Environmental DNA monitoring of fish communities at the Hywind Tampen floating offshore wind farm

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Summary

Environmental impact assessment and regular environmental monitoring are prerequisites for the construction, operation, and decommissioning of offshore wind farms (OWFs). Molecular approaches are increasingly being considered as a possible complement or alternative to currently used marine baseline and monitoring methods, both for water column and seafloor organism studies. The following report shows the results of a study where two molecular environmental DNA (eDNA) methods – metabarcoding and ddPCR quantitative assays – have been used to characterize the water column at the Hywind Tampen floating OWF (FOWF) based on filtered 20 m and bottom water samples from within, upstream, and downstream from the FOWF as well as three reference stations further away from the FOWF. The aim of this study was to gain further information on the performance of eDNA water samples as a method to monitor impact on the pelagic ecosystem. More specifically, this work builds upon a similar study at the Hywind Scotland Pilot Park in 2021 (Ray et al., 2022), aiming to address gaps identified in that study such as temporal stability of the results, eDNA degradation and current transport in the water through sampling the same stations at three different times. Metabarcoding was employed for a community view of a) fish fauna specifically, using the MiFish primer set, and b) a universal eukaryote dataset based on 18S V1-V2 primers. Quantitative assays were employed for two commercially important pelagic fish species: mackerel and herring. The study aimed to investigate the following three research questions:

- RQ1: Is there any persistent significant difference (i.e. over the three time points) in a) pelagic fish, b) demersal fish and c) plankton composition between the FOWF area and outside the FOWF?
- RQ2: Using two pelagic fish species as a ddPCR demonstration, can the influence of currents be seen in the abundances estimates from stations upstream, in, and downstream of the wind farm?
- RQ3: What is the overlap in species composition between species detected by previous net and ROV studies and species detected in the current study MiFish dataset?

We were able to recreate surface (20 m) and bottom water community data for both fishes (MiFish) and total eukaryote (18S V1-V2) communities using an upstream, FOWF, downstream, and reference sampling approach, sampled at three timepoints T0, T1 (+24h), and T2 (+7d). This study design allowed both study of changes in community composition based on area and over time. As in the previous Hywind Scotland study (Ray et al., 2022), samples proved distinct and were able to distinguish local conditions at the sampling site and depth. The 18S data was dominated by calanoid copepods, with fewer dinoflagellate (especially *Karenia*) sequences than in the previous Hywind Scotland data, representing different seasonal conditions with regards to algal blooming relative to the previous study.

While less taxa can be identified to low (i.e. species or genus) taxonomic rank, the larger number of organisms in the 18S data makes it more sensitive in discerning patterns between stations (i.e. beta diversity).

The MiFish metabarcoding marker detected 36 fish species in the area, including relevant commercially important species. The majority of the demersal or mesopelagic species were detected in the bottom water samples, demonstrating that the marker picked up local variation in community structure. The 20 m samples, on the other hand, indicated high abundances of schooling pelagic fish species. These results show a similarly high level of species detection as the previous MiFish Hywind Scotland study. Ground truthing using demersal fish species reported by recent net and ROV studies in the area (de Jong et al., 2022; Palm et al., 2023) showed that the MiFish marker detects reported fish species except elasmobranchs (sharks and rays), which were not detected in the MiFish data; a known deficiency that can be mitigated by the use of special primers suitable for this taxonomic group. In a couple of cases, MiFish only resolved to genus, rather than species, level (N. Dunn et al., 2022). Conversely, the MiFish bottom water data also picked up 13 species not reported from the previous capture surveys, representing species that are difficult to capture using nets due to size or behavior.

eDNA transport by ocean currents was investigated through comparison of T0 data with downstream T1 (+24h) data in order to assess similarities due to eDNA current transport. Using this approach, we could not observe any patterns indicating an effect of eDNA current transport within the data from this study. Metabarcoding multivariate analyses did not show any T0 and downstream T1 (+24h) clustering. For quantitative mackerel ddPCR data, considering only T0 and downstream T1 stations, both time and region had a significant effect on eDNA concentrations, but in the herring ddPCR data, neither factor had a significant effect. This result could be related to both eDNA current transport, the movement of fish during this period, or a combination of both. We assess that the impact of current transport does not significantly confound the interpretation of data for individual species or community compositions derived from eDNA water samples under the conditions of this study.

Overall, the study confirms the utility of eDNA samples as a powerful tool in monitoring OWF installations. A number of potential impacts of OWFS on fish have been suggested in the scientific literature, both negative (noise, vibrations, pollution, oceanographic) or positive (reef and fish-aggregating device effects, fisheries closure). While differences in local community composition due to either depth or time were evident in the metabarcoding data, we could not establish any clear effect – either positive or negative – on fish or plankton communities due to the Hywind Tampen FOWF in this study. Considering that Hywind Tampen was only partially installed and is a floating turbine OWF with a limited number of turbines in a deep-water area, this was expected and may serve as a baseline. It is possible that effects such as for instance mooring structure organism epigrowth means that any effects will be more prevalent at a later date. A follow-up study after some years of operation would yield additional information on any positive or negative impact when the structures have been present in the water for an extended period of time.

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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.
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 – 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

Several potential impacts of offshore wind farms (OWFs) on the pelagic environment have been hypothesized. Though an area of active research, the magnitude and relative importance of impact on the pelagic ecosystem during the operational phase of OWFs are not well known. Possible impact from OWFs could include both increased aggregation of finfish around man-made structures due to the fish aggregating device effect (FAD), artificial reef effects or closure to fishing, or other factors such as changed primary production caused by increased upper ocean mixing or turbine noise (Dorrell et al., 2022; Floeter et al., 2017; Slavik et al., 2019). The relative importance of these effects is not well-established but are expected to vary from case to case based on local conditions and type and number of OWF structures. This is especially true for the emerging technology using floating turbines such as those of Hywind Tampen, where very few studies are available (Farr et al., 2021).

Fishing activities are restricted in most OWFs, and even more so for floating OWFs (FOWFs). This has been shown to have a positive impact on abundance and diversity of the demersal fish assemblage (Bergström et al., 2013). The main drivers for the positive effects are the increase in habitat heterogeneity, the artificial reef effect, and the removal of the bottom trawling from the area (Bergström et al., 2014; Stenberg et al., 2015). Based on this, OWFs have been suggested to act as marine protected areas in coastal zone management practices (Hammar et al., 2015; Inger et al., 2009). The pelagic fish community is normally assessed using pelagic trawl in combination with sonar. Because of the restrictions on use of fishing gear inside FOWFs, monitoring is challenging and there are thus less studies available that have successfully measured any impact (Methratta & Dardick, 2019).

Environmental DNA (eDNA) comprises molecular methods that use DNA from environmental samples to detect and characterize local communities and keystone species as an alternative to more invasive methods such as fish capture. Such eDNA methods are still in development, necessitating further validation to assess the applicability in marine monitoring. Recent research compared eDNA based data with trawl and sonar data showing a strong correlation and concluded that eDNA based methods are a good proxy for fish assessments (Shelton et al., 2022; Stoeckle et al., 2021), however methods calibration through ground truthing is still in an early phase in particular when applied to open ocean ecosystems (Kirtane et al., 2021).

In 2021, NORCE conducted a marine water eDNA survey at the Hywind Scotland Pilot Park off Peterhead, UK (Ray et al., 2022) using metabarcoding to detect local fish species and ddPCR assays to quantify DNA concentrations of Atlantic mackerel and Atlantic herring. Results from this study were promising: metabarcoding eDNA data was able to provide station-level resolution of 26 different finfish species as well as data on pelagic community structure (Dahlgren et al., 2023; Hestetun et al., 2023), and the ddPCR assays were able to provide Atlantic mackerel and herring gene copy abundance estimates. While this showed the potential of eDNA as a monitoring parameter, lack of time series data and ground-truthing from fisheries surveys means that the eDNA approach needs further validation and development into best practices.

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 (environmental monitoring region IV) along a NW-SE bottom slope gradient towards the Norwegian trench (Fig. 1). The purpose of Hywind Tampen is to supply nearby oil and gas installations – Snorre B and Gullfaks A – with electrical power in lieu of currently used gas generators, estimated to reduce CO_2 emissions by some 200.000 tons per year as

well as reducing NO_X emissions from these installations. As of May 2023, eight of the planned eleven turbines have been put in place, with the final three turbines currently being installed. Equinor is interested in further validating eDNA as a parameter for mapping fish biodiversity. For this reason, Equinor requested a follow-up eDNA study at the Hywind Tampen floating OWF site building on the results from the Hywind Scotland study (Ray et al., 2022). Notably, the Hywind Tampen FOWF is also subject to a separate baseline study by Equinor and the Institute of Marine Research (IMR) to determine impact from the Hywind FOWF, including the capture surveys by IMR used to ground truth the MiFish data here (de Jong et al., 2022; Palm et al., 2023). A separate eDNA study connected to the IMR-led WindSYS project, conducted by the Norwegian Institute for Nature Research (NINA), is expected within 2024-2025.

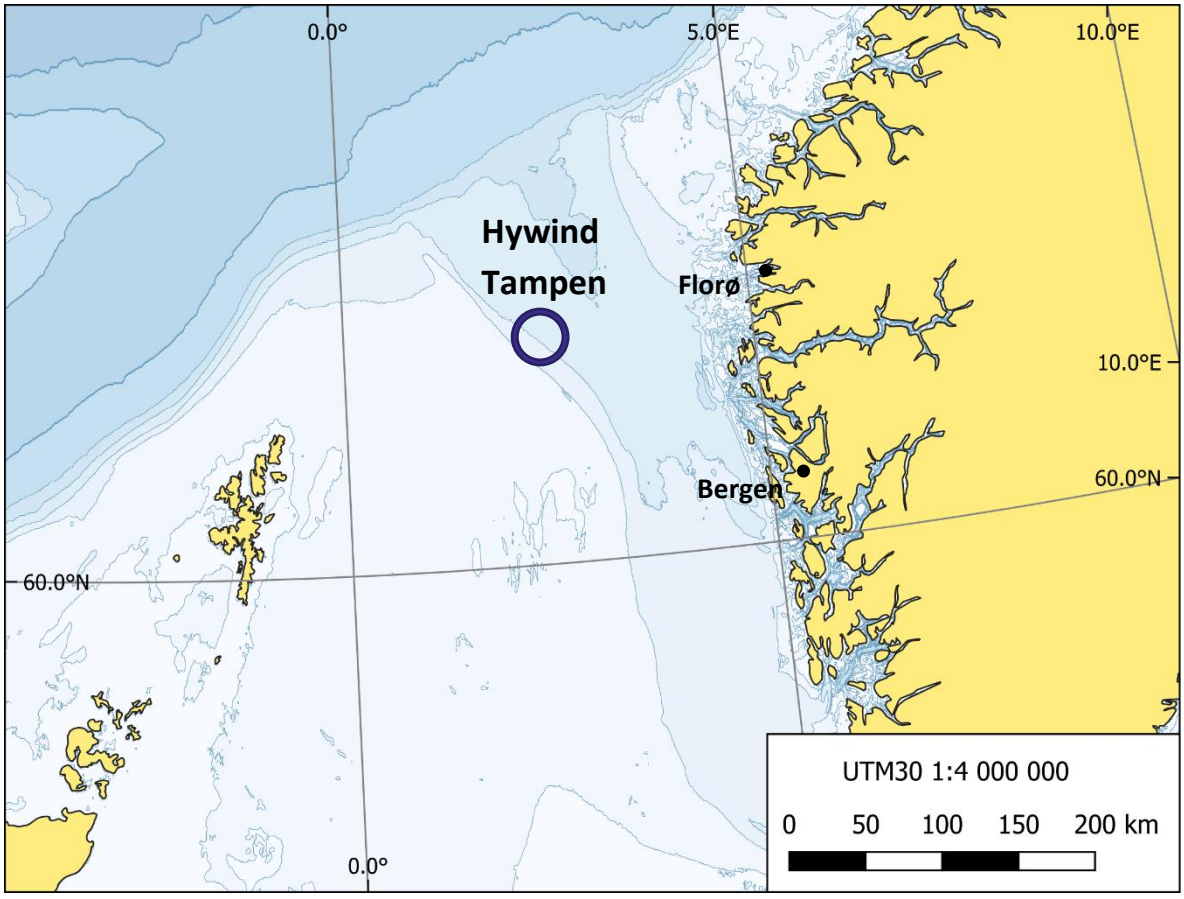

Figure 1. Map showing the general location of the Hywind Tampen FOWF.

This study's main aim is further validation of water sample eDNA as a monitoring tool in the context of OWF environmental management. Building on the recommended further directions for research in the Hywind Scotland work, this study aims to include investigations into the stability of the eDNA signal in the light of the prevailing current direction at Hywind Tampen by revisiting designated sampling stations three times over the course of a week. A recent Equinor/IMR catch study (de Jong et al., 2022) also allows the opportunity to do partial ground truthing about the number of demersal finfish detectable using eDNA. The study aims can thus be defined through three research questions:

- RQ1: Is there any persistent significant difference (i.e. over the three time points) in a) pelagic fish, b) demersal fish and c) plankton composition between the Hywind FOWF area and outside the FOWF?
- RQ2: Using two pelagic fish species as a ddPCR demonstration, can the influence of currents be seen in the abundances estimates from stations upstream, in, and downstream of the WF?
- RQ3: What is the overlap in species composition between species detected by de Jong et al. in the 2022 demersal gillnet/seine survey and species detected in the current eDNA study?

2. Materials and methods

2.1. Study area

The Hywind Tampen floating offshore wind farm (FOWF) is located in the Tampen area in the Northwestern part of the North Sea, around 140 km west of the town of Florø (Fig. 1). The first turbines of the Hywind Tampen FOWF were installed in the spring of 2022, with eight of the eleven planned turbines operational at the time of the sampling for this study. The total height of each 8 MW turbine is 190 m, with a rotor diameter of 167 m. The floating fundaments extend down to around 90 m depth. Each turbine is fastened to the seafloor with three 800 m anchor lines. The FOWF is situated over a sloping seafloor in a NW-SE direction, and water depths in the area are around 260-300 m. The seafloor in the area is composed of sandy soft bottom with scattered rocks. No coral or sponge occurrences are known from the area, but the area is used by bentho-pelagic or semi-pelagic spawning species such as gadoids including cod, saithe, haddock, and Norway pout (Equinor, 2019).

2.2. Field sampling

The sampling design of the Hywind Tampen study included seven stations upstream, in and downstream of the FOWF, and three reference stations further south-east of the FOWF. While the reference stations were only sampled once (T0), the remaining stations were sampled three times, at T0, 24 hours later (T1), and one week later (T2) (Fig. 2; Table 1).

The samples for the Hywind Tampen study were collected as part of the environmental monitoring cruise to the oil and gas installations in the area on the 2nd – 16th of May 2023 onboard the Atlantic Offshore vessel *Ocean Response* chartered by Equinor on behalf of relevant offshore operators. Akvaplan-niva, contracted to perform the standard environmental monitoring sampling program, were also available to assist with water sampling for the Hywind Tampen eDNA study. Cruise mobilization took place at the Mongstad Coast Center Base (Mongstad CCB), north of Bergen starting 08:00 on May 2nd. An unfurnished 20-foot container was chartered from Mongstad CCB and furnished with equipment sent from NORCE the week before into a makeshift lab onboard.

Equipment and working surfaces were decontaminated with 5% (v/v) sodium hypochlorite, sodium hydroxide solution (household bleach) prior to commencement of work and between sampling stations to reduce ambient and carryover eDNA contamination. Water was collected using a weighted 7.5L-Niskin bottle on a center hanger deployed individually on a winch and closed at desired sampling depth using metal messengers deployed from deck. Two Niskin bottles were alternated to minimize time between deployments. The contents of each Niskin bottle were dispensed into a cleaned 10 L plastic jerry can that had been rinsed with sample water prior to filling. Triplicate 2 L water subsamples were filtered in parallel through 0.45 µm Sterivex PES filters using one of two Masterflex peristaltic pumps with a multi-channel pump head and pumping speed of 400 rpm. Subsamples from 20 m depth and bottom water (5 m off the seafloor) were filtered simultaneously using a two-pump set-up to maximize throughput at each sampling station (Fig. 3).

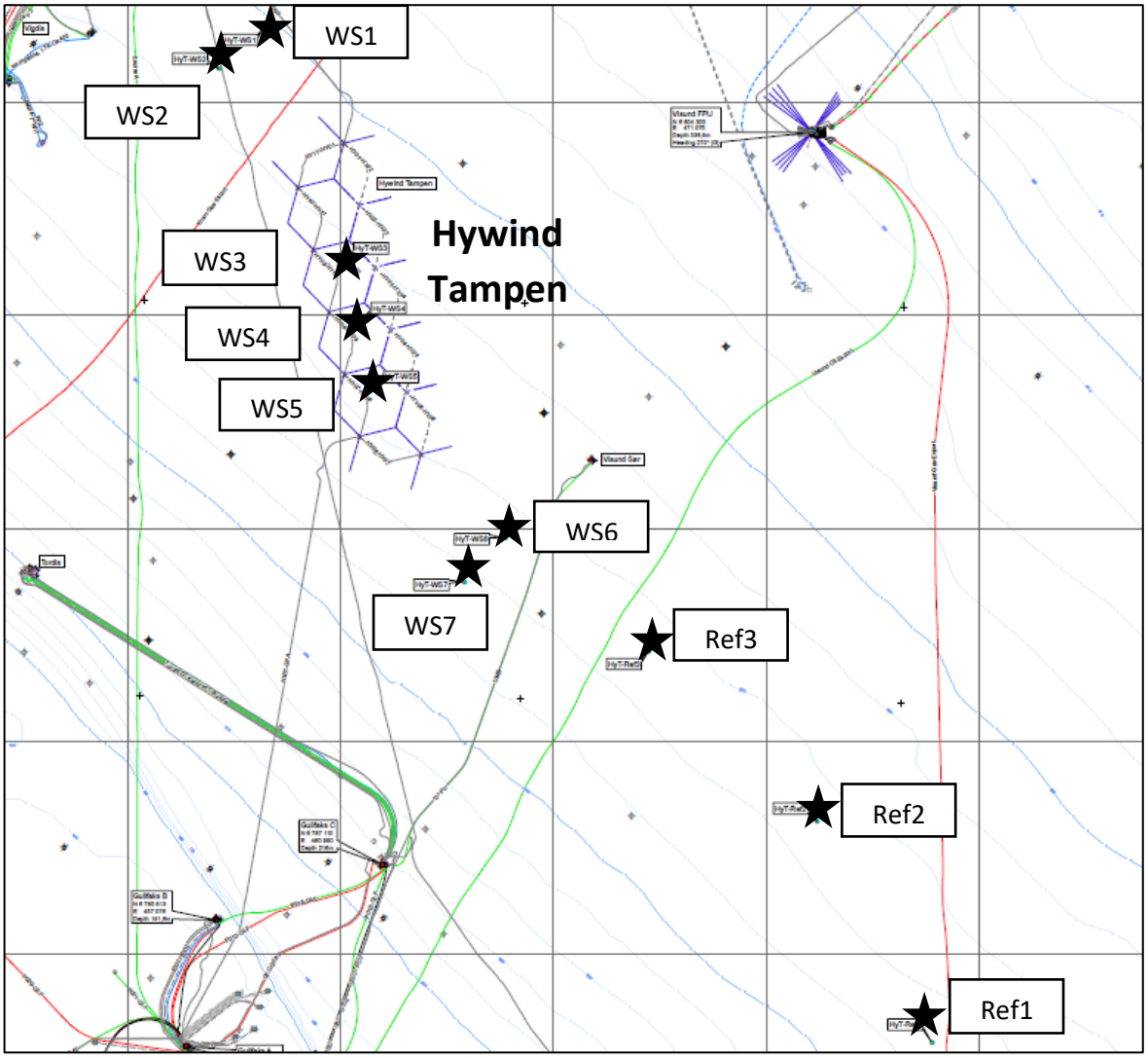

Figure 2. Placement of Hywind Tampen water sampling stations. Stations are divided into upstream (WS1-2), OWF (WS3-5), downstream (WS6-7) and reference stations (Ref1-3) along the prevailing slope into the Norwegian trench in this area and following the dominating current direction.

Pump tubing was decontaminated between water samples by filling with 5% (v/v) bleach solution, emptying, flushing with 200 mL distilled water, emptying, and then flushing with approx. 100 mL of the next water sample prior to filter attachment and sample filtration. Filtering speed for all samples was approximately 125 mL min^{-1}. Outflow volume from each filter was recorded. Excess water was expelled from filters using a 60 mL syringe. Air and water control ("blank") samples were collected at each station to control for ambient and carry-over contamination, respectively. 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 the last of the distilled water when cleaning the tubing through a 0.45 µm Sterivex PES filter. Finally, filters were capped, placed individually inside sterile 50 mL polypropylene tubes, and immediately frozen at -20°C on board.

To identify stratification depths and differences in oceanographic properties between the sampling stations and time points, CTD profiles were taken at each station for each time point. Two SAIV SD204

CTD instruments were used to gather CTD data, one instrument brought by NORCE, and a separate instrument from Akvaplan-niva. To maximize the information from CTD deployment, for the first (T0) and third (T2) round of samples (as well as reference stations Ref2-3), the Akvaplan-niva CTD was used as this had a mounted fluorescence sensor. For the second round (and Ref1), the NORCE CTD was used as this had a mounted turbidity sensor.

Table 1. Positions of the Hywind Tampen stations sampled in this study (UTM30).

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

Figure 3. Simultaneous filtration of collected water samples inside the on-deck container using peristaltic pumps powered from electricity to the container. Triplicate 2L-water samples (brown bottles) from each sampling depth were filtered through 0.45 µm Sterivex filters using four-channel pump heads and subsequently frozen. Filter outflow was collected in 2L-beakers, and outflow volume was measured and recorded.

2.3. Lab processing

Lysis of filtered particles was conducted inside Sterivex filters to minimize contamination and maximize lysis efficiency. Frozen filters were put in ATL buffer, and 60 microliters of 20 mg mL^{-1} Proteinase K (QIAGEN) were added to each thawed filter. Filters were tightly capped and incubated at 56°C with

gentle rotation overnight. Lysate was aspirated from Sterivex filters using sterile 5 mL syringes and lysate volume was recorded. One milliliter 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 one modification: 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).

Table 2. Primers and probes used in the study.

Oligo name	5'-3' DNA sequence	Final conc.	Function	Reference
ddPCR *Scomber scombrus* (Atlantic mackerel)				
Scosco_CYBF14517	TTCCCTGCTTGGTCTCTGTT	400 nM	forward	(Knudsen et al., 2019)
Scosco_CYBR14597	GGCGACTGAGTTGAATGCTG	800 nM	reverse	
Scosco_CYBP14541*	TTCCCAAATCCTCACAGGACTATTC	200 nM	probe	
ddPCR *Clupea harengus* (Atlantic herring)				
Cluhar_CYBF14928	CCCATTTGTGATTGCAGGGG	200 nM	forward	(Knudsen et al., 2019)
Cluhar_CYBR15013	CTGAGTTAAGTCCTGCCGGG	1000 nM	reverse	
Cluhar_CYBP14949*	TACTATTCTCCACCTTCTGTTCCTC	300 nM	probe	
Metabarcoding 18S (V1-V2) ribosomal RNA gene				
SSU_F04mod	GCTTGWCTCAAAGATTAAGCC	240 nM	forward	(Sinniger et al., 2016)
SSU_R22	CCTGCTGCCTTCCTTRGA	240 nM	reverse	
Metabarcoding MiFish				
MiFish-U-F	GTCGGTAAAACTCGTGCCAGC	300 nM	forward	(Miya et al., 2015)
MiFish-U-R	CATAGTGGGGTATCTAATCCCAGTTTG	300 nM	reverse	
*** ddPCR probes were modified at the 5'-end with the 6-FAM fluorophore for *S. scombrus* and with HEX fluorophore for *C. harengus*, both probes at the 3'-end with the BHQ1 fluorescence quencher**				

2.4. Droplet digital PCR analysis

Quantitative molecular detection was conducted using a DX200 droplet digital PCR (ddPCR) system (Bio-Rad) with published assays targeting the mitochondrial cytochrome B gene (*cytB*) of either Atlantic mackerel (*Scomber scombrus*) or Atlantic herring (*Clupea harengus*) (Knudsen et al. 2019) (Table 2). ddPCR master mixes were prepared in a template-free pre-PCR laboratory room inside a class II biosafety cabinet with laminar air flow using UV-treated plastics. Template DNA was added to pre-prepared ddPCR master mixes while working inside a second, class II biosafety cabinet inside a separate lab purposed for DNA/RNA work. Both labs have positive pressure HEPA-filtered ventilation to reduce exterior airborne contamination.

The assays for Atlantic mackerel and Atlantic herring were multiplexed into one PCR reaction using probes with different reporter dyes attached. For each sample, triplicate 20 µL ddPCR assays consisted of primers and probe (Table 2), 1X ddPCR Supermix for probes (Bio-Rad) and 5 µL undiluted template. The PCR amplification program for the multiplexed reaction consisted of an initial denaturation at 95°C for 10 min, followed by 45 cycles of 94°C for 30 sec and 57 °C for 60 sec, and a final denaturation at 98°C for 10 min. Ultrapure water was added instead of template DNA for ddPCR negative (no template) controls. PCR reactions were emulsified using a droplet generator (Bio-Rad) according to manufacturer

instructions. After a brief equilibration to room temperature, droplet fluorescence was read using a droplet reader (Bio-Rad) with default settings for FAM and HEX detection. Determination of positive and negative droplets was determined using NTCs and positive controls of Atlantic herring and mackerel DNA. Absolute target gene copies per microliter in ddPCR reactions were normalized to copies L^{-1} seawater.

Statistical analysis and visualization of ddPCR results were conducted in the R statistical computing environment (R Core Team, 2020). Data visualization was done using the base (R Core Team, 2020) and ggplot2 (Wickham, 2016) packages. Single-factor (area, region or time) explanatory power on ddPCR results (copies L^{-1}) was tested using stats::kruskal.test() with default parameters, followed by a post-hoc Dunn's test (O. J. Dunn, 1964) of multiple comparisons using a Bonferroni adjusted error rates using stats::dunn.test().

2.5. Metabarcoding

As in the previous Hywind Scotland study (Ray et al., 2022), PCR amplification was done using two metabarcoding markers: The MiFish universal fish 12S rRNA gene primer pair MiFish-U-F and MiFish-U-R (Miya et al., 2015), specifically to capture fish communities in the area, and 18S V1-V2 universal eukaryote sequences using primers SSU_F04mod (Cordier pers. comm.) and SSU_R22 (Sinniger et al., 2016), to capture a broad range of eukaryote single-celled and animal diversity (Table 2). PCR amplification was done with adapter-linked primers using the KAPA3G Plant PCR kit (KAPA Biosystems) at annealing temperatures 65 °C and 57 °C for MiFish and 18S primers respectively. 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 contamination due to sample processing. Sequencing was performed on an Illumina MiSeq instrument using v3 with 300 bp chemistry at the Norwegian Sequencing Centre (University of Oslo, Norway).

Initial quality check of sequence fastq files was done using FastQC v0.11.8 (Andrews, 2010). Cutadapt v1.18 (Martin, 2011) was used to trim primer sequences from fastq files, and dada2 (Callahan et al., 2016) was used to filter, denoise, merge forward and reverse sequence reads and create ASV tables and fasta files for each dataset. ASV tables were curated using a custom script similar to the UNCROSS algorithm (Edgar, 2016), and decontam (Davis et al., 2018) to identify any probable contaminant sequences, which were subsequently removed from the entire datasets. Taxonomy was assigned using CREST4 with the Silvamod 1.48 database for the 18S dataset, and blastn with a 95% threshold against the MitoFish v3.96 database (Iwasaki et al., 2013) for the MiFish 12S dataset.

Multivariate analysis, including Hellinger transformation, Bray-Curtis dissimilarity, non-metric multidimensional scaling (NMDS) and cluster plots, PERMANOVA and SIMPER analyses were done using the R vegan package v 2.5-7 (Oksanen et al., 2019). Data visualization was done using the ggplot2 package (Wickham, 2016).

3. Results

3.1. CTD measurements

CTD measurements were done for each sampling event to record oceanographic conditions to investigate water mass properties. Measurements included temperature, salinity, oxygen concentration, fluorescence (T0, T2) and turbidity (T1). CTD figures for temperature are shown in Figure 4; other parameters are found in Appendix A. CTD measurements were generally similar across stations in the area. The CTD parameters showed a split between surface water masses (lower temperatures and salinity, higher oxygen concentrations and fluorescence) and deeper water at around 20-40 m depth. These differences were less pronounced at T2 compared to T0 and T1, except for fluorescence, which showed increased surface primary production at the later T2 timepoint. Temperatures also decreased at greater (>150 m) depth.

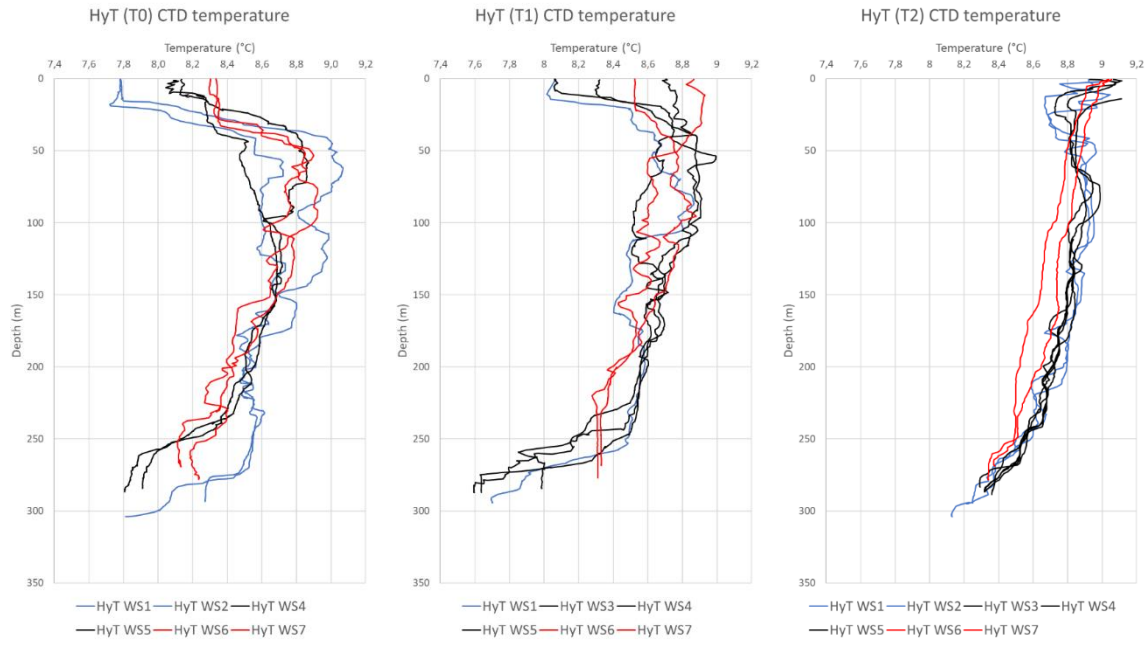

Figure 4. Temperature CTD profiles of stations WS1-7 at time T0-T3 showing a thermocline at 20-40 m depth and gradually reduced temperature below 150 m. Differences are less pronounced at the last sampling time (T2) one week after initial sampling.

3.2. MiFish metabarcoding results

The total number of raw sequences from the MiFish dataset was 40 595 936 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, extraction and PCR controls. Bioinformatic filtering, denoising, merging and chimera detection, reduced this to 32 424 173 sequences; after uncross and decontam additional filtering, 32 105 905 sequences remained distributed over 985 ASVs. Taxonomic assignment using the MitoFish v396 database yielded 36 fish species (Appendix B-C).

The most abundant species in the entire MiFish dataset was Atlantic herring (*Clupea harengus*), followed by blue whiting (*Micromesistius poutassou*), Atlantic mackerel (*Scombrus scombrus*), saithe (*Pollachus virens*), and silvery cod (*Gadiculus thori*) (Fig. 5; Table 3).

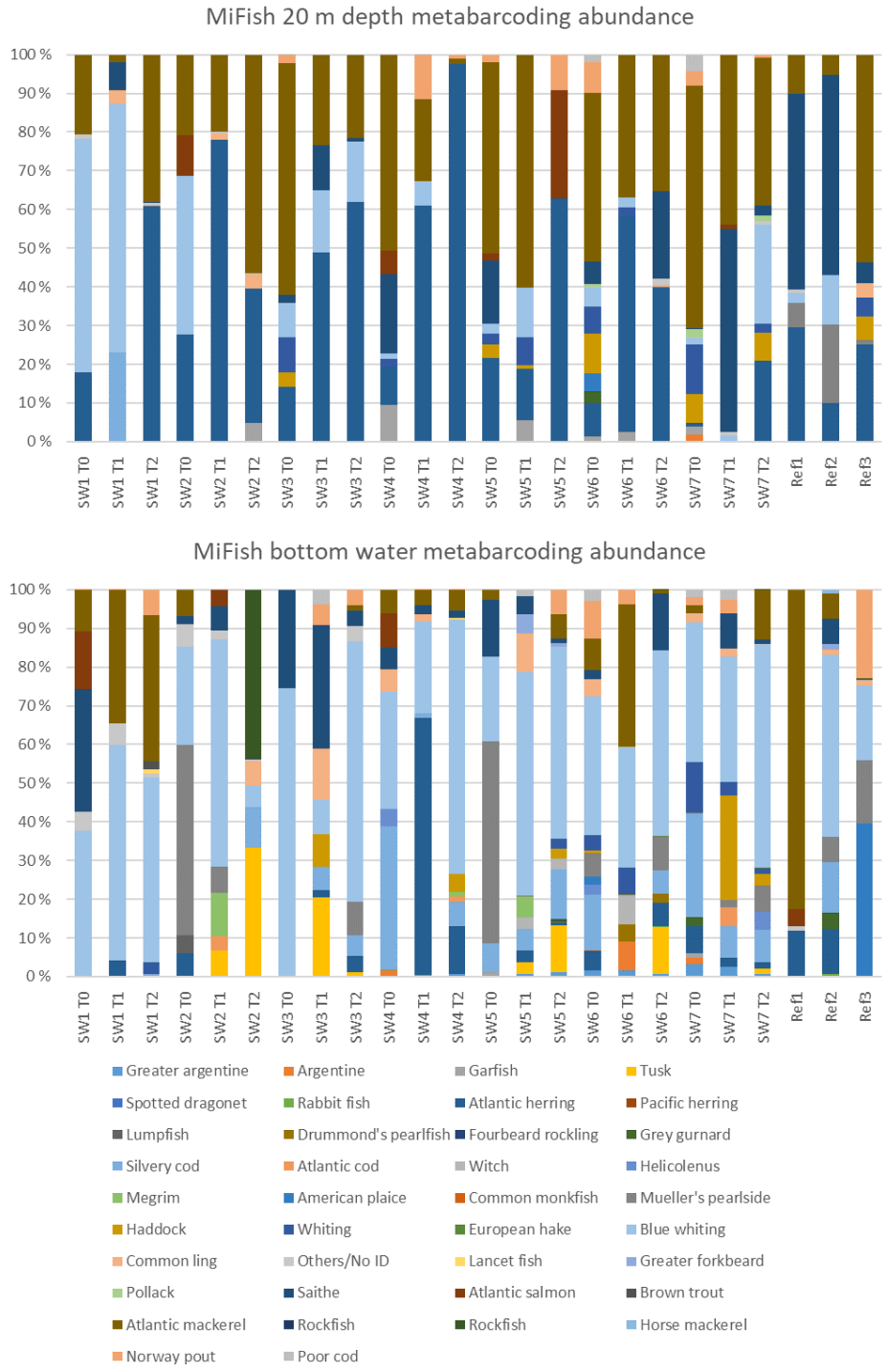

Figure 5. Relative abundance of all identified species in the MiFish dataset at sample level and sorted by depth.

Table 3. Absolute number of sequence reads for the 20 fish species with the highest number of sequences in the MiFish dataset, as identified by the MitoFish 3.96 database.

Name	Total	20 m	Bottom	Name	Total	20 m	Bottom
Atlantic herring	7934799	7069311	865488	**Tusk**	441477	0	441477
Blue whiting	7655464	2420587	5234877	**Atlantic salmon**	410842	256489	154353
Atlantic mackerel	6938791	5598278	1340513	**American plaice**	295610	43063	252547
Saithe	2334577	1425755	908822	**Garfish**	189901	175708	14193
Silvery cod	1199149	159816	1039333	**Rockfish**	163370	0	424
Mueller's pearlside	1140728	163621	977107	**Poor cod**	149378	55110	94268
Norway pout	603894	229592	374302	**Megrim**	113579	0	113579
Whiting	560804	370073	190731	**Witch**	89707	0	89707
Haddock	521203	293652	227551	**Greater argentine**	78466	0	78466
Common ling	442580	79514	363066	**Argentine**	76509	15839	60670

Identified species included both demersal species, with greater diversity and numbers in the bottom water samples, and pelagic schooling species at large abundances. The mesopelagic schooling pearlside was also abundant, especially in bottom water samples (Fig. 5; Table 3).

Two incongruities were noted in the taxonomic identification of sequences from the MiFish data: At one deep-water station (SW3 T0), an ASV was identified as the freshwater species gudgeon (*Gobio gobio*). Given the improbability of this species being present at this location (or DNA transported through natural means), this result was discarded and merged with other sequences where no ID could be made. One ASV had highest affinity to Pacific, rather than Atlantic herring. This is likely intraspecific variation within Atlantic herring not represented in the Mitofish database, but the result is retained here for reference for future studies.

Pairwise similarities at species level between samples were calculated using the Bray-Curtis index with Hellinger-transformed data. The resultant similarities have been visualized using NMDS plots to show clustering of samples based on depth (Fig. 6) or timepoint, separately for 20 m and bottom water samples (Fig. 7-8).

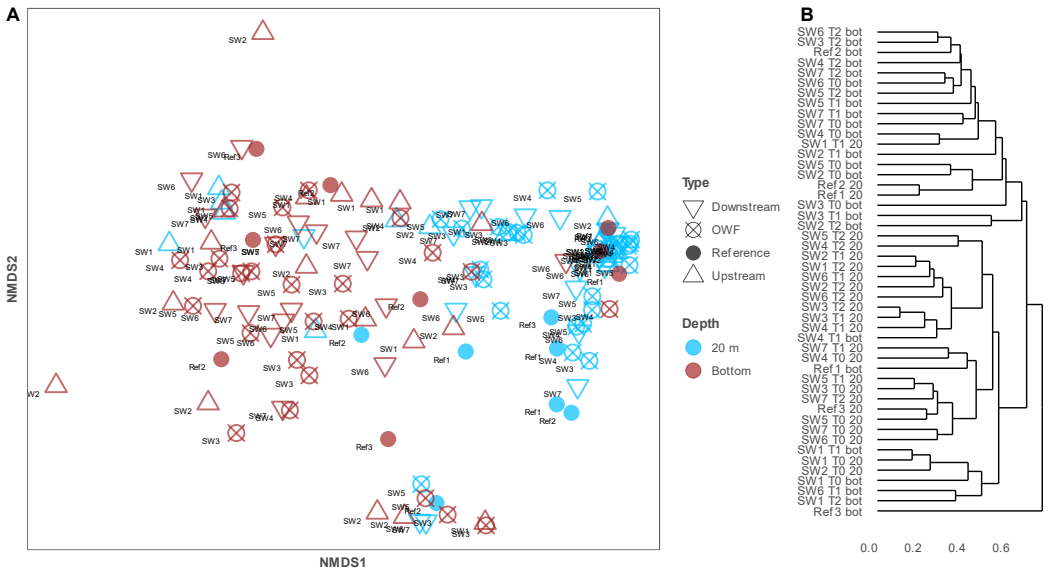

Figure 6. MiFish marker (A) NMDS analysis of both 20 m (light blue) and bottom water stations (brown) at sample level (three samples per station and depth), and (B) cluster analysis at station level, showing relative similarities in fish community composition.

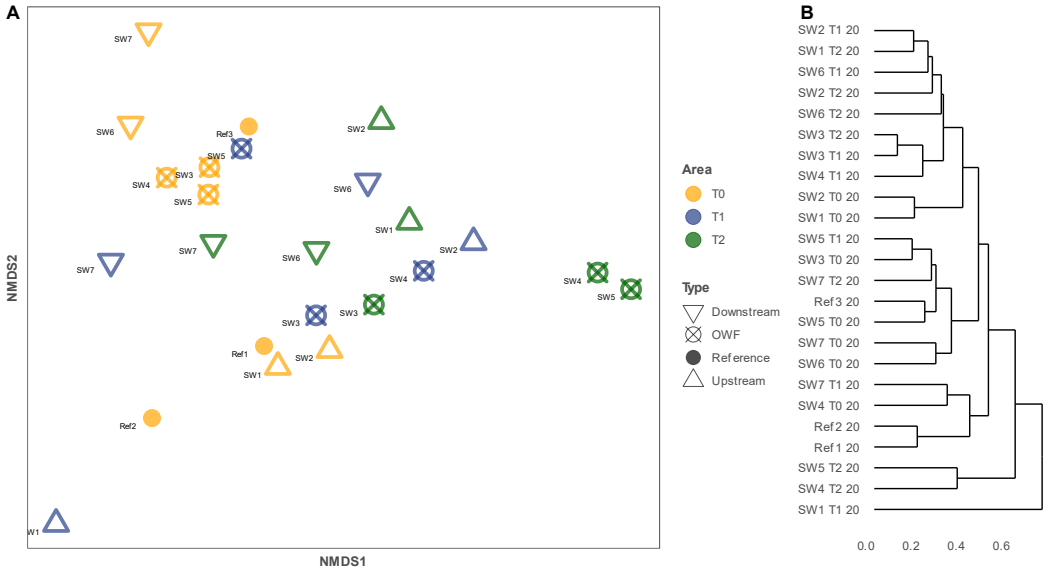

Figure 7. MiFish marker (A) NMDS and (B) cluster analysis of 20 m depth water stations at station level showing relative similarities in fish community composition across stations and time points.

Figure 8. MiFish marker (A) NMDS and (B) cluster analysis of bottom water stations at station level showing relative similarities in fish community composition across stations and time points.

The NMDS analysis in Figure 6 revealed evident clustering based on depth, showing that recovered fish species were different at surface vs bottom samples. For NMDS analyses showing location type (upstream, OWF, downstream, reference) or timepoint (T0-T2), 20 m T0 upstream stations were close to T1 OWF stations, but no clear pattern of any eDNA current drift was seen (Fig. 7). No evident pattern could be found in the bottom fish community to support impact due to either FOWF or eDNA current transport (Fig. 8).

PERMANOVA analysis of the entire MiFish dataset showed significant differences for depth (F = 32.748; p = 0.001), and weaker but still significant differences based on location (F = 3.510; p = 0.001). Only a barely significant result was found based on time point, indicating stable conditions over time (F = 1.939; p = 0.036). For 20 m samples specifically, there was a weak but significant difference based on both location (F = 4.065; p = 0.001) and time (F = 3.422; p = 0.002). For bottom samples, differences were weaker than at 20 m for both location (F = 2.624; p = 0.001) and time (F = 1.559; p = 0.05). SIMPER analysis of depth differences showed that blue whiting explained 17% of the observed differences, followed by Atlantic herring at 16%, Atlantic mackerel at 15%, saithe at 8%, silvery cod at 6%, pearlside at 5% and all remaining species at close to 32% in total.

Biodiversity estimates were calculated using the Shannon index H_{ln} for both 20 m depth and bottom water samples. In general, diversity was slightly higher towards the south-east stations, but there were no obvious diversity trends in Shannon values across either time or the type of station (Table 4).

A previous fish capture survey in the area by the Institute of Marine Research (IMR), funded by Equinor, deployed demersal gillnets in a transect perpendicular to the slope of the seafloor in the area (de Jong et al., 2022). Additional studies were performed in 2023 (Palm et al., 2023). Comparing the catch and ROV data thus provided an opportunity to do general ground truthing of the MiFish eDNA data from this survey. The 36 fish species detected in this study were cross-checked against reported species from the catch studies mentioned above, which reported 31 species: 24 species overlapped in both studies. Of the seven reported species not detected in the MiFish eDNA dataset, three were skates and two

were sharks, indicating underrepresentation of elasmobranch taxa in the eDNA approach used here (the Holocephali rabbit fish *Chimaera monstrosa* was on the other hand reported in both datasets). Of the species detected only in the MiFish dataset were several small species, such as lancetfish, not typically caught with gillnets (Fig. 9; Appendix D).

Table 4. MiFish Shannon values (H_{ln}) calculated separately for timepoints and station type, as well as area and time averages.

20 m	T0	T1	T2	Area avg	Bottom	T0	T1	T2	Area avg
Upstream	1.64	1.16	1.23	1.34	**Upstream**	2.05	1.75	1.84	1.88
OWF	2.02	1.69	0.95	1.55	**OWF**	1.93	2.15	2.06	2.05
Downstream	2.39	1.32	1.97	1.90	**Downstream**	2.87	2.61	2.27	2.59
Ref	1.88				**Ref**	1.80			
Time avg	2.02	1.39	1.38		**Time avg**	2.29	2.17	2.06	

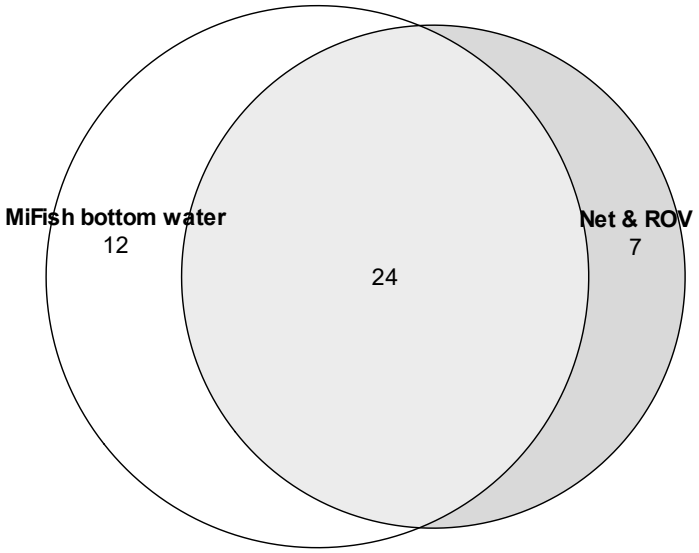

Figure 9. Euler diagram showing the overlap in species identified in the MiFish eDNA samples and species reported from either the 2022 Hywind Tampen area standing net capture study (de Jong et al., 2022) or the ROV survey from the same area the following year (Palm et al., 2023). Two instances of genus-level identification in the MiFish dataset, *Sebastes* sp. and *Helicolenus* sp., have been synonymized with the species *Sebastes norvegicus* and *Helicolenus dactylopterus* in the catch/ROV datasets for this comparison (see also Appendix D).

3.3. 18S rRNA metabarcoding results

For the 18S rRNA V1-V2 universal eukaryote dataset, the total number of raw sequences were 30 508 331 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, extraction and PCR controls. Bioinformatic filtering, denoising, merging and chimera detection reduced this to 23 017 157 sequences; after uncross and decontam additional filtering, 19 720 584 sequences remained distributed over 5539 ASVs. Removing ASVs with 10 reads or less from

the dataset reduced the number of ASVs to 3264. Taxonomic assignment of these ASVs after abundance filtering using CREST4 with the SilvaMod 1.48 database yielded 643 taxonomic groups at various level of resolution.

Kingdom level abundance showed a good spread of different eukaryote taxa. In the 20 m samples, Metazoa (multicellular animals) constituted the second most abundant kingdom, followed by Stramenopiles, then Haptophyta. In bottom water samples, Stramenopiles was the second most abundant kingdom, followed by choanoflagellates, then Metazoa (Fig. 10), meaning metazoan fraction of the total dataset was higher in the 20 m samples relative to bottom water.

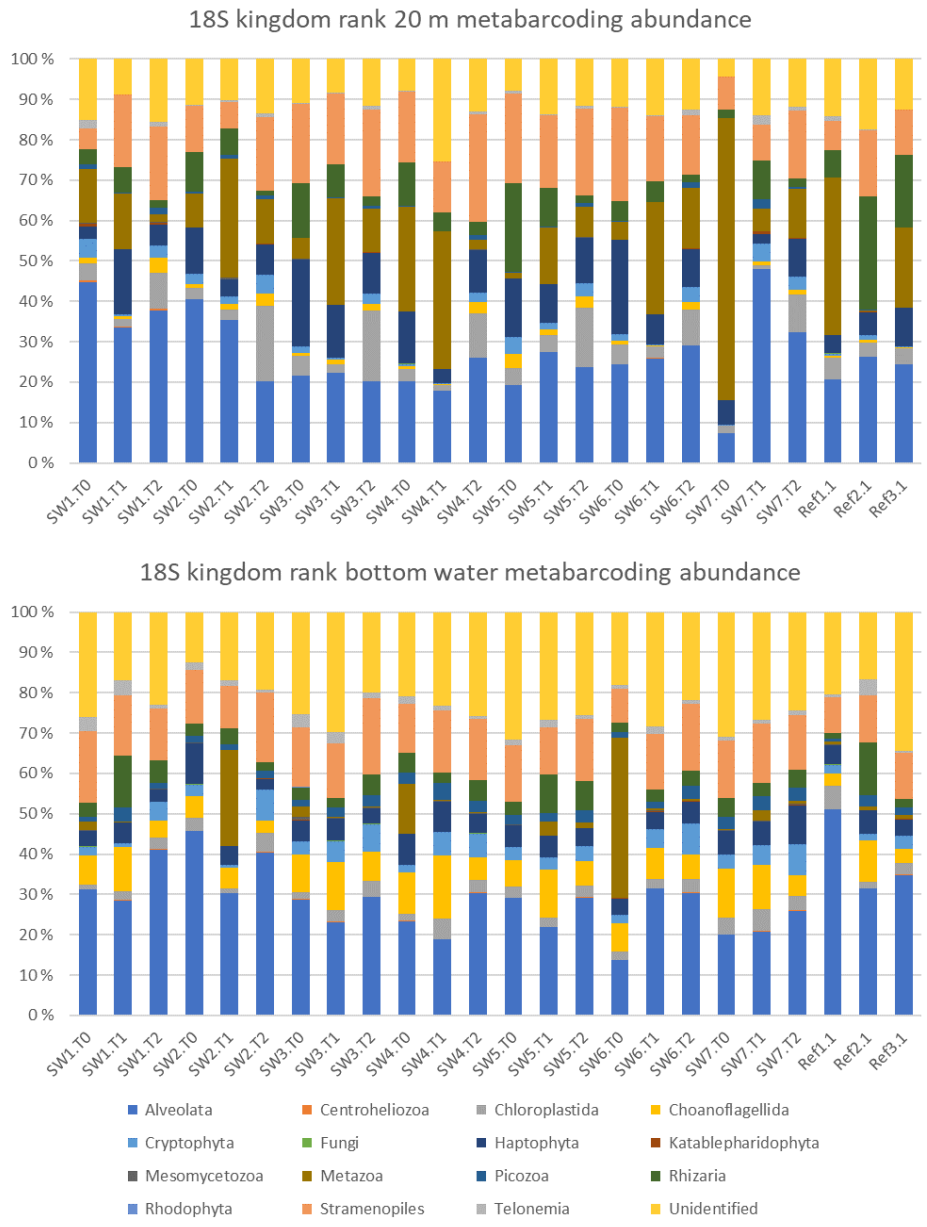

Figure 10. Relative abundance at kingdom level recovered in the 18S dataset from 20 m depth and bottom water samples.

Looking specifically at the metazoan kingdom (multicellular animals) in the 18S dataset at phylum rank, arthropods are clearly most abundant (>90%) owing largely to the number of calanoid sequences found. The second most abundant metazoan phylum was Chordata in both 20 m and bottom water, followed by annelids (bottom water) and echinoderms (Fig. 11).

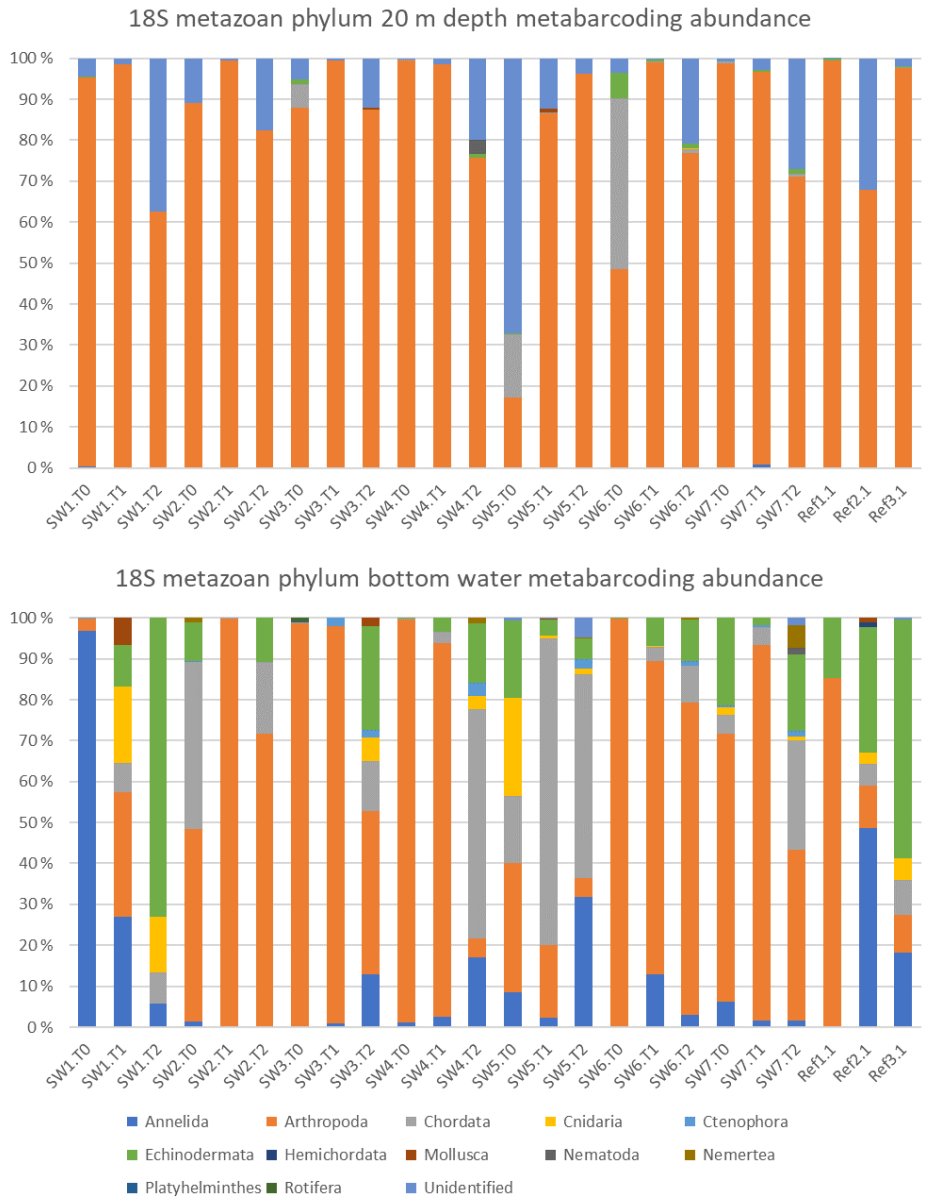

Figure 11. Metazoan relative abundance in the 18S rRNA dataset at phylum level.

Pairwise similarities between 18S V1-V2 amplicon ASV-level communities at the different samples were calculated using the Bray-Curtis index with Hellinger-transformed data. The resultant similarities have been visualized using NMDS (non-metric multidimensional scaling) plots to show clustering of samples based on depth or farm vs. reference area (Fig. 12).

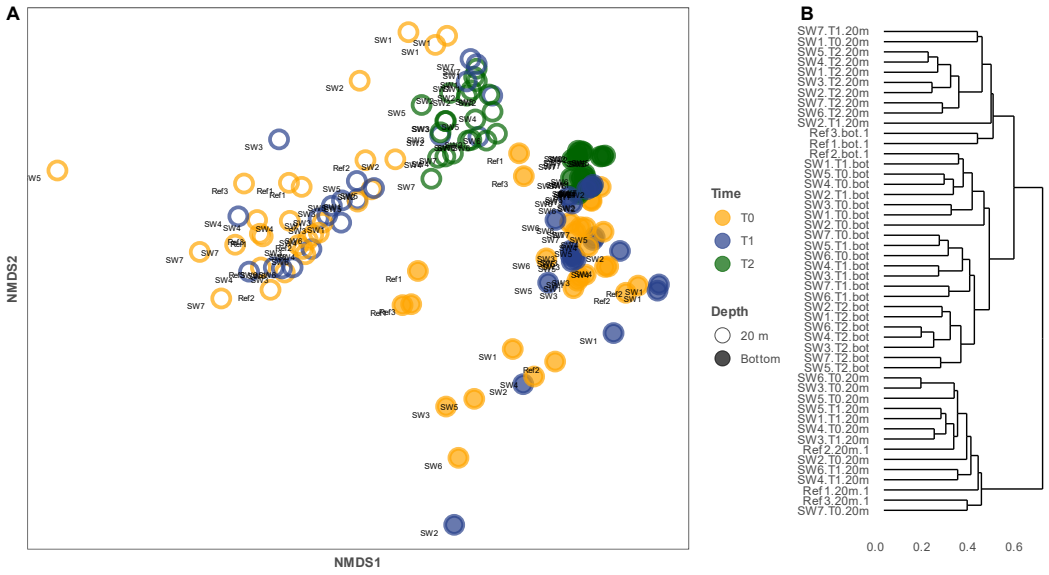

Figure 12. NMDS plot of 18S data Bray-Curtis pairwise similarity showing stations color coded by time, and with symbols indicating depth.

The NMDS analysis demonstrated clear separation in two main clusters based on depth. Within each cluster, T0 and T1 samples were more similar to each other, while T2 (one week later) were tightly clustered in each end of the main depth-based clusters, showing some change in general community composition with time.

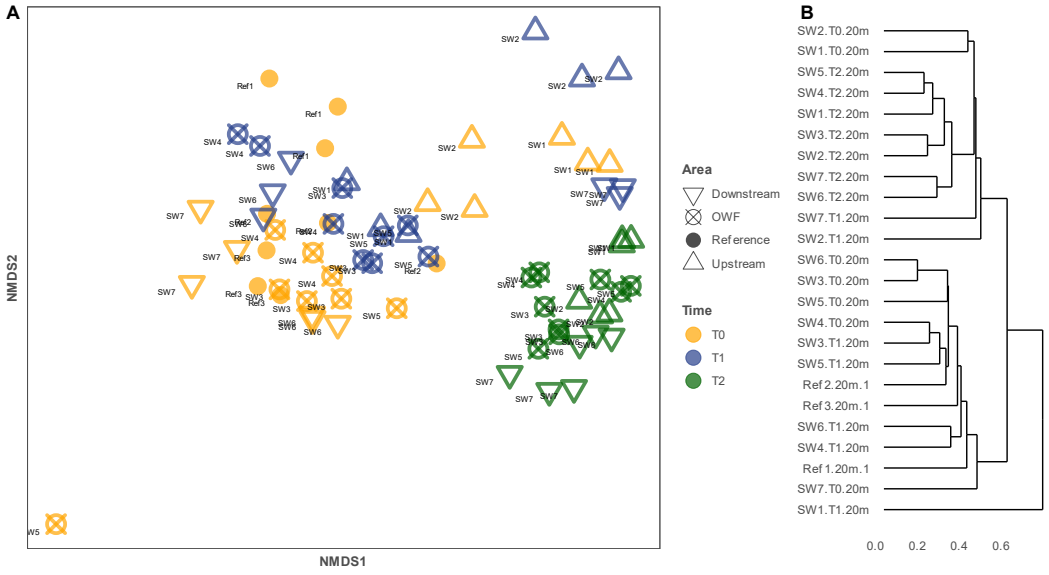

Figure 13. NMDS plot of 18S data Bray-Curtis pairwise similarity showing 20 m depth stations color coded by time, and with symbols indicating placement with regards to the Hywind FOWF.

Looking at 20 m 18S data only with samples coded by station type, there was no indication of current transport of extracellular DNA influencing the community composition between upstream and OWF

sites, rather upstream, OWF, and downstream sites clustered together across T0 and T1 while T2 samples were separate from both (Fig. 13). Time proved a stronger predictor of community composition (PERMANOVA F = 18.403; p = 0.001) than station category (PERMANOVA F = 8.073; p = 0.001). Similarly results were evident for the bottom water samples (Fig. 14).

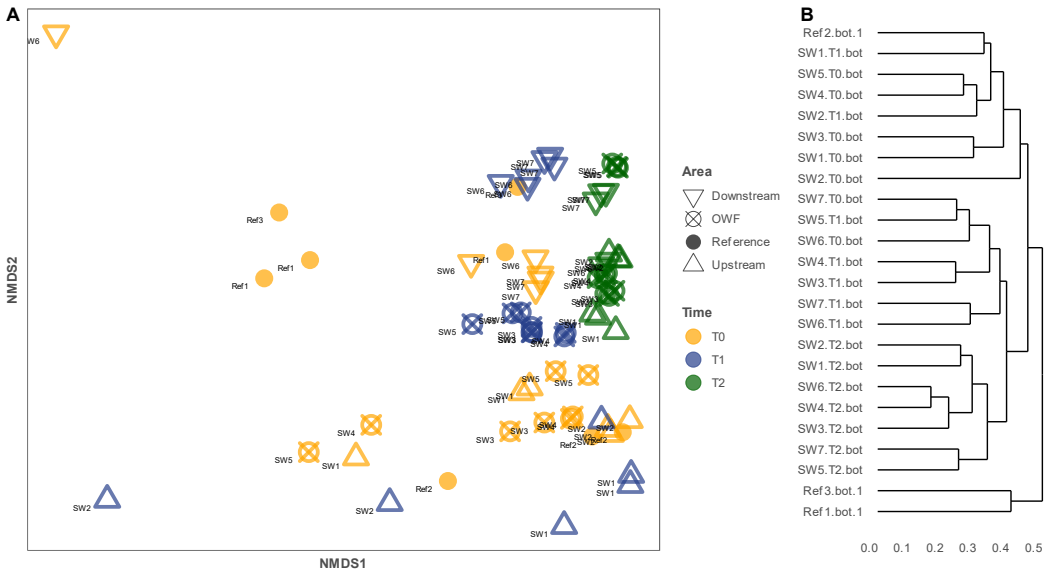

Figure 14. NMDS plot of 18S data Bray-Curtis pairwise similarity showing 20 m depth stations color coded by time, and with symbols indicating placement with regards to the Hywind FOWF.

3.4. Droplet digital PCR results

In total, we performed 271 multiplexed ddPCR reactions to quantify eDNA of Atlantic mackerel (*Scomber scombrus*) and Atlantic herring (*Clupea harengus*) at 20 m depth. Sampling controls for air blanks, water blanks and extraction controls (n = 55) all yielded 0 copies L^{-1} for both fish targets (data not shown). eDNA signal for mackerel ranged from 0 (non-detected; N = 56 samples) to 808.9 copies L^{-1}, which occurred at HyT-SW6, downstream of the OWF at T0 (Fig. 15A). For herring, ddPCR results ranged from 0 (non-detected; N = 63) to 1600 copies L^{-1} in sample HyT-SW4, in the OWF at T2 (Fig. 15B). Detection rates for filtered eDNA samples were 74% for mackerel (160 positive detections from 216 samples analyzed) and 71% (153 positive detections from 216 samples analyzed) for herring.

ddPCR quantification revealed that the mackerel eDNA signal varied between Hywind Tampen stations and with time sampled (Fig. 16). Mackerel eDNA concentration inside the wind park was not significantly different than in the reference area when all time points are considered (Kruskal-Wallis chi-squared = 3.01, df = 1, *p-value* = 0.08), but significantly higher if only T0 samples are considered (Kruskal-Wallis chi-squared = 14.92, df = 1, *p-value* = <0.01). For the Hywind Tampen stations where samples were collected at different time points, time was observed to have a significant effect on mackerel eDNA quantities (Kruskal-Wallis chi-squared = 33.87, df = 2, *p-value* = <0.01). For Atlantic herring, a similar variation with time was seen (Fig. 15) and significant differences between Hywind Tampen stations and reference stations were observed when all time points were considered (Kruskal-Wallis chi-squared = 7.78, df = 1, *p-value* = 0.01), however not if only T0 samples are used (Kruskal-Wallis chi-squared = 1.67, df = 1, *p-value* = 0.20).

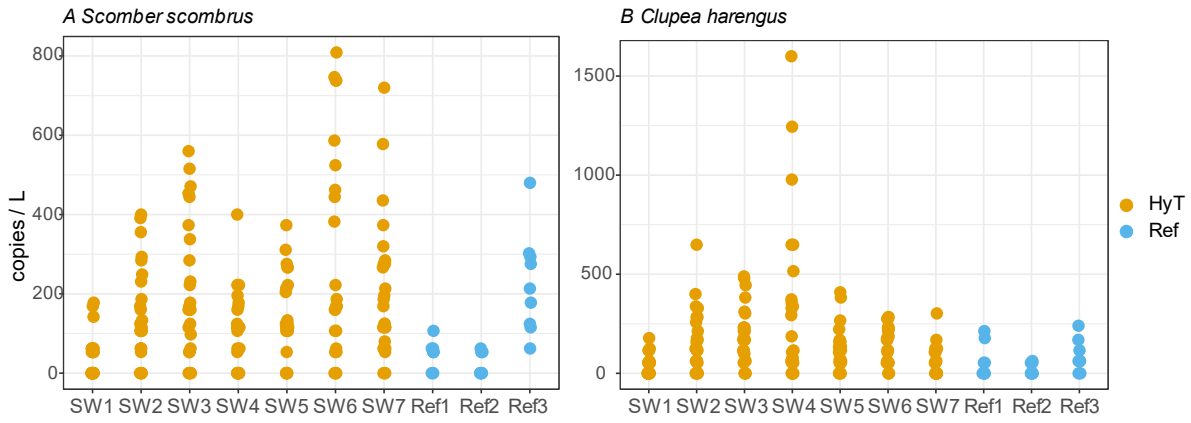

Figure 15. Scatterplots showing ddPCR quantification results for (A) Atlantic mackerel (*Scomber scombrus*) and (B) Atlantic herring (*Clupea harengus*) at the ten sampling stations investigated in this study. Station name/number are shown on the x-axis. ddPCR results (y-axis) are shown as target gene copies per liter of seawater.

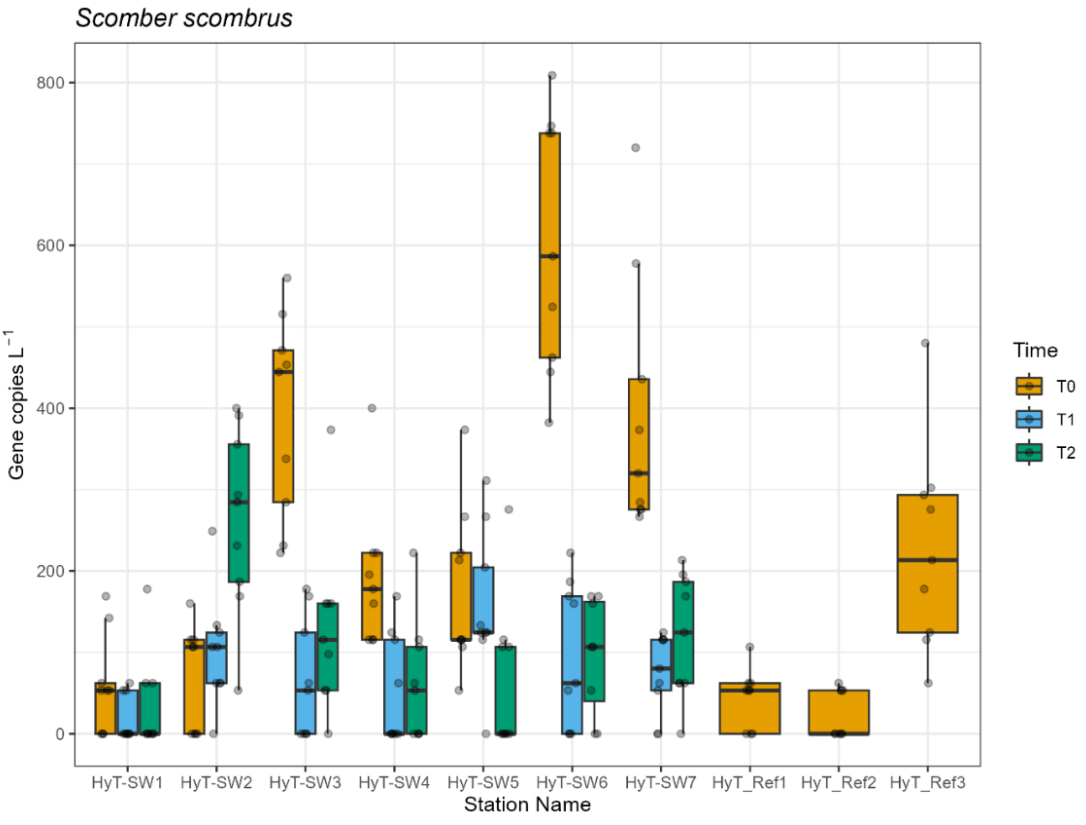

Figure 16. Box-and-whisker plots summarizing ddPCR results for Atlantic mackerel at 20 m depth between the wind park (HyT, yellow bars) and reference area (Ref), and different sampling times (T0, T1, T2).

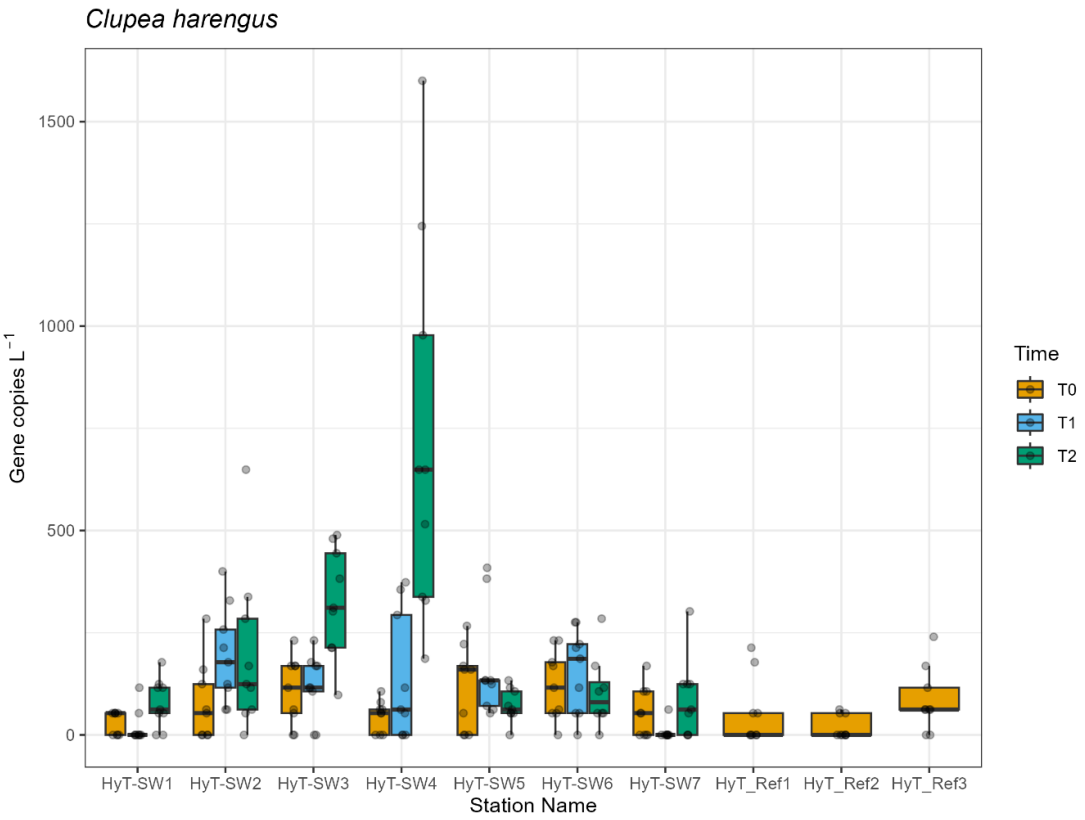

Figure 17. Box-and-whisker plots summarizing ddPCR results for Atlantic herring at 20 m depth in the wind park (HyT, yellow bars) and reference area (Ref), and different sampling times (T0, T1, T2).

To determine if water movement had an influence on eDNA detection between T0 and T1 (24 hours), we grouped sites into 3 regions, the FOWF and upstream and downstream (Fig. 18). Visually it is difficult to determine if there is a movement of eDNA signal from upstream into the FOWF, or from the FOWF to downstream within 24 hours. Between regions, there was a significant difference for mackerel eDNA (Kruskal-Wallis chi-squared = 25.92, df = 2, *p-value* = <0.01) between all sites (Dunn-test *p-value* = <0.01), however for herring this was not significant between regions (Kruskal-Wallis chi-squared = 4.69, df = 2, *p-value* = 0.10). When considering only T0 and T1, there was a significant difference between mackerel eDNA detection (Kruskal-Wallis chi-squared = 29.04, df = 1, *p-value* = <0.01) but not for herring eDNA (Kruskal-Wallis chi-squared = 1.99, df = 1, *p-value* = 0.16).

For both fish species, we detected a moderate but significant positive correlation between the number of gene copies in the ddPCR assays and the relative abundance of these species in the MiFish metabarcoding data (Fig. 19). The correlation was slightly stronger for herring (Pearson correlation = 0.52, *p value* = <0.01) than mackerel (Pearson correlation = 0.47, *p value* = <0.01).

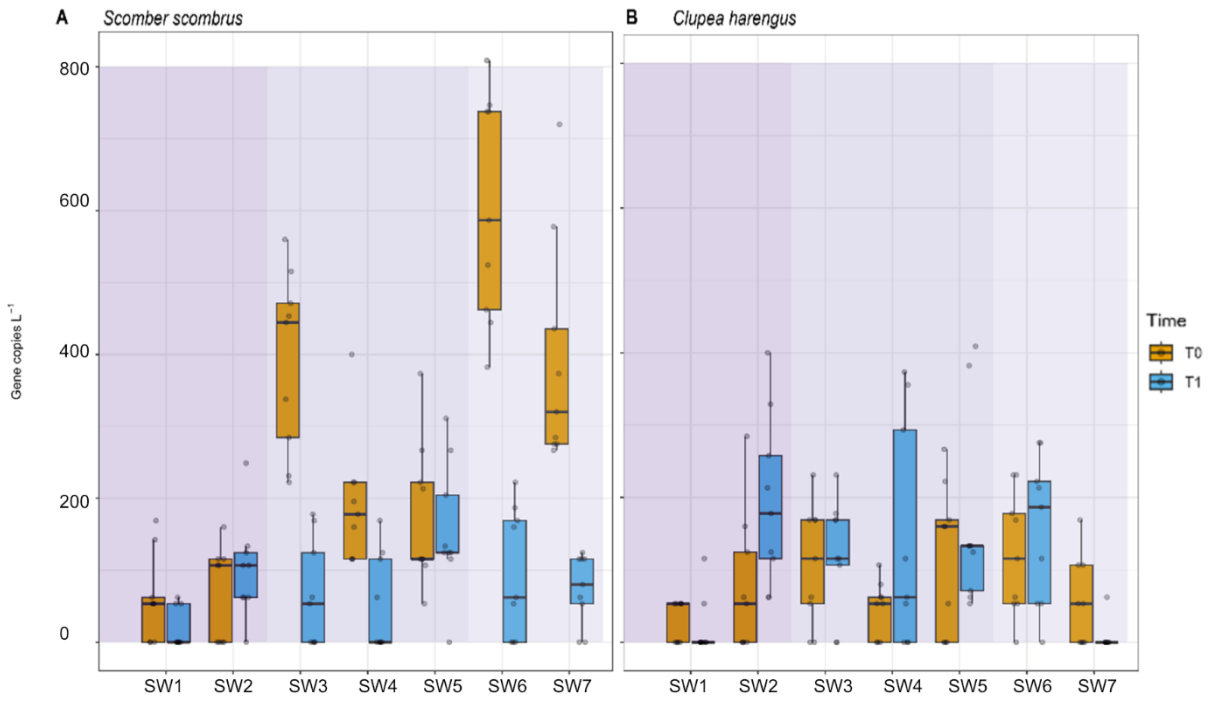

Figure 18. Box-and-whisker plots summarizing ddPCR results for Atlantic mackerel (A) and herring (B) at 20 m depth between the wind park stations at T0 and T1. Purple shading indicates theoretical water direction from dark (upstream) to light (downstream) purple (left to right).

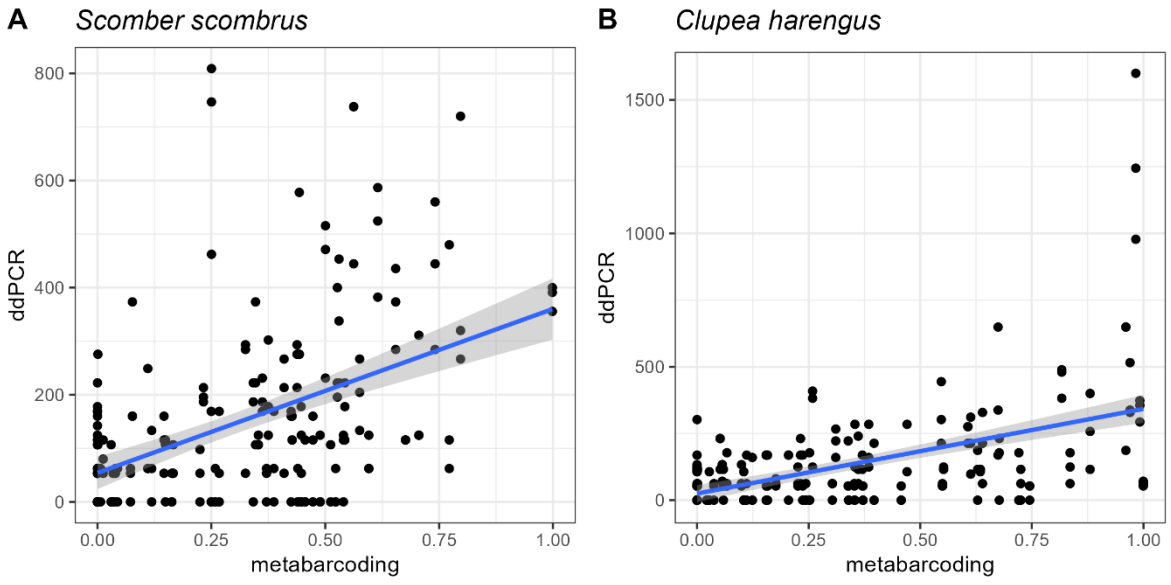

Figure 19. Dot plot comparing the quantification of Atlantic mackerel (A) and herring (B) eDNA from ddPCR and the relative abundance from MiFish metabarcoding results. Blue line is the linear regression between the two data sets with grey shaded area showing the standard error.

4. Discussion and conclusions

The Hywind Tampen study builds on an earlier pilot study of Hywind Scotland using the same methodology (Hestetun et al., 2023; Ray et al., 2022). While that study showed the ability of the chosen MiFish marker to detect a variety of fish species from the area, and the ability of eDNA data to discriminate between stations and depths, the inclusion of only one timepoint in that study made it impossible to attribute patterns to persistent effects due to the FOWF in the area or any current transport of free eDNA, a major consideration for including the three timepoints in the current Hywind Tampen study. An additional benefit for the current study was the ability to use previous fish capture data (de Jong et al., 2022; Palm et al., 2023) to provide ground truthing of the recovered eDNA community data in this report as no such data was available for the Hywind Scotland study.

4.1. Are there persistent differences between FOWF and non-FOWF fish and plankton communities?

Stations were categorized as either upstream, FOWF, downstream, or reference stations, along the NW-SE bottom slope in the area. CTD measurements did not detect any notable differences in oceanographic conditions between stations, rather slight seasonal change in temperature over time from T0 to T2 (one week later) that were consistent over stations. Similar to the previous Hywind Scotland study, there were significant differences in species composition between surface (20 m) and bottom water samples, reflecting the distinct natural communities at these different depths. In the 18S data, Alveolata was the most abundant kingdom-rank taxon across most stations (containing abundant dinoflagellate clades) but did not exhibit the same dominance as in the previous Hywind Scotland study (Ray et al., 2022), which identified a bloom of the dinoflagellate *Karenia* at the time of sampling.

MiFish differences due to area category and time, while significant, were very weak in both 20 m and bottom water samples, however. MiFish Shannon biodiversity index values were also similar between station groupings and time, with only a very weak positive trend towards the South-East detected. 18S differences were somewhat stronger, especially with regards to time. Still, the overall picture from the data is that the Hywind Tampen FOWF does not seem to have any particular impact on the measured communities. The ddPCR results for the two pelagic schooling species Atlantic mackerel and herring also did not show strong correlation between the FOWF and reference sites. In both cases, there was a stronger correlation with sampling time, showing that the concentrations of these species varied in the area over time according to the movement of the schools. Thus we could not see clear influence of the FOWF, either positive or negative, in any of our data.

It is important to note, however, that turbines were still being installed at the Hywind Tampen FOWF during the time of sampling, and installed turbines had been present for only a short time. Another factor is the precise distance of sampling stations to the turbines. While sampling had to be done at a safety distance from the turbines Wilhelmsson et al. (2006) showed that FAD effects might be detectable only at very close range to the structures. Potentially, eDNA water movement could detect FAD effects further away from the turbines than the SCUBA dive visual counts in that study, though it is difficult to quantify the extent of any such increased detection radius. In addition, the floating construction of the FOWF and comparatively high water depth in the area (266-300 m) could very well mean that any influence on demersal communities is light. Due to the recent construction, it is also likely that there was less growth on anchoring structures than would be expected after a number of

years in operation. A follow-up study after a certain number of years of operation would shed more light on these caveats.

4.2. Is there any detectable influence from water current transport of eDNA between stations?

To investigate any significant current transport of eDNA, T0 and T1 (T0+24h) data were investigated for similarities in T0 concentrations and T1 concentrations further downstream, with station distances being roughly correlated with an estimated 24h prevailing current speed distance. Any significant current transport of eDNA would imply similarities in organism composition results in T0 concentrations and T1 concentrations further downstream and would need to stand out against the background dynamics of the pelagic communities in the area to be detectable using this design.

A qualitative assessment of pairwise similarities in the metabarcoding datasets did not reveal any consistent clustering of T0 and downstream T1 station data in either the MiFish or 18S datasets. However, in contrast to quantitative assay data such as ddPCR data, metabarcoding abundance data is imprecise, so any subtle effects from current transport could be lost in the data.

For the 20 m ddPCR data it was also difficult to determine a significant water current influence on eDNA quantification. For mackerel, considering only T0 and T1, both time and region had a significant effect on eDNA concentrations, however for herring neither factor appeared significantly different. This could be related to many factors, including physical oceanography (current speed, vertical water transport) or the movement of fish during this period. For mackerel the detection was generally lower at T1 than T0, which could be a consequence of eDNA signal dilution within the water column.

In summary, we thus could not observe any consistent patterns indicating impact of current transport within the data from this study. Disentangling any potential effects of currents from the dynamics of local organism communities presents challenges. However, we assess that the impact of current transport does not significantly seem to confound the interpretation of data for individual species or community compositions derived from eDNA water samples under the conditions at the Hywind Tampen FOWF.

4.3. MiFish completeness and missing taxa

The 12S MiFish marker dataset identified 36 fish species, including a variety of pelagic and demersal species, with a greater diversity of species in the bottom samples, and fewer but very abundant schooling species in the 20 m samples. This mirrors the performance of this marker from the 26 species reported in the previous Hywind Scotland study (Ray et al., 2022).

For this study, we had the opportunity to ground truth MiFish results with fish species previously reported during demersal capture and ROV work (de Jong et al., 2022; Palm et al., 2023). This allowed us to investigate whether certain taxa or taxonomic groups were missing from the MiFish dataset that could be due to biases in the marker.

Of the abundant species identified as commercially important in the previous net study, i.e. ling, hake, saithe, cod, and whiting (de Jong et al., 2022), all were detected in the MiFish dataset, at large to medium abundances, implying good utility of this marker for these fishes. Of the 36 fish species

detected from bottom water samples in this study, 24 were reported from these previous studies. Of the 12 remaining species only detected by MiFish eDNA most represent a mix of species too small to be caught by the 2022 net-based approach, or with a behavior that makes them less likely to be caught by this method. Conversely, seven previously reported species were not detected in the MiFish dataset. Five of these were elasmobranchs, highlighting a known deficiency of the MiFish primer set in this taxonomic group in particular (Miya et al., 2015). Thus, for future studies, the current eDNA approach used in this study should be supplemented by the MiFish-E primer pair (N. Dunn et al., 2022; Miya et al., 2015) designed to overcome this limitation.

In terms of taxonomic resolution, the MiFish sequences were able to resolve the majority of fishes to species level. This is in congruence with comparative studies of eDNA fish markers that show a high level of resolution for this marker (Polanco et al., 2021). In a couple of cases, sequences were equally similar to several matching species, such as was the case for *Helicolenus* sp. and *Sebastes* sp. (likely these sequences represent blackbelly rosefish, *H. dactylopterus* and Atlantic redfish, *S. norvegicus*). Similarly, Atlantic halibut and blackbellied angler were not detected, possibly as they could not be resolved against other flatfish and the European angler, respectively. In contrast, MiFish assignments also included Pacific herring (*Clupea pallasii*), a sister species to the Atlantic herring (*C. harengus*), from which it diverged approximately 2 Mya. Isolated Pacific and hybrid herring populations are found in the White Sea and Balsfjord (Pettersson et al., 2023); at Hywind Tampen, intraspecific variation within Atlantic herring populations not represented in the Mitofish database is a more likely interpretation, however.

The MiFish data also contained a number of sequences belonging to the freshwater species gudgeon (*Gobio gobio*). These hits are examples of false positives since the likelihood of the presence of the species at Tampen is non-existent. We regard these sequences as the result from lab or sampling gear contamination, stressing the need to continue work to improve sampling and lab decontamination practeses and controlls.

5. Conclusions

- At 36 fish species, the MiFish dataset provides a largely comprehensive overview of the fish communities at the Tampen site, albeit with a notable gap in detecting elasmobranchs – a known limitation of the primer pair used. In a few instances, the taxonomic resolution was insufficient for identifying taxa to species level. On the other hand, 13 species not detected in previous catch and ROV studies were present in the MiFish dataset.

- The 18S eukaryote dataset was dominated by calanoid copepods, and there was no presence of the *Karenia* algal bloom observed at the previous Hywind Scotland study (Ray et al., 2022). Although the 18S data offers less taxonomic detail, particularly concerning economically significant groups like those identified with MiFish primers, it encompasses a broader spectrum of organisms. This broader scope enhances its sensitivity to shifts in community structure, making it a valuable tool for detecting patterns of change between stations.

- Our findings indicate no clear effect of current transport on eDNA community composition or ddPCR abundance data, suggesting that at the Hywind Tampen site, such transport is not substantial enough to interfere with data interpretation.

- Temporal and depth variations in the eDNA samples effectively reflect local environmental conditions, consistent with findings from the previous Ray et al. 2022 study. This ability to monitor changes in species composition (and for ddPCR: gene copy abundances) over time provides additional validation of the eDNA methodology for monitoring.

- Overall, the eDNA data suggests that the Hywind Tampen FOWF does not exert a discernible impact, be it positive or negative, on the composition of eukaryote organisms in a broad sense (18S), for fishes in particular (MiFish), or through the more precise abundance estimates gained from ddPCR measurements of Atlantic mackerel and herring. Given that the OWF has a relatively small number of turbines, floating construction, is newly installed, and situated in deep water, we asses no measurable impact at the community level at the time of study. However, subsequent studies, conducted after several years of operation, would provide evidence to whether these observations hold true over time.

6. Acknowledgements

We would like to thank Equinor for collaboration and support to conduct this study, in particular Kari Mette Murvoll, Anita Skarstad, Ane Kjølhamar, Lars Petter Myhre, Rolf Sundt, Linn Hocking, and Mari-Anne Hetle. For excellent help and assistance during the 2023 monitoring cruise where field work was conducted, we would like to thank Akvaplan-niva personnel onboard, led by Rune Palerud, and the *Ocean Response* crew and master.

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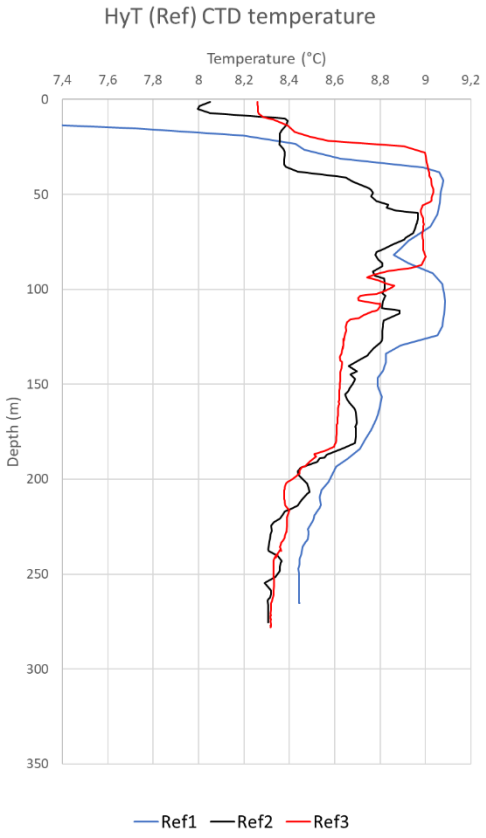
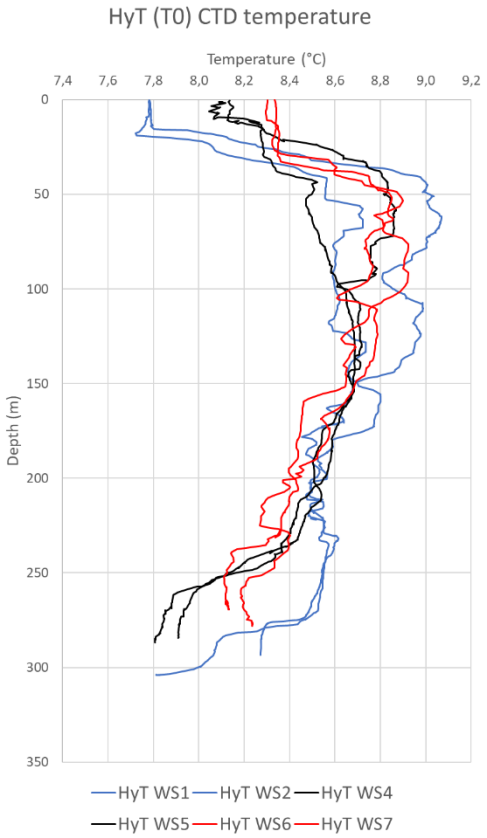
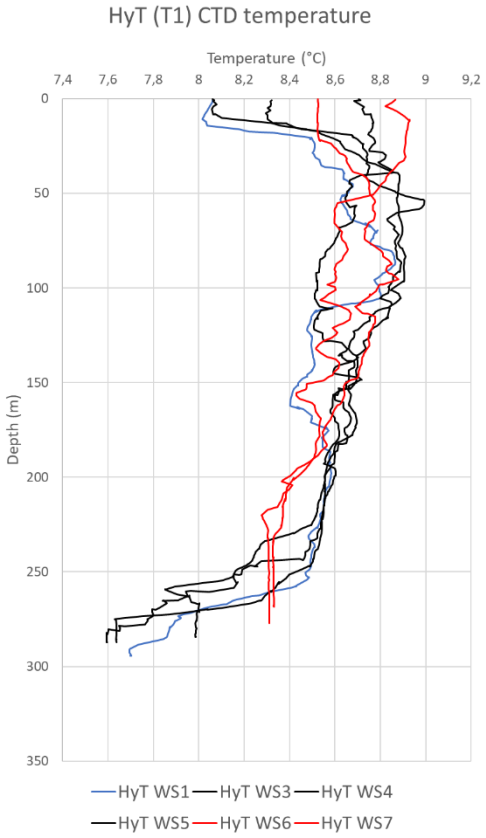
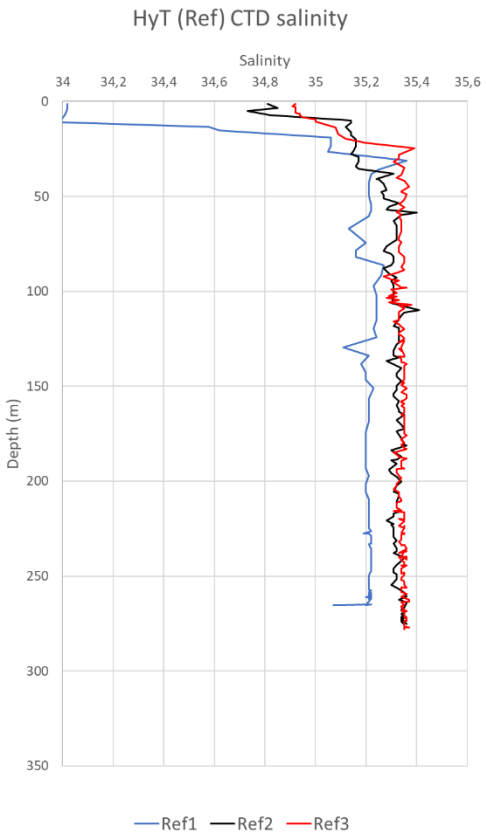
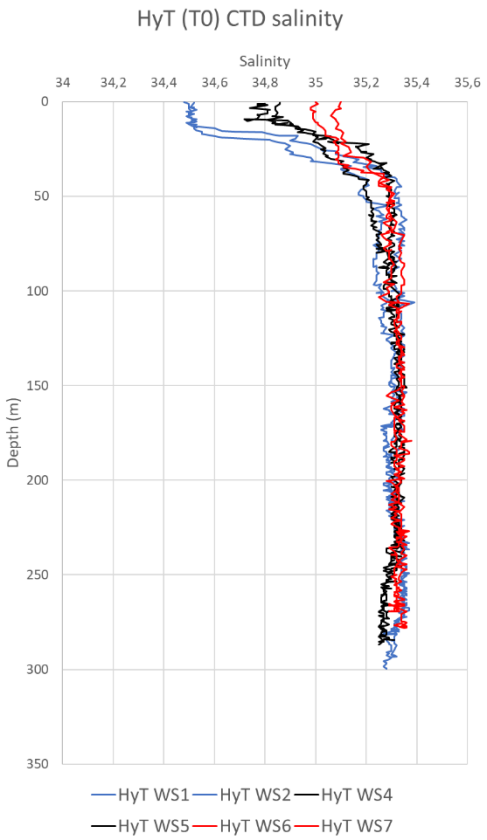
Appendix A: CTD series measurements

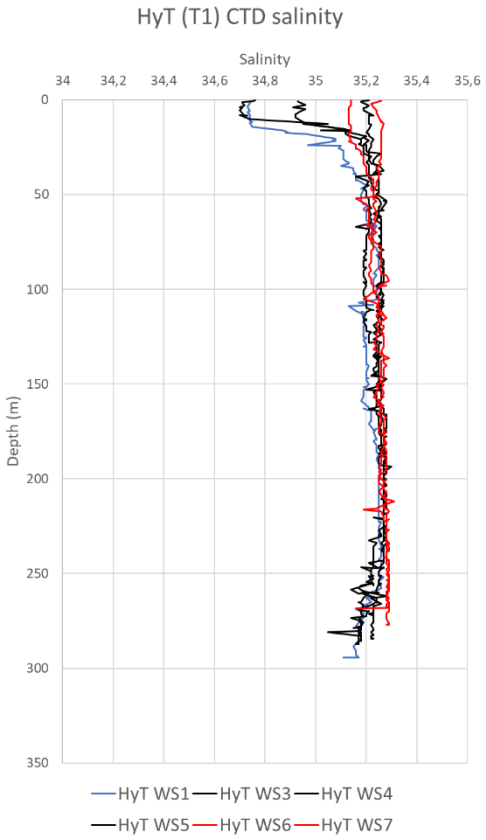


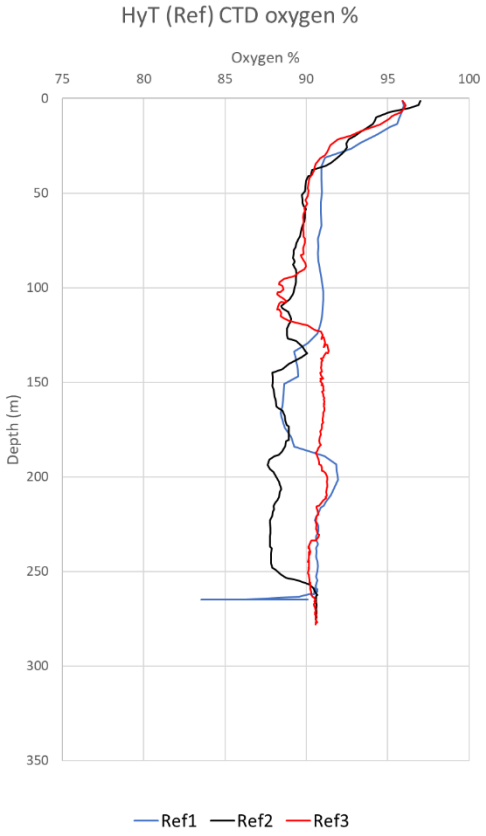


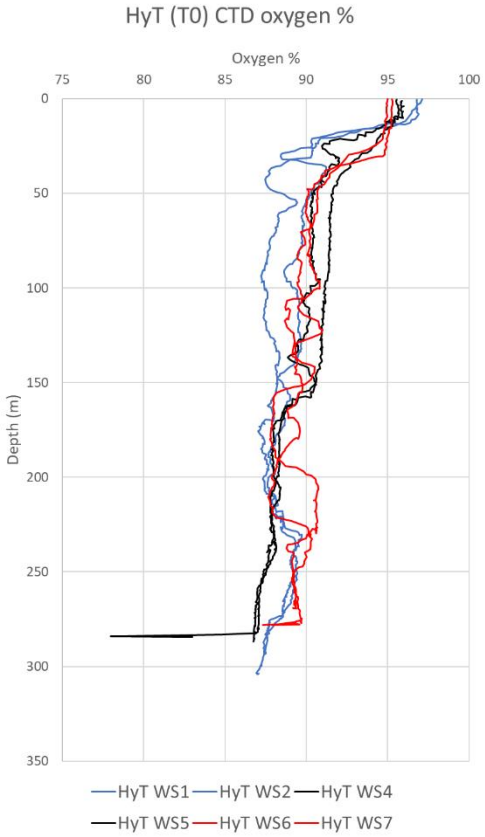


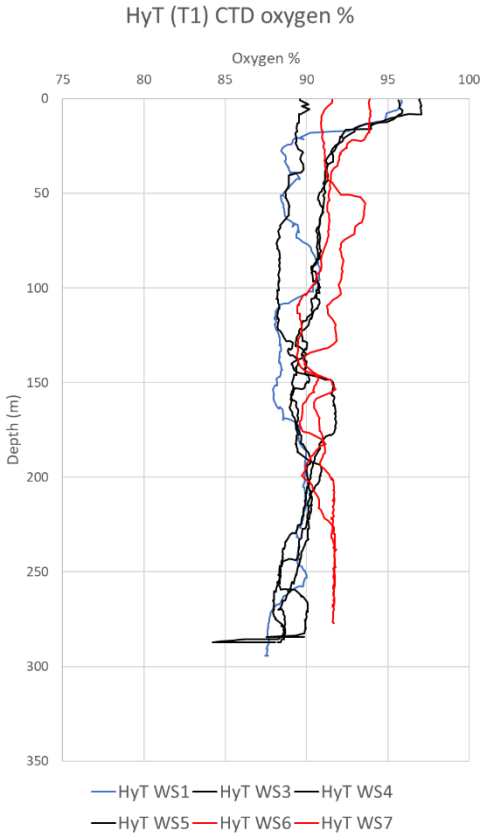

HyT (Ref) CTD salinity
Salinity
34 34,2 34,4 34,6 34,8 35 35,2 35,4 35,6
0 50 100 150 200 250 300 350
Depth (m)
Ref1 Ref2 Ref3

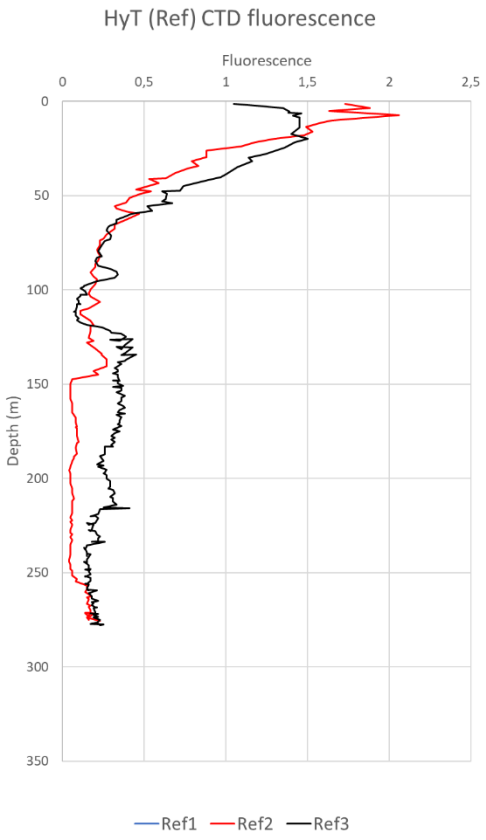

HyT (T0) CTD salinity
Salinity
34 34,2 34,4 34,6 34,8 35 35,2 35,4 35,6
0 50 100 150 200 250 300 350
Depth (m)
HyT WS1 HyT WS2 HyT WS4
HyT WS5 HyT WS6 HyT WS7

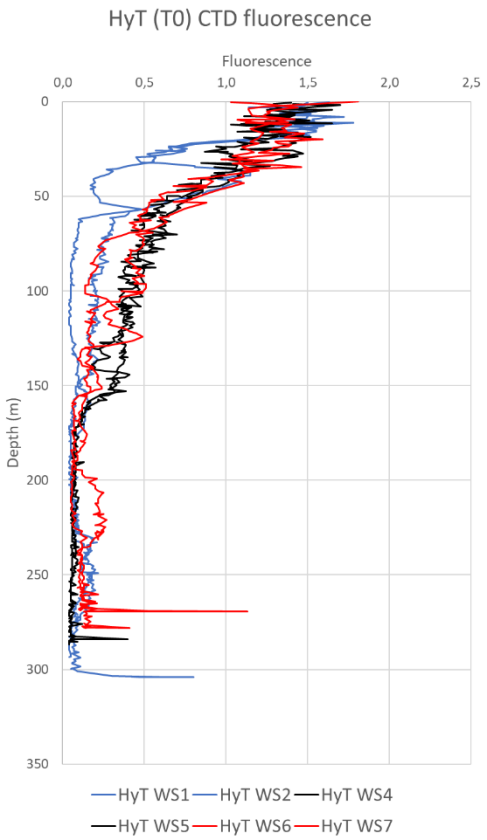

HyT (T1) CTD salinity
Salinity
34 34,2 34,4 34,6 34,8 35 35,2 35,4 35,6
0 50 100 150 200 250 300 350
Depth (m)
HyT WS1 HyT WS3 HyT WS4
HyT WS5 HyT WS6 HyT WS7

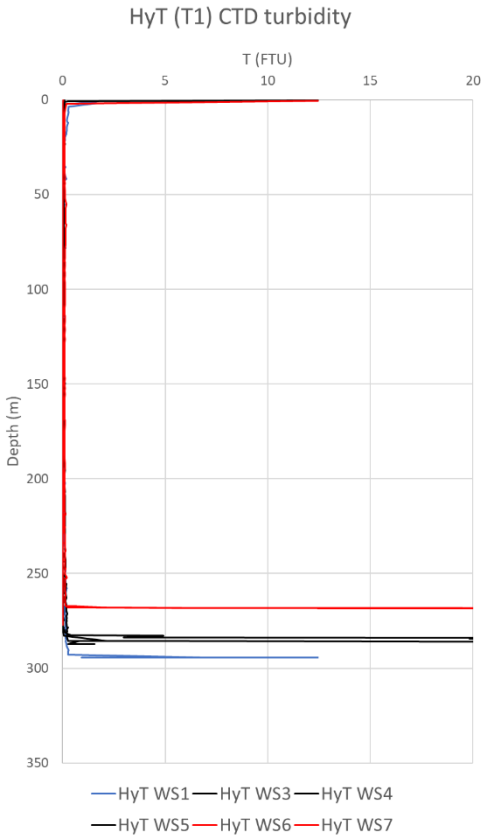

HyT (Ref) CTD oxygen %
Oxygen %
75 80 85 90 95 100
0 50 100 150 200 250 300 350
Depth (m)
Ref1 Ref2 Ref3


HyT (T0) CTD oxygen %
Oxygen %
75 80 85 90 95 100
0 50 100 150 200 250 300 350
Depth (m)
HyT WS1 HyT WS2 HyT WS4
HyT WS5 HyT WS6 HyT WS7


HyT (T1) CTD oxygen %
Oxygen %
75 80 85 90 95 100
0 50 100 150 200 250 300 350
Depth (m)
HyT WS1 HyT WS3 HyT WS4
HyT WS5 HyT WS6 HyT WS7


HyT (Ref) CTD fluorescence
Fluorescence
0
0,5
1
1,5
2
2,5
Depth (m)
0
50
100
150
200
250
300
350
Ref1
Ref2
Ref3


HyT (T0) CTD fluorescence
Fluorescence
0,0
0,5
1,0
1,5
2,0
2,5
Depth (m)
0
50
100
150
200
250
300
350
HyT WS1
HyT WS2
HyT WS4
HyT WS5
HyT WS6
HyT WS7


HyT (T1) CTD turbidity
T (FTU)
0
5
10
15
20
Depth (m)
0
50
100
150
200
250
300
350
HyT WS1
HyT WS3
HyT WS4
HyT WS5
HyT WS6
HyT WS7

Appendix B. 20 m MiFish data

English	Norwegian	SW1 T0	SW1 T1	SW1 T2	SW2 T0	SW2 T1	SW2 T2	SW3 T0	SW3 T1	SW3 T2	SW4 T0	SW4 T1	SW4 T2
Greater argentine	Vassild	0	0	0	0	0	0	0	0	0	0	0	0
Argentine	Strømsild	0	0	0	0	0	0	0	0	18	0	0	0
Garfish	Horngjel	0	0	0	10	0	28918	780	9	21	67928	0	0
Tusk	Brosme	0	0	0	0	0	0	0	0	0	0	0	0
Spotted dragonet	Flekket fløyfisk	0	0	0	0	0	0	0	0	0	0	0	0
Rabbit fish	Havmus	0	0	0	0	0	0	0	0	0	3	0	0
Atlantic herring	Sild	120996	0	254501	203743	515300	212823	92622	329546	261393	69737	218511	884376
Pacific herring	Stillehavssild	0	0	0	0	0	0	0	0	0	173	0	0
Lumpfish	Rognkjeks	0	0	0	0	0	0	0	0	0	0	0	0
Drummond's pearlfish	Snyltefisk	0	0	0	0	0	0	0	0	0	0	0	0
Fourbeard rockling	Firetrådet tangbrosme	0	0	0	0	0	0	0	0	0	0	0	0
Grey gurnard	Knurr	0	0	0	0	0	0	0	0	0	0	0	0
Silvery cod	Sølvtorsk	0	159802	0	0	0	0	0	0	0	0	14	0
Atlantic cod	Torsk	0	0	0	0	0	0	0	0	0	0	0	0
Witch	Smørflyndre	0	0	0	0	0	0	0	0	0	0	0	0
Helicolenus	Helicolenus	0	0	0	0	0	0	0	0	0	0	0	0
Megrim	Glassvar	0	0	0	0	0	0	0	0	0	0	0	0
American plaice	Gapeflyndre	0	0	0	0	0	4	0	0	0	0	0	0
Common monkfish	Breiflabb	0	0	0	0	0	0	0	0	0	0	0	0
Mueller's pearlside	Laksesild	0	0	234	0	0	0	0	0	45	0	0	0
Haddock	Hyse	0	0	0	0	0	0	24668	0	0	0	0	0
Whiting	Hvitting	0	0	0	0	0	0	58402	0	0	16003	0	0
European hake	Lysing	0	0	0	0	0	0	0	0	0	0	0	0
Blue whiting	Kolmule	406997	444924	431	301453	0	0	56752	108485	655294	9102	21604	0
Common ling	Lange	0	24546	0	0	12029	21733	0	0	0	0	0	0
No_hit	Ingen_ID	8225	0	2740	245	1808	2054	984	62	7	472	212	0
Lancet fish	Stor lysprikkfisk	0	0	0	0	0	0	0	0	0	0	0	0
Greater forkbeard	Skjellbrosme	0	0	0	0	0	0	0	0	0	0	0	0
Pollack	Lyr	0	0	0	0	0	0	0	0	0	0	0	0
Saithe	Sei	0	49236	984	0	0	51	14270	79033	41613	147228	11	0
Atlantic salmon	Laks	0	0	0	77894	0	0	0	21	0	43631	0	0
Brown trout	Ørret	0	0	0	0	0	0	0	0	0	0	0	0
Atlantic mackerel	Makrell	138328	13484	159143	153229	132124	343850	392467	157602	906529	363414	75913	12012
Rockfish	Uer	0	0	0	0	0	0	0	0	0	0	0	0
Rockfish	Uer	0	0	0	0	0	0	0	0	0	0	0	0
Horse mackerel	Hestmakrell	0	0	0	0	0	0	0	0	0	0	0	0
Norway pout	Øyepål	0	0	0	0	0	0	14738	0	0	0	41439	9534
Poor cod	Sypike	0	0	0	0	0	0	23	0	0	8	5	0

English	Norwegian	SW5 T0	SW5 T1	SW5 T2	SW6 T0	SW6 T1	SW6 T2	SW7 T0	SW7 T1	SW7 T2	Ref1	Ref2	Ref3
Greater argentine	Vassild	0	0	0	0	0	0	0	0	0	0	0	0
Argentine	Strømsild	0	0	0	0	0	0	15821	0	0	0	0	0
Garfish	Horngjel	21	38016	0	13125	10174	0	16691	0	0	0	0	15
Tusk	Brosme	0	0	0	0	0	0	0	0	0	0	0	0
Spotted dragonet	Flekket fløyfisk	0	0	0	0	0	0	0	0	0	0	0	0
Rabbit fish	Havmus	0	0	0	0	0	0	0	0	0	0	0	0
Atlantic herring	Sild	171580	92774	264875	80822	224342	172746	7861	61	117146	233023	52410	135586
Pacific herring	Stillehavssild	0	0	0	0	0	0	0	0	0	0	0	0
Lumpfish	Rognkjeks	0	0	0	0	0	0	0	0	0	0	0	0
Drummond's pearlfish	Snyltefisk	0	0	0	0	0	0	0	0	0	0	0	0
Fourbeard rockling	Firetrådet tangbrosme	0	0	0	0	0	0	566	0	0	0	0	0
Grey gurnard	Knurr	0	0	0	27255	0	0	0	0	0	0	0	0
Silvery cod	Sølvtorsk	0	0	0	0	0	0	0	0	0	0	0	0
Atlantic cod	Torsk	0	0	0	0	0	0	0	0	0	0	0	0
Witch	Smørflyndre	0	0	0	0	0	0	0	0	0	0	0	0
Helicolenus	Helicolenus	0	0	0	0	0	0	0	0	0	0	0	0
Megrim	Glassvar	0	0	0	0	0	0	0	0	0	0	0	0
American plaice	Gapeflyndre	0	0	11	43048	0	0	0	0	0	0	0	0
Common monkfish	Breiflabb	0	0	0	0	0	0	0	0	0	0	0	0
Mueller's pearlside	Laksesild	0	0	0	0	0	1424	0	18	0	49549	105874	6477
Haddock	Hyse	27877	7475	0	96262	20	0	63622	0	41240	0	0	32488
Whiting	Hvitting	22948	51032	0	66001	8969	0	108774	0	12200	0	0	25744
European hake	Lysing	0	0	0	0	0	0	0	0	0	0	0	0
Blue whiting	Kolmule	19735	88779	0	45408	10173	0	15526	2628	144320	20427	67400	1149
Common ling	Lange	0	0	0	0	0	1636	0	0	0	0	0	19570
No_hit	Ingen_ID	48	0	451	1447	0	7105	1052	2039	5393	8173	276	6
Lancet fish	Stor lysprikkfisk	0	0	0	0	0	0	0	0	0	0	0	0
Greater forkbeard	Skjellbrosme	0	0	0	0	0	0	0	0	0	0	0	0
Pollack	Lyr	0	0	0	6996	9	0	18134	0	7656	0	0	0
Saithe	Sei	129175	0	0	54035	0	98518	2242	95867	14119	400514	270529	28330
Atlantic salmon	Laks	14977	31	117569	0	0	0	0	2366	0	0	0	0
Brown trout	Ørret	0	0	0	0	0	0	0	0	0	0	0	0
Atlantic mackerel	Makrell	391638	421040	140	407824	149005	152658	535347	80215	215202	79326	27715	290073
Rockfish	Uer	0	0	0	0	0	0	0	0	0	0	0	0
Rockfish	Uer	0	0	0	0	0	0	0	0	0	0	0	0
Horse mackerel	Hestmakrell	0	0	0	0	0	0	0	0	0	0	0	0
Norway pout	Øyepål	15635	0	38933	74358	0	0	30593	0	4362	0	0	0
Poor cod	Sypike	0	0	0	18165	0	0	36909	0	0	0	0	0

Appendix C. Bottom water MiFish data

English	Norwegian	SW1 T0	SW1 T1	SW1 T2	SW2 T0	SW2 T1	SW2 T2	SW3 T0	SW3 T1	SW3 T2	SW4 T0	SW4 T1	SW4 T2
Greater argentine	Vassild	0	0	0	0	0	0	0	0	0	0	0	3234
Argentine	Strømsild	0	0	0	0	0	0	0	0	0	11178	0	0
Garfish	Horngjel	0	0	0	0	0	0	0	0	0	0	0	0
Tusk	Brosme	15	24	0	45	25733	123772	0	120132	6080	27	0	26
Spotted dragonet	Flekket fløyfisk	0	0	0	0	0	0	0	0	0	0	0	0
Rabbit fish	Havmus	0	0	11	0	0	0	8	0	0	702	2881	0
Atlantic herring	Sild	15	28513	212	38533	0	6	1445	10784	20407	0	407347	51064
Pacific herring	Stillehavssild	0	0	0	0	0	0	0	0	0	0	0	0
Lumpfish	Rognkjeks	0	0	0	28816	0	0	0	7	0	0	0	0
Drummond's pearlfish	Snyltefisk	0	0	0	0	0	0	0	0	6	0	0	0
Fourbeard rockling	Firetrådet tangbrosme	0	0	0	0	0	0	0	0	0	0	0	0
Grey gurnard	Knurr	0	0	85	0	0	0	0	0	11	0	0	0
Silvery cod	Sølvtorsk	23	87	1357	0	44	39599	0	36508	26232	256088	6907	25626
Atlantic cod	Torsk	0	7	0	0	14096	0	0	0	0	0	0	6108
Witch	Smørflyndre	0	0	0	0	7	10	0	0	0	0	0	0
Helicolenus	Helicolenus	6	18	0	0	7	0	0	0	0	29919	0	0
Megrim	Glassvar	18	8	0	0	42271	0	0	10	0	0	0	4075
American plaice	Gapeflyndre	0	2	0	0	0	0	0	0	19	0	0	0
Common monkfish	Breiflabb	0	0	0	0	0	0	0	0	0	0	0	82
Mueller's pearlside	Laksesild	12	43	123	313649	25859	0	0	75	42696	0	0	0
Haddock	Hyse	0	0	0	0	0	0	0	49224	0	0	0	19599
Whiting	Hvitting	0	0	8937	0	26	0	0	0	0	0	0	0
European hake	Lysing	0	0	0	0	0	0	0	0	0	0	0	0
Blue whiting	Kolmule	141973	384057	138531	162079	223642	20512	259337	52391	333652	208402	146234	272257
Common ling	Lange	0	14	30	0	35	23808	48	77216	0	40823	10343	0
No_hit	Ingen_ID	18694	37127	2612	37344	8947	1734	125682	36	20662	46	0	14
Lancet fish	Stor lysprikkfisk	0	0	3295	0	0	0	0	0	0	0	0	1535
Greater forkbeard	Skjellbrosme	0	0	32	0	0	0	0	8	0	0	0	0
Pollack	Lyr	0	0	0	0	0	0	0	0	0	0	0	0
Saithe	Sei	119193	0	61	12597	23882	0	132531	188463	18793	38661	14188	8114
Atlantic salmon	Laks	56195	42	0	0	16295	0	0	0	0	60776	0	0
Brown trout	Ørret	0	0	5930	0	0	0	0	0	0	0	0	0
Atlantic mackerel	Makrell	39915	238383	109635	43862	0	0	0	0	7590	42099	25170	22106
Rockfish	Uer	0	0	0	0	0	424	0	0	0	0	0	0
Rockfish	Uer	0	0	0	0	0	163127	0	0	72	0	0	25
Horse mackerel	Hestmakrell	0	0	17	0	0	0	0	0	0	0	0	0
Norway pout	Øyepål	231	23	18975	0	0	0	0	31800	19450	0	48	164
Poor cod	Sypike	0	0	0	16	6	0	0	22106	0	0	0	0

English	Norwegian	SW5 T0	SW5 T1	SW5 T2	SW6 T0	SW6 T1	SW6 T2	SW7 T0	SW7 T1	SW7 T2	Ref1	Ref2	Ref3
Greater argentine	Vassild	0	8531	4127	15129	9248	4372	19250	11401	3174	0	0	0
Argentine	Strømsild	0	0	0	0	40861	0	8631	0	0	0	0	0
Garfish	Horngjel	7052	3	0	0	0	0	7138	0	0	0	0	0
Tusk	Brosme	49	39150	42031	0	0	78240	0	0	6153	0	0	0
Spotted dragonet	Flekket fløyfisk	0	0	0	5	0	0	0	0	3	0	0	0
Rabbit fish	Havmus	0	4	20	0	0	1162	77	0	0	0	3697	0
Atlantic herring	Sild	19	41809	2153	49480	0	38644	39817	10863	7591	53751	63035	0
Pacific herring	Stillehavssild	0	0	0	1321	0	0	0	0	0	0	0	0
Lumpfish	Rognkjeks	8	4	0	0	0	0	0	0	0	0	0	0
Drummond's pearlfish	Snyltefisk	0	0	1079	0	24382	15831	0	0	4	3	0	0
Fourbeard rockling	Firetrådet tangbrosme	0	0	648	0	0	0	0	0	0	0	0	0
Grey gurnard	Knurr	0	0	1909	0	0	0	13990	0	0	0	24084	0
Silvery cod	Sølvtorsk	42841	72636	43971	145436	0	38334	153761	38862	40356	0	70665	0
Atlantic cod	Torsk	0	0	0	0	0	0	0	22230	0	0	0	0
Witch	Smørflyndre	0	40115	9523	0	40052	0	0	0	0	0	0	0
Helicolenus	Helicolenus	8	23	0	23860	0	0	0	0	21754	0	0	0
Megrim	Glassvar	0	67197	0	0	0	0	0	0	0	0	0	0
American plaice	Gapeflyndre	0	0	0	21181	0	0	0	0	0	0	0	231345
Common monkfish	Breiflabb	0	0	0	0	0	0	0	0	0	0	0	0
Mueller's pearlside	Laksesild	300613	3503	0	61017	0	55280	2413	9590	31496	0	36064	94674
Haddock	Hyse	24	0	8964	6072	2123	0	0	126588	14940	17	0	0
Whiting	Hvitting	0	0	9544	38599	37673	0	74461	15917	5574	0	0	0
European hake	Lysing	0	0	0	0	307	1813	0	0	1534	0	0	0
Blue whiting	Kolmule	126314	757360	171810	355855	169885	308909	208135	151849	271625	0	256199	113869
Common ling	Lange	49	130311	276	43948	0	0	12684	9371	0	0	7658	6452
No_hit	Ingen_ID	0	899	6	0	799	33	961	0	149	5149	163	1556
Lancet fish	Stor lysprikkfisk	0	0	0	0	0	0	0	0	0	0	0	0
Greater forkbeard	Skjellbrosme	0	63076	2899	0	0	40	0	0	0	0	8466	0
Pollack	Lyr	0	0	0	0	0	0	0	0	0	0	0	0
Saithe	Sei	83306	61789	4253	25123	346	95033	0	42259	5477	0	34753	0
Atlantic salmon	Laks	0	0	0	0	0	0	0	0	0	21045	0	0
Brown trout	Ørret	0	0	0	0	0	0	0	0	0	0	0	0
Atlantic mackerel	Makrell	15648	0	21431	80699	201491	6154	12097	0	61023	375724	35986	1500
Rockfish	Uer	0	0	0	0	0	0	0	0	0	0	0	0
Rockfish	Uer	0	0	0	0	17	20	0	0	41	7	0	61
Horse mackerel	Hestmakrell	0	0	0	0	0	0	0	3	0	0	5371	0
Norway pout	Øyepål	0	0	22278	97365	20989	0	12312	16406	0	0	0	134261
Poor cod	Sypike	19	20914	0	27445	0	0	11218	12534	0	10	0	0

Appendix D. MiFish and capture study species composition

Checklist of fish species in the bottom samples from the current study against 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. Note the relative lack of elasmobranch species in the MiFish eDNA dataset. 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.

English name	Scientific name	MiFish abundance	de Jong 2022/2023	Comment
Thorny skate	*Amblyraja radiata*		3	Skate
Greater argentine	*Argentina silus*	69218	1	
Argentine	*Argentina sphyraena*	19809		
Garfish	*Belone belone*	14193		
Tusk	*Brosme brosme*	441477	15	
Spotted dragonet	*Callionymus maculatus*	8		
Rabbit fish	*Chimaera monstrosa*	8562	58	
Atlantic herring	*Clupea harengus*	865488	1	
Pacific herring	*Clupea pallasii*	1321		Likely *C. harengus* intraspecific variation.
Common skate	*Dipturus intermedius/batis*		6	Skate
Lumpfish	*Cyclopterus lumpus*	28835		
Drummond's pearlfish	*Echiodon drummondi*	16923	ROV	
Fourbeard rockling	*Enchelyopus cimbrius*	648		
Grey gurnard	*Eutrigla gurnardus*	40079	3	
Silvery cod	*Gadiculus argenteus*	1039333	ROV	
Atlantic cod	*Gadus morhua*	42441	215	
Blackmouth catshark	*Galeus melastomus*		35	Shark
Righteye flounder	*Glyptocephalus cynoglossus*	49655	1	
American plaice	*Hippoglossoides platessoides*	252547	8	
Atlantic halibut	*Hippoglossus hippoglossus*		3	Possibly lack of resolution.
Blackbelly rosefish	*Helicolenus dactylopterus*		ROV	
	Helicolenus sp.	75595		Only resolved to genus level.
Megrim	*Lepidorhombus whiffiagonis*	113579	9	
Shagreen skate	*Leucoraja fullonica*		3	Skate
Cuckoo ray	*Leukoraja naevus*		1	Skate
Blackbellied angler	*Lophius budegassa*		1	Possibly lack of resolution.
European angler	*Lophius piscatorius*	82	20	
Mueller's pearlside	*Maurolicus muelleri*	977107		
Atlantic haddock	*Melanogrammus aeglefinus*	225428	34	
Whiting	*Merlangius merlangus*	153058	141	
European hake	*Merluccius merluccius*	3347	84	
Blue whiting	*Micromesistius poutassou*	5064992	41	

Common ling	*Molva molva*	363066	589	
Lancet fish	*Notoscopelus kroyeri*	4830		
Greater forkbeard	*Phycis blennoides*	74521	2	
Atlantic pollock	*Pollachius pollachius*	0*	69	*Present in MiFish 20 m data.
Saithe	*Pollachius virens*	908476	158	
Atlantic salmon	*Salmo salar*	154353		
Brown trout	*Salmo trutta*	5930		
Atlantic mackerel	*Scomber scombrus*	1139022	47	
Atlantic redfish	*Sebastes norvegicus*		1	
	Sebastes sp.	163777		Only resolved to genus level.
Spiny dogfish	*Squalus acanthias*		8	Shark
Horse mackerel	*Trachurus trachurus*	5391		
Norway pout	*Trisopterus esmarkii*	353313	ROV	
Poor cod	*Trisopterus minutus*	94268		