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Metabarcoding of sediment eDNA communities at the Hywind Scotland OWF – A pilot study

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Summary

Environmental impact assessment and regular environmental monitoring of marine life are prerequisites for the construction, operation, and decommissioning of offshore wind farms (OWFs). Molecular methods such as metagenomics, quantitative PCR or metabarcoding, are increasingly being considered as a possible complement or alternative to currently used marine baseline and monitoring methods, both for pelagic (open water) and benthic (seafloor) organism studies. Metabarcoding is one such molecular method that uses DNA sequence differences between species in specific so-called marker genes to identify organism biodiversity in a bulk specimen sample containing multiple animals, or environmental sample from water or sediment. While a simplification, a list of unique gene sequence variants (variously referred to as ASVs or OTUs in the literature) in a metabarcoding dataset can be conceptually considered similar to different species in a classical species list.

The following report is an assessment of the performance of such metabarcoding data – directly from DNA in sediment samples – in sediment impact monitoring as a complement to the current standard using species lists based on identification of >1 mm softbottom macrofauna. While recent eDNA studies have become more numerous, cumulative experience in particular habitats is still low: Thus this report should be considered an exploratory pilot study.

In metabarcoding, there are many gene markers available, which target different parts of the organism community in the sample (bacteria, protists, multicellular organisms etc.). Here, we sequenced two gene markers: the metabarcoding 18S rDNA ribosomal marker, regions V1-V2 (targeting eukaryotes in general) and the metazoan (animal) cytochrome oxidase subunit I (COI) marker. The 18S marker was expected to provide data on sequences from several protist groups, meiofauna (animals <1 mm) and some macrofauna (>1 mm); the COI marker is a well-established macrofaunal marker employed as the main animal marker in the Barcode of Life initiative, and it has the highest taxonomic coverage in online databases, such as GenBank, which allows more sequence variants to be identified to known species.

The aims were to (i) study 18S V1-V2 eukaryote and COI metazoan benthic community composition; (ii) assess the performance of the chosen eDNA sampling design, sample type, replicate number and molecular markers in detecting environmental impact; and (iii) compare how the metabarcoding results compare to morphological taxonomy of 1 mm sieved macrofauna from the same stations. The metabarcoding sediment samples were collected together with standard macrofauna samples at the Hywind OWF during spring 2022.

The study comprises 15 sampling stations at 110-120 m depth from two Hywind turbines (12 samples) and three reference stations: Sediment was collected at the same time as the standard monitoring parameters (processed separately by DNV) using van Veen grab sampling, frozen and transported to the NORCE lab in Bergen, where after DNA extraction the 18S rDNA V1-V2 and COI markers were amplified and subject to high-throughput metabarcode sequencing. Resultant sequence data was denoised, filtered and clustered using a custom dada2 and swarm pipeline to produce relevant ASV and OTU tables of community composition. These data are presented here together with the 1 mm sieved macrofauna monitoring data processed by DNV.

The DNV morphological dataset comprised 10 010 specimens from 212 species, with major phyla including annelids, mollusks, arthropods, and echinoderms. Biotic index values showed very good

to good conditions for all stations and no environmental impact was detected. nMDS analysis showed that station replicates mostly separated into distinct clusters based on the separate sampling areas.

From a 25 million read dataset, the post-filtering 18S rDNA V1-V2 metabarcoding dataset produced 1 473 OTUs following a 2*10-5 abundance cutoff, mostly from the SAR supergroup (Stramenopiles, Alveolata, Rhizaria), but with a 16% 380 OTU metazoan fraction. High-level taxonomic groups were mostly consistent across stations. Shannon index biodiversity values were consistent in the total dataset and for single-celled organisms, intermediately consistent for meiofauna, and least consistent for macrofauna, indicating incomplete sampling for this fraction. Area nMDS separation was less clear than in the morphological data.

From an initial 37 million raw sequences, 1.6 million sequences could be assigned as metazoans in the COI dataset comprising 118 OTUs, the rest representing non-target bacteria or unidentifiable sequences. Metazoan sequences included nine phyla, unevenly distributed over the stations in the dataset. Shannon and sensitivity index values were variable across replicates and stations in the dataset, indicating insufficient sampling effort relative to the targeted organism group, and no clear pattern emerged from the nMDS analysis.

In the absence of detected environmental impact in any of these three datasets, we focused our analysis of the comparative performance of the datasets on sampling comprehensiveness and variability of the data relative to replicates and stations used in the study sampling design. Based on our results, we found that the morphological dataset was better able to show fine-scale differences in organism composition in the data based on the three areas sampled, while metabarcoding data was a bit noisier in this regard. The COI dataset exhibited the most variability between sites, followed by the 18S macrofaunal data. This shows that the sediment volumes used here could be insufficient for these “larger” animals. Single-cell protist and <1 mm meiofaunal 18S data were more similar between samples, but even so could not group the samples by area as well as the morphological data.

We conclude that i) while sediment eDNA has previously showed robust performance in detecting environmental impact on benthic communities, the sampling coverage used in this study did not provide equal-quality data to morphology in detecting small changes in species composition between closely related unaffected sites; ii) taxonomic coverage in online identification databases is still unable to identify large parts of metabarcoding datasets to low level (e.g., genus/species); iii) to lessen the effects of sample variability and increase the resolution of the metabarcoding data, a combination of concentrating on small/single-cell organisms and increasing sample effort, fine-tuning bioinformatic parameters, and further development of metabarcoding-specific biotic indices, is expected to raise metabarcoding data quality.

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1. Introduction

The Hywind Scotland Pilot Park is a floating offshore wind farm (OWF) situated at the Buchan Deep, east of Peterhead, UK (Fig. 1). As part of the planning and consenting process, some baseline and monitoring surveys have been performed. The wind farm, being in world's first floating offshore wind farm, also represents an interesting location for research activities regarding possible environmental implications of floating offshore wind. As part of a research project, NORCE conducted a benthic eDNA study to evaluate the usability of sediment metabarcoding in benthic impact assessment, complementing a separate study using current standard morphological methodology (Møskeland, Fjukmoen, and Melsom 2023) and a pelagic eDNA report from the same installation (Ray et al. 2022). The purpose of the benthic eDNA study was to act as a pilot study to evaluate the usability of sediment metabarcoding in benthic impact assessment.

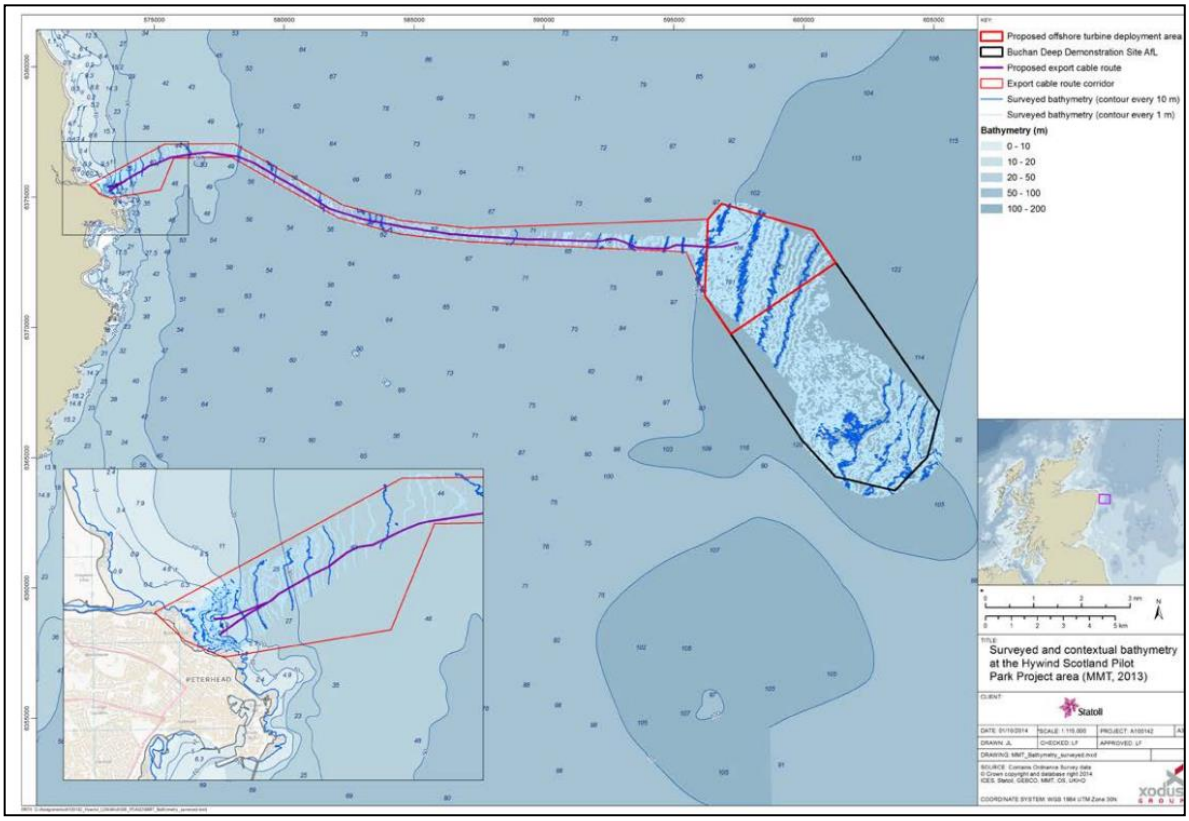


Figure 1. *Map of the Hywind Scotland Pilot Project wind farm area. Source: Hywind Scotland Pilot Park Environmental Statement* (Statoil 2015).

Like other man-made structures in the ocean, OWFs may have a variety of effects on local organism communities, which can range from increased artificial substrate availability for colonization, shelter and feeding (the so-called artificial reef effect), the tendency of finfish to aggregate around man-made structures (the fish aggregating device – FAD effect), to increased noise, turbulence and electromagnetic fields that may cause adverse effects (Bergström et al. 2014). In the case of benthic communities, OWFs introduce hard substrate suitable for colonization into areas where such substrate may be naturally scarce (i.e., soft bottom habitats), leading to changes in total organism community composition and resulting trophic interactions (Degraer et al. 2020). Potentially, OWF structures can act as steppingstones of invasive organisms across otherwise unreachable distances (Lloret et al. 2022). As a floating OWF, Hywind Scotland fundaments do not reach all the way to the

seafloor, however the floating fundaments, that reach 80 m down into the water column, and anchoring structures, still represent significant area for colonization. A previous study found clear bands on zonation of the fundaments, with a littoral upper band, and sublittoral organisms on the deeper part of the fundaments (Karlsson et al. 2022). Growth of fouling organisms in particular mussels and amphipods, has been shown to significantly increase secondary production in a windfarm in the southern North Sea, leading to carbon assimilation and increased flux of organic carbon to the seafloor (Mavraki et al. 2020). To which degree these changes affect seafloor communities below the structures at Hywind Scotland has not previously been studied.

Methods based on environmental DNA (eDNA), including metabarcoding, have increasingly become common in the scientific literature in the recent decade. The potential of metabarcoding in characterizing organism communities has been recognized for some time (Taberlet et al. 2012; Bik et al. 2012). Sources of eDNA can be filtered water, sediment or other substrate, or organism bulk samples retrieved from the appropriate environment by e.g., sieving or scraping sediment or hard substrate. Several recent studies have sought to employ metabarcoding of marine sediment as a complement to existing biological softbottom impact parameters based on >1 mm macrofauna, which currently form the basis of softbottom monitoring in the North Sea and elsewhere (Aylagas et al. 2018; Lanzén et al. 2021; Mauffrey et al. 2021). Depending on the metabarcoding genetic marker employed, different parts of the organism community can be targeted, including all metazoans (animals), meio- (<1mm) or macrofauna, single-celled eukaryotes (protists) or even prokaryotes.

There are numerous methodological issues that need to be satisfactorily resolved in order to mature metabarcoding towards regulatory implementation (Zinger et al. 2019), and proof-of-concept studies such as this one aim to showcase the methodology in real-life situations in order to build experience with the data type, paving the way for future routine implementation. Direct eDNA extraction from sediment – rather than bulk community samples – represents a less work-intensive approach. Lanzén et al. (2021) showed the potential of sediment samples in characterizing local environmental impact through changes in total eukaryote community composition as observed using the 18S V1-V2 ribosomal DNA (rDNA) marker (Sinniger et al. 2016). In the case of macrofauna in particular, more high-quality eDNA data are obtainable through bulk organism samples – crushed and mixed tissue from all animals in an e.g. sieved sample – rather than directly from sediment, but in a sediment-based eDNA study, adding the macrofaunal marker cytochrome oxidase subunit I (COI), provides additional data with little extra effort, though this marker is more vulnerable to uneven distribution of DNA in the sediment compared to single-celled eukaryotes.

In this study, we used molecular metabarcoding of the 18S V1-V2 rDNA and COI markers from benthic sediment eDNA samples to (i) measure the 18S V1-V2 eukaryote and COI metazoan benthic community composition of eDNA extracted from the Hywind Scotland sediment samples; (ii) assess the performance of the chosen eDNA sampling design, sample type, replicate number and molecular markers in detecting anthropogenic impact; and (iii) how metabarcoding results compare to morphological taxonomy of 1 mm sieved macrofauna from the same sampling stations.

The 18S V1-V2 marker is commonly used in the scientific literature, and was chosen as it was expected to provide data on sequences from several protist groups, meiofauna (animals <1 mm) and some macrofauna (>1 mm) (Sinniger et al. 2016); the COI marker is the standard macrofauna marker in the Barcode of Life initiative when barcoding animals directly, which means that it has the

highest coverage in online databases, such as GenBank, that allow sequence variants to be identified to known species (Andújar et al. 2018).

In this report, the specific list of stations and the sampling design at Hywind Scotland have been moved to the results section, with materials and methods being reserved for general methodological topics. Similarly, strictly environmental assessment of Hywind Scotland based on a combination of eDNA and morphotaxonomic data is found at the end of the results section, while the discussion contains a treatment on the applicability of the eDNA approach in benthic monitoring. These choices were made to increase consistency with the structure of existing routine monitoring reports and anticipates a similar structure being used in future cases where several installations or areas will be examined in the same report.

2. Materials and methods

2.1. Study area

The Hywind Scotland floating OWF started operation in 2017 and is located 25 km east of Peterhead at the Buchan Deep. It is composed of five floating turbine units with a hub height of 82-101 m and rotor diameter of 154 m, placed 800-1600 m apart, each moored by three anchors with 600-1200 m mooring radius, and is further connected by 33 kV inter-array cables connected to the Peterhead Grange Substation. The following information is adapted from the Hywind Scotland Environmental Statement (Statoil 2015).

The water depth is 100-120 in the OWF area, with a tidally driven predominantly north-south wave direction. Bottom conditions are a blend of sand and gravel with scattered boulders (defined as "circalittoral fine sand"), no significant contamination levels measured, with megafauna including sparse hermit crabs, brittle stars (*Ophiura* sp.), hydroids and anemones on the scattered hard substrate. Main infaunal species include polychaetes *Scoloplos armiger*, *Spiophanes bombyx* and *Owenia fusiformis* and echinoderms *Ophiura affinis*, *Amphiura filiformis*, *Echinocyamus pusillus* and *Spatangus* sp. (Duranovic et al. 2013).

2.2. Field sampling

benthic samples in this report were collected by DNV on May 11.-12., 2022. The sediment sampling equipment used on the cruise was a standard 0.1 m^2 surface area van Veen grab sampler for the acquisition of morphotaxonomy and physicochemical parameters. A separate report based on standard environmental parameters including 1 mm macrofauna data, total organic carbon (TOC) and grain size distribution data is available from DNV (Møskeland, Fjukmoen, and Melsom 2023). Data from that report have been included here to investigate the correlation of the macrofauna and eDNA datasets, in order to assess the performance of the eDNA environmental parameters.

Samples for eDNA were collected as part of this existing sampling design, i.e., by subsampling the undisturbed surface of the van Veen grab replicates. Commonly, a combination grab with a dedicated side chamber allows the simultaneous sampling of morphotaxonomical and other parameters, depending on the planned parameters at a particular sampling station. The eDNA sampling design is similar to that of Lanzén et al. (2021): Three sediment subsamples were collected and pooled together in a single 50 mL Falcon tube for each grab sample, approximately 30 g sediment in total. Sampling equipment was thoroughly cleaned between samples using a 20% household bleach solution, and disposable gloves was used during sampling. Sediment subsamples were kept frozen at –20 °C until the end of the field cruise and subsequently transported to NORCE lab facilities in Bergen for extraction of sediment eDNA (Fig. 2).

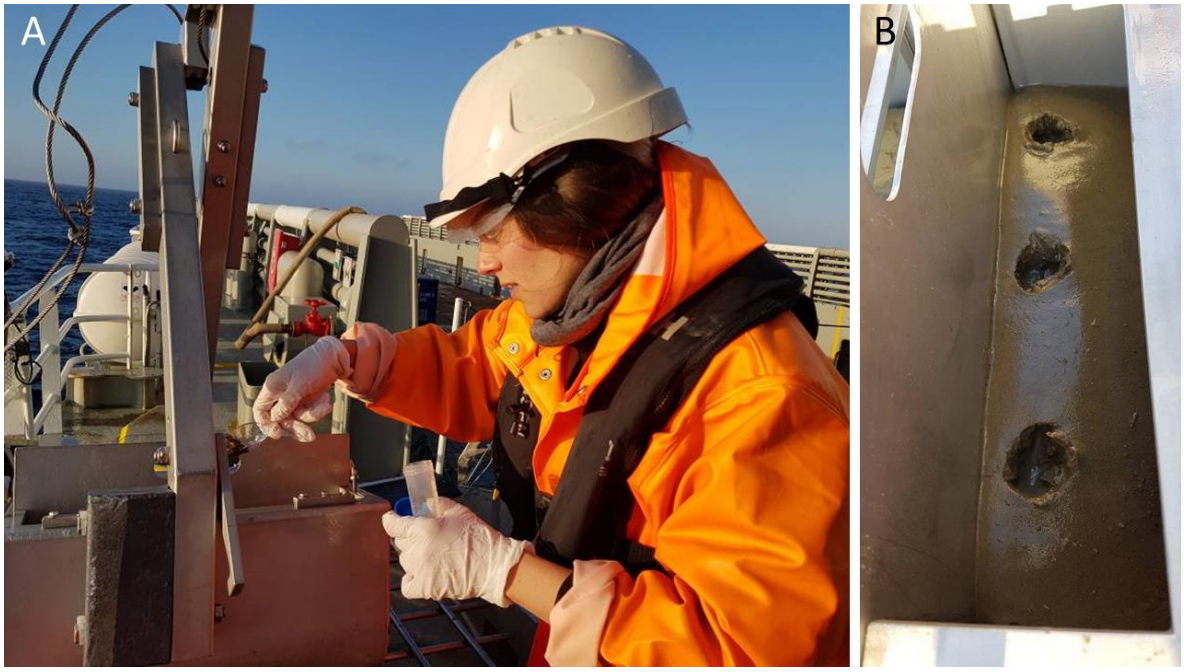


Figure 2. *A) Sediment eDNA sampling from van Veen grab sampler into a 50 mL tube for subsequent freezer storage, B) sediment subsamples were pooled from three locations from the top 2 cm of the grab surface, here taken from the side chamber of a two-chamber grab sampler.* Photo: Jon Hestetun.

2.3. Lab processing

DNA was extracted from all sample replicates similar to the protocol used in Hestetun et al. (2021): Sediment samples were thawed at +4 °C and stirred by hand for approximately one minute prior to DNA extraction. Three 0.5 g sediment replicates were made from each sediment sample and extracted using Qiagen PowerSoil tubes and C1 solution. Extraction steps included homogenization using a Precellys homogenizer (3 x 4000 rpm for 40 s). After centrifugation (10K rpm for 1 min) a Qiagen QIAsymphony SP robot (DSP DNA kit, Tissue LC protocol) was used for remaining extraction steps. Extract concentrations were measured using a Qubit 3.0 fluorometer (Thermo Fisher Scientific).

Extract replicates from each sediment sample were pooled prior to PCR amplification. Amplification involved a single PCR and custom nested barcode approach with individual tags at both library and sample level. Two molecular markers were amplified: the 350-400 bp 18S rRNA gene V1-V2 region, capturing both multicellular metazoans and a broad range of single-cell eukaryotes, and 313 bp of cytochrome c oxidase subunit I (COI), targeting metazoans only. For 18S rRNA, the primers SSU_F04mod (5'-GCTTGWCTCAAAGATTAAGCC-3') (Cordier pers. comm.) and SSU_R22 (5'-CCTGCTGCCTTCCTTRGA-3') (Sinniger et al. 2016) were used; for COI, the primers mlCOIintF-XT (5'-GGWACWRGWTGRACWITITAYCCYCC-3') (Wangensteen et al. 2018) and jgHCO2198 (5'-TAIACYTCIGGRTGICCRAARAAYCA-3') (Geller et al. 2013). Annealing temperatures were set to 57 °C and 45 °C for 18S and COI respectively. COI primer concentration was tripled relative to 18S to account for the higher number of ambiguous bases in the COI primers. Extraction and PCR negative controls were used to detect contamination during processing. After amplification, PCR products

were sent to Novogene for subsequent ligation and high throughput sequencing on the Illumina NovaSeq platform.

2.4. Bioinformatic analysis

Demultiplexing at library level was done by Novogene. Additional multiplexing of each library-level file into individual sample raw fastq files and removal of primer sequences were done using an in-house demultiplexing pipeline. Individual sample fastq files were subsequently denoised and pairwise merged using dada2 with a NovaSeq-adapted error profile (Callahan et al. 2016). SWARM v2.2.1 (Mahé et al. 2015) was used to derive OTUs from dataset sequences, using a d parameter of 3 for COI, and 1 for 18S data. Taxonomy was assigned using CREST4 4.2.6 with the SilvaMod 1.38 database for 18S data, and MIDORI 246 for COI data (Lanzén et al. 2012). Cross contamination filtering was done using a custom function analogous to UNCROSS (Edgar 2016) and decontam (Davis et al. 2018), and additional abundance filtering at $2*10^{-5}$ relative abundance for OTUs over the entire dataset.

Multivariate analysis, including 4^{th} root transformation, Bray-Curtis dissimilarity, and non-metric multidimensional scaling (nMDS) were done using Primer 7 (Clarke and Gorley 2015). Species accumulation curves were done using the specaccum function (2000 permutations) in the R vegan package v 2.5-7 (Oksanen et al. 2019). Euler diagrams were done using the R eulerr package v 7.0.0 (Larsson J 2022).

2.5. Biotic indices

Impact on offshore benthic communities is typically assessed from 1 mm macrofauna through a combination of alpha- and beta diversity parameters, and biotic sensitivity indices (Norwegian Environment Agency 2020). Common parameters for alpha diversity include number of species (richness), number of specimens, and the species distribution (diversity), the latter often measured using the Shannon index (H'). In accordance with Norwegian regulations, Shannon index values in this report are calculated using $\log_2$. Notably, Shannon index values do not translate across datasets, i.e., the morphological, 18S and COI in this report, and should only be used for internal comparison within each dataset.

Biotic sensitivity indices also consider sensitivity values or eco groups assigned to individual species. In Europe, the Azti Marine Biotic Index (AMBI) is the most used sensitivity index (Borja, Franco, and Pérez 2000), but there are also two Norwegian indices, the indicator species index (ISI) and the Norwegian Sensitivity Index (NSI) (Rygg 2002). These indices are dependent on matching species in the dataset, meaning that they can also be applied to OTUs in metabarcoding that can be identified to species level. However, as metabarcoding taxonomy databases are incomplete this will only be usable for parts of the metabarcoding data. Still, applying existing biotic indices on COI metabarcoding data (as the COI marker has the least incomplete taxonomy data) has been used in certain studies with some success (Hestetun, Lanzén, and Dahlgren 2021; Lejzerowicz et al. 2015), and these indices have thus been calculated for COI data here, using the R BBI package (Cordier and Pawlowski 2018).

3. Hywind Scotland results

3.1. General description

The Hywind Scotland OWF is located at the Buchan Deep at 100-120 m depth. Bottom conditions at all sampled stations were described as grayish brown sand during sampling (defined as “circalittoral fine sand”) The van Veen grab sampling stations at the Hywind OWF is listed in Table 1, and their relative placement in relation to the Hywind turbines is shown in Figure 3.

Table 1. *Benthic stations at Hywind Scotland sampled with van Veen grab part of this study. Station names are composed of turbine number, transect order and direction. Latitude and longitude are given in WGS84 decimal degrees, heading gives the direction (in degrees) of the corresponding transect, and distance signifies the distance of the corresponding station from the turbine center.*

Name	Date	Lat	Long	Depth	Heading	Distance
HS1-1 NE	2022-05-11	57.4845	-1.3321	117.5	18	25
HS1-2 NE	2022-05-11	57.4851	-1.3317	117.5	18	100
HS1-3 NE	2022-05-11	57.4860	-1.3312	118.0	18	200
HS1-1 SW	2022-05-11	57.4842	-1.3327	116.5	255	25
HS1-2 SW	2022-05-11	57.4841	-1.3339	116.5	255	100
HS1-3 SW	2022-05-11	57.4839	-1.3355	117.5	255	200
HS2-1 NE	2022-05-12	57.4910	-1.3518	111.5	19	25
HS2-2 NE	2022-05-12	57.4916	-1.3513	112.5	19	100
HS2-3 NE	2022-05-12	57.4924	-1.3507	112.5	19	200
HS2-1 SE	2022-05-11	57.4906	-1.3517	113.0	140	25
HS2-2 SE	2022-05-11	57.4901	-1.3509	114.0	140	100
HS2-3 SE	2022-05-11	57.4894	-1.3498	116.0	140	200
Ref-1	2022-05-12	57.5022	-1.3711	106.0	NA	NA
Ref-2	2022-05-12	57.4973	-1.3454	119.5	NA	NA
Ref-3	2022-05-11	57.4717	-1.3435	117.5	NA	NA

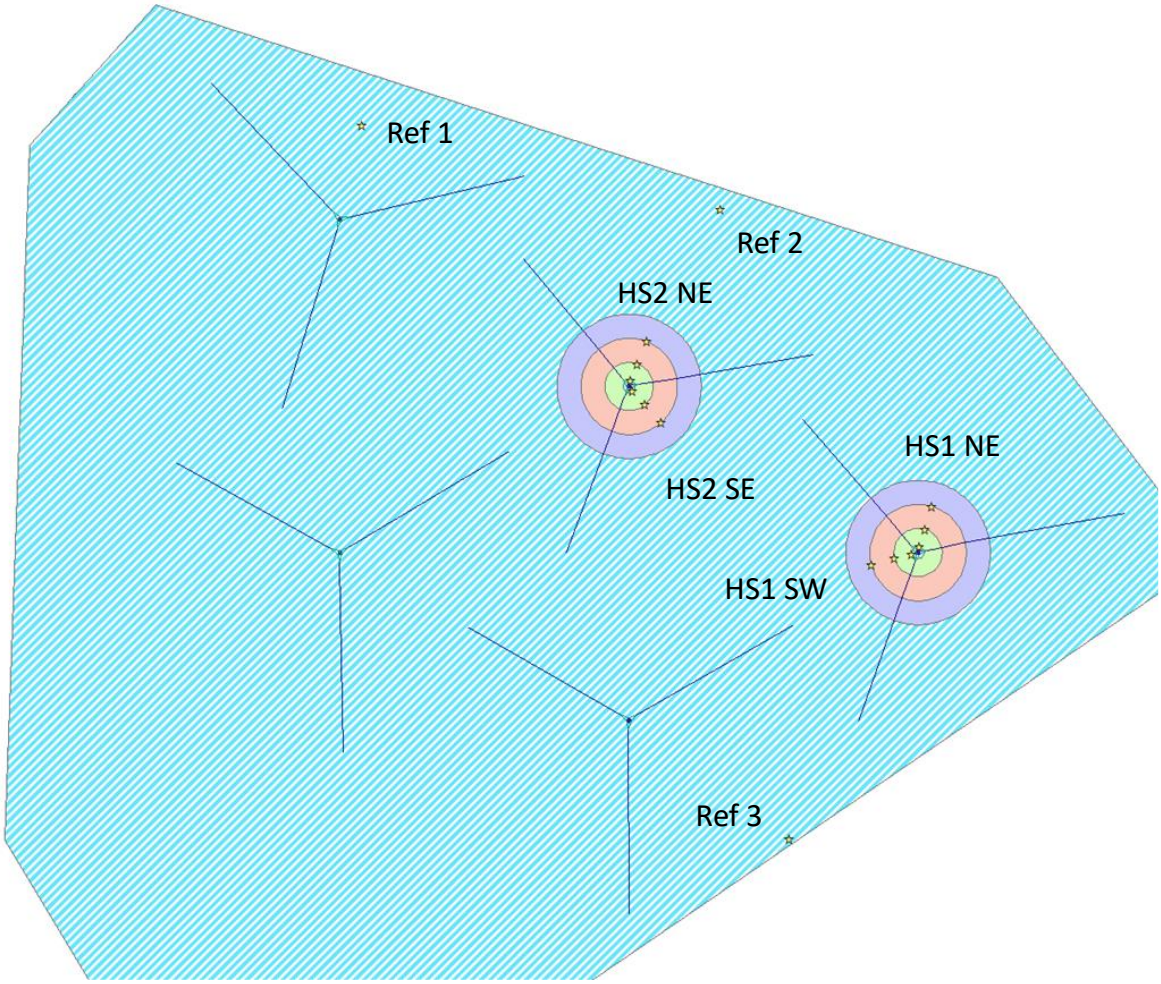


Figure 3. *Detailed map showing the placement of sampling stations relative to Hywind turbines. Each of the two turbines HW1 and HW2 were given two transects of sampling stations: For each of the transects HS1 NE, SW and HS2 NE, SE, three stations were sampled at approximately 25, 100 and 200 m distance.*

3.2. Previous environmental data

A benthic baseline study was done prior to the establishment of the OWF (Duranovic et al. 2013), The turbine area was classified as offshore circalittoral sand, with no special features noted. While minor *Sabellaria spinulosa* reefs were noted in the general area, none were registered in the turbine area itself. Chemical parameters generally exceeded "low" OSPAR environmental assessment criteria (EAC) threshold levels for most metals. No hydrocarbon (THC, PAHs) impact was detected (Duranovic et al. 2013).

Colonization of turbines and moorings was assessed in 2020. This visual study confirmed high degree of colonization and colonization patterns consistent with an intermediate colonization succession stage, and clear depth zonation in species composition (Karlsson et al. 2022).

Plankton and fish community dynamics and composition were studied in two recent studies deploying an autonomous surface vessel (Sailbuoy) and water column eDNA (Ramasco 2022; Ray et

al. 2022). The Sailbouy study did report increased production of primary and secondary producers (phyto- and zooplankton) in the OWF and attributed this to potential increased upwelling in the OWF area due to water column mixing, though it did not conclude that there was a consistent increase in fish biomass. The eDNA report did show some increased abundance of annelids and echinoderms in the OWF at 10 m, possibly related to larval dispersal, and while herring was more abundant in the OWF relative to the study reference area, no clear pattern in the total fish community was evident, and the single-point nature of that study means it could not support any conclusions regarding persistent patterns.

3.3. Morphological results

Morphological data based on 1 mm sieve fraction macrofauna were collected, processed, and analyzed by DNV (Møskeland, Fjukmoen, and Melsom 2023). These species lists were also analyzed here to provide a comparison to the eDNA results in this report.

The whole morphological dataset contained 10 010 specimens in 212 separate species. This comprised eight separate phyla, with Annelida as the most abundant phylum, followed by Arthropoda and Mollusca (Fig 4).

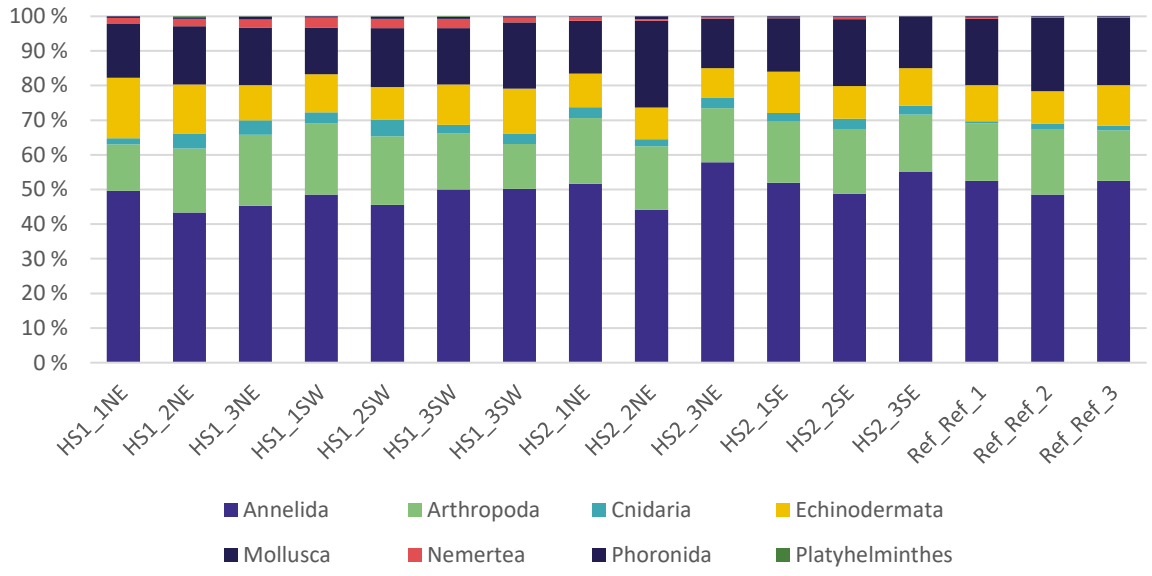


Figure 4. *The relative abundance at phylum level shown separately for each station in the DNV Hywind Scotland morphological dataset. The three rightmost stations are reference stations.*

Table 2. *The twenty most abundant metazoan OTUs in the Hywind DNV morphological dataset.*

Species	Phylum	Sum	Percentage
Scoloplos armiger	Annelida	1 763	17.61%
Amphiura filiformis	Echinodermata	531	6.44%
Kurtiella bidentata	Mollusca	519	2.93%
Ophiuroidea juv.	Echinodermata	475	2.76%
Bathyporeia elegans	Arthropoda	473	2.83%
Eudorellopsis deformis	Arthropoda	363	2.23%
Ophelia borealis	Annelida	327	2.06%
Spiophanes kroyeri	Annelida	314	2.02%
Cerianthus lloydii	Cnidaria	263	1.72%
Antalis	Mollusca	238	1.59%
Chaetozone	Annelida	223	1.51%
Ennucula tenuis	Mollusca	210	1.45%
Harpinia antennaria	Arthropoda	173	1.21%
Diplocirrus glaucus	Annelida	155	1.10%
Abra prismatica	Mollusca	154	1.10%
Lanice conchilega juv.	Annelida	134	0.97%
Nemertea	Nemertea	132	0.96%
Prionospio fallax	Annelida	128	0.94%
Thracia villosiuscula	Mollusca	126	0.94%
Lanice conchilega	Annelida	114	0.86%

The 20 most common taxa in the dataset are given in Table 2, consisting of a mix of common macrofaunal phyla including annelids, arthropods, echinoderms, and mollusks.

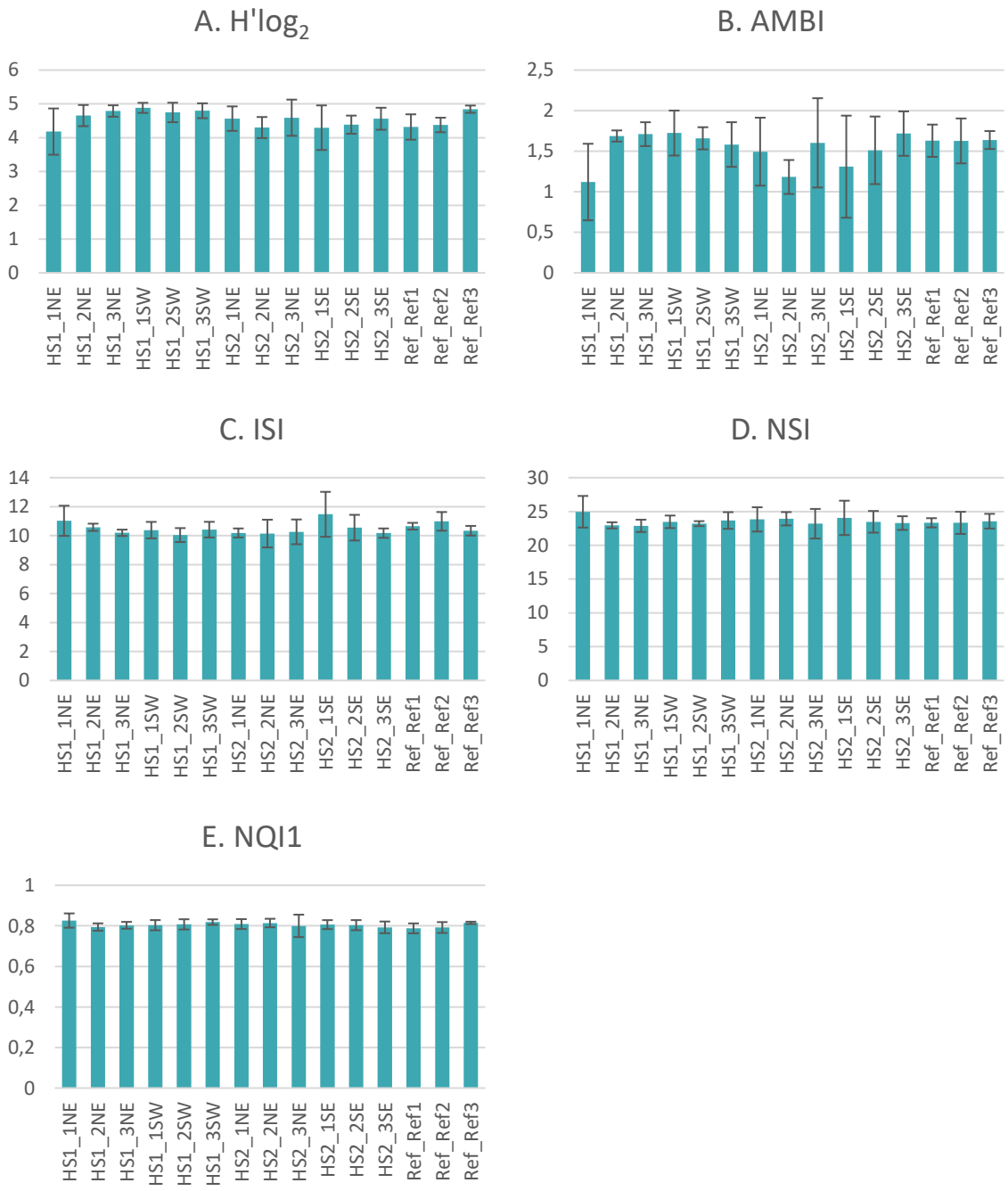


Figure 5. *Biotic index values from the DNV morphological dataset including A) Shannon diversity (H'log$_2$), B) AMBI, C) ISI, D) NSI, and E) NQI1, with standard deviation based on station inter-replicate variation.*

Shannon and biotic sensitivity index values including AMBI, ISI and NSI were calculated on the dataset, and are shown in Figure 5.

Bray-Curtis pairwise dissimilarities between morphological samples at the grab level were plotted from a fourth root transformed dataset using an nMDS plot (Fig. 6). The morphological data showed a separation between the three main areas in the dataset, i.e., the two turbines and the references stations. These results echo the similar analysis (at station level) in the DNV report (Møskeland, Fjukmoen, and Melsom 2023).

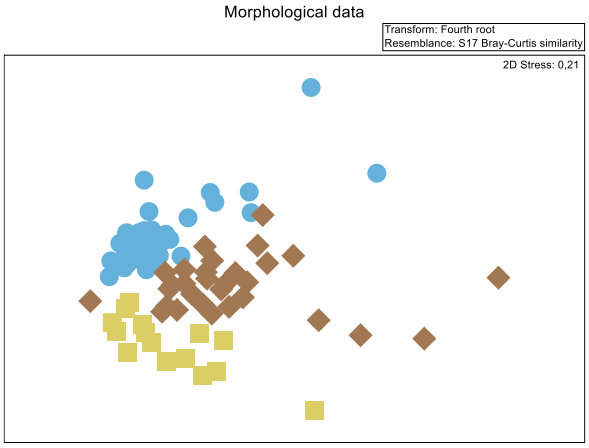


Figure 6. *Bray-Curtis dissimilarities from fourth root transformed data of the DNV morphological data plotted using multivariate nMDS with nMDS1 and nMDS2 as axes. Blue circles represent Hywind turbine 1, brown diamonds turbine 2, and yellow squares reference stations.*

The species accumulation curve (Fig. 7) shows the average species increase for each new station in the morphological dataset. The continued rise of the curve shows that more species would be expected with increased sampling beyond the dataset analyzed here.

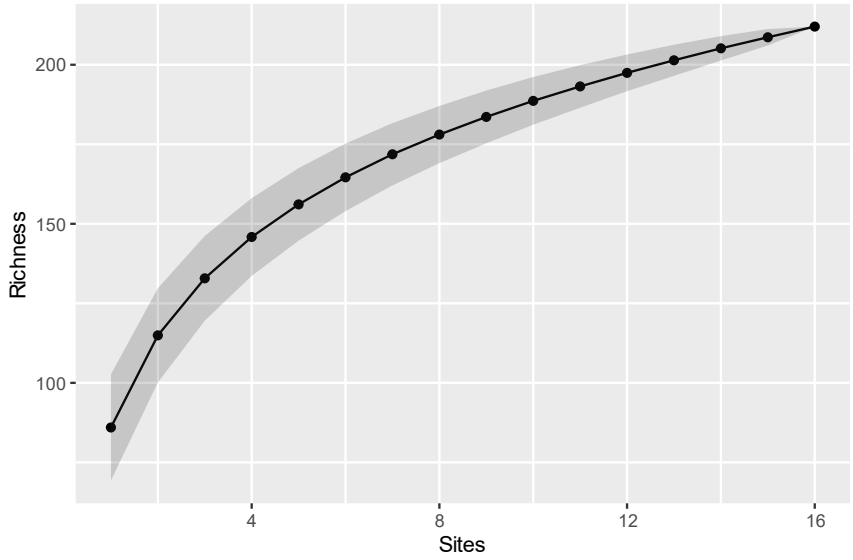


Figure 7. *Average increase in species richness (Y) with increased number of stations (X) in the morphological dataset based on 2000 permutations. The shaded area shows between-station variability in species increase for individual permutations.*

3.4. Metabarcoding 18S results

After initial demultiplexing and primer trimming, the 18S rRNA V1-V2 universal eukaryote dataset contained 28 201 476 reads over 51 data replicates: 36 OWF and 9 reference sample replicates (3 for each sampling station) and 6 negative controls. Bimera removal after dada2 processing reduced the number of reads to 28 189 209. Length (350-400 bp), UNCROSS and decontam filtering reduced the number of reads to 25 522 825, distributed over 14 614 ASVs. After SWARM clustering, decontam and UNCROSS filtering, 10 019 OTUs were identified from ASVs, reduced to 1473 OTUs

following a $2*10^{-5}$ abundance cutoff. Taxonomic assignment of these OTUs after abundance filtering, using CREST4 with the SilvaMod 1.38 database, yielded 444 taxonomic groups at various level of resolution.

For the whole dataset, read abundance at high taxonomic level showed that the protist SAR supergroup clades Stramenopiles (30%), Alveolata (24%), and Rhizaria (19%) comprised the majority of the dataset. Metazoa comprised 16% of the dataset, Chloroplastida 4%, six other clades 2%, finally 5% that could not be assigned to any taxon (Fig. 8).

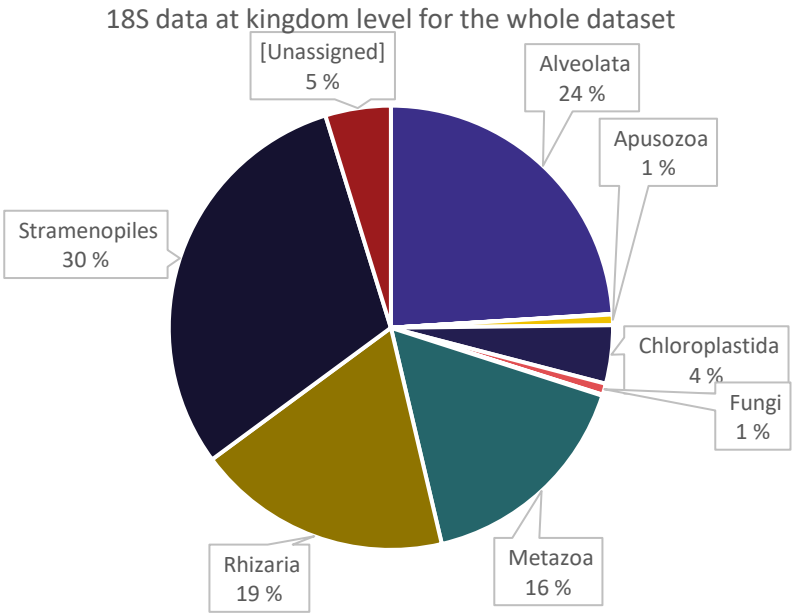


Figure 8. *18S V1-V2 read abundance for major clades in the whole Hywind dataset, shown as percentages.*

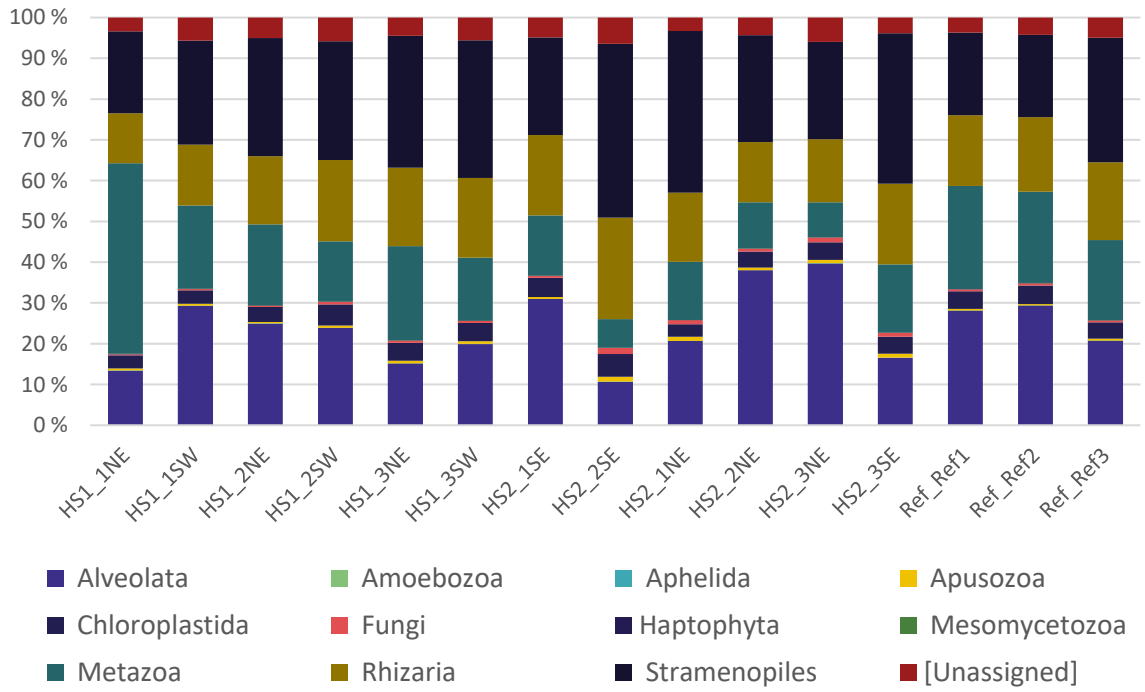


Figure 9. *18S V1-V2 relative abundance of higher-level taxonomic groups (roughly "kingdom" level) shown separately for each station in the Hywind Scotland dataset. The three rightmost stations are reference stations.*

The total number of sequences varied between samples (Appendix A), but relative abundance at high taxonomic level remained steady through the dataset, with the most variation found in metazoan reads (Fig. 9).

Table 3. *The twenty most abundant OTUs in the total Hywind 18S V1-V2 dataset.*

OTU	Clade	Identity	Sum	Percentage
OTU_1	Stramenopiles	Oomycota	4 025 625	15.80%
OTU_2	Alveolata	*Pelagostrobilidium*	3 814 667	14.98%
OTU_3	Stramenopiles	Oomycota	1 707 290	6.70%
OTU_7	Rhizaria	TAGIRI1-lineage X sp.	760 708	2.99%
OTU_5	[Unassigned]	[Unassigned]	510 211	2.00%
OTU_6	Metazoa	unclassified Thoracostomopsidae	489 835	1.92%
OTU_8	Metazoa	*Oncholaimus*	376 548	1.48%
OTU_10	Metazoa	*Temora longicornis*	348 977	1.37%
OTU_11	Chloroplastida	Pycnococcaceae	292 151	1.15%
OTU_13	Rhizaria	*Cryothecomonas*	259 310	1.02%
OTU_12	Rhizaria	Euglyphida	247 166	0.97%
OTU_25	Rhizaria	Mataza-lineage X sp.	205 922	0.81%
OTU_15	Alveolata	Choreotrichida	202 397	0.79%
OTU_16	Metazoa	*Anomalocardia auberiana*	197 857	0.78%
OTU_20	Rhizaria	*Cryothecomonas* sp.	190 841	0.75%
OTU_17	Alveolata	Suessiaceae	174 626	0.69%
OTU_18	Stramenopiles	Labyrinthulaceae X	154 618	0.61%
OTU_19	[Unassigned]	[Unassigned]	154 381	0.61%
OTU_24	Metazoa	*Abatus cavernosus*	141 776	0.56%
OTU_21	Chloroplastida	Dolichomastigaceae-B	135 119	0.53%

The twenty most abundant OTUs in the entire eukaryote dataset included SAR protists (Stramenopiles, Alveolata, and Rhizaria), two nematodes, a calanoid copepod, a mollusk and one echinoderm, in addition to two unclassified OTUs (Table 3). This data is shown at station level in Appendix B.

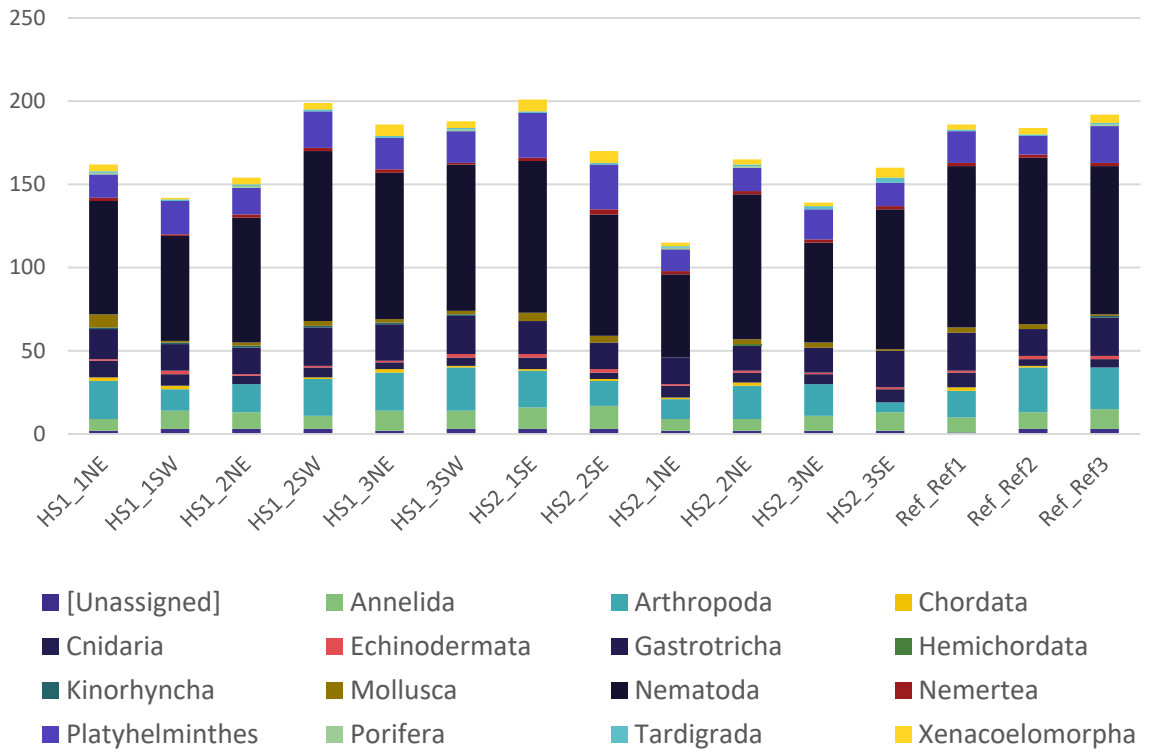


Figure 10. *Absolute richness (number of unique OTUs) in the metazoan fraction of the 18S rDNA V1-V2 dataset, shown at phylum level for each station in the data.*

Looking more specifically at the metazoan part of the 18S dataset, there were 380 unique OTUs in the dataset. Richness (the number of unique OTUs) for each station was relatively even for all stations in the dataset and across metazoan phyla. The phyla with the highest number of unique OTUs included nematodes, arthropods, gastrotrichs, nemerteans, annelids, and cnidarians (Figure 10).

Table 4. *The twenty most abundant metazoan OTUs in the Hywind 18S V1-V2 dataset.*

OTU	Phylum	Taxon	Total	Percentage
OTU_6	Nematoda	unclassified Thoracostomopsidae	489 835	12.07%
OTU_8	Nematoda	*Oncholaimus*	376 548	10.56%
OTU_10	Arthropoda	*Temora longicornis*	348 977	10.94%
OTU_16	Mollusca	*Anomalocardia auberiana*	197 857	6.96%
OTU_24	Echinodermata	*Abatus cavernosus*	141 776	5.36%
OTU_31	Nematoda	Monhysterida	105 908	4.23%
OTU_32	Nemertea	*Cephalothrix filiformis*	104 087	4.34%
OTU_57	Nematoda	unclassified Oncholaimidae	100 586	4.39%
OTU_50	Arthropoda	Maxillopoda	97 683	4.46%
OTU_36	Arthropoda	*Halacaroides antoniazziae*	92 555	4.42%
OTU_52	Platyhelminthes	*Cheliplana* cf. *orthocirra*	64 167	3.21%
OTU_113	Nematoda	*Calomicrolaimus parahonestus*	59 591	3.08%
OTU_66	Arthropoda	*Leptocythere*	57 513	3.06%
OTU_62	Nematoda	Araeolaimida	54 268	2.98%
OTU_68	Arthropoda	Calanoida	49 431	2.80%
OTU_78	Arthropoda	Harpacticoida	40 788	2.38%
OTU_95	Nematoda	Araeolaimida	36 537	2.18%
OTU_112	Nematoda	Monhysterida	35 814	2.19%
OTU_88	Nematoda	Enoplida	35 105	2.19%
OTU_96	Nematoda	*Bathylaimus*	32 100	2.05%

The twenty most abundant metazoans in the 18S V1-V2 dataset are given in Table 4. Nematodes constituted the majority of the metazoan reads, with some abundant OTUs belonging to other phyla including calanoid arthropods, Mollusca, flat- and roundworms.

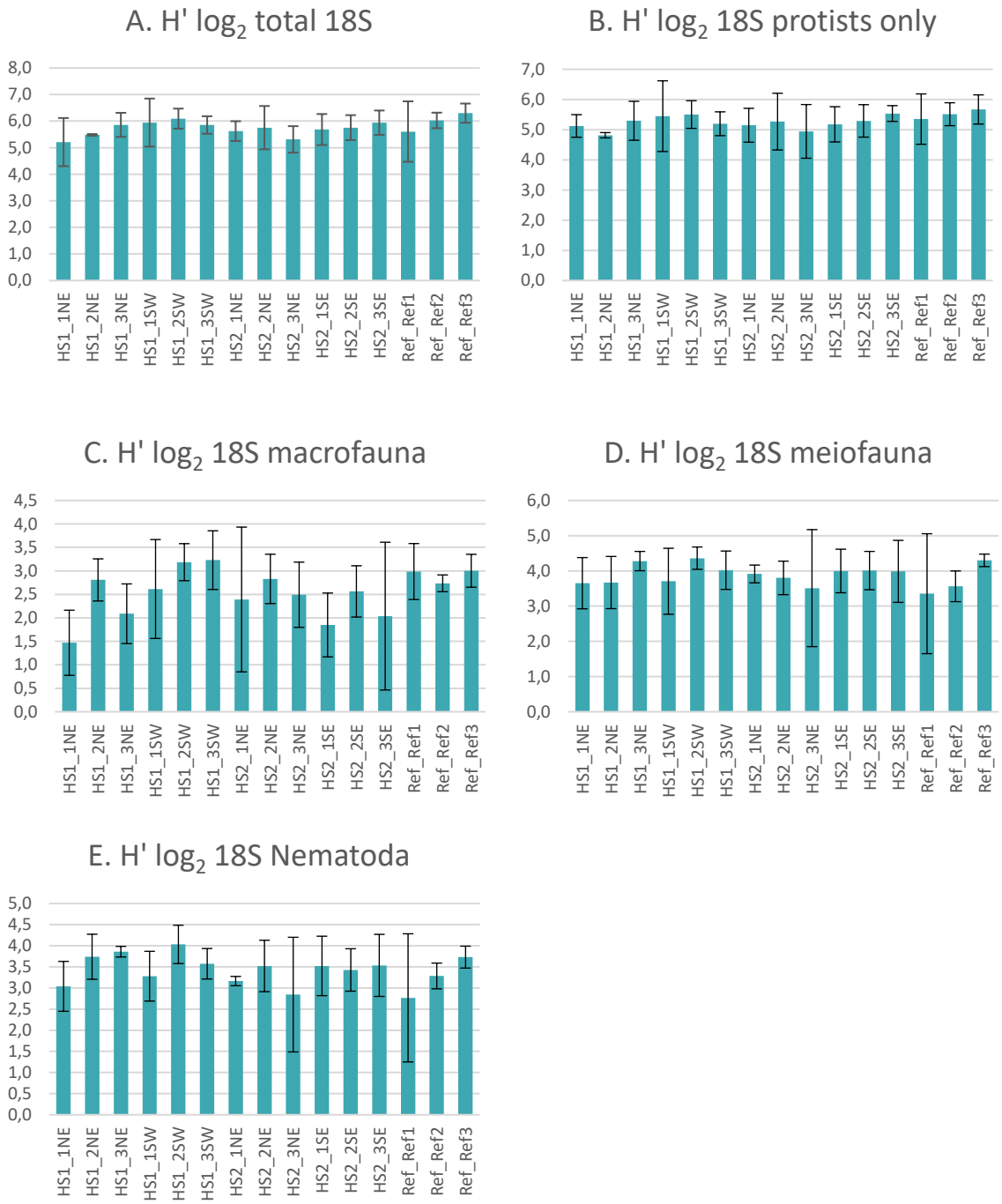


Figure 11. *Shannon index log_2-values calculated for A) the full 18S dataset, B) protist (non-metazoan) taxa only, C) macrofaunal metazoan phyla only, D) meiofaunal metazoan phyla only, and E) nematodes only, with standard deviation based on station inter-replicate variation.*

Shannon (H'log_2) values were calculated for the whole 18S V1-V2 dataset, and separately for protists only (also including Chloroplastida), macrofauna, meiofauna, and nematodes only (Fig 11). Note that the values cannot be compared across datasets.

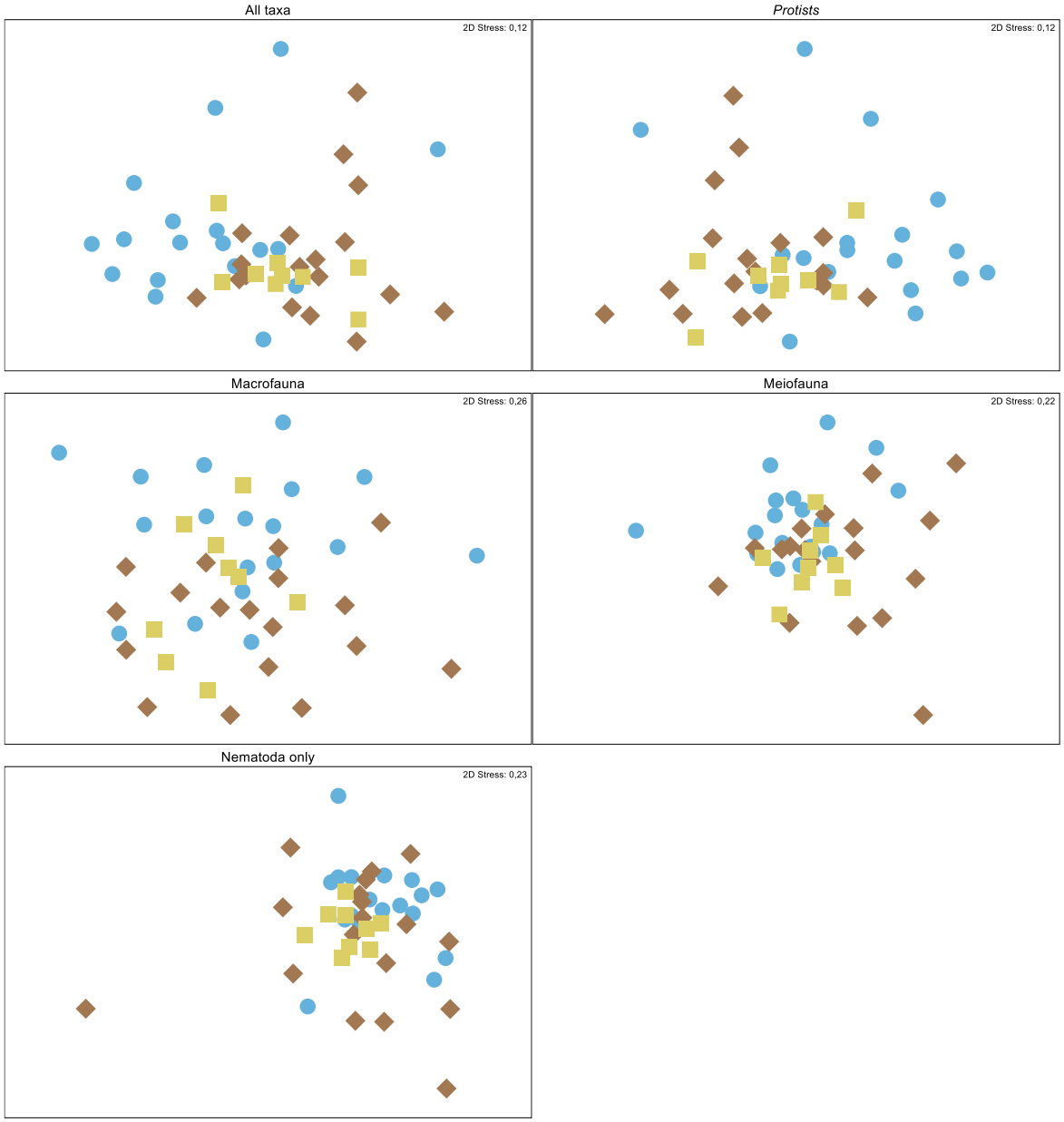


Figure 12. *Bray-Curtis dissimilarities from fourth root transformed data of (A) total, (B) protists, (C) macrofaunal phyla, (D) meiofaunal phyla, and (E) nematodes only plotted using multivariate nMDS with nMDS1 and nMDS2 as axes. Blue circles represent Hywind turbine 1, brown diamonds turbine 2, and yellow squares reference stations.*

Similarity between the 18S OTU tables of the individual stations and their replicates were plotted, using Bray-Curtis dissimilarity based on fourth root transformed data, using nMDS multivariate analysis. As with Shannon calculations, this was done separately on different parts of the dataset (Fig. 12).

The species accumulation curve for the complete 18S rDNA V1-V2 dataset (Fig. 13) shows the average OTU increase for each new station in the data. The steep initial and subsequently flattening curve shows that many OTUs in the dataset is detected even from a subset of stations in the dataset.

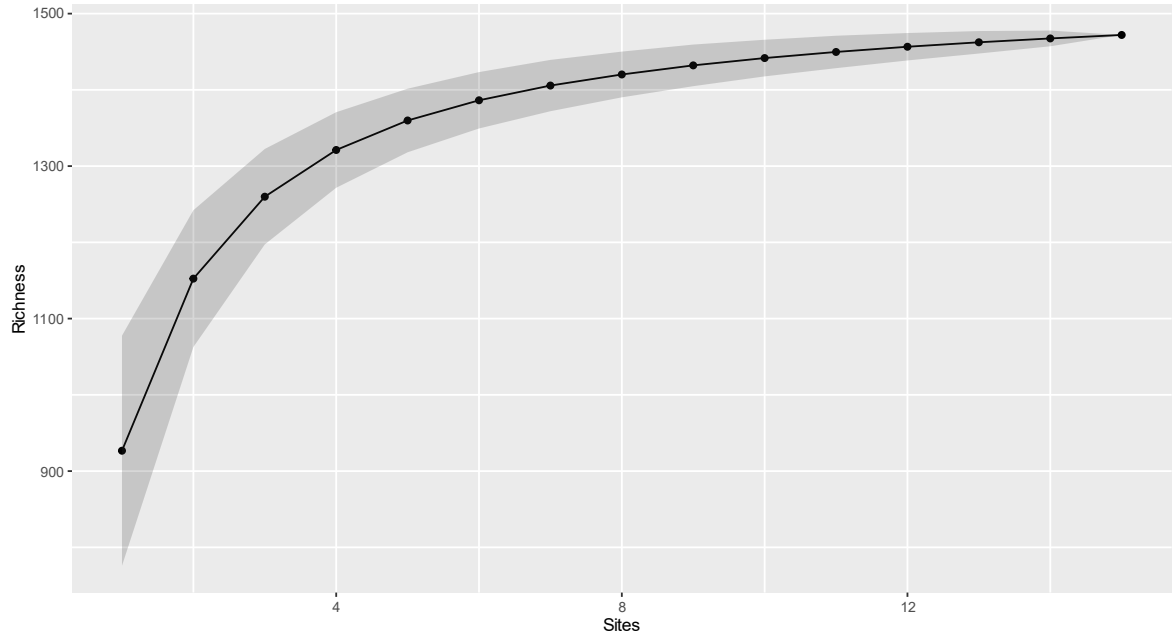
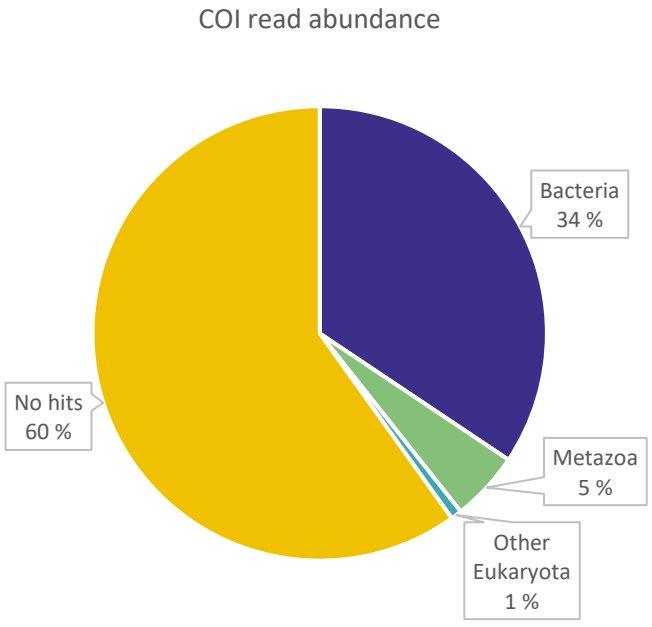


Figure 13. *Average increase in OTU richness (Y) with increased number of stations (X) in the 18S rDNA V1-V2 dataset based on 2000 permutations. The shaded area shows between-station variability in species increase for individual permutations.*

3.5. Metabarcoding COI results

After initial demultiplexing and primer trimming, the COI dataset contained 37 635 255 reads over 51 data replicates: 36 OWF and 9 reference sample replicates (3 for each sampling station) and 6 negative controls. Bimera removal after dada2 processing reduced the number of reads to 37 635 197. UNCROSS and decontam filtering reduced the number of reads to 35 383 820. Dada2 yielded 51 952 ASVs, reduced to 37 545 OTUs with SWARM (d=3). The $2*10^{-5}$ relative abundance filter reduced the number of OTUs to 2339 and the number of reads to 31 823 186.



The majority of COI data were either unassigned (19 854 826 reads, 1495 OTUs) or bacterial (11 403 024, 686 OTUs) sequences. Eukaryotes included non-metazoan (259 619 reads, 39 OTUs) sequences in addition to metazoan sequences. As non-metazoans are non-target for the COI marker, non-metazoan reads were removed from the COI dataset, leaving 1 643 687 reads distributed across 118 OTUs for further analysis (Fig. 14).

Figure 14. *Relative read abundance of metazoan and non-metazoan fractions of the Hywind COI dataset.*

There were nine phyla in the metazoan COI dataset. With the exception of the meiofaunal groups nematodes and gastrotrichs, these represented the macrofaunal groups that are targeted using the COI marker (Fig. 15).

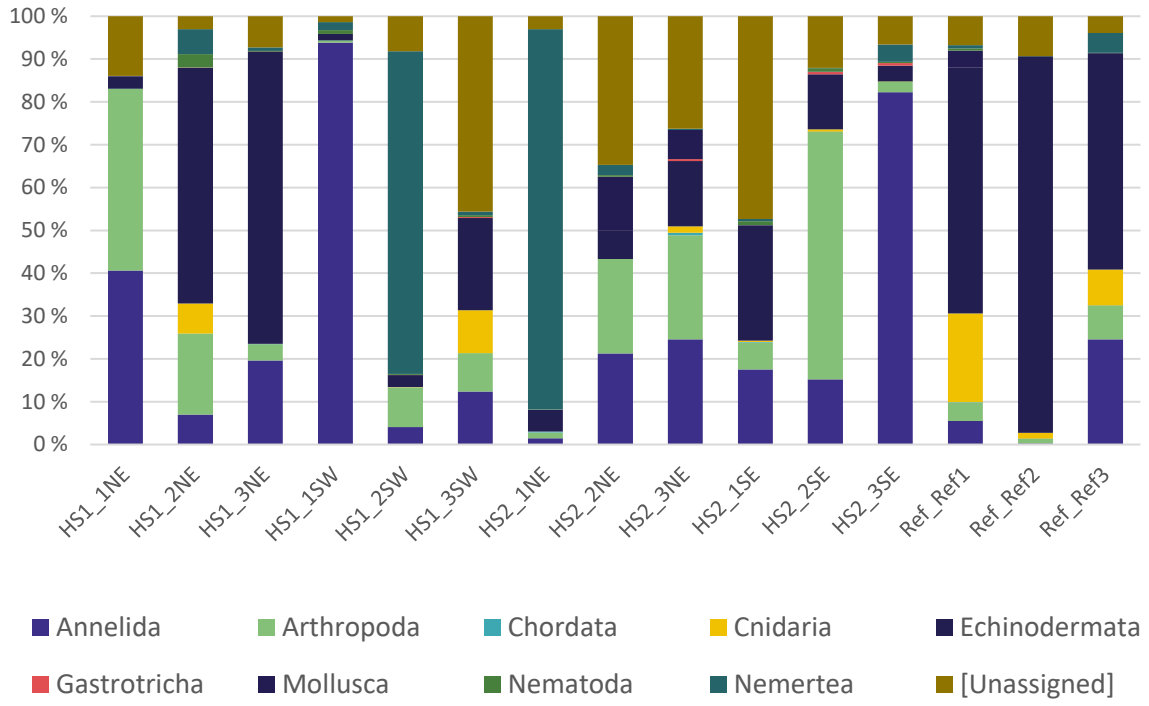


Figure 15. *COI relative abundance of phylum-level taxonomic groups shown separately for each station in the Hywind Scotland dataset. The three rightmost stations are reference stations.*

The twenty most abundant OTUs in the COI dataset included the macrofaunal phyla Echinodermata, Annelida, Arthropoda and Cnidaria as well as Nemertea (Table 5). This data is shown at station level in Appendix C.

Table 5. *The twenty most abundant metazoan OTUs at phylum level or below in the Hywind COI dataset.*

OTU	Phylum	Taxon	Total	Percentage
OTU_19	Echinodermata	*Echinocardium cordatum*	253 924	20.10%
OTU_22	Annelida	*Aricidea minuta*	188 751	14.94%
OTU_27	Nemertea	Enopla	173 845	13.76%
OTU_32	Annelida	Polychaeta	125 916	9.97%
OTU_43	Echinodermata	*Echinocardium flavescens*	105 942	8.39%
OTU_49	Arthropoda	*Bathyporeia tenuipes*	89 639	7.10%
OTU_103	Nemertea	Cephalotrichidae	59 181	4.69%
OTU_121	Annelida	*Grania postclitellochaeta*	53 431	4.23%
OTU_131	Arthropoda	Amphipoda	34 704	2.75%
OTU_226	Echinodermata	*Amphiura filiformis*	31 231	2.47%
OTU_287	Arthropoda	*Ameira* sp. n.	20 948	1.66%
OTU_205	Arthropoda	Arthropoda	20 584	1.63%
OTU_255	Annelida	*Nerillidium*	17 410	1.38%
OTU_319	Arthropoda	*Haloschizopera* sp. n.	16 171	1.28%
OTU_281	Cnidaria	*Halcampa* cf. *duodecimcirrata*	14 935	1.18%
OTU_432	Annelida	Cirratulidae	14 362	1.14%
OTU_354	Nemertea	Tubulanidae	12 269	0.97%
OTU_409	Cnidaria	Actiniaria	10 152	0.80%
OTU_412	Annelida	Terebellidae	10 097	0.80%
OTU_428	Annelida	*Glycera alba*	9 583	0.76%

The number of unique COI metazoan OTUs identified to at least phylum level at each station is shown in Figure 16. Arthropoda was the most species-rich phylum, followed by the annelids. Meiofauna, while less prominent in the 18S V1-V2 data, was still present as nematodes or gastrotrichs.

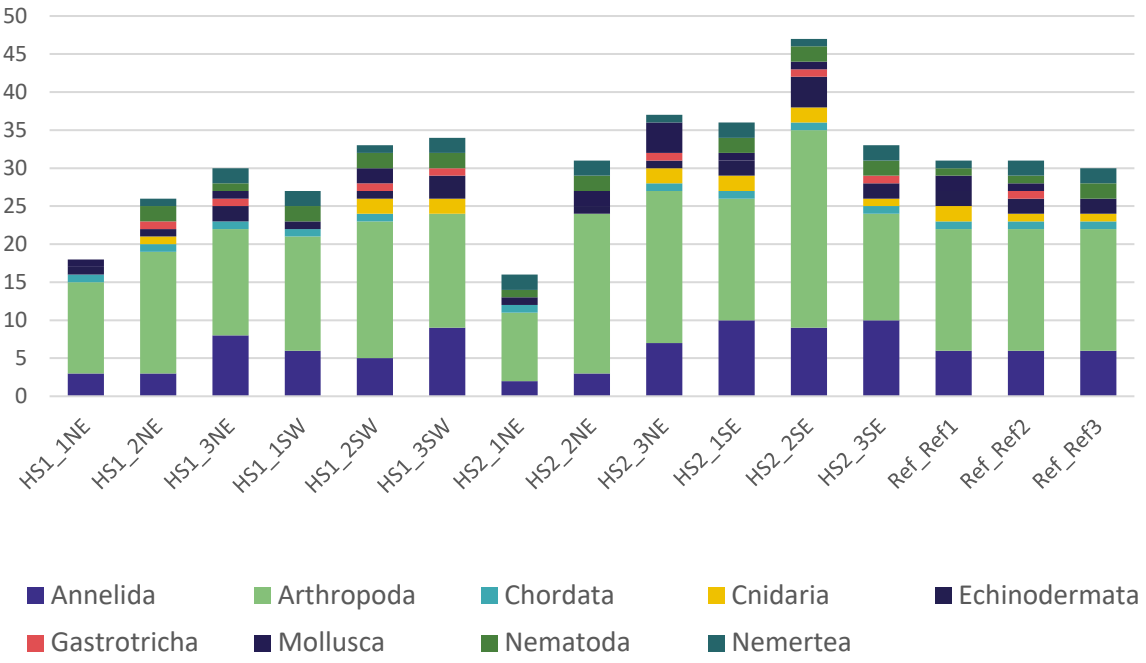


Figure 16. *The number of unique OTUs classified to at least phylum level in the Hywind COI dataset, shown separately for each station in the dataset.*

In addition to Shannon diversity, it is possible to calculate index values for OTUs that are identifiable as species included in existing morphological biotic indices such as AMBI, NSI, ISI or NQI, based on read abundance values and species identity. Note that this only uses a small part of the COI dataset given that most OTUs in the COI data cannot be identified to species level due to gaps in the Midori sequence database (Fig. 17).

Figure 17. *Biotic index values from the COI metazoan dataset including A) Shannon diversity (H'log$_2$), B) AMBI, C) ISI, D) NSI, and E) NQI1, with standard deviation based on station inter-replicate variation.*

Pairwise Bray-Curtis dissimilarities of the metazoan OTUs from the COI dataset were calculated between samples at the station replicate level based on fourth root transformed data and plotted using an nMDS plot (Fig. 18).

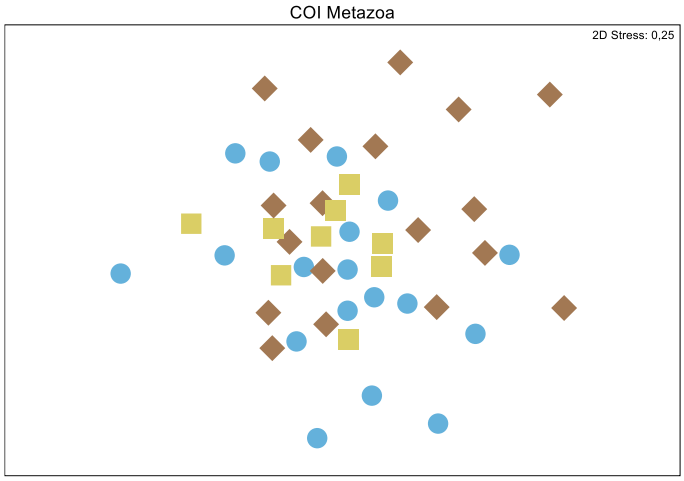


Figure 18. *Bray-Curtis dissimilarities from fourth root transformed data of COI metazoans plotted using multivariate nMDS with nMDS1 and nMDS2 as axes. Blue circles represent Hywind turbine 1, brown diamonds turbine 2, and yellow squares reference stations.*

The species accumulation curve for the complete COI (Fig. 19) shows the average OTU increase for each new station in the data. Compared to the morphological and 18S data, the relatively large, shaded area shows more variability in species increase for individual permutations, signifying higher variability between data for individual stations.

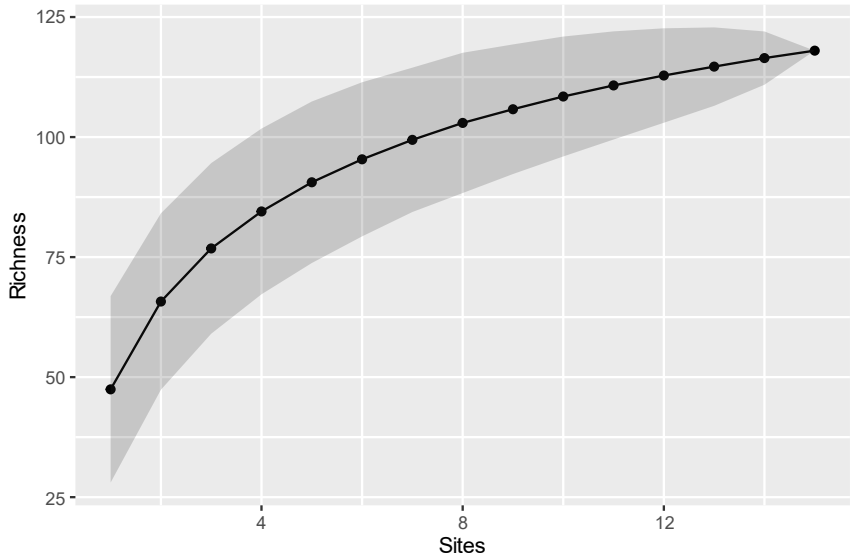


Figure 19. *Average increase in OTU richness (Y) with increased number of stations (X) in the COI metazoan dataset based on 2000 permutations. The shaded area shows between-station variability in species increase for individual permutations.*

3.6. Taxonomic overlap

Overlap in the taxonomic groups between the morphological, COI and metazoan part of the 18S datasets is shown in Figure 20. The overlap is highest at higher taxonomic level, and lowest at genus and species level. The number of taxa exclusive to the morphological dataset also increases with lower taxonomic level, likely as a result of a lower number of sequences being identifiable to a lower

taxonomic level in the metabarcoding datasets. Some taxa exclusive to one or both metabarcoding datasets are meiofauna that are not part of the morphological dataset scope.

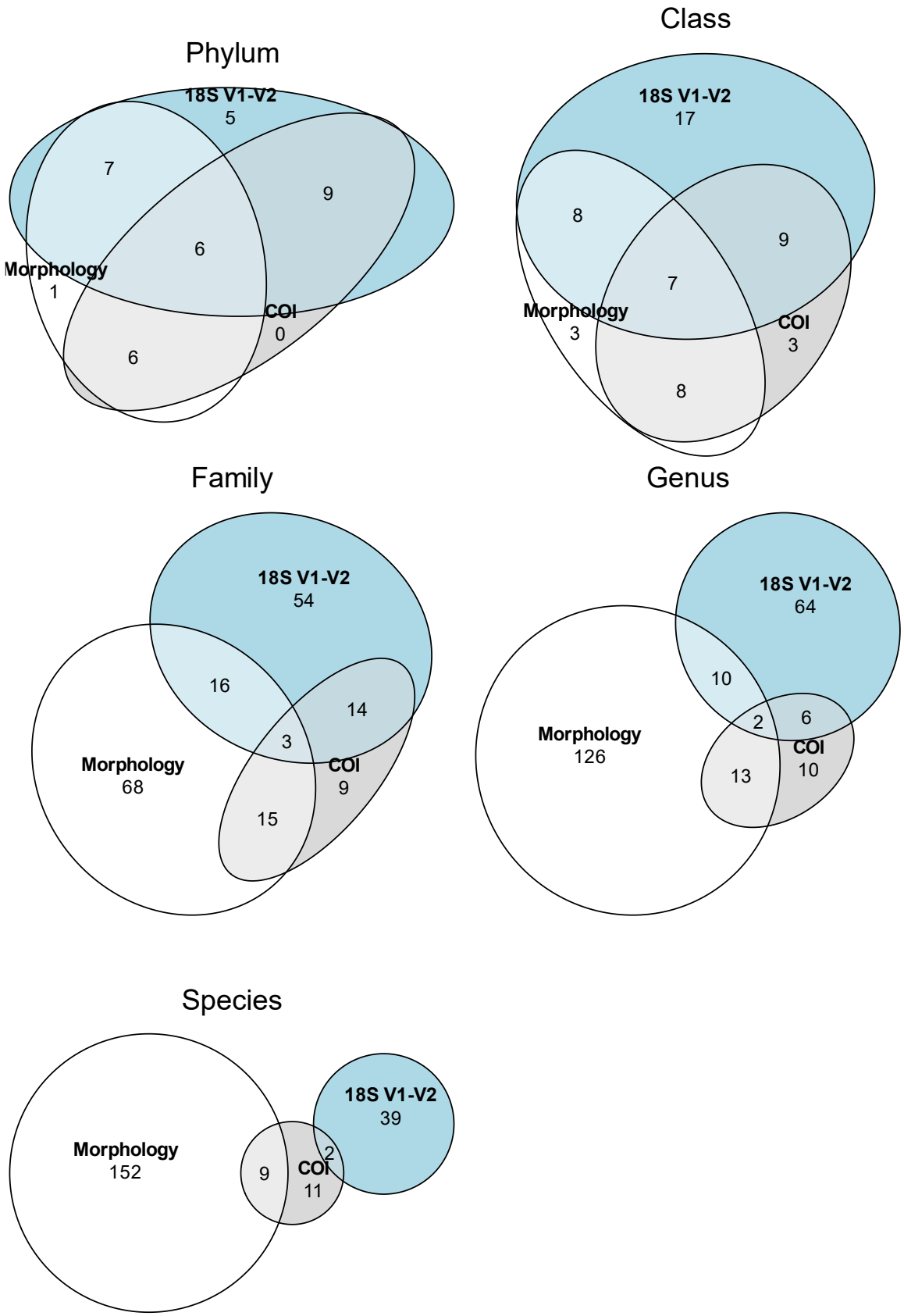


Figure 20. *Euler diagrams showing the relative degree of overlap in taxa at phylum to species level between morphological identification and taxonomic assignment of sequences for the COI and metazoan portion of the 18S V1-V2 metabarcoding datasets.*

3.7. Hywind eDNA environmental assessment

The Norwegian implantation of the water directive gives environmental quality standards (EQS) for benthic fauna parameters in different areas. This can provide a reference for assessing environmental conditions of morphological soft bottom fauna. Applicable values have been assessed here using the "water type N 1-2", Southern North Sea (Veileder 02:2018) (Table 6). In the morphological dataset, except HS1_1NE (4.18) Shannon H'log_2 values were all between 4.2 and 5, which gives a "very good" EQS for all stations in the dataset ("good" for HS1_NE1). ISI values were all between 8.5-13.2 (very good), NSI values all between 20-25 ("good"), NQI values all between 0.75-0.94 ("very good"), finally ES_{100} values were all between 29-58 ("very good"). Thus, the morphological dataset does not indicate any impact on benthic fauna at any of the Hywind stations (Table 7).

Table 6. *EQS values of biotic index and biodiversity parameters of benthic soft bottom fauna from the Norwegian Water Directive implementation part of Table 9.22* (Veileder 02:2018 2018).

Index	Water type N 1-2 (Southern North Sea Exposed/semi-exposed coastal)				
	Very good	Good	Moderate	Bad	Very bad
NQI1	0.94-0.75	0.75-0.66	0.66-0.51	0.51-0.32	0.32-0
H'log_2	6.3-4.2	4.2-3.3	3.3-2.1	2.1-1	1-0
ES_{100}	58-29	29-20	20-12	12-6	6-0
ISI_{2012}	13.2-8.5	8.5-7.6	7.6-6.3	6.3-4.6	4.6-0
NSI	30-25	25-20	20-15	15-10	10-0

Table 7. *Classification of Hywind morphological data in accordance with above EQS values. Stations are color coded according to classification: blue = "very good", green = "good" EQS status.*

	H'(log_2)		ISI		NSI		NQI1		ES_{100}	
Station	**Avg**	**St.dev**	**Avg**	**St.dev**	**Avg**	**St.dev**	**Avg**	**St.dev**	**Avg**	**St.dev**
HS1_1NE	4.18	0.68	11.02	1.04	24.96	2.34	0.83	0.04	34	8
HS1_2NE	4.65	0.31	10.58	0.25	22.95	0.46	0.79	0.02	35	5
HS1_3NE	4.79	0.17	10.20	0.22	22.88	0.91	0.80	0.02	38	3
HS1_1SW	4.88	0.15	10.38	0.57	23.47	0.93	0.80	0.03	39	2
HS1_2SW	4.75	0.29	10.04	0.48	23.20	0.37	0.81	0.03	38	4
HS1_3SW	4.80	0.22	10.41	0.54	23.66	1.23	0.82	0.01	40	3
HS1_3SWex	4.56	0.36	10.18	0.31	23.83	1.80	0.81	0.02	35	7
HS2_1NE	4.30	0.31	10.14	0.96	23.92	0.99	0.81	0.02	30	8
HS2_2NE	4.59	0.54	10.26	0.85	23.19	2.19	0.80	0.06	36	5
HS2_3NE	4.30	0.66	11.47	1.55	24.06	2.54	0.81	0.02	32	12
HS2_1SE	4.38	0.27	10.55	0.89	23.47	1.60	0.80	0.02	35	7
HS2_2SE	4.56	0.33	10.17	0.32	23.28	1.01	0.79	0.03	36	4
HS2_3SE	4.32	0.38	10.65	0.23	23.33	0.68	0.79	0.02	33	7
Ref_Ref1	4.38	0.22	10.99	0.64	23.31	1.64	0.79	0.03	32	7
Ref_Ref2	4.84	0.11	10.34	0.33	23.57	1.09	0.81	0.01	40	2
Ref_Ref3	4.61	0.28	10.03	0.43	23.00	0.99	0.79	0.02	38	3

EQS values do not exist for metabarcoding data, and thus environmental assessment of this data must be based on observed difference in community structure between e.g. stations close to the turbines vs reference stations and between turbines. Here, we did not see a pattern indicating consistent differences in community composition based on these factors in either the 18S or COI datasets (Figs. 12, 18). Based on the metabarcoding data in this report, we could thus not detect any environmental impact at the sampled stations.

Biotic index values were calculated for parts of the COI dataset identifiable to lower taxonomic level (i.e., genus, species). (This was not done for 18S data, as species-level metazoan identification is low for non-COI markers.) The unstable OTU abundances between replicates and stations evident in the COI data in particular, translated to these values, however, and the results should be interpreted rather as incomplete sampling towards the measured communities (see discussion) rather than a reflection of actual community environmental status.

4. Discussion and conclusions

4.1. Data resolution and ability to discriminate sites

Previous studies have shown that sediment eDNA datasets have the ability to detect changes in benthic community composition due to anthropogenic impact such as oil and gas extraction (Lanzén et al. 2021; 2016; Mauffrey et al. 2021; Cordier et al. 2019; Laroche et al. 2018) and aquaculture (Pawlowski et al. 2014; 2016). Yet, to act as a reliable complement to existing morphological data, eDNA metabarcoding datasets need to provide a consistent and high-resolution overview of the studied communities on par with that of morphological datasets. Even in previous studies, metabarcoding data has often displayed increased inter-sample variation/noise compared to morphological data, a finding that is also clear in the results from this report. For example, in Lanzén et al. (2021), xy-plots of impact parameters showed a higher degree of outliers relative to morphological data when correlated to impact parameters (e.g. Figs. 2, 4-5 in that study).

The Hywind site does not feature environmental impact detectable by the community composition data in this report from either the morphological or metabarcoding data. This assessment corroborates the DNV monitoring report (using the DNV morphological data also re-analyzed here) (Møskeland, Fjukmoen, and Melsom 2023). For this reason, we did not pursue a more detailed gradient analysis based on distance to turbine, and the comparative evaluation of the metabarcoding and morphological datasets thus turns towards evaluating the relative degree to which the different datasets are able to provide a stable and high-resolution picture of the targeted organisms at the stations sampled. These results can be intuitively interpreted from the stability in abundance between stations in the datasets (Figs. 4, 9, 15) and by comparison of nMDS plots for the different datasets (Figs. 6, 12 and 18). Here, the morphological data provides a more consistent separation between the turbines and reference sites than both metabarcoding datasets.

The COI dataset is especially noisy with regards to sample and station heterogeneity, a finding that is mirrored by the macrofaunal portion of the 18S rDNA dataset when viewed in isolation (Figs. 11C, 17A). In general, while it does give more information than simple presence-absence data only, metabarcoding abundance should be interpreted with caution, as it is heavily dependent on a number of factors including primer bias, and the mix of organism DNA in the sample (Zinger et al. 2019; Deagle et al. 2014). Additionally, assessing macrofauna through sediment eDNA (rather than bulk community samples) has been shown to be susceptible to heterogeneous distribution of both DNA and the actual animals themselves (Hestetun, Lanzén, and Dahlgren 2021; Hestetun, Lanzén, Skaar, et al. 2021), a simple matter of sample size vs the size of the targeted community. Hestetun, Lanzén, and Dahlgren (2021) showed that this effect was lessened when applying biotic indices to COI data, however we did not see a similar increase in performance relative to basic alpha diversity data here. While we included COI here due to its higher potential for taxonomic identification and relation to current morphological practices, the results underscore that sediment eDNA studies benefit from using microorganism markers to increase the sampling coverage of the data. Accordingly, this heterogeneity is lessened in the meiofauna (and nematode only) parts of the 18S dataset in this report, and especially for microorganisms (Fig. 11A-B).

Species accumulation curves show some convergence when looking at the total number of samples for both morphology and both metabarcoding datasets. Notably, the high variance in the COI accumulation curve shows the higher variability between replicates and samples for this marker

(Fig. 19). These curves do not immediately imply lower sampling coverage for the metabarcoding data than for morphology (compare Figs. 7, 13, 19). Yet, as the species accumulation curves consider richness only, not abundance, the greater propensity of metabarcoding to detect even small quantities of DNA from organisms, could provide a slightly false image regarding sampling completeness from considering these curves alone. Thus, even when considering microorganisms specifically (using the particular 18S V1-V2 marker in this report), the whole-grab macrofaunal data from a single grab replicated provides better coverage than a single sediment replicate, even when considering the higher heterogeneity of animals vs. microorganisms. As such, the results in this report highlight that a more comprehensive sampling approach is needed to increase the resolution and representativeness of the metabarcoding data, in practice through more sediment per sample and a higher number of replicate samples per station. Alternatively, that eDNA is extracted from e.g., elutriated meiofauna or even sieved macrofauna, depending on which organisms are targeted.

4.2. Metabarcoding macrofauna performance and taxonomic coverage

Comparing the taxonomic identifications of metazoan macrofaunal phyla in the different datasets, there was high overlap at higher taxonomic level (phylum, class), but little overlap at lower taxonomic level (family, genus, species) (Fig. 20). This is partly because the markers target different parts of the organism community: For instance, 18S metazoan data is skewed towards meiofaunal groups, especially nematodes, while COI targets mostly macrofauna. Hence, for the 18S data, the metazoan part of the dataset was dominated by meiofauna, especially nematodes, but also other meiofaunal phyla such as Gastrotricha and Xenacoelomorpha.

Another clear result at lower taxonomic level was the increasing number of morphological species identified relative to assignable metabarcoding sequences, starting at family level, and increasingly prevalent at genus and species level (Fig. 20). This highlights the incomplete nature of current taxonomic database coverage: Many common OTUs in both 18S and COI datasets were only identifiable to higher taxonomic level (Tables 4-5) representing organisms where no close relatives are present in online databases. Furthermore, 18S identifications at species level should be interpreted with caution: Species-level metazoan 18S OTUs, such as *Temora longicornis*, *Anomalocardia auberiana*, and *Abatus cavernosus*, are unexpected given known morphological biodiversity in the general area. This is most likely due to the conserved nature of the 18S V1-V2 marker, which underestimates true diversity (Tang et al. 2012), and thus the most likely explanation for these cases is that the sequences represent related species with similar marker sequence to the actual species at the sites. As taxonomic databases grow with the addition of barcoded specimens, coverage of macrofauna is expected to increase (Hestetun et al. 2020). Targeted barcoding by piggybacking on existing environmental surveys (through preservation in ethanol and subsequent barcoding of typical fauna from the area) could be used to expedite this process.

4.3. Recommendations for further development

While this study highlights some limitations of the sampling design and approach used here, it is important to emphasize that this is mostly a question of incomplete sampling and partial lack of taxonomic information in online databases rather than the data type itself. As with other data types there is a trade-off between efficiency and cost-effectiveness, and high-quality data. Due to the lack of detected environmental impact in the Hywind dataset, the relative performance of metabarcoding data in this report is related specifically to baseline community characterization

rather than impact detection, where studies have shown the ability of metabarcoding data to detect shifts at impacted sites (e.g. Laroche et al. 2018; Lanzén et al. 2021). While the sediment sampling design chosen here did provide coherent community composition data, the ability of this data to shifts in community composition between the sites was not as sensitive as that of the morphological dataset, i.e. the sediment volume used for the metabarcoding data was a bit low.

In contrast to eDNA from the water column, sediment eDNA distribution varies even at small spatial scales. This heterogeneity can either be handled by concentrating on organisms with more even small-scale distribution, such as single-celled eukaryotes or prokaryotes, by increasing sampling effort, or a combination of both. While comprehensive macrofaunal datasets may be difficult without extracting DNA from sieved bulk samples rather than directly from sediment, elutriation of meiofauna – i.e. floating off animals from sediment using water – could be a good compromise (Brannock and Halanych 2015), and a publication comparing this method to that of sieved fauna and direct sediment extraction is currently in prep at NORCE. Another approach is to develop biotic indices directly on non-macrofauna metabarcoding data as has been trialed in multiple studies, an approach that is less reliant on taxonomical identification but would allow this data to be evaluated on its own merit (Lanzén et al. 2021; Keeley, Wood, and Pochon 2018; Aylagas, Borja, and Rodríguez-Ezpeleta 2014). This would allow direct use of protist, prokaryote or meiofauna data in calculating sensitivity, and decrease reliance of macrofauna. The findings in this report reflect the main conclusions from the NORCE 2018-2021 MetaMon project (Hestetun, Lanzén, Bagi, et al. 2021): An increased focus on markers that target single-celled organisms (such as other 18S and prokaryote 16S markers), with increased sampling effort, represent the most promising direction towards developing metabarcoding as a distinct methodology for benthic environmental monitoring.

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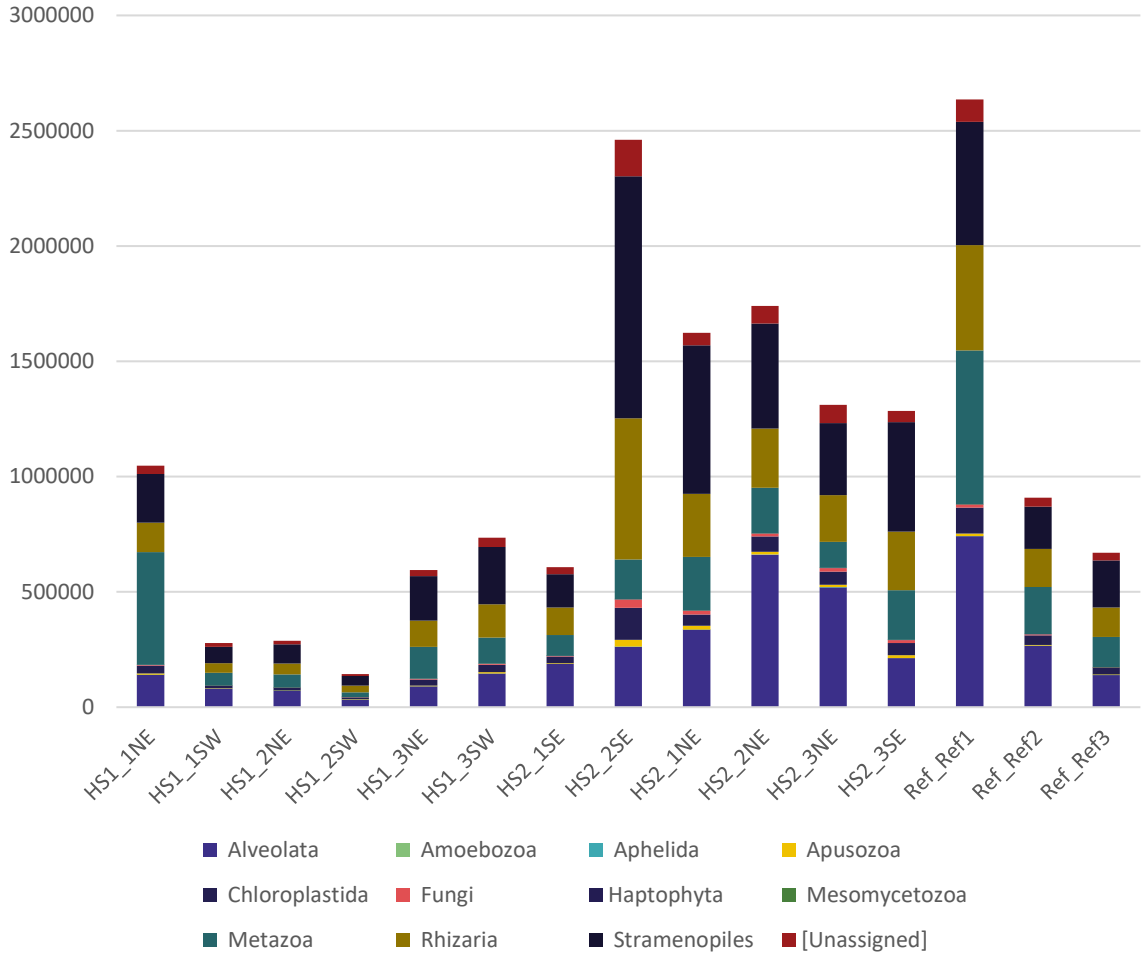
Appendix A. *Absolute number of 18S V1-V2 reads at high (~"kingdom") level per station.*



Appendix B. *Twenty most abundant OTUs in the 18S V1-V2 dataset shown at station level.*

OTU	Clade	Identity	HS1_1NE	HS1_2NE	HS1_3NE	HS1_1SW	HS1_2SW	HS1_3SW	HS2_1NE	HS2_2NE	HS2_3NE	HS2_1SE	HS2_2SE	HS2_3SE	Ref_Ref1	Ref_Ref2	Ref_Ref3
OTU_1	Stramenopiles	Oomycota	280701	62474	135663	100099	104282	184541	529102	298030	160316	101012	697527	649763	404540	118008	199567
OTU_2	Alveolata	Pelagostrobilidium	281551	74583	62959	66705	170288	133197	238227	560875	481971	218061	349949	182889	674013	206096	113303
OTU_3	Stramenopiles	Oomycota	160568	20473	41921	31673	36831	65173	254316	137578	90621	43220	308885	252804	161163	40959	61105
OTU_7	Rhizaria	TAGIRI1-lineage X sp.	36961	8349	20339	18054	35004	28498	47030	80733	46788	39181	142003	67106	123014	41462	26186
OTU_5	[Unclassified]	[Unclassified]	56957	8926	10496	12367	29066	26893	17360	41015	49434	19389	134178	21350	48399	15978	18403
OTU_6	Metazoa	unclassified Thoracostomopsidae	25560	112	0	0	678	189	539	0	0	4362	0	0	435697	22698	0
OTU_8	Metazoa	Oncholaimus	0	4055	20615	4219	2913	14420	19995	53596	0	14006	15364	20238	133781	41098	32248
OTU_10	Metazoa	Temora longicornis	116762	2679	17172	16272	330	4827	89764	17358	15669	9344	37479	0	8299	12819	203
OTU_11	Chloroplastida	Pycnococcaceae	24035	4159	9214	8617	9946	11172	14251	27341	18877	10555	60567	26198	40455	14742	12022
OTU_13	Rhizaria	Cryothecomonas	6763	1819	6328	6712	8295	8091	19452	30696	8346	10235	47673	25058	53550	15784	10508
OTU_12	Rhizaria	Euglyphida	23406	6303	8316	8455	9024	10107	18998	16300	8509	5492	39889	23475	40684	13744	14464
OTU_25	Rhizaria	Mataza-lineage X sp.	19188	3806	6989	5840	8308	9492	19716	18917	10333	8313	32774	20005	24668	8903	8670
OTU_15	Alveolata	Choreotrichida	0	409	4129	5965	27210	2255	27490	17528	681	7778	1136	12927	69743	14140	11006
OTU_16	Metazoa	Anomalocardia auberiana	197821	0	0	0	0	0	0	0	0	36	0	0	0	0	0
OTU_20	Rhizaria	Cryothecomonas sp.	8173	1583	4761	5270	6053	6323	16045	18850	4440	7455	44001	23077	28063	10192	6555
OTU_17	Alveolata	Suessiaceae	3416	690	4709	10002	7695	5796	22313	19294	3680	4204	24806	17478	26530	11221	12792
OTU_18	Stramenopiles	Labyrinthulaceae X	7341	2053	4155	2760	4563	6208	17863	10517	7439	4336	24861	18191	29274	7255	7802
OTU_19	[Unclassified]	[Unclassified]	14125	2637	5998	2144	5726	5589	16486	10356	9015	5921	18670	12309	26689	9479	9237
OTU_24	Metazoa	Abatus cavernosus	7846	3661	554	608	532	5795	715	69	7275	2441	982	97196	11342	886	1874
OTU_21	Chloroplastida	Dolichomastigaceae-B	6556	1385	3457	5053	4224	3447	6590	15666	7861	6101	27464	12965	24075	6190	4085

Appendix C. *Twenty most abundant metazoan OTUs identified at least to phylum level in the COI dataset shown at station level.*

OTU	Phylum	Taxon	HS1_1NE	HS1_2NE	HS1_3NE	HS1_1SW	HS1_2SW	HS1_3SW	HS2_1NE	HS2_2NE	HS2_3NE	HS2_1SE	HS2_2SE	HS2_3SE	Ref_Ref1	Ref_Ref2	Ref_Ref3
OTU_19	Echinodermata	Echinocardium cordatum	0	0	16574	0	0	1828	0	0	0	0	11100	2123	16840	191687	13772
OTU_22	Annelida	Aricidea minuta	0	0	3579	426	0	1097	0	0	432	4738	16390	160229	0	372	1488
OTU_27	Nemertea	Enopla	0	0	0	0	0	0	173845	0	0	0	0	0	0	0	0
OTU_32	Annelida	Polychaeta	0	0	0	125251	0	0	0	0	0	665	0	0	0	0	0
OTU_43	Echinodermata	Echinocardium flavescens	1886	22311	76	2015	1780	1030	10068	4773	28032	2071	1285	5384	24523	352	356
OTU_49	Arthropoda	Bathyporeia tenuipes	0	0	0	0	0	0	0	0	0	0	89639	0	0	0	0
OTU_103	Nemertea	Cephalotrichidae	0	2361	74	104	52253	112	869	318	550	219	251	240	571	16	1243
OTU_121	Annelida	Grania postclitellochaeta	25002	2804	114	0	132	213	2824	0	8744	117	12338	102	780	166	95
OTU_131	Arthropoda	Amphipoda	0	5317	0	0	16	0	0	2521	0	0	26585	265	0	0	0
OTU_226	Echinodermata	Amphiura filiformis	0	0	0	0	0	922	0	0	0	11978	18331	0	0	0	0
OTU_287	Arthropoda	Ameira sp. n.	1019	0	0	0	0	0	0	0	19929	0	0	0	0	0	0
OTU_205	Arthropoda	Arthropoda	12571	16	132	75	520	80	0	343	865	17	4798	593	87	406	81
OTU_255	Annelida	Nerillidium	0	0	0	0	0	537	0	0	16873	0	0	0	0	0	0
OTU_319	Arthropoda	Haloschizopera sp. n.	11365	332	10	54	8	456	961	206	1465	88	572	20	58	503	73
OTU_281	Cnidaria	Halcampa cf. Duodecimcirrata	0	0	0	0	35	0	0	0	0	47	0	0	14853	0	0
OTU_432	Annelida	Cirratulidae	0	0	0	1029	2443	71	0	0	0	872	31	6680	3113	64	59
OTU_354	Nemertea	Tubulanidae	0	0	139	2459	0	27	0	1398	0	66	0	8089	0	35	56
OTU_409	Cnidaria	Actiniaria	0	2862	0	0	26	1754	0	0	0	132	13	0	57	2966	2342
OTU_412	Annelida	Terebellidae	0	0	31	0	0	0	0	10061	0	0	0	0	0	5	0
OTU_428	Annelida	Glycera alba	0	0	0	0	0	0	0	0	9583	0	0	0	0	0	0