Hestetun et al. (2024) and the 2022 Tampen catch study by De Jong et al., with additional species mentioned in de Jong et al. 2023 Tampen ROV transect descriptions (marked as "ROV"). Read and catch abundances are given for the total study samples as a very rough estimate of detection efficacy. All species recovered in catch and ROV studies not in eDNA data are present in the MitoFish database, so non-detection in the MiFish dataset here thus implies either not present, less relative release of eDNA in water from certain taxa, or potential primer bias. An asterisk notes presence in 20 m data for the MiFish datasets.

Scientific name	MiFish-U/E (this study)	MiFish-U Hestetun et al. 2024	de Jong 2022/2023	Comment
Amblyraja radiata	100		3	Skate
Argentina silus	81159	69218	1	
Argentina sphyraena	39405	19809		
Belone belone	0*	14193		
Brosme brosme	344571	441477	15	
Callionymus maculatus	6530	8		
Chimaera monstrosa	9028	8562	58	
Clupea harengus	667057	865488	1	
Clupea pallasii		1321		Likely *C. harengus* intraspecific variation.
Cyclopterus lumpus	56025	28835		
Dipturus intermedius/batis	8		6	Skate
Echiodon drummondi		16923	ROV	
Enchelyopus cimbrius	3861	648		
Etmopterus spinax	71			
Eutrigla gurnardus	12574	40079	3	
Gadiculus argenteus	830889	1039333	ROV	
Gadus morhua	108040	42441	215	
Galeus melastomus	3		35	Shark
Gasterosteus aculeatus	2855			
Glyptocephalus cynoglossus	38675	49655	1	
Helicolenus dactylopterus			ROV	
***Helicolenus* sp.**	8217	75595		Only resolved to genus.
Hippoglossoides platessoides	71656	252547	8	
Hippoglossus hippoglossus			3	
***Hippoglossus* sp.**	0*			Only resolved to genus.

Lamna nasus	2896			
Lepidorhombus whiffiagonis	23249	113579	9	
Leucoraja fullonica			3	Skate
Leucoraja naevus			1	Skate
Lophius budegassa			1	Possibly lack of resolution.
Lophius piscatorius	1351	82	20	
Maurolicus muelleri	868590	977107		
Melanogrammus aeglefinus	327111	225428	34	
Merlangius merlangus	170951	153058	141	
Merluccius merluccius	942	3347	84	
Micromesistius poutassou	3671804	5064992	41	
Microstomus kitt	205			
Molva dypterygia	21559			
Molva molva	171247	363066	589	
Notoscopelus kroyeri		4830		
Phycis blennoides	21564	74521	2	
Pollachius pollachius	128	0*	69	*Present in 20 m data.
Pollachius virens	327558	908476	158	
Salmo salar	122253	154353		
Salmo trutta		5930		Possibly misassignment.
Scomber scombrus	916684	1139022	47	
Sebastes norvegicus			1	
***Sebastes* sp.**	71251	163777		Only resolved to genus level.
Squalus acanthias			8	Shark
***Squalus* sp.**	2953			
Trachurus trachurus	29478	5391		
Trisopterus esmarkii	205317	353313	ROV	
Trisopterus minutus	192946	94268		

Appendix C – 20 m data

Name	Ref1 T0	Ref2 T0	Ref3 T0	SW1 T0	SW1 T1	SW1 T2	SW2 T0	SW2 T1	SW2 T2	SW3 T0	SW3 T1	SW3 T2
Amblyraja radiata	0	0	0	0	0	0	0	0	0	0	0	0
Argentina silus	0	0	0	0	0	0	0	0	0	0	0	0
Argentina sphyraena	0	0	0	0	0	0	0	0	0	0	0	0
Belone belone	0	0	4543	0	0	30	0	0	107	43315	11	18216
Brosme brosme	0	0	0	0	0	0	0	0	0	0	0	0
Callionymus maculatus	0	0	0	0	0	0	0	0	0	0	0	0
Chimaera monstrosa	0	0	0	0	0	0	0	0	0	0	0	0
Clupea harengus	27372	0	181131	144354	48100	30965	146340	237650	147900	91251	152691	174502
Cyclopterus lumpus	0	29278	0	0	0	10	0	0	0	0	0	0
Dipturus sp.	0	0	0	0	0	0	0	0	0	0	0	0
Enchelyopus cimbrius	0	0	0	0	0	8	0	0	0	6261	0	0
Etmopterus spinax	0	0	0	0	0	0	0	0	0	0	0	0
Eutrigla gurnardus	0	0	0	0	0	0	0	0	3	0	0	0
Gadiculus argenteus	0	0	0	0	60487	0	0	0	0	0	0	0
Gadus morhua	9291	0	0	0	0	0	0	0	0	0	0	0
Galeus melastomus	0	0	0	0	0	0	0	0	0	0	0	0
Gasterosteus aculeatus	0	0	0	0	0	0	0	0	0	0	0	0
Glyptocephalus cynoglossus	0	0	0	0	0	0	0	0	0	0	0	0
Helicolenus sp.	0	0	0	0	0	0	0	0	0	0	0	0
Hippoglossoides platessoides	0	0	26	0	0	0	19146	0	0	0	0	0
Hippoglossus sp.	10974	0	0	0	0	0	0	0	0	0	0	10
Lamna nasus	0	0	0	0	0	0	0	0	0	0	0	0
Lepidorhombus whiffiagonis	0	0	0	0	0	0	0	0	0	0	0	0
Lophius sp.	0	0	0	0	0	0	0	0	0	0	0	0
Maurolicus sp.	0	44570	0	0	0	0	0	0	11801	0	0	0
Melanogrammus aeglefinus	12605	0	11915	0	0	0	0	0	0	12989	13	7251
Merlangius merlangus	0	0	0	0	0	0	0	0	0	9037	0	0
Merluccius merluccius	0	0	0	0	0	0	0	0	0	0	0	0
Micromesistius poutassou	64144	174423	0	234428	132383	78061	45100	0	45	0	20838	62431
Microstomus kitt	0	0	0	0	0	0	0	0	0	0	0	0
Molva dypterygia	0	0	0	0	0	0	0	0	0	0	0	0
Molva molva	0	0	9962	0	0	0	0	8187	0	0	0	0
Phycis blennoides	0	0	0	0	0	0	0	0	0	0	0	0
Pollachius pollachius	0	0	0	0	0	0	0	0	0	0	0	0
Pollachius virens	200376	102470	6738	0	0	0	0	2679	169	32	0	85307
Salmo salar	0	0	0	0	0	0	0	0	27	0	0	0
Scomber scombrus	46560	0	269054	0	0	140237	162631	41503	258906	223069	179544	90594
Sebastes sp.	0	0	0	0	48925	0	0	0	0	0	3	0
Squalus sp.	0	0	0	0	0	0	0	0	0	0	0	0
Trachurus sp.	3352	0	0	0	0	0	0	0	0	0	0	0
Trisopterus esmarkii	0	0	0	0	0	33635	0	0	0	0	0	8155
Trisopterus minutus	0	0	0	0	0	0	0	0	8444	17208	0	0

Name	**SW4 T0**	**SW4 T1**	**SW4 T2**	**SW5 T0**	**SW5 T1**	**SW5 T2**	**SW6 T0**	**SW6 T1**	**SW6 T2**	**SW7 T0**	**SW7 T1**	**SW7 T2**
Amblyraja radiata	0	0	0	0	0	0	0	0	0	0	0	0
Argentina silus	0	0	86	0	0	0	52	0	0	0	0	0
Argentina sphyraena	0	0	0	0	0	0	0	0	0	0	0	0
Belone belone	75400	0	0	48608	16	0	24304	0	0	0	0	0
Brosme brosme	0	0	36	0	0	0	0	0	0	0	0	0
Callionymus maculatus	0	0	0	0	0	0	0	0	0	0	0	0
Chimaera monstrosa	0	0	0	0	0	0	0	0	2515	0	0	0
Clupea harengus	7958	67920	437422	172306	166525	80223	9823	216070	63907	44025	67872	58925
Cyclopterus lumpus	0	0	14	0	0	12	0	0	0	0	0	0
Dipturus sp.	0	0	0	0	0	0	0	0	0	0	0	0
Enchelyopus cimbrius	0	0	0	0	0	0	0	0	0	0	0	0
Etmopterus spinax	0	0	0	0	0	0	0	0	0	0	0	0
Eutrigla gurnardus	0	0	5	0	0	0	0	0	0	0	0	0
Gadiculus argenteus	0	0	0	0	0	0	223	0	0	0	0	0
Gadus morhua	0	0	0	0	0	0	0	6796	0	0	0	0
Galeus melastomus	0	0	0	0	0	0	0	0	0	0	0	0
Gasterosteus aculeatus	0	0	9	0	0	0	0	0	0	0	0	0
Glyptocephalus cynoglossus	0	0	0	0	0	0	0	0	13585	0	0	0
Helicolenus sp.	0	0	0	0	0	0	0	0	0	0	0	0
Hippoglossoides platessoides	0	0	0	0	0	0	0	7	0	0	41	0
Hippoglossus sp.	0	0	0	0	0	0	0	0	0	0	0	0
Lamna nasus	0	0	3	0	0	0	0	0	0	0	8	0
Lepidorhombus whiffiagonis	0	0	0	0	0	0	0	0	0	0	0	0
Lophius sp.	0	0	0	604	0	0	2	0	0	0	0	0
Maurolicus sp.	0	0	0	0	0	0	0	0	0	10867	2658	14541
Melanogrammus aeglefinus	0	0	244	63171	0	0	49175	0	12250	17843	0	0
Merlangius merlangus	633	0	54	0	6246	137	53444	12158	27986	85838	0	2217
Merluccius merluccius	0	0	0	0	0	0	0	0	0	0	0	0
Micromesistius poutassou	72200	88180	1706	0	38536	60430	371	30160	65184	46844	36028	113660
Microstomus kitt	0	0	0	0	0	0	0	0	0	0	0	0
Molva dypterygia	0	0	0	0	0	0	0	0	0	0	0	0
Molva molva	0	0	0	0	0	0	11176	0	0	0	30833	15
Phycis blennoides	0	0	0	0	0	0	0	0	0	0	0	0
Pollachius pollachius	0	0	0	0	0	0	0	0	0	11932	0	0
Pollachius virens	98842	63413	160	0	24380	0	47543	0	114685	16344	4936	22830
Salmo salar	20496	0	0	0	0	0	0	0	0	0	0	9739
Scomber scombrus	313774	73814	44416	144421	119188	111248	365328	104381	56255	308629	61233	56350
Sebastes sp.	7	0	0	0	5	0	4	0	59	0	0	0
Squalus sp.	0	0	0	0	0	0	0	0	0	0	0	0
Trachurus sp.	0	0	0	0	0	0	0	0	0	0	0	0
Trisopterus esmarkii	0	0	7334	27803	0	34994	10239	0	0	2291	153542	18824
Trisopterus minutus	0	0	148	0	0	0	17126	0	0	11978	0	0

Appendix D – Bottom water data

Name	Ref1 T0	Ref2 T0	Ref3 T0	SW1 T0	SW1 T1	SW1 T2	SW2 T0	SW2 T1	SW2 T2	SW3 T0	SW3 T1	SW3 T2
Amblyraja radiata	0	0	0	0	0	0	0	0	0	0	0	0
Argentina silus	0	0	13	0	0	0	0	0	16501	0	0	0
Argentina sphyraena	5	0	0	0	0	0	0	0	259	0	0	4375
Belone belone	0	0	0	0	0	0	0	0	0	0	0	0
Brosme brosme	206	0	0	0	29	0	0	26	61912	0	134961	0
Callionymus maculatus	0	0	82	0	0	0	0	0	0	0	0	4
Chimaera monstrosa	0	1367	0	0	0	0	0	0	5	0	0	0
Clupea harengus	0	11783	0	0	105043	0	0	0	29716	76	25329	15927
Cyclopterus lumpus	0	0	0	0	5	0	55906	0	0	0	11	0
Dipturus sp.	0	0	0	0	0	0	0	0	0	0	0	0
Enchelyopus cimbrius	0	0	0	0	0	0	0	0	0	0	0	0
Etmopterus spinax	0	36	0	0	0	0	0	0	0	0	0	0
Eutrigla gurnardus	0	0	12574	0	0	0	0	0	0	0	0	0
Gadiculus argenteus	0	35864	53	0	61	0	0	30162	33599	17	0	65167
Gadus morhua	82	0	0	0	0	0	16349	10384	0	0	46620	0
Galeus melastomus	0	0	0	0	0	0	0	0	0	0	0	0
Gasterosteus aculeatus	0	0	0	0	0	0	0	0	0	0	0	0
Glyptocephalus cynoglossus	3	0	12	0	0	0	0	0	0	0	0	0
Helicolenus sp.	0	0	0	0	0	0	0	0	0	0	0	0
Hippoglossoides platessoides	0	0	0	13922	0	17597	14934	0	4	0	0	0
Hippoglossus sp.	0	0	0	0	0	0	0	0	0	0	0	0
Lamna nasus	0	0	0	0	0	0	0	0	0	0	0	0
Lepidorhombus whiffiagonis	0	7285	0	6	2	0	0	15924	0	0	0	0
Lophius sp.	0	0	0	0	0	0	0	0	0	0	0	0
Maurolicus sp.	0	32353	195728	0	6	0	206534	96730	18993	3	0	121
Melanogrammus aeglefinus	0	13327	0	0	0	0	0	0	0	0	0	0
Merlangius merlangus	0	0	0	0	0	41146	0	0	0	0	0	0
Merluccius merluccius	0	0	0	0	0	0	0	0	0	0	0	0
Micromesistius poutassou	54294	207950	37383	59229	35249	41358	0	0	57017	101494	72748	225108
Microstomus kitt	0	0	0	0	0	0	0	0	0	0	0	0
Molva dypterygia	0	4643	0	0	0	0	0	0	0	0	0	16916
Molva molva	0	0	14132	0	0	0	0	0	0	0	7632	0
Phycis blennoides	0	0	5	0	0	0	0	0	0	0	0	0
Pollachius pollachius	0	0	0	0	0	0	0	0	0	0	0	0
Pollachius virens	45893	41026	11841	75555	65	0	0	0	0	0	36037	26012
Salmo salar	0	0	0	20216	15	0	49073	0	50	0	0	0
Scomber scombrus	0	11783	85667	0	96503	60736	45284	18154	52950	29	120573	2879
Sebastes sp.	0	0	9	0	9	8	0	0	71185	0	0	10
Squalus sp.	0	2953	0	0	0	0	0	0	0	0	0	0
Trachurus sp.	0	19186	0	0	0	9	0	0	0	0	0	15
Trisopterus esmarkii	0	0	52196	0	40692	0	0	0	0	0	0	3790
Trisopterus minutus	0	0	0	0	0	92454	0	0	0	0	0	0

Name	SW4 T0	SW4 T1	SW4 T2	SW5 T0	SW5 T1	SW5 T2	SW6 T0	SW6 T1	SW6 T2	SW7 T0	SW7 T1	SW7 T2
Amblyraja radiata	0	0	0	0	0	0	0	100	0	0	0	0
Argentina silus	0	1465	15717	0	7140	4481	12262	0	7325	16255	0	0
Argentina sphyraena	0	0	15403	0	0	43	2304	4	11	66	4031	12904
Belone belone	0	0	0	0	0	0	0	0	0	0	0	0
Brosme brosme	0	0	20655	57	21837	51204	0	12196	31375	10113	0	0
Callionymus maculatus	0	0	17	0	4	0	0	2	0	0	6421	0
Chimaera monstrosa	2591	0	223	0	85	185	284	0	2902	1010	0	376
Clupea harengus	33446	216817	40481	0	19361	11840	45608	68961	59	0	29540	13070
Cyclopterus lumpus	9	0	0	19	0	0	0	0	0	0	75	0
Dipturus sp.	0	0	0	0	0	0	0	0	0	0	8	0
Enchelyopus cimbrius	0	0	0	0	0	3861	0	0	0	0	0	0
Etmopterus spinax	0	0	7	0	0	28	0	0	0	0	0	0
Eutrigla gurnardus	0	0	0	0	0	0	0	0	0	0	0	0
Gadiculus argenteus	170465	15320	21648	0	44847	38904	106945	55383	33778	128671	25768	24237
Gadus morhua	0	0	0	0	39	6086	3159	0	0	19924	0	5397
Galeus melastomus	0	0	0	0	3	0	0	0	0	0	0	0
Gasterosteus aculeatus	0	0	2855	0	0	0	0	0	0	0	0	0
Glyptocephalus cynoglossus	0	0	0	0	4913	18483	0	15259	5	0	0	0
Helicolenus sp.	0	0	0	0	0	10	0	0	0	8207	0	0
Hippoglossoides platessoides	0	33	1280	0	15756	0	26332	0	0	0	0	28207
Hippoglossus sp.	0	0	0	0	0	0	0	0	0	0	0	0
Lamna nasus	0	0	2892	4	0	0	0	0	0	0	0	0
Lepidorhombus whiffiagonis	0	0	0	0	0	0	0	0	2	0	0	30
Lophius sp.	0	0	0	0	226	0	1125	0	0	0	0	0
Maurolicus sp.	68893	0	51656	73813	45599	4820	0	0	13892	17042	147	42260
Melanogrammus aeglefinus	0	2487	82973	0	0	4178	30477	36684	39813	33224	73651	10297
Merlangius merlangus	0	0	0	0	6405	13150	14743	43270	0	17581	25757	8899
Merluccius merluccius	0	0	0	0	0	0	0	0	184	0	711	47
Micromesistius poutassou	212888	88361	899711	76670	273426	221287	194615	153893	119124	108300	213934	217765
Microstomus kitt	0	0	0	0	0	0	14	0	0	0	191	0
Molva dypterygia	0	0	0	0	0	0	0	0	0	0	0	0
Molva molva	0	0	10088	0	20468	0	31939	22451	0	20006	34495	10036
Phycis blennoides	0	0	0	0	3523	18036	0	0	0	0	0	0
Pollachius pollachius	0	0	0	0	0	128	0	0	0	0	0	0
Pollachius virens	8747	0	0	46935	2632	4898	8218	24	11216	0	0	8459
Salmo salar	44905	0	0	0	0	0	0	0	0	0	60	7934
Scomber scombrus	0	59933	72220	148413	0	32138	21342	17	45102	0	16232	26729
Sebastes sp.	0	0	0	0	0	0	0	15	15	0	0	0
Squalus sp.	0	0	0	0	0	0	0	0	0	0	0	0
Trachurus sp.	0	0	0	10263	0	0	0	0	5	0	0	0
Trisopterus esmarkii	0	0	0	42119	3830	16094	2868	0	20648	23080	0	0
Trisopterus minutus	0	10454	74752	0	0	6607	0	0	0	0	0	8679

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