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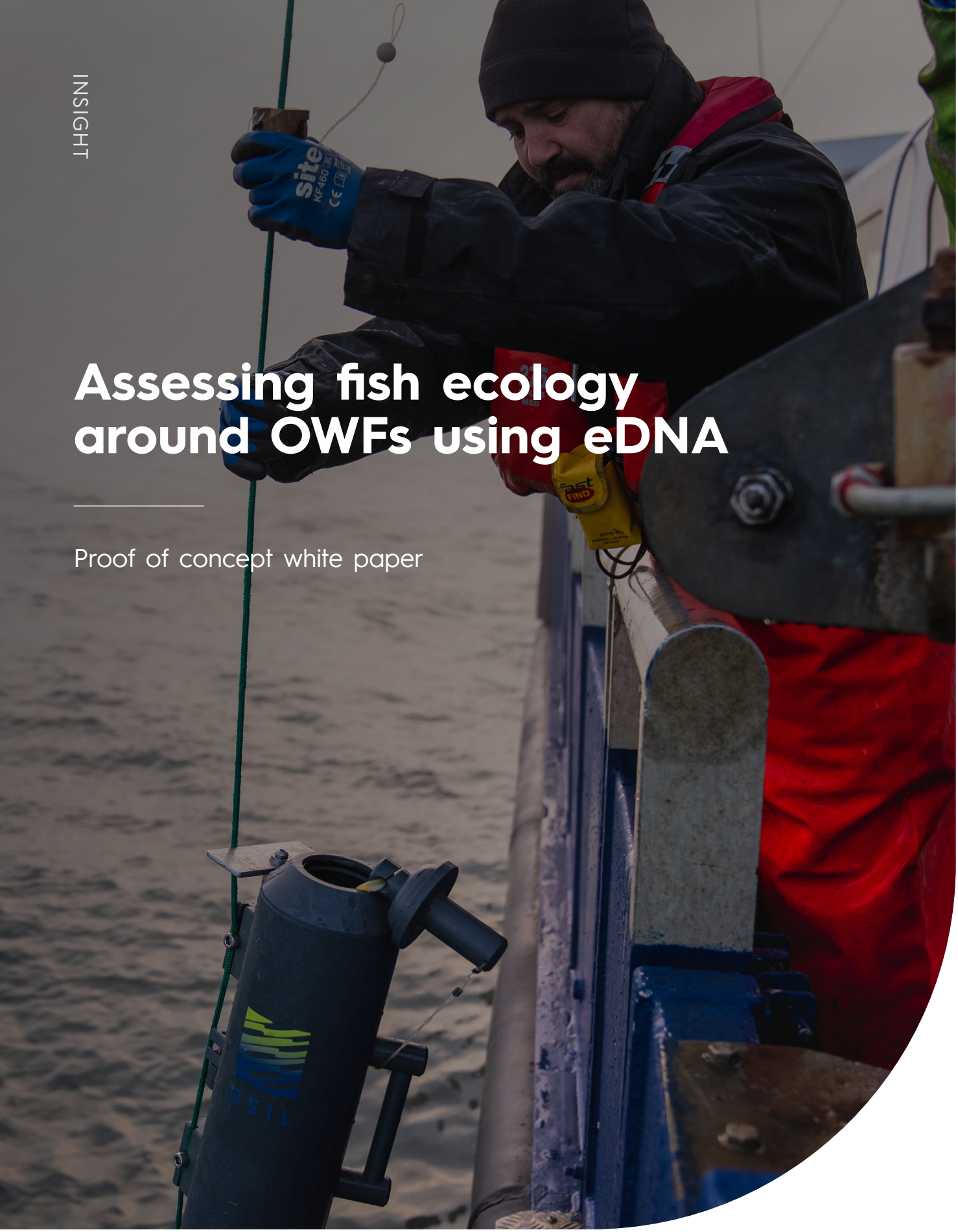

Assessing fish ecology around OWFs using eDNA

Proof of concept white paper

NATURE METRICS
DNA-BASED MONITORING

Offshore Wind Growth Partnership

EDF renewables

natural power

Document history

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Executive Summary

Proof-of-concept that environmental DNA (eDNA) sampling and analysis can be used commercially around offshore renewable energy developments as an innovative environmental survey technique to improve or replace traditional fish ecology surveys.

Governments and the energy industry are rapidly transitioning to renewable sources of energy, placing increasing pressure on existing supply chain for offshore wind developments. This includes satisfying the environmental and consenting requirements under EIA legislation whilst balancing limited resources such as vessel and staff availability. Innovative methods are needed such as using eDNA for the collection of biological data at greater frequencies without an increase in survey effort.

To provide an evidence base for the potential acceptance of eDNA based methods as a valid alternative to conventional fish trawl surveys, both methods were conducted in parallel at an offshore wind installation. Seasonal co-located trawls and eDNA sampling were carried out at four locations around the Blyth Offshore Demonstrator, with eDNA sampling at two additional locations within the turbine array, which was not possible to survey with trawl sampling. eDNA samples were analysed using fish, vertebrate (fish, mammals and birds) and invertebrate assays. This report focusses on fish ecology and marine mammal occurrence, with comparative analysis of the fish and vertebrate assays presented. Investigation into the invertebrate communities is ongoing and will be reported on subsequently.

The study found that eDNA methods can be used to practically sample the fish ecology offshore whilst working around a commercial OWF.

eDNA consistently detected a greater number of species than trawl data (54 species detected by eDNA, compared to 26 species in trawls). Species missed by trawls included smaller species and bottom-dwellers that are not often captured due to biases associated with this fishing gear. Conversely, trawls did capture species not detected to species level by concurrent eDNA samples, including gurnard and species of elasmobranchs. Despite this, these were detected to family level by eDNA. The most abundant species identified were consistent across the trawl data and eDNA as well as being in line with historical trends at the site.

When data from the fish and vertebrate assay and trawl abundance were simplified to a presence/absence metric there was a strong statistical difference in community composition found between the methods, likely due to greater numbers of species detected by eDNA. In addition, using eDNA removes issues around uncertainty in the data from gear selectivity and potential human error in the misidentification of species.

Seasonal and spatial patterns in species occurrence and community composition were similar between the trawl and eDNA based sampling methodologies, with broad seasonal similarities to historic trends. Multivariate analysis showed the same hierarchical clustering and similar analysis of similarities (ANOSIM) results for the trawl data and eDNA fish assay (for stations outside the turbine area). This indicates that eDNA methods not only pick up individual species trends but can also be used to calculate ecological diversity metrics and track seasonal and spatial differences in community composition.

Utilising eDNA based sampling techniques allowed surveying of areas adjacent to turbines, not normally accessible by trawl surveys. The species detected from these samples supports the hypothesis that the artificial habitat created by turbines may be providing shelter and food for fish, with some reef associated species at higher relative densities compared to stations outside of the turbine area.

Four marine mammal taxa (Minke whale, harbour porpoise, bottlenose dolphin and white sided/white beaked dolphin) were identified by the vertebrate assay. The information on the seasonal occurrences of key mammal species can be used to inform targeted mitigation measures, and the information provided by eDNA can complement that of dedicated site surveys for marine mammals that are largely restricted to visual observations at the surface.

The study demonstrates eDNA based surveys are a market-ready solution to optimise consenting phase surveys of offshore wind site development, as well as ongoing monitoring and targeted mitigation strategies. Replacing traditional survey methods for assessing fish communities around OWFs with eDNA sampling provides greater opportunities for developments to collect the data required as a larger pool of vessels becomes accessible to undertake the survey work and can be combined with other site-based activities (e.g., site investigation work). This could greatly reduce the costs, resource consumption (e.g., fuel) and risks of delays to surveys and therefore to subsequent consents.

In conclusion, the adoption of the method has the potential for huge benefits to the industry by providing more efficient, affordable, and scalable consenting and site survey solutions. This could speed up the development of OWFs and reduce costs for developers/operators, ultimately reducing the cost of overall energy production.

Regulator and stakeholder acceptance of eDNA methods for use in offshore baseline setting and monitoring will be a key step towards accelerating and improving environmental monitoring for future offshore wind development.

1. Project Overview

Natural Power, alongside Project partners NatureMetrics and EDF Renewables, were successful in securing funding via an Offshore Wind Growth Partnership (OWGP) Innovation Grant for 50% of the Project costs for an environmental DNA (eDNA) Fish Ecology Research Project. This 18-month Project involved surveying using both traditional trawl and eDNA survey methods around a commercial offshore wind farm to trial the viability of eDNA sampling and provide a method for such data collection for fish ecology assessments.

Four surveys were conducted over 12 months beginning in March 2022 and continuing in each concurrent season (quarter), mirroring the typical compliance frequency for fish ecology monitoring (Natural Power, 2021). The data from the concomitant trawls and eDNA samples were compared to assess the effectiveness of the eDNA method. For eDNA analysis, two typical assays used by NatureMetrics for 'fish' and 'invertebrate' were tested as well as a new 'marine vertebrate' assay currently in the research and development (R&D) stage. Comparative analysis of the 'fish' and 'marine vertebrate' assays have been presented in this report. The R&D 'marine vertebrate' assay also provided results on marine mammal species which are included in this report.

An initial review of invertebrate eDNA results proved encouraging and the project scope was extended, in August 2022, to allow for ground truthing of the eDNA data. Results from the 'invertebrate' assay will be investigated in detail and presented in a subsequent report.

The Project was carried out at Blyth Offshore Demonstrator, where pre- and post-construction monitoring surveys using traditional methods have been conducted for over a decade. The 2022 dataset was also compared against the historical fish community data from the site to investigate alignment with known long-term ecological trends. Additional sampling within the turbine array where trawling could not be conducted due to safety reasons was also undertaken, with the view to obtaining information on how the area in the vicinity of the turbines is utilised by fish species.

1.1. Project Drivers

Governments and the energy industry are rapidly transitioning to renewable sources of energy in response to climate change. This has led to ambitious time-bound goals, with the overall aim of Net Zero by 2050 (GovUK, 2022). One key target is deriving 50 gigawatts (GW) of energy from offshore wind by 2030 (Department for Energy Security and Net Zero and Department for Business and Trade, 2023). The Crown Estate's Offshore Wind Leasing Round (Round 4) will bring approximately 8 GW of additional capacity around England and Wales (Crown Estate, 2023), and Crown Estate Scotland's ScotWind leasing round aims to provide as much as 27.6 GW of offshore wind capacity delivered between 2027-2032 (Crown Estate Scotland, 2023). This places increasing pressure on existing supply chains, not only for offshore wind infrastructure, but also to satisfy the environmental and consenting requirements for offshore developments. With current resource limitation (e.g., vessel availability and specialist staff), innovative methods of obtaining biological data at greater frequencies without an increase in surveying effort are essential to meet demand. Furthermore, as Offshore Wind Farm (OWF) developments move into deeper offshore waters, current survey techniques can become more challenging or infeasible, potentially limiting the amount of targeted sites-specific data that can be obtained.

Environmental DNA methods provide a non-invasive solution which can outperform conventional methods for a variety of terrestrial and marine biological surveys (Fediajevaite *et al*. 2021). Despite this, offshore environmental surveys using eDNA are relatively new, and have typically been conducted in nearshore areas (e.g., Ely *et al*. 2021; Monuki, Barber, and Gold 2021; Mynott and Marsh 2020) or around oil and gas platforms (Alexander *et al*. 2022; Mauffrey *et al*. 2021; Cordier *et al*. 2019). Such studies have shown that eDNA often captures additional species compared to traditional methods, including those which are ecologically important in environmental assessments for consent applications and ongoing monitoring (Mynott & Marsh, 2020).

There is a developing body of literature comparing eDNA based surveys with conventional surveys for fish ecology (e.g., Alexander *et al*. 2022; Port *et al*. 2016; Thomsen *et al*. 2016; Stoeckle, Ausubel, and Coogan 2022). However, to date only one other study has investigated the potential application of eDNA methods around offshore wind infrastructure, and concluded it was a powerful future tool but recommended comparative studies of eDNA and trawl data to further understanding (Ray *et al*. 2023).

1.2. Aims & Objectives

The overall aim of the project was to compare eDNA based methods with conventional fish trawl surveys with a view to identifying whether using eDNA can support or replace traditional methods for environmental baseline setting and compliance monitoring. This was evaluated throughout the project from survey design, method development and statistical analysis of resulting datasets to answer a number of key questions provided below:

1. Can eDNA samples be practically obtained offshore whilst working around a commercial OWF?
2. Does the number of species and/or species composition differ between the two methods?
3. Can seasonal trends be identified in eDNA data and if so, do they align with trawl data (present and historic)?
4. Can spatial trends be identified in eDNA data and if so, do they align with trawl data (present and historic)?
5. Does eDNA data identify any differences in fish community composition in the vicinity of the turbines from that of trawl stations? If so, does it support the theory that artificial reef habitat is having a positive effect on fish ecology in the area?
6. Can eDNA provide data on other species groups such as invertebrates and marine mammals?
7. Which eDNA assay ('fish' or 'marine vertebrate' (henceforth referred to as 'vertebrate')) preforms the best?
8. Does eDNA sampling reduce survey costs and sampling effort?*

*NB. Reference to savings in survey cost and sampling effort (question 8) have been referred to throughout the report qualitatively.

1.3. Report Scope

This report has been produced as a key output of the OWGP Innovation Grant. The purpose of the funding is to support projects which result in market-ready technologies, products, and services to accelerate offshore wind site development during the consenting phase. The study aims to identify a potential solution to overcome a current challenge associated with offshore wind site development through the investigation of an innovative environmental survey technology to feed into the assessment process. This report focuses primarily on fish ecology assessment, but also includes reference to data obtained on invertebrates (investigated in detail in a subsequent report), as well as marine mammals which could feed into future research.

2. Sampling Design & Methodology

2.1. Survey Design

The Project was carried out at Blyth Offshore Demonstrator (BOD), located approximately 5 km off the coast of Blyth, northeast England. Pre- and post-construction monitoring surveys at the site using traditional trawl methods have been conducted for over a decade as part of the Marine License condition.

Three trawl locations were selected from those conducted during the pre/post-construction monitoring at BOD ensuring coverage of a range of habitats and depths representative of the site conditions. The existing monitoring station numbering has been retained in this report. Nearshore and offshore locations were chosen as previous results indicated that fish catch composition changed with depth (Natural Power, 2021). A new trawl station as close to the turbines as practically possible was also selected to compare species composition within the turbine area (from eDNA samples) with the nearby trawl station.

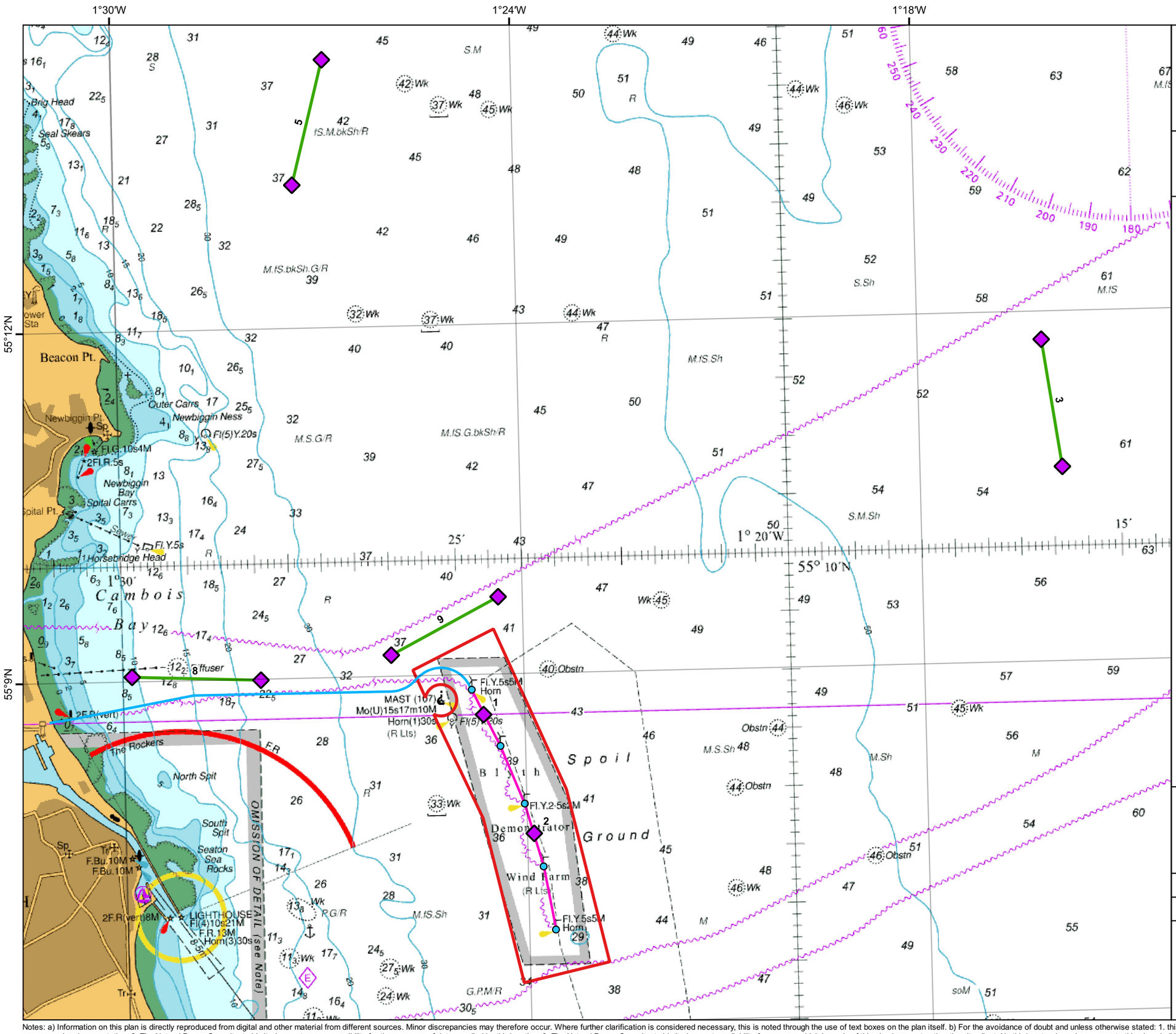
Survey locations were Station 8 (nearshore along the cable route), 3 (offshore, at the greatest depth), 5 (furthest north and slightly inshore) and Station 9 (new station closest to the turbines) as illustrated in **Figure 2.1**. Water samples for eDNA analysis were collected from within the BOD array at eDNA stations 1 and 2 (where trawling could not be conducted due to safety risks of snagging gear), and at the beginning and end of each trawl sampling station.

The different taxa targeted by both survey techniques undergo seasonal migrations as well as changes in biological activity (e.g., spawning) that are considered to alter the quantity of eDNA produced. Therefore, sampling was conducted quarterly to align with previous survey frequency (Natural Power, 2021) and to capture this seasonal variation.

The surveys took place during 2022 on the below dates within the pre-defined seasonal sampling windows:

- Winter: 28th March
- Spring: 24th May
- Summer: 2nd September
- Autumn: 12th December

Additional sampling took place on 5th January 2023 to conduct benthic invertebrate sampling for the extension of the project, as well as obtaining one remaining set of eDNA samples from Station 8 which were not collected in the autumn survey due to equipment failure.



Project:

Ecological Assessment Around Offshore Wind Farms Using eDNA

Title:

Figure 2.1: Blyth Trawl and eDNA Survey Design

Key

- Blyth Offshore Demonstrator Array 2 project area boundary
- Turbine
- Anemometry mast
- Inter array cable
- Export cable
- Fish ecology trawl
- eDNA water sampling station

Scale @ A3: 1:60,000

Coordinate System: WGS 84 / UTM zone 30N

Graticules: WGS 84

0 0.5 1 1.5 2 km

N

Date: 02-11-23	Prepared by: JO	Checked by: ME
Ref: GB200769_M_109_B		

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2.2. Sampling Methodology

2.2.1. Traditional techniques

An otter trawl was used with a commercially comparable net (80 mm mesh in the main body and cod-end with the foot rope using 15-20 cm rubber hoppers). The net was towed for 30 minutes at approximately 3.5 knots. Any reduction of the tow duration was recorded on the deck logs stating why the tow was hauled early. Coordinates and times (GMT) for the beginning and end of each trawl were recorded using a handheld GPS or the vessel GPS (preferred). Depth of water (m) was recorded from the vessels system, as well as the prevailing weather conditions and sea state.

For each haul, the catch was unloaded into fish boxes/bins and photographed with a waterproof label showing the sample number. Any unidentifiable or unusual specimens were also photographed for later identification/verification. All individuals in the catch were enumerated and measured where appropriate in accordance with Annex IV of EU Regulation 2019/1241; total length for fish and elasmobranch species (width for skates and rays); carapace width for crabs (length for lobsters) and mantle length for squid and identified to species level. All macro-invertebrate species were enumerated, with sub-sampling undertaken if large quantities were captured (as specified in Boyd *et al*. 2016). Following identification and enumeration all specimens were returned to the sea.

2.2.2. Environmental DNA field sampling

At the beginning and end of each tow, three 5 L replicate water samples for eDNA analysis were collected using a Niskin bottle. The vessel was positioned relative to the wind/tide/current to avoid the cable leading under the vessel and the Niskin bottle was deployed from the side of the vessel, avoiding the propellor. There was no vessel transiting during the deployment. Taking above into consideration, where possible, the vessel was orientated into the tide when taking the samples, to minimise any contamination from the trawl catch.

The bottle was lowered on a dyneema cable, moved up and down a few times to flush the inside and the sample taken c.1 meter above the seabed to capture the near-benthic eDNA without disturbing the seafloor. The correct length of cable was payed out using the depth on the vessel sounder and meter markings on the cable. Three 5 L replicates were also taken at two sample stations (eDNA Stations 1 and 2) as close to the turbines as possible. The coordinates and time (GMT) when the sample was taken was recorded using the NatureMetrics app or a handheld GPS. Depth of water (m) was recorded from the vessels system.

A clean set of gloves was worn between each sample to prevent contamination. Sampling kits with all the equipment required for eDNA sampling were provided by NatureMetrics. Once on board the water sample was transferred from the Niskin bottle to a single-use sterile sample bag for filtering each replicate.

A peristatic pump (vampire sampler) was used to filter the water samples. One end of a length of tubing was connected to the filter and the tubing was fed through the pump. The other end of the tubing was then placed into the sample bag and the pump was turned on to filter the sample (**Figure 2.2** and **Figure 2.3**). Once the sample bag was empty, any water remaining in the tubing was pumped through to ensure the complete sample had passed through the filter. A preservation buffer was then added to the filter and caps added to both ends of the filter. The filter and completed data sheet were stored in the corresponding specimen bag labelled with the station and sample number for analysis in the laboratory.

Figure 2.2: Environmental DNA field sampling

Figure 2.1: Environmental DNA field sampling

The Niskin bottle and buckets were cleaned with spray bleach and flushed with deionized water between each sample station.

During each survey, two field control samples were taken (towards the beginning and end of the survey) to test for contamination that may have occurred during sampling. Field controls were collected by filling the cleaned Niskin bottle with deionized water. The deionized water was then emptied into a sample bag and the water was filtered following the above procedure.

2.2.3. Environmental DNA Laboratory Analysis

Technical details of the eDNA laboratory analysis have been provided in **Appendix A**, with an overview summarised below.

DNA Extraction, Amplification & Sequencing

In the laboratory, DNA was extracted from each filter and a DNA extraction blank was processed with each batch to assess potential contamination in the extraction process. DNA was then purified and quantified.

Two typical assays used by NatureMetrics for 'fish' and 'invertebrate' were tested as well as a vertebrate assay. A genetic barcode region was amplified using primers specific to the assay for each sample. The amplified DNA was then sequenced to identify unique genetic sequences.

Bioinformatics

The raw sequence data was processed and compared to genetic reference databases of species through a bioinformatics process to generate an output list of taxa detected in each sample for ecological analysis.

For each assay, assignments were made to the lowest possible taxonomic level, using similarity thresholds >90%. A country-based sense-checking step was also implemented in line with the Global Biodiversity Information Facility (GBIF) occurrence records for the United Kingdom. All taxonomic units with species-level identifications were queried against the International Union for Conservation of Nature (IUCN) Red List to obtain global threat status.

The number of reads assigned to each species per sample during the taxonomic assignment against the reference database (i.e., read count) (as in Muri *et al*. 2016) was used for down-stream analysis.

3. Data analysis

As sequence read counts (henceforth referred to as 'read counts') from eDNA and abundance of fauna in traditional trawl datasets vary in their units and scale of capture, it was decided to analyse the two datasets separately. Each dataset is processed and analysed with univariate and multivariate techniques (see below **Section 3.3 - 3.4** for details) separately based on the read count and abundance information respectively. Both datasets were then simplified to presence / absence of occurrence of each species at a station during each survey and combined to allow simple multivariate comparisons to be made.

3.1. Trawl data processing

Raw trawl data were imported into R programming software (R Core Team, 2021). The counts of any species sub-sampled during trawl surveys were raised by the appropriate amount in order to provide the total abundance in the whole sample. Catch data were then standardised to 30-minute trawls using a standardisation factor to account for differences in sampling effort.

3.2. eDNA data processing

All analysis for eDNA was conducted using the 'vertebrate' and 'fish' assays separately for comparison. Results from the 'invertebrate' assay will be investigated in detail in a subsequent report.

Read count datasets were filtered to remove reads that were identified as likely contaminants using the 'decontam' package (Davis *et al*. 2018) in R. This filtering used prevalence testing which flags species as potential contaminants if read counts in samples are similar to those in control runs (field blanks). Where a species was deemed to be a potential contaminant (using the default probability threshold of 0.1), it was removed from the dataset for all stations within that month. Decontamination was carried out for all surveys separately. Seven species were removed during the decontamination process across the three assays, including John dory (*Zeus faber)* from the Fish Assay in the May 2022 survey (p = 0.066, prevalence = 4), and brill (*Scophthalmus rhombus)* in the Fish Assay from December 2022 (p=0.083, prevalence=3). Other contaminants were non-fish species (e.g., *Acanthogorgiidae*, unidentified duck species (*Antatidae*) and, marine worms (*Spionidae*)).

Additionally, only species identified to species-level were retained for analysis (except for a skate species which was identified through bioinformatics as either the cuckoo ray (*Leucoraja naevus)* or shagreen ray *(Leucoraja fullonica)* which was included in the analysis as *Leucoraja sp*.). Additionally, freshwater species Stone loach (*Barbatula barbatula*) and Minnow (*Phoxinus phoxinus*) were removed from the dataset as their eDNA was likely to be present in the vicinity from either freshwater input or deposited by predator species, rather than the fish being present. This is also the case for three-spined stickleback (*Gasterosteus aculeatus*) which is commonly found in brackish water and was also removed due to uncertainty around whether these records indicate marine presence, however they are very tolerant of salinity changes and some populations are known to be anadromous (Kottelat,& Freyhof, 2007). Taking a cautious approach, this species was also removed due to uncertainty around whether these records indicate marine presence.

As part of the decontamination process, the mammal species blue whale (*Balaenoptera musculus*) which was recorded in the spring vertebrate assay data, was removed from the dataset as it only appeared in one of the three station replicates samples. It seemed unlikely that this result could have been possible, however, following a sighting of two blue whales in the central North Sea just north of Newcastle in 2020, there is growing evidence to suggest their presence in shallow waters of the central North Sea (Lavallin *et al.* 2023).

Seasonal variation in occurrence from historic trawls and drop-down video at the site is known, with a seasonal signal for haddock (*Melanogrammus aeglefinus*), whiting (*Merlangius merlangus*), plaice (*Pleuronectes platessa*), grey gurnard (*Eutrigla gurnadus*), dab (*Limanda limanda*), lough rough dab (*Hippoglossoides platessoides*), and lemon sole (*Microstomus kitt*) (Natural Power, 2021). Seasonal occurrences were visualised for these species from eDNA, with read counts considered to provide an indication of the extent of occurrence (see caveats listed in the discussion of this report) to investigate whether eDNA showed a similar seasonal trend.

3.3. Univariate analysis

The following species diversity indices were calculated for both trawl and eDNA data:

- Number of Species (S) (Taxa): provides the number of species present in a sample, with no indication of relative abundances;
- Effective species: the number of equally abundant species needed to obtain the same mean proportional species abundance as that observed in the survey data;
- Number of individuals / read counts (n) (Abundance): provides the total number of individuals or read counts counted;
- Species Diversity - Shannon-Wiener index (H'): measures the uncertainty in predicting the identity of the next species withdrawn from a sample. Typically between 1.5 and 3.5, a lower value shows lower diversity;
- Species Richness - Margalef's index (d): measures the number of species present for a given number of individuals. The higher the index, the greater the diversity;
- Pielou's evenness (J'): shows how evenly the individuals in a sample are distributed. J' is a range of zero to one. The less variation in the samples, the higher J' is.

These univariate indices enable the reduction of large datasets into useful metrics, which can be used to accurately describe community structures. However, where eDNA read counts are used, it should be noted that read counts are not directly linked to abundance, and indices that incorporate abundance may be less reliable with eDNA compared to trawls. Regardless, results are presented equally for both methods for comparison.

3.4. Multivariate analyses

Multivariate analysis is an effective method for detecting subtle changes in species community datasets. Multivariate analyses were calculated in R using the vegan package (Oksanen *et al*. 2022). Due to the partially skewed nature of species data, and its varying abundances, a square root transformation was applied to normalise the trawl data distribution, and fourth root to eDNA read counts - reducing dominant effects of highly abundant taxa. A Bray-Curtis resemblance matrix was applied to the transformed infauna data.

To cluster stations based on the similarity profiles (SIMPROF) of community composition, hierarchical clustering and permutation testing were utilized to identify the coherence of groups of stations. This process effectively creates a dendrogram of similarity between stations and descends nodes while testing for significant multivariate structure within the node until the total number of significant stations is identified.

During the summer survey, trawl sampling at station 8 was not possible due to the presence of static fishing gear in the region. It was however possible to collect eDNA samples at the planned trawl start and end locations. In order to reduce potential effects from this unbalanced design (Anderson & Walsh, 2013), station 8 was removed prior to ANOSIM and permutational multivariate analysis of variance (PERMANOVA) tests when using the trawl data (see below).

ANOSIM was used to determine whether there was a significant difference between community composition between surveys, stations, and between stations within and outside the turbine development area in the case of the

eDNA dataset. This uses a Bray-Curtis dissimilarity matrix to determine whether there is a greater difference in the mean ranks between groups than those within groups, where groups are variables such as station and survey. The resultant R statistic quantifies that difference, with values of 0 representing random groupings (I.e., there is no significant influence of group on species composition), and values of closer to –1 or +1 showing a stronger influence of groups.

PERMANOVA was used to determine whether the same variables tested with ANOSIM affected community composition. PERMANOVA is a semiparametric method that partitions multivariate variation within dissimilarity measures. This tests whether the centroid and spread of dissimilarity differs between groups (e.g., stations, surveys, location), and permutes with random draws from the dataset to calculate the probability of the given groups explaining variation in composition.

3.5. Comparison

3.5.1. Data processing

In order to compare the species occurrence in eDNA and trawls directly, the differences in species detected by the two methods were investigated. Venn diagrams were constructed with the Venn Diagram package in R (Chen 2022), to visualize the species that were detected uniquely by each sampling methodology or shared in both datasets. The list of species picked up by one method but not the other is presented to determine whether key species are missed or conversely detected; or whether there is a pattern in those differences. Stations 1 and 2 were omitted from eDNA analysis for this element, as they were not sampled by trawls.

3.5.2. Multivariate

As the units and scale of trawl abundance and eDNA read counts are not equal, these measures were transformed to be presence/absence of each species in each sample. Due to the binary nature of this occurrence, Jaccard index of dissimilarity was adopted as a measure of distance. The Jaccard measure calculates the proportion of species that are shared between pairs of samples. This can be written as

$$1 - \frac{a}{a + b + c}$$

Where a is the number of species present in both samples, b is the number of species present in x but not y, and c is the number of species that are present in y but not x.

Due to station 8 being missed during the summer trawl survey, this station was removed from eDNA and trawl datasets in all seasons for multivariate analysis to improve comparability. Using the Jaccard distances, ANOSIM was used to determine whether species composition varied by sampling methodology. Non-Metric Multidimensional Scaling (NMDS) plots were produced to examine the similarity between sampling methods.

4. Method Development

A key aspect of the Project was to trial how the eDNA method could be practically implemented at sea, whilst working on a commercial offshore wind farm site. **Table 4.1** outlines the lessons learnt and improvements made throughout the Project.

Table 4.1: Lessons learnt during eDNA and fish ecology surveys.

Observation	Details	Suggested improvement	Action
Use of 3 L sample bag for eDNA samples	As the sample bags were smaller than the water samples taken, each sample had to be processed in two halves which increased the time required to get through each sample.	If possible, larger sample bags would be used to increase processing efficiency.	NatureMetrics supplied 5.4 L bags which greatly increased efficiencies.
Use of one Niskin bottle to collect eDNA water samples	More than one Niskin bottle could be deployed to improve time efficiency. Furthermore, due to the first observation listed above, the Niskin bottle could not be redeployed until the full sample had been processed.	Numerous Niskin bottles could be deployed at each station to increase processing efficiency.	The efficiencies gained from the larger sample bag meant a second Niskin bottle wasn't needed. The whole sample was emptied into the sample bag and the Niskin redeployed.
Replacement of batteries in the vampire sampler	The vampire sampler battery depleted fairly quickly and needed replacing often (approximately every two samples). NatureMetrics did inform us of this requirement and chargers were plugged in inside the wheelhouse.	The batteries charged within the required amount of time so did not hold up the process overall. No improvement suggested for the next survey.	As per the first survey the batteries were replaced at approximately every two samples. No real time delays were caused.
Sample recording including the use of the NatureMetrics app	Sample recording became quite busy when eDNA sample processing was simultaneous with the fish trawl processing. Some glitches with the app led to slight delays e.g., barcode scanning issue when attempting to use a tablet but the app worked on one mobile phone.	As the survey progressed, staff developed a routine for sample recording which improved efficiencies.	Feedback passed on to the developers to add changes to V3 of the app. Making sure there was a white background behind the barcode seemed to improve its success when using a mobile phone.
Issue with vampire sampler (VS) setting	The VS setting moved between 1 and 2. Setting 2 (pulse) was then accidentally selected which caused the tubing to bubble.	Ensure the VS is always on setting one.	None required.

Observation	Details	Suggested improvement	Action
Preferred bucket size corresponds to the sample bag being used.	A clear plastic square bucket with lid worked well for the 3L sample bag, whereas large black buckets are preferable for the 5.4L bags.	Select bucket to match sample bags provided.	None required.
Issue with the vampire sampler motor	Vampire sampler failure due to an issue with the motor which prevented the water being drawn up the tubing into the filter.	Replacement VS sent. Samples successfully filtered and preserved.	Issue logged with NatureMetrics. No follow up action required.

Despite a few initial tweaks to improve efficiencies, the methods were successfully implemented, and no issues were significant enough to impact the completion of the surveys to meet the project aims.

As such the Project has concluded that eDNA samples can be practically obtained offshore whilst working around a commercial OWF.

5. Results

5.1. Species Occurrence and Community Composition

A total of 26 fish species and 1,483 individuals were captured during the 2022 trawl surveys. The most abundant species of fish were whiting, haddock, dab, plaice, and long rough dab (**Table 5.1**). This aligns with the post-construction compliance monitoring results whereby in year 3, the most abundant species from the trawl catches across seasons were dab, plaice and haddock followed by long rough dab (Natural Power, 2021). A total of 59 species of fish were detected in the fish assay (including within turbine Stations 1 and 2) and 54 species were detected in the fish assay from trawl stations alone (**Table 5.2**). Read counts indicate the most abundant species of fish were haddock, long rough dab, and dab, followed by whiting, lemon sole and plaice.

There were 42 species of fish identified in the vertebrate assay (including within turbine Stations 1 and 2) and 41 fish species detected in the vertebrate assay from the trawl stations alone (**Appendix B, Table B.1**). Read counts indicate the most abundant species of fish were cod (*Gadus morhua)*, long rough dab, lemon sole and hake (*Merluccius merluccius*).

There are several species that were not detected by one sampling method, which were detected by the other method. Nineteen species were recorded using both the trawl and fish assay eDNA method, whilst 35 species were unique to the eDNA data and seven species unique to the trawls (**Figure 5.1**).

When using the fish assay, the species not detected by eDNA, that were present in the trawl data were: red gurnard *(Aspitriglia cuculus),* tub gurnard *(Chelidonichthys lucerna),* grey gurnard *(Eutrigla gurnardus),* anglerfish *(Lophius sp.),* thornback ray *(Raja clavata),* cuckoo ray *(Raja naevus)* and small spotted catshark *(Scyliorhinus canicula).* However, anglerfish was identified to species level using eDNA as European angler/common monkfish (*Lophius piscatorius)*. In addition, a *Triglidae* species sequence was frequently detected in the fish assay data, which is likely to be one or several of the gurnard species identified (which share the same fish assay (zero-radius Operational Taxanomic Unit) (ZOTU) sequence). A ray was identified in the vertebrate assay as either the cuckoo or shagreen ray (L*eucoraja naevus/Leucoraja fullonica*) and was included in the final vertebrate assay dataset as *Leucoraja* sp. **(Appendix B)**.

Trawls did not catch the following species that were detected by eDNA (using the fish assay): Atlantic wolfish *(Anarhichas lupus),* European eel *(Anguilla anguilla),* spotted dragonet *(Callionymus maculatus),* Yarell's blenny *(Chirolophis ascanii),* five-bearded rockling (*Ciliata mustela*), Northern rockling *(Ciliata septentrionalis),* herring *(Clupea harengus),* crystal goby *(Crystallogobius linearis),* goldsinny (*Ctenolabrus rupestris),* lumpsucker *(Cyclopterus lumpus),* bass *(Dicentrarchus labrax),* lesser weaver *(Echiichthys vipera),* fourbeard rockling (*Enchelyopus cimbrius),* anchovy *(Engraulis encrasicolus),* witch (*Glyptocephalus cynoglossus*), sea snail *(Liparis liparis),* Montagu's sea snail *(Liparis montagui),* shanny *(Lipophrys pholis),* anglerfish, thickback sole *(Microchirus variegatus),* bull rout *(Myoxocephalus Scorpius),* European smelt *(Osmerus eperlanus),* butterfish *(Pholis gunnellus,* Norwegian topknot *(Phrynorhombus norvegicus),* pollack *(Pollachius pollachius),* saithe *(Pollachius virens),* sand goby *(Pomatoschistus minutus),* tadpole fish *(Raniceps raninus),* sea trout *(Salmo trutta),* pilchard *(Sardina pilchardus),* Atlantic mackerel *(Scomber scombrus),* brill *(Scophthalmus rhombus),* sea scorpion *(Taurulus bubalis),* Atlantic horse mackerel *(Trachurus trachurus)* and bib *(Trisopterus luscus).*

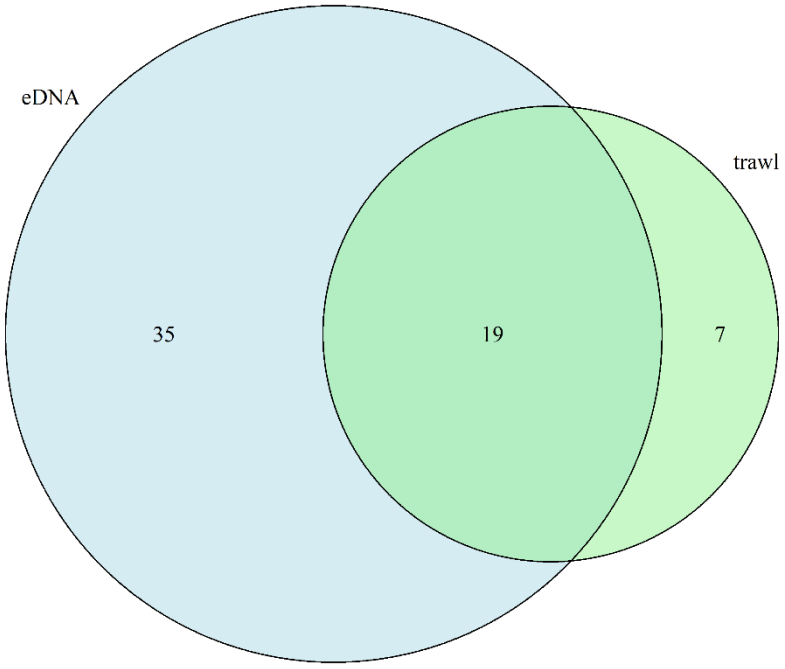


Figure 5.1: Venn diagram showing the number of species detected by eDNA (using the fish assay) and trawls.

For the vertebrate assay, thirteen species were common to both the trawl and vertebrate assay eDNA method, whilst 28 species were unique to the eDNA data and 13 species unique to the trawls (**Appendix B, Figure B.1**). Again, gurnard species were present in the trawl but not the eDNA when using the vertebrate assay as well as species such as haddock, whiting and plaice (which were not identified to species level in this assay). Species such as Atlantic salmon *(Salmo salar)* and sea trout and bottom dwelling fish species such as rockling, goby and shanny were present in the eDNA data, but not the trawl. A full breakdown of species by method using the vertebrate assay is provided in (**Appendix B, Table B.1**).

There was a significant difference between community composition between methods when read counts from the fish assay and trawl abundance were simplified to presence or absence at each station and dissimilarity was calculated by Jaccard dissimilarity index (ANOSIM P = 0.001; R = 0.9615) (**Figure 5.2**). Similarly, a significant difference was found between eDNA (using the vertebrate assay) and trawl abundance (ANOSIM P = 0.002; R=0.606) (**Appendix B, Figure B.2**).

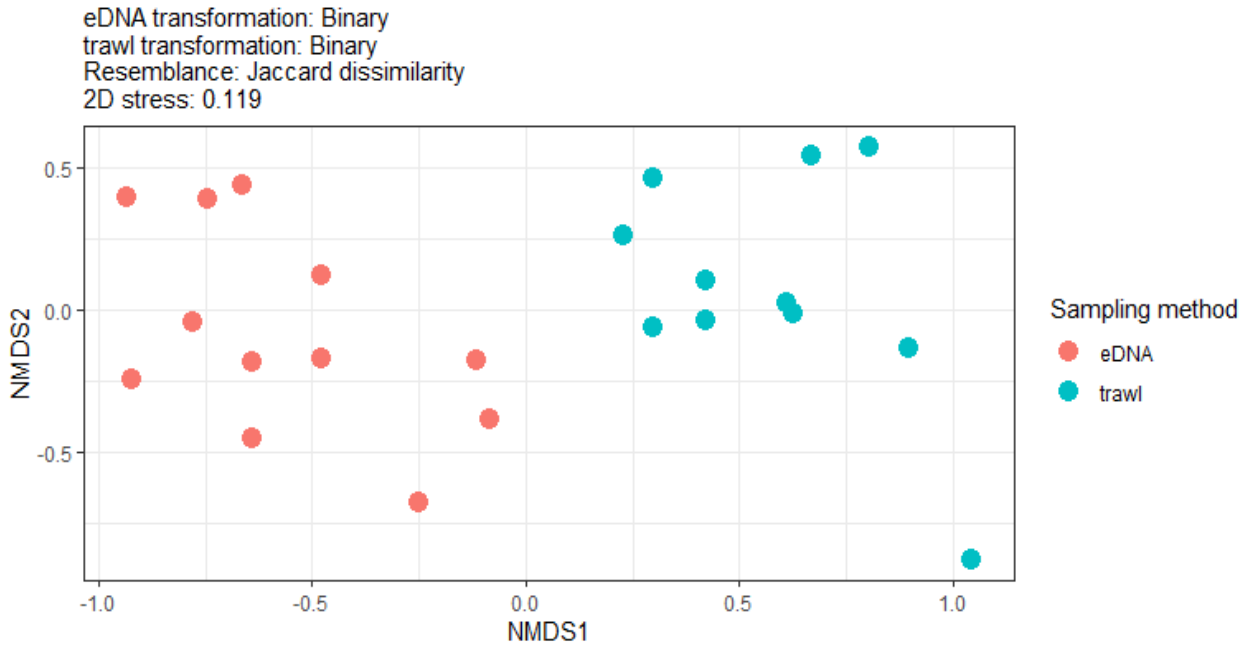


Figure 5.2: **NMDS plot showing Jaccard dissimilarity between samples from eDNA and trawl data after presence/absence transformation – using fish assay.**

The NMDS plot (**Figure 5.2**) clearly shows the dissimilarity with no overlap between the two methods. This is expected given the greater number of species detected using the fish assay eDNA results and the trawl catch data. However, to investigate the species driving this dissimilarity between sampling methods, redundancy analysis was conducted with constrained ordination. This allowed the investigation and visualization of species that were significantly different between methods, filtered to only include those that explained over 45% of variation for the fish assay (**Figure 5.3**) and vertebrate assay (**Appendix B, Figure B.3**).

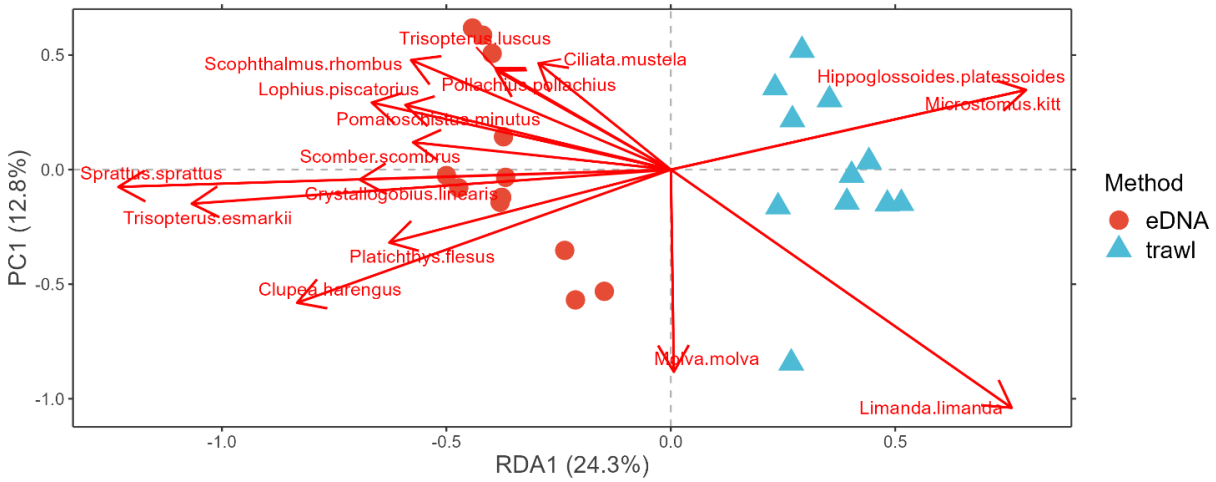


Figure 5.3: **Ordination showing species driving dissimilarity between sampling method – fish assay.**

Figure 5.3 suggests the main differences in species composition between the methods is that flat fish and ling are more dominant in the trawl method results and whilst sprat *(Sprattus sprattus)* and herring are common in the eDNA results, they are not present or are only present in small numbers in the trawl catches. Bib and Norway pout are also driving the dissimilarity between methods, as are five bearded rockling and sand goby. This may reflect fish that can easily escape trawl nets, either due to being smaller or evading capture (e.g., by hiding in crevices), but are being captured by the eDNA method (fish assay).

There is more overlap between the methods when comparing community composition at stations using trawl catch data and the vertebrate assay eDNA data, although the results remain significantly different (**Appendix B, Figure B.2**). Species driving dissimilarity are also flat fish for the trawls and to an extent cod, common dragonet and Norway pout in the vertebrate assay eDNA (**Appendix B, Figure B.2**).

5.2. Seasonal Trends

Seasonal fluctuations in abundances of certain fish species in the area can be seen in the trawl sampling results (**Table 5.1**) and in the eDNA results (**Table 5.2, Appendix B, Table B.2**)

The 2022 trawl results found whiting and haddock in much greater abundances in the autumn, long rough dab and lemon sole in highest abundances in winter, while dab and plaice were less abundant in winter than in the other seasons and most abundant in spring (**Table 5.1, Figure 5.4**).

Table 5.1: Standardised abundance of fish species captured per season, rounded to the nearest whole number.

Common name	Latin name	Winter	Spring	Summer	Autumn
Whiting	*Merlangius merlangus*	33	1	18	360
Haddock	*Melanogrammus aeglefinus*	70	2	6	278
Dab	*Limanda limanda*	39	105	52	80
Plaice	*Pleuronectes platessa*	11	60	37	39
Long rough dab	*Hippoglossoides platessoides*	55	30	21	20
Grey gurnard	*Eutrigla gurnardus*	3	23	5	21
Lemon sole	*Microstomus kitt*	15	12	8	6
Pogge	*Agonus cataphractus*	8	1	0	8
Cod	*Gadus morhua*	3	3	2	5
Flounder	*Platichthys flesus*	4	4	0	0
Angler fish	*Lophius sp.*	3	2	2	0
Cuckoo ray	*Raja naevus*	0	0	0	4
Sole	*Solea solea*	0	1	0	3
Turbot	*Scophthalmus maximus*	1	0	1	1
Small-spotted catshark	*Scyliorhinus canicula*	0	0	0	3
Norway pout	*Trisopterus esmarkii*	0	0	0	3
Ling	*Molva molva*	0	0	0	2
Red gurnard	*Aspitriglia cuculus*	0	1	0	0
Common dragonet	*Callionymus lyra*	1	0	0	0
Tub gurnard	*Chelidonichthys lucerna*	1	0	0	0

Common name	Latin name	Winter	Spring	Summer	Autumn
Hake	*Merluccius merluccius*	0	0	1	0
Red mullet	*Mullus surmuletus*	1	0	0	0
Thornback ray	*Raja clavata*	1	0	0	0
Sprat	*Sprattus sprattus*	1	0	0	0
Poor cod	*Trisopterus minutus*	1	0	0	0
John Dory	*Zeus faber*	1	0	0	0

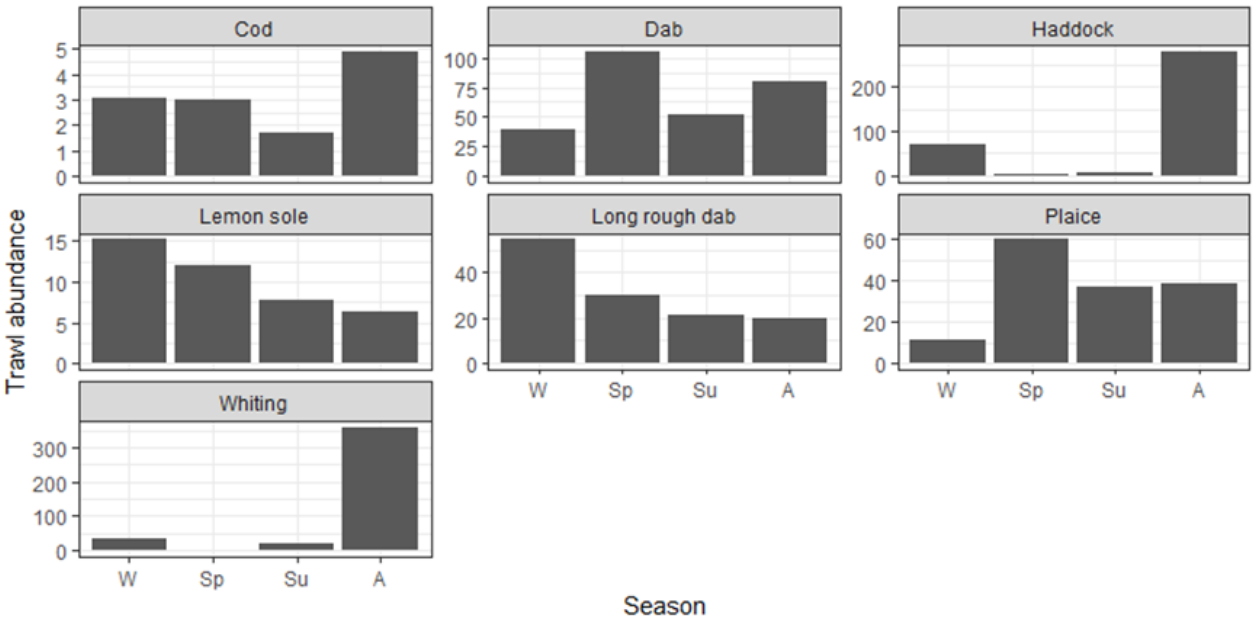


Figure 5.4: Abundance of key species that were investigated for seasonal signal in trawls (W = winter, Sp = spring, Su = summer, A = autumn).

Seasonal trends from the eDNA data are largely consistent with those of the trawl data, particularly the fish assay data. Whiting was present in the highest relative abundances during autumn in the trawl and fish assay data. Cod and haddock were most abundant in the autumn trawls, but most prevalent in winter in the fish assay results (**Table 5.2, Figure 5.5**). Whiting and haddock were not captured by the vertebrate eDNA assay (**Appendix B, Table B.2, Figure B.4**). Dab were most abundant in the spring trawls and had the highest relative abundances in spring and summer in both the eDNA fish and vertebrate assays. Long rough dab were most abundant in winter and spring in the trawl and both fish and vertebrate assay results. Plaice were most abundant in spring in the trawl and fish assay data but was not detected using the vertebrate assay.

Table 5.2: **eDNA occurrence of fish species identified using the Fish Assay. Numbers presented are read counts from all stations except station 1 and 2.**

Common name	Latin name	Winter	Spring	Summer	Autumn	Total
Haddock	*Melanogrammus aeglefinus*	473708	85685	19782	364160	943335
Long rough dab	*Hippoglossoides platessoides*	468317	328721	57135	69833	924006
Dab	*Limanda limanda*	0	327788	302342	138345	768475
Whiting	*Merlangius merlangus*	101084	34130	108321	222491	466026
Lemon sole	*Microstomus kitt*	107494	111902	11614	28042	259052
Plaice	*Pleuronectes platessa*	46459	114103	50771	20228	231561
Atlantic herring	*Clupea harengus*	0	139411	15776	11694	166881
Crystal goby	*Crystallogobius linearis*	186	137886	24392	0	162464
Sprat	*Sprattus sprattus*	56720	29026	33228	27277	146251
Turbot	*Scophthalmus maximus*	16662	5092	81759	9575	113088
Atlantic mackerel	*Scomber scombrus*	1226	963	62039	0	64228
Cod	*Gadus morhua*	23027	2980	8737	26811	61555
Brill	*Scophthalmus rhombus*	55255	0	4250	0	59505
Flounder	*Platichthys flesus*	0	0	31343	13144	44487
Sole	*Solea solea*	2785	8544	6730	13120	31179
Sand goby	*Pomatoschistus minutus*	1649	1402	26045	0	29096
Red mullet	*Mullus surmuletus*	22631	0	22	0	22653
Norway pout	*Trisopterus esmarkii*	1501	1815	8066	6149	17531
Angler fish	*Lophius piscatorius*	4658	134	10628	305	15725
Ling	*Molva molva*	0	46	0	12766	12812
Bib	*Trisopterus luscus*	9627	653	474	0	10754
Hake	*Merluccius merluccius*	16	0	9992	0	10008
Butterfish	*Pholis gunnellus*	2462	4682	801	611	8556
Lumpsucker	*Cyclopterus lumpus*	7582	93	18	0	7693
Pollack	*Pollachius pollachius*	877	0	0	5171	6048
Bull rout	*Myoxocephalus scorpius*	787	677	1911	1450	4825
John Dory	*Zeus faber*	4754	0	11	0	4765
Norwegian topknot	*Phrynorhombus norvegicus*	589	1656	1082	0	3327
Poor cod	*Trisopterus minutus*	1181	332	981	0	2494
Yarrell's blenny	*Chirolophis ascanii*	151	0	888	1313	2352

Common name	Latin name	Winter	Spring	Summer	Autumn	Total
Fourbeard rockling	*Enchelyopus cimbrius*	38	1632	547	0	2217
Atlantic wolffish	*Anarhichas lupus*	55	1693	0	0	1748
Saithe	*Pollachius virens*	0	45	520	1087	1652
Northern rockling	*Ciliata septentrionalis*	1152	278	85	0	1515
Common dragonet	*Callionymus lyra*	572	673	202	0	1447
European pilchard	*Sardina pilchardus*	0	1249	0	27	1276
Witch	*Glyptocephalus cynoglossus*	30	1179	0	0	1209
Thickback sole	*Microchirus variegatus*	0	71	818	316	1205
Atlantic horse mackerel	*Trachurus trachurus*	15	0	0	938	953
Sea scorpion	*Taurulus bubalis*	0	826	0	0	826
Sea trout	*Salmo trutta*	0	0	441	281	722
Five-bearded rockling	*Ciliata mustela*	174	0	0	469	643
Anchovy	*Engraulis encrasicolus*	0	0	0	594	594
Shanny	*Lipophrys pholis*	314	0	0	0	314
Pogge	*Agonus cataphractus*	0	0	0	259	259
Sea snail	*Liparis liparis*	0	205	0	0	205
Lesser weever	*Echiichthys vipera*	0	201	0	0	201
European smelt	*Osmerus eperlanus*	0	0	0	201	201
Goldsinny	*Ctenolabrus rupestris*	0	127	0	41	168
Spotted dragonet	*Callionymus maculatus*	0	0	113	0	113
European eel	*Anguilla anguilla*	78	0	0	0	78
Bass	*Dicentrarchus labrax*	0	43	0	0	43
Montagu's sea snail	*Liparis montagui*	37	0	0	0	37
Tadpole fish	*Raniceps raninus*	0	0	32	0	32

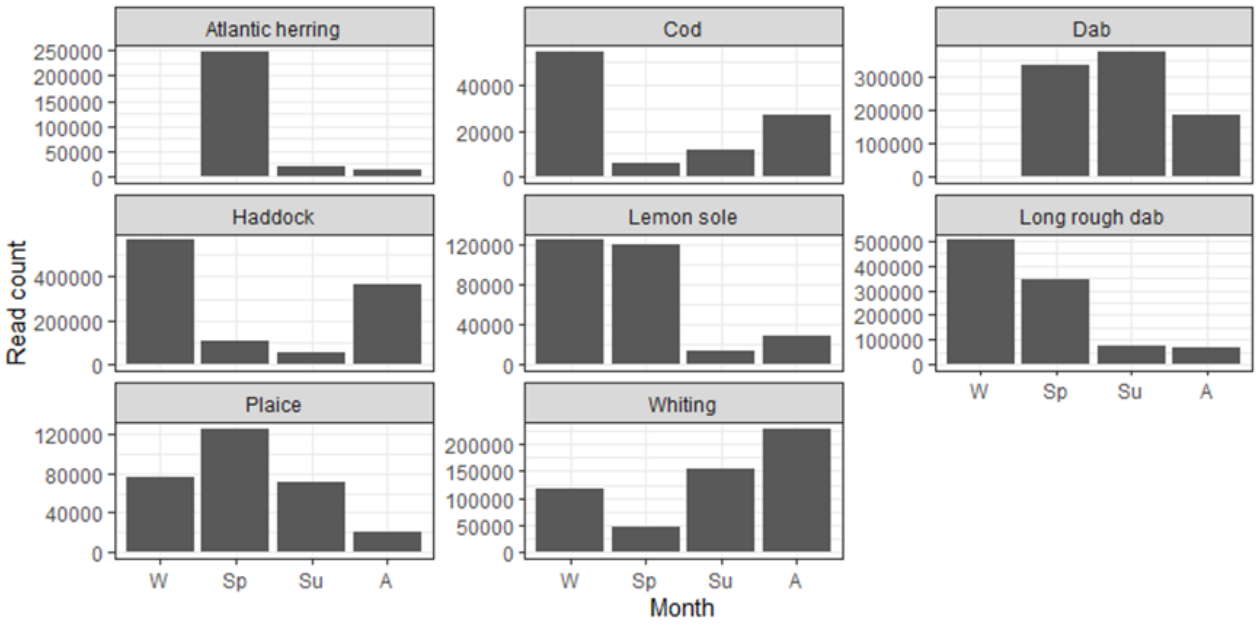


Figure 5.5: Temporal variation in read counts from the Fish Assay in species known to have a seasonal signal at the site.

When looking at univariate analysis for the trawl data; diversity, effective species and evenness indices values are lowest in the autumn at all trawl stations other than Station 8 which is lowest in spring (**Figure 5.6**). Conversely, richness values are lowest in spring at trawl Stations 3, 5, and 8 and lowest in autumn at trawl Station 9 (**Figure 5.6**). This is reflective of the seasonal and migratory variation in species occurrence; for offshore stations 3, 5 and 9 diversity indices are lower in the autumn due to the large abundances of whiting and haddock. Whereas diversity indices for the inshore Station 8 are lowest in spring where the catch was dominated by dab with some flounder (*Platichthys flesus*) and plaice with only one other fish species present.

Multivariate analysis was used to determine whether there was a significant difference between community composition for the trawl data between surveys and both ANOSIM and PERMANOVA found that community structure differs significantly between seasons (ANOSIM; P = 0.002; R2 = 0.5741) (PERMANOVA; P < 0.001; R = 0.57605; F = 7.5402).

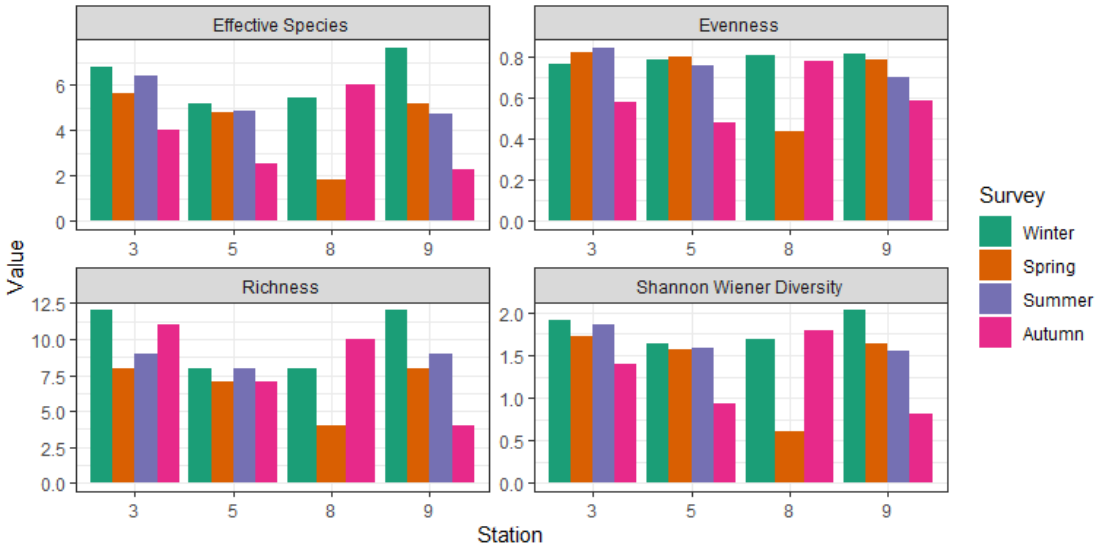


Figure 5.6 Univariate indices of community composition in trawl data, for each season and station.

Fish and vertebrate assay univariate analysis across seasons shows diversity values are greatly reduced at Stations 1 and 2 in autumn (**Figure 5.7, Appendix B, Figure B.5**). Diversity values for Station 9, which lies closest to Stations 1 and 2, are also reduced in autumn in the fish assay and invertebrate assay data and in the trawl data (**Figure 5.6**). This is likely due to a reduced number of taxa with high relative abundance (dab, haddock and whiting) recorded within the turbine stations and much higher abundance of haddock and whiting at Station 9 in the trawl data in autumn (**Table 5.1**).

When comparing eDNA and trawl univariate results over seasons, Station 8 has lower diversity values in the spring than in other seasons and this is not reflected in the eDNA assay results. The trawl catch was dominated by dab with some flounder while plaice was the only other fish species recorded in the trawl catch. This is similar to the historical data at BOD, whereby post-construction year 3 monitoring had only dab and plaice present in the trawl catch at Station 8. The eDNA method however detected 31 species at Station 8 in spring (fish assay) and 21 fish species in the vertebrate assay. These species included dab and plaice but also several species that are not generally captured using the otter trawl method such as crystal goby (*Crystallogobius linearis*) and sand eel species (*Ammodytes*) as well as haddock, herring, sprat, poor cod (*Trisopterus minutus*), bib, sea scorpion and bull rout.

As with the seasonal changes seen in the trawl data, multivariate analysis showed statistically significant difference between community composition between surveys in the eDNA data. When using the fish assay, ANOSIM suggested that community composition in eDNA varies significantly between seasons (P = 0.001; R = 0.6395), This is supported by PERMANOVA, with survey season a significant predictor (P = 0.001; R2 = 0.439; F = 5.716) is significant. When using the vertebrate assay, ANOSIM suggested that community composition in eDNA varies significantly between seasons (P = 0.001; R = 0.8307), as does PERMANOVA (P < 0.001; R = 0.573; F=14.966).

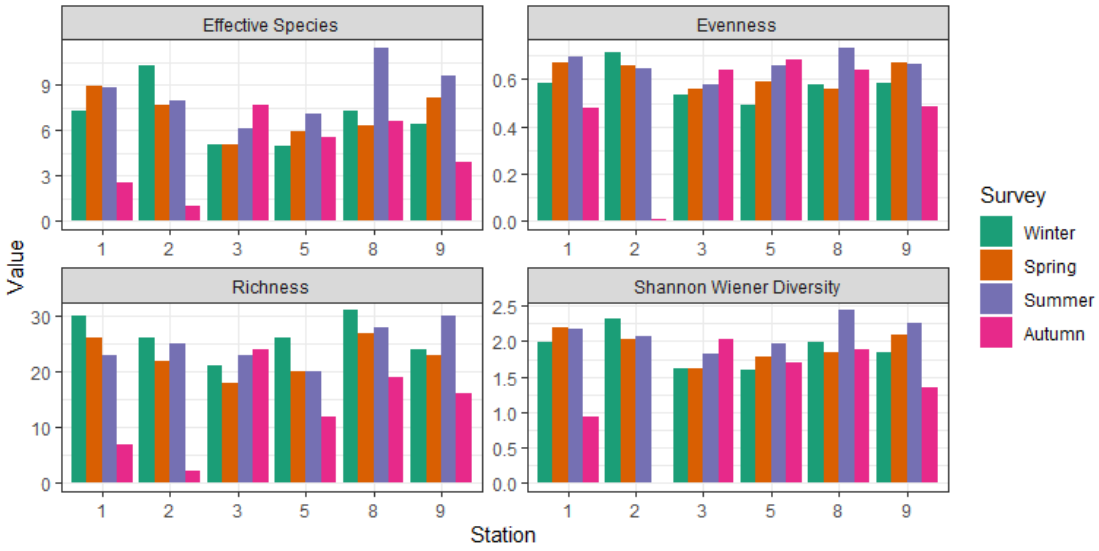


Figure 5.7: **Variation in univariate indices in eDNA data by season and station, using the Fish Assay**

5.3. Spatial Trends

Univariate analyses of the trawl catch data shows diverse and species rich communities across all trawl stations (**Table 5.3, Figure 5.8**),

Table 5.3: **Univariate metrics of diversity from trawl data for each station.**

Station	No. Taxa	Abundance	Shannon-Wiener Diversity	Richness	Evenness	Effective Species Number
3	17	321.33	2.01	2.77	0.71	7.45
5	13	466.67	1.58	1.95	0.62	4.85
8	14	359.09	1.76	2.21	0.67	5.79
9	16	335.39	1.95	2.58	0.70	7.03

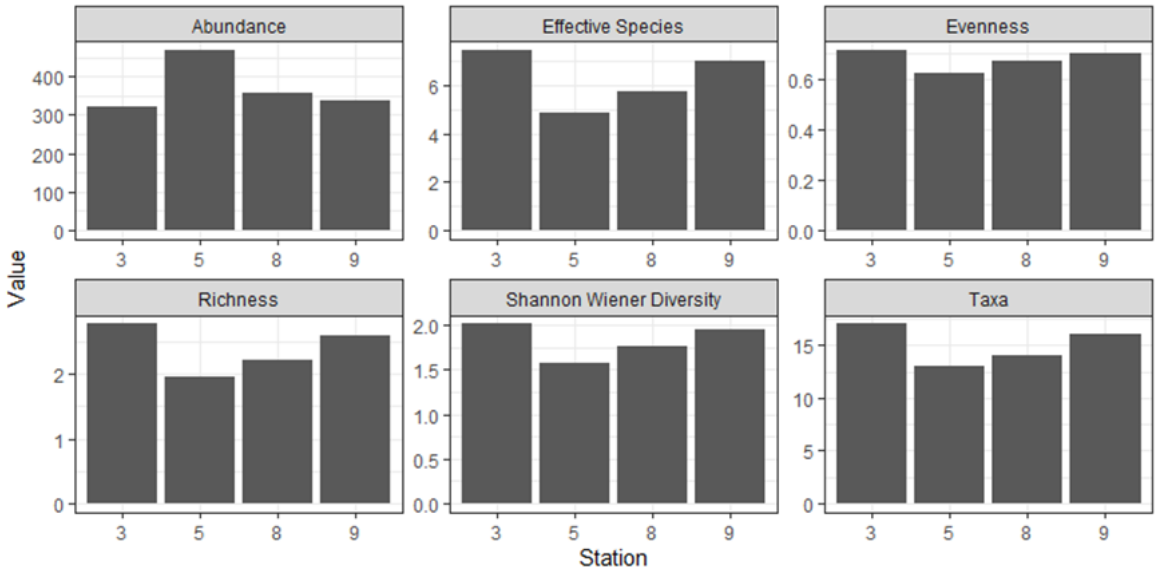


Figure 5.8: Univariate indices of community composition in trawl data for each station.

Station 5 has the greatest abundance and lowest diversity scores of the four trawl stations. Station 3, followed by Station 9 have the lowest abundances and highest diversity scores of the trawl stations (**Figure 5.8**).

Univariate analysis of the eDNA datasets included Stations 1 and 2, despite these stations not being present in the trawl data. The fish assay detected more fish taxa and number of read counts than the vertebrate assay overall and at each station (**Table 5.4, Appendix B, Table B.3**). Stations 8 and 9 have the highest number of taxa in the fish assay data, whereas stations 3 and 8 have the highest number of taxa in the vertebrate assay data. Univariate diversity indices: Shannon-Wiener, Richness, Evenness and Effective species number are all higher when using the fish assay than the vertebrate assay, however both sets of eDNA results indicate a diverse and species rich community across the project area (**Figure 5.9, Appendix B, Figure B.6**).

Table 5.4: Univariate measures of community structure, using the Fish Assay

Station	No. Taxa	No. Read Counts	Shannon-Wiener Diversity	Richness	Evenness	Effective Species Number
1	42	2722.35	2.53	5.18	0.68	12.55
2	39	3174.50	2.66	4.71	0.73	14.33
3	40	4041.63	2.15	4.70	0.58	8.58
5	36	4617.78	2.20	4.15	0.61	9.04
8	42	3552.65	2.59	5.02	0.69	13.33
9	43	4237.69	2.36	5.03	0.63	10.56

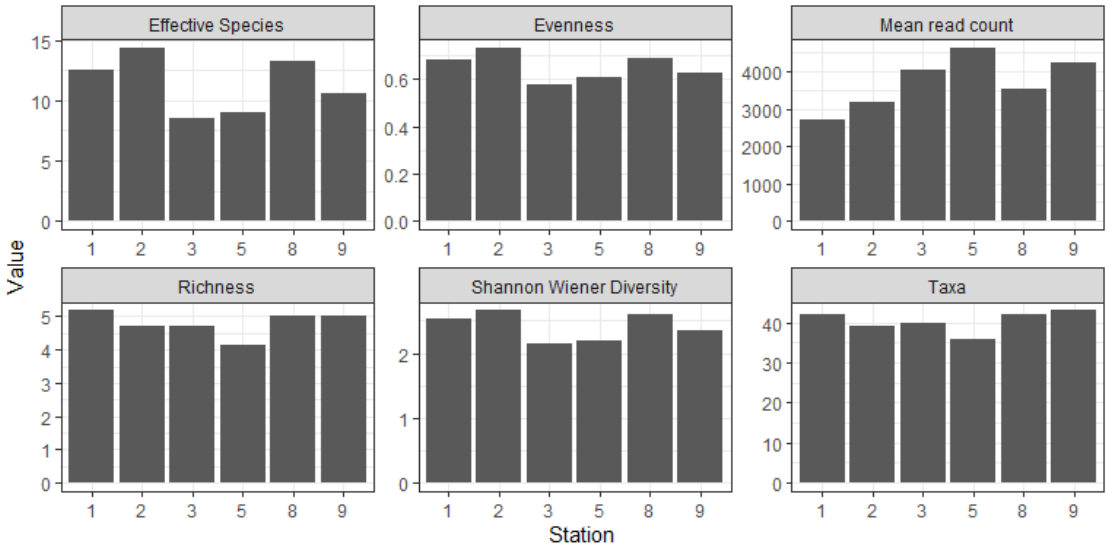


Figure 5.9: Univariate indices of community composition in eDNA data, using the Fish Assay

When comparing eDNA univariate results with those of the trawl, results are broadly similar; diversity values are highest at stations 3 and 9 in the trawl data and at 8 and 9 in the eDNA fish assay data (excluding within turbine stations 1 and 2) and species richness lowest at station 5 in both datasets. Diversity and evenness are slightly greater at station 3 than 5 in the fish assay results. However, station 5 has the lowest number of species in both the trawl and fish assay results. (**Figures 5.8 and 5.9,**).

When using hierarchical clustering (SIMPROF) based on Bray-Curtis dissimilarity, two significant clusters were identified from the trawls. Station 8 (which is the closest inshore) was clustered on its own, with a separate cluster containing stations 9, 5 and 3. Within the cluster of three stations, there was greater similarity between species composition at stations 9 and 5 (**Figure 5.10**). Station 3 is least similar to the other stations in the cluster and this station lies furthest offshore. Station 8 has the least similarity to the other trawl stations, and this may be expected due to the shallower waters supporting a slightly different community than the deeper offshore locations and is consistent with the findings of the historical data (Natural Power, 2021). Stations 5 and 9 shared most similarity; Station 5 is further north and slightly inshore and Station 9 was not trawled previously but was included in this Project to provide a trawl station as close to the turbines as possible.

Multivariate analysis to explore whether there was a significant difference between community composition between stations found no significant difference between stations (ANOSIM: P =0.054; R2 = 0.22); whereas PERMANOVA did ($P < 0.001$; $R = 0.534$; $F = 4.716$).

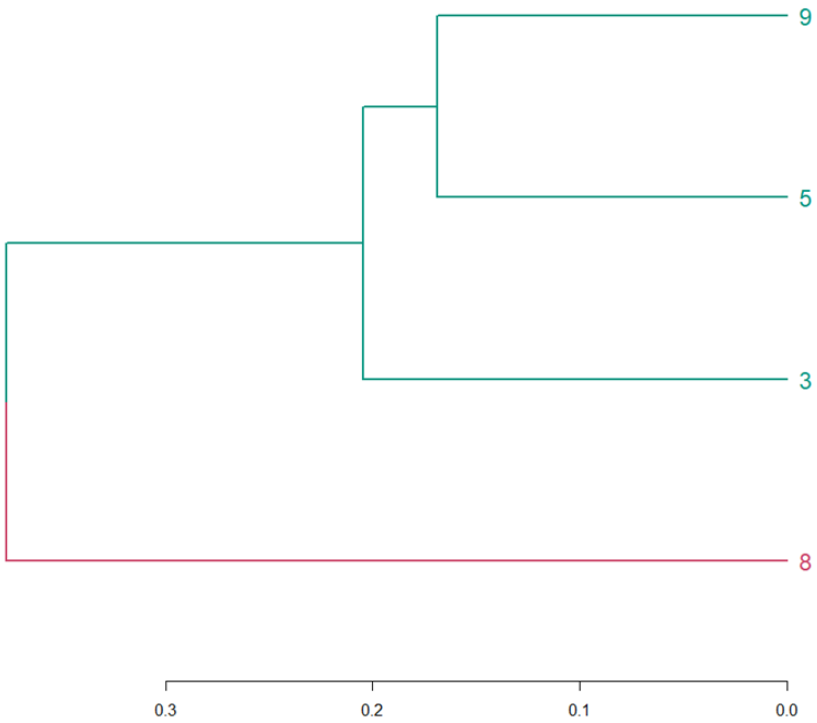


Figure 5.10: Station clustering in trawl data.

Hierarchical clustering (SIMPROF) of eDNA stations, using the fish assay, found three significant clusters. Station 1 and 2 (within the area occupied by turbines and therefore not sampled by trawls) were clustered separately from the rest. Station 8 (the closest to shore) was more closely related to the other stations outside of the turbine development area but was clustered alone. Stations 3 (furthest offshore), 5 (furthest north), and 9 were clustered together (**Figure 5.10**). This mirrors the hierarchical clustering (SIMPROF) of stations from the trawl data for the trawl stations (**Figure 5.11**).

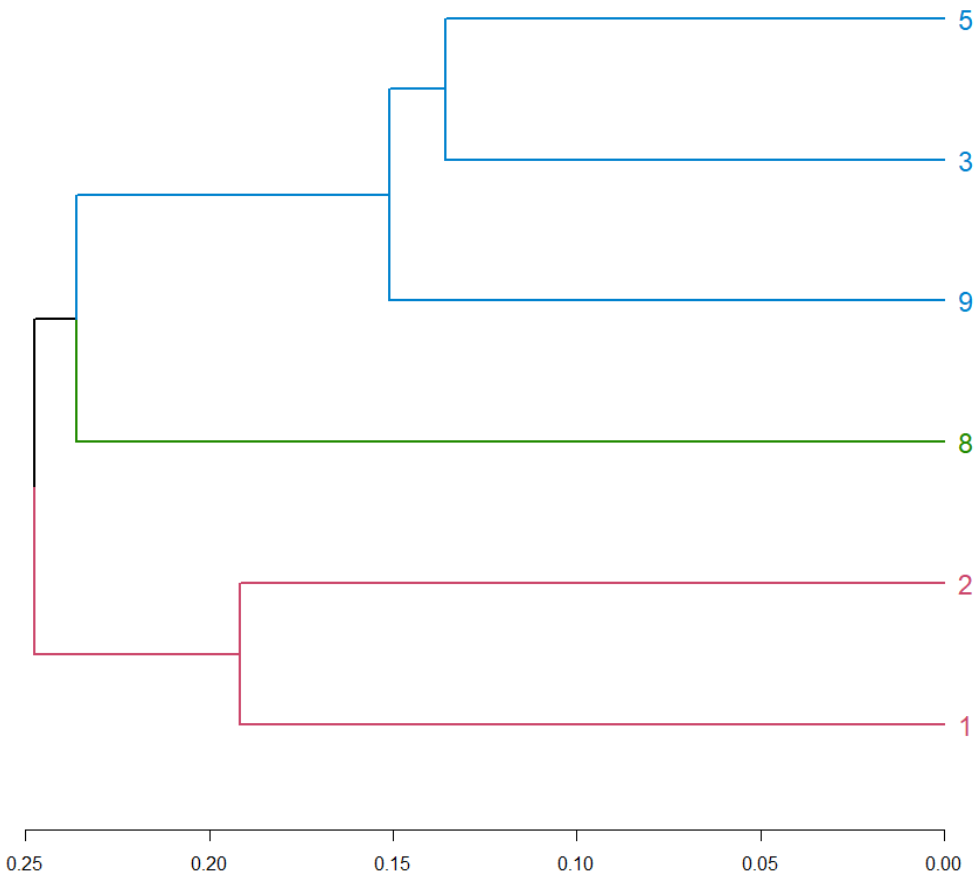


Figure 5.11: Hierarchical clustering of sampling stations identified from the fish assay dataset.

When using the fish assay, ANOSIM suggested that community composition in eDNA varies significantly between stations within the array area (Stations 1 and 2) and those outside (Stations 3,5, 8, 9) (P=0.002; R=0.303), but not between stations overall (P = 0.458; R = 0.005). This is supported by PERMANOVA, with station found not to be a significant predictor (P = 0.544; R2 = 0.097; F=0.943), whereas location (P = 0.005; R2 = 0.081; F=3.157) was significant. When using the vertebrate assay, hierarchical clustering resulted in Stations 1 and 2 clustered together, as were Stations 3 and 5. However, in comparison to the fish assay, Stations 8 and 9 were also clustered together (**Appendix B, Table B3, Figure B.7**).

For Multivariate analysis., when using the vertebrate assay, ANOSIM suggested that community composition in eDNA varies significantly between stations within and outside the turbine development area (P=0.02; R=0.238), but not between stations (P = 0.260; R=0.0647). Conversely, PERMANOVA suggests that station is a significant predictor (P =0.001; R2 = 0.138; F=2.715), as well as location (P < 0.001; R2 = 0.108; F = 8.474).

5.4. Within Turbine Stations

Given the assumptions and caveats surrounding using read counts as a proxy for abundance (**see Section 6. Discussion**), and different numbers of stations within the development area (Stations 1 & 2) and those outside (all others), data were simplified in an attempt to identify whether species occurring within these two areas differ biologically or ecologically. The mean read count of each species was calculated per area, and then normalized for each area so that the value represents the percentage mean contribution of the read counts of that species.

Table 5.5 shows relative occurrence of species within the area for the fish assay which can be used to compare between areas for each species. For example, Five-bearded rockling (*Ciliata mustela*) contributes 0.14% of mean read counts outside of the turbine area, whereas this species contributes 13.26% at stations within – suggesting higher levels of occurrence within turbines.

Table 5.5: The percentage of mean read counts from the Fish Assay within the development area (Stations 1 & 2) and those outside (Stations 3,5, 8, and 9) for each species.

Common name	Latin name	Outside	Within
Pogge	*Agonus cataphractus*	0.29	0.00
Atlantic wolffish	*Anarhichas lupus*	0.98	0.11
European eel	*Anguilla anguilla*	0.04	0.00
Mediterranean scaldfish	*Arnoglossus laterna*	0.00	0.04
Common dragonet	*Callionymus lyra*	0.20	0.06
Spotted dragonet	*Callionymus maculatus*	0.13	0.27
Yarrell's blenny	*Chirolophis ascanii*	0.53	3.43
Five-bearded rockling	*Ciliata mustela*	0.14	13.30
Northern rockling	*Ciliata septentrionalis*	0.21	0.59
Atlantic herring	*Clupea harengus*	4.66	14.24
Crystal goby	*Crystallogobius linearis*	4.54	0.27
Goldsinny	*Ctenolabrus rupestris*	0.06	0.97
Lumpsucker	*Cyclopterus lumpus*	0.86	0.55
Bass	*Dicentrarchus labrax*	0.05	0.00
Lesser weever	*Echiichthys vipera*	0.11	0.08
fourbeard rockling	*Enchelyopus cimbrius*	0.28	0.19
Anchovy	*Engraulis encrasicolus*	0.66	0.00
Cod	*Gadus morhua*	1.21	3.10
Witch	*Glyptocephalus cynoglossus*	0.27	0.38
Blackbelly rosefish	*Helicolenus dactylopterus*	0.00	0.59
Long rough dab	*Hippoglossoides platessoides*	12.90	4.57
Dab	*Limanda limanda*	15.06	11.38
Sea snail	*Liparis liparis*	0.23	0.00
Montagu's sea snail	*Liparis montagui*	0.04	0.00

Common name	Latin name	Outside	Within
Shanny	*Lipophrys pholis*	0.18	0.00
Angler fish	*Lophius piscatorius*	0.61	0.16
Muller's pearlside	*Maurolicus muelleri*	0.00	0.50
Haddock	*Melanogrammus aeglefinus*	13.01	9.93
Whiting	*Merlangius merlangus*	6.85	5.35
Hake	*Merluccius merluccius*	1.12	0.00
Norway bullhead	*Micrenophrys lilljeborgii*	0.00	1.80
Thickback sole	*Microchirus variegatus*	0.15	0.08
Lemon sole	*Microstomus kitt*	4.02	2.17
Ling	*Molva molva*	4.77	0.30
Red mullet	*Mullus surmuletus*	1.58	0.00
Bull rout	*Myoxocephalus scorpius*	0.49	0.81
European smelt	*Osmerus eperlanus*	0.22	0.02
Butterfish	*Pholis gunnellus*	0.56	0.52
Norwegian topknot	*Phrynorhombus norvegicus*	0.46	0.68
Flounder	*Platichthys flesus*	1.66	2.74
Plaice	*Pleuronectes platessa*	3.45	4.50
Pollack	*Pollachius pollachius*	0.29	0.19
Saithe	*Pollachius virens*	0.31	0.00
Sand goby	*Pomatoschistus minutus*	1.48	2.22
Tadpole fish	*Raniceps raninus*	0.04	0.82
Atlantic salmon	*Salmo salar*	0.00	0.15
Sea trout	*Salmo trutta*	0.27	0.47
European pilchard	*Sardina pilchardus*	0.71	0.00
Atlantic mackerel	*Scomber scombrus*	2.39	1.93
Turbot	*Scophthalmus maximus*	3.24	1.07
Brill	*Scophthalmus rhombus*	3.02	1.11
Sole	*Solea solea*	0.99	1.83
Sprat	*Sprattus sprattus*	2.27	4.68
Sea scorpion	*Taurulus bubalis*	0.31	0.45
Atlantic horse mackerel	*Trachurus trachurus*	0.35	0.00
Norway pout	*Trisopterus esmarkii*	0.42	0.33
Bib	*Trisopterus luscus*	0.63	0.39
Poor cod	*Trisopterus minutus*	0.23	0.69
John Dory	*Zeus faber*	0.44	0.00

The species which occur in greater relative abundance within the turbines than outside include a variety of bottom dwelling flat fish, such as flounder (*Platichthys flesus)*, plaice, witch (*Glyptocephalus cynoglossus*) and other bottom dwelling fish species which prefer rocky, reefy or sandy habitats such as goldsinny (*Ctenolabrus rupestris)*, bull rout (*Myoxocephalus scorpius*), fourbearded rockling (*Enchelyopus cimbrius*) and Norway bullhead (*Micrenophrys lilljeborgii*).

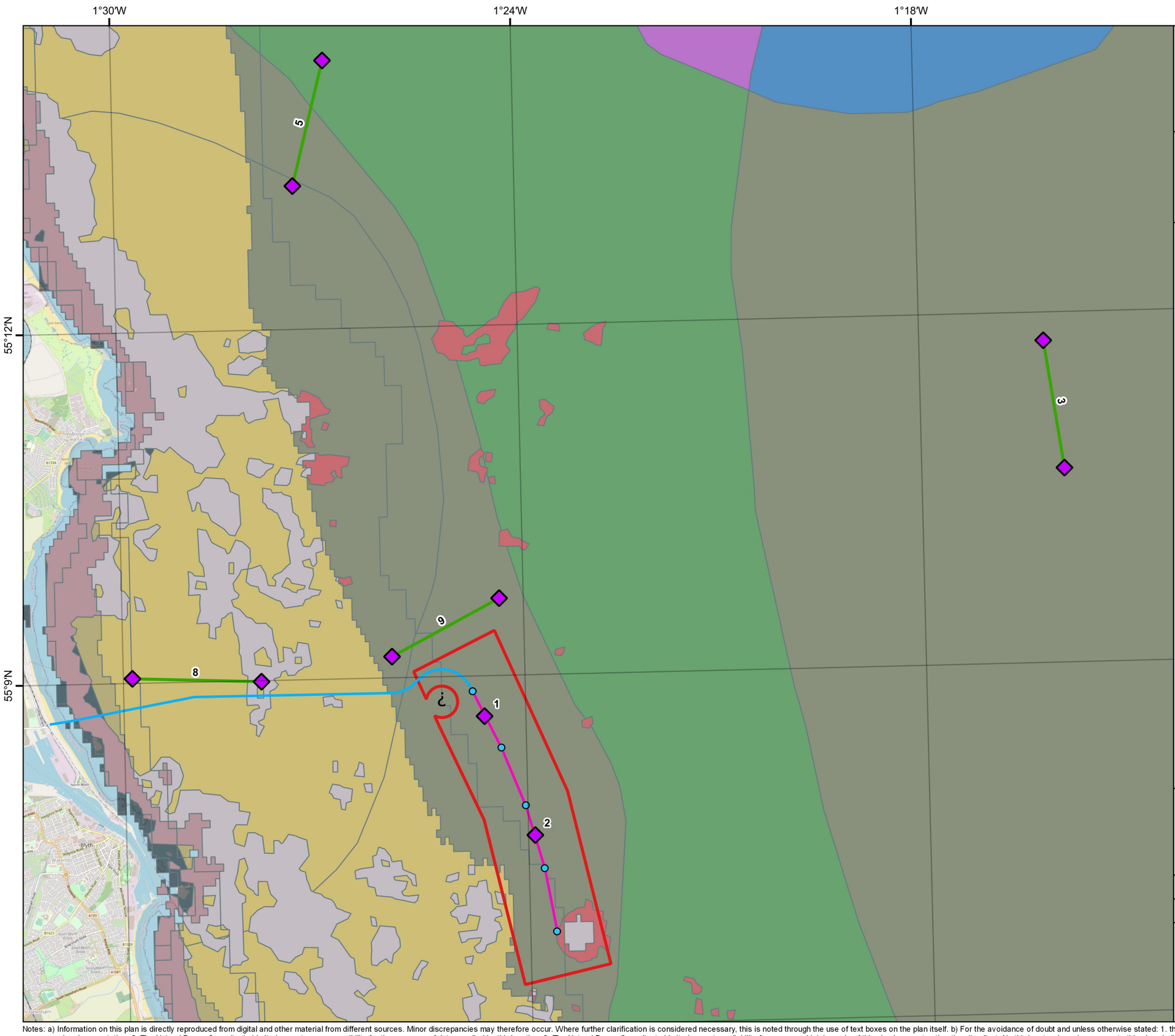
There is also a group of pelagic fish which, from the fish assay, occur in greater relative abundances inside the turbines than outside and include cod, poor cod (*Trisopterus minutus*), Atlantic salmon *(Salmo salar)*, sea trout (*Salmo trutta*), herring (*Clupea harengus*) and sprat (*Sprattus sprattus*). The predicted habitat type in the turbine area is mud (**Figure 5.12**), however, many of the species found in greater relative abundances here prefer coarser sediment types. For example, the goldsinny inhabits rocks or algae (MarLIN), Norway bullhead inhabits hard bottoms or algae (GBIF), the four-bearded rockling dwells on muddy sand between patches of hard substrate (Fishbase) and the bull rout is usually found on rocky substrate with sand and mud (MarLIN).

Seasonal trends inside and outside the turbine stations

Cod were found in greatest abundances in the trawl catches in Autumn (**Table 5.6**). The fish assay shows an overall greater abundance of cod in the winter and greater percentage mean contribution within the turbines (9.4%) than outside the turbine area (1.42%) (**Table 5.6**). This coincides with the spawning period for cod in the area which is January to April (Ellis *et al*. 2012). Conversely, haddock were found in greater relative abundance out with the turbines in autumn, winter and spring but at greater relative abundance within the turbines in summer (**Table 5.6**).

Atlantic herring (*Clupea harengus*) spawning grounds lie close to the BOD site and spawn August-October in this region (Ellis *et al*., 2012, Coull *et al*. 1998). Herring are found at greater relative abundance within the turbines in spring, whilst sprat are found in much greater abundances within the turbines in summer (**Table 5.6**).

Similar trends are seen when comparing results from the vertebrate assay with cod and poor cod greater at the within turbine stations than out with (**Appendix B, Tables B.4, B.5**). However, herring and sprat were not identified to species level in the vertebrate assay. This is also true for bottom dwelling species five-bearded rockling, fourbeard rockling, goldsinny, and Norway bullhead.



Project:

Ecological Assessment Around Offshore Wind Farms Using eDNA

Title:

Figure 5.12: Benthic Habitat Types Across Blyth Trawl and eDNA Project Area

Key

- Blyth Offshore Demonstrator Array 2 project area boundary
- Turbine
- Anemometry mast
- Inter array cable
- Export cable
- Fish ecology trawl
- eDNA water sampling station

Benthic habitats (EUSeaMap, 2021)

- Circalittoral mixed sediment
- Offshore circalittoral mixed sediment
- Circalittoral mud
- Infralittoral mud
- Offshore circalittoral mud
- Circalittoral rock and biogenic reef
- Infralittoral rock and biogenic reef
- Offshore circalittoral rock and biogenic reef
- Offshore circalittoral coarse sediment
- Offshore circalittoral sand
- N/A

Scale @ A3: 1:60,000

Coordinate System: WGS 84 / UTM zone 30N

Graticules: WGS 84

0 0.5 1 1.5 2 km

N

Date: 21-07-23	Prepared by: JO	Checked by: ME
Ref: GB200769_M_110_A		

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Table 5.6: Seasonal eDNA occurrence of fish species compared between turbine area (B) and outside (O) - fish assay. (W = winter, Sp = spring, Su = summer, A = autumn)

Common name	Latin name	W O	W B	Sp O	Sp B	Su O	Su B	A O	A B
Pogge	*Agonus cataphractus*	0.00	0.00	0.00	0.00	0.00	0.00	0.30	0.00
Atlantic wolffish	*Anarhichas lupus*	0.08	0.23	2.35	0.06	0.00	0.00	0.00	0.00
European eel	*Anguilla anguilla*	0.06	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Mediterranean scaldfish	*Arnoglossus laterna*	0.00	0.00	0.00	0.00	0.00	0.08	0.00	0.00
Common dragonet	*Callionymus lyra*	0.81	0.00	0.23	0.11	0.13	0.06	0.00	0.00
Spotted dragonet	*Callionymus maculatus*	0.00	0.00	0.00	0.00	0.22	0.48	0.00	0.00
Yarrell's blenny	*Chirolophis ascanii*	0.21	0.00	0.00	4.60	0.85	0.00	0.76	0.00
Five-bearded rockling	*Ciliata mustela*	0.06	17.13	0.00	0.00	0.00	0.00	0.55	0.00
Northern rockling	*Ciliata septentrionalis*	0.33	1.19	0.39	0.13	0.08	0.98	0.00	0.00
Atlantic herring	*Clupea harengus*	0.00	0.00	10.76	33.51	2.15	1.36	1.70	0.00
Crystal goby	*Crystallogobius linearis*	0.07	0.43	10.08	0.47	2.74	0.13	0.00	0.00
Goldsinny	*Ctenolabrus rupestris*	0.00	0.00	0.09	1.30	0.00	0.00	0.05	0.00
Lumpsucker	*Cyclopterus lumpus*	1.54	0.83	0.06	0.47	0.03	0.00	0.00	0.00
Bass	*Dicentrarchus labrax*	0.00	0.00	0.06	0.00	0.00	0.00	0.00	0.00
Lesser weever	*Echiichthys vipera*	0.00	0.00	0.14	0.00	0.00	0.14	0.00	0.00
fourbeard rockling	*Enchelyopus cimbrius*	0.05	0.52	0.76	0.08	0.21	0.27	0.00	0.00
Anchovy	*Engraulis encrasicolus*	0.00	0.00	0.00	0.00	0.00	0.00	0.69	0.00
Cod	*Gadus morhua*	1.42	9.43	0.69	1.56	0.98	0.97	2.84	0.00
Witch	*Glyptocephalus cynoglossus*	0.04	0.68	0.41	0.11	0.00	0.00	0.00	0.00
Blackbelly rosefish	*Helicolenus dactylopterus*	0.00	0.76	0.00	0.00	0.00	0.00	0.00	0.00
Long rough dab	*Hippoglossoides platessoides*	28.91	9.02	19.85	4.76	5.19	8.04	6.25	0.06
Dab	*Limanda limanda*	0.00	0.00	19.79	2.98	27.46	25.17	12.39	67.39
Sea snail	*Liparis liparis*	0.00	0.00	0.28	0.00	0.00	0.00	0.00	0.00
Montagu's sea snail	*Liparis montagui*	0.05	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Shanny	*Lipophrys pholis*	0.22	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Angler fish	*Lophius piscatorius*	0.60	0.06	0.09	0.00	1.35	0.47	0.36	0.00
Muller's pearlside	*Maurolicus muelleri*	0.00	0.64	0.00	0.00	0.00	0.00	0.00	0.00

Common name	Latin name	W O	W B	Sp O	Sp B	Su O	Su B	A O	A B
Haddock	*Melanogrammus aeglefinus*	28.03	23.24	5.67	6.76	1.80	12.31	28.27	10.09
Whiting	*Merlangius merlangus*	6.24	4.46	2.79	4.09	9.84	15.57	17.27	20.77
Hake	*Merluccius merluccius*	0.02	0.00	0.00	0.00	2.12	0.00	0.00	0.00
Norway bullhead	*Micrenophrys lilljeborgii*	0.00	0.00	0.00	4.79	0.00	0.03	0.00	0.00
Thickback sole	*Microchirus variegatus*	0.00	0.00	0.10	0.00	0.22	0.15	0.37	0.00
Lemon sole	*Microstomus kitt*	6.94	5.11	7.06	2.65	1.30	1.02	2.97	0.00
Ling	*Molva molva*	0.00	0.32	0.06	0.59	0.00	0.00	7.43	0.00
Red mullet	*Mullus surmuletus*	2.14	0.00	0.00	0.00	0.04	0.00	0.00	0.00
Bull rout	*Myoxocephalus scorpius*	0.28	0.28	0.24	1.48	3.65	0.91	0.84	0.00
European smelt	*Osmerus eperlanus*	0.00	0.03	0.00	0.00	0.00	0.00	0.23	0.00
Butterfish	*Pholis gunnellus*	0.87	0.89	0.93	0.00	0.38	0.29	0.36	0.00
Norwegian topknot	*Phrynorhombus norvegicus*	0.28	0.83	0.77	0.92	1.03	0.00	0.00	0.00
Flounder	*Platichthys flesus*	0.00	0.00	0.00	0.00	2.99	5.86	1.53	0.21
Plaice	*Pleuronectes platessa*	2.87	7.37	6.89	4.53	4.84	8.45	2.62	1.42
Pollack	*Pollachius pollachius*	0.07	0.25	0.00	0.00	0.00	0.00	1.20	0.00
Saithe	*Pollachius virens*	0.00	0.00	0.06	0.00	0.50	0.00	0.42	0.00
Sand goby	*Pomatoschistus minutus*	0.47	0.63	0.97	0.56	3.31	5.87	0.00	0.00
Tadpole fish	*Raniceps raninus*	0.00	0.39	0.00	1.45	0.06	0.00	0.00	0.00
Atlantic salmon	*Salmo salar*	0.00	0.19	0.00	0.00	0.00	0.00	0.00	0.00
Sea trout	*Salmo trutta*	0.00	0.61	0.00	0.00	0.84	0.00	0.16	0.00
European pilchard	*Sardina pilchardus*	0.00	0.00	1.73	0.00	0.00	0.00	0.03	0.00
Atlantic mackerel	*Scomber scombrus*	0.35	0.80	0.27	3.99	5.92	2.41	0.00	0.00
Turbot	*Scophthalmus maximus*	1.58	1.73	1.77	1.70	13.00	0.57	1.39	0.00
Brill	*Scophthalmus rhombus*	8.72	1.58	0.00	0.67	0.62	2.06	0.00	0.00
Sole	*Solea solea*	0.79	3.62	0.91	1.42	0.92	3.32	5.09	0.00
Sprat	*Sprattus sprattus*	3.66	4.76	2.12	11.37	3.34	2.50	2.65	0.00
Sea scorpion	*Taurulus bubalis*	0.00	0.57	0.38	0.00	0.00	0.00	0.00	0.00
Atlantic horse mackerel	*Trachurus trachurus*	0.02	0.00	0.00	0.00	0.00	0.00	0.55	0.00
Norway pout	*Trisopterus esmarkii*	0.19	0.17	0.25	0.92	0.96	0.45	0.72	0.00
Bib	*Trisopterus luscus*	0.91	0.71	0.91	0.44	0.30	0.05	0.00	0.06

Common name	Latin name	W O	W B	Sp O	Sp B	Su O	Su B	A O	A B
Poor cod	*Trisopterus minutus*	0.42	0.54	0.09	1.54	0.62	0.00	0.00	0.00
John Dory	*Zeus faber*	0.61	0.00	0.00	0.00	0.02	0.00	0.00	0.00

5.5. Application to other Species Groups

Marine mammal data

The following marine mammal species were identified by the vertebrate assay – minke whale (*Balaenoptera acutorostrata*), white-sided/white-beaked dolphin (*Lagenorhynchus* species), bottlenose dolphin (*Tursiops truncatus*), and harbour porpoise (*Phocoena phocoena*) (**Table 5.7**).

Table 5.7: Marine mammal species occurrence in eDNA data

Common name	Latin name	Winter	Spring	Summer	Autumn
Minke whale	*Balaenoptera acutorostrata*	0	0	216	0
White-sided/ White-beaked dolphin	*Lagenorhynchus acutus /Lagenorhynchus albirostris*	88	0	256	539
Harbour porpoise	*Phocoena phocoena*	7003	3643	1353	1303
Bottlenose dolphin	*Tursiops truncatus*	3408	429	0	0

6. Discussion

Suitability of the eDNA method whilst working at an OWF

This study compared the occurrence of fish species in concurrent water samples for eDNA sequencing and trawls around a commercial offshore wind farm. In terms of method development and suitability whilst working offshore at a commercial wind farm site, eDNA sampling was successfully implemented. A number of lessons learnt have been outlined to improve efficiencies, but none of these were significant enough to impact the completion of the surveys to meet the project aims.

During one of the surveys, a trawl stationed could not be sampled by trawl due to the presence of static fishing gear however it was possible to collect eDNA samples from the trawl start and end locations. Furthermore, eDNA samples were collected from stations within the turbine locations, where it has never been possible to trawl due to the health and safety risk of gear snagging. The method developed during this study can be used to practically sample the fish ecology offshore whilst working around a commercial OWF.

Species occurrence and community composition between methods

eDNA consistently detected a greater number of species compared to traditional methods, including smaller fish species, migratory species, and bottom-dwellers that are not often captured in trawl gear due to biases associated with gear selectivity and limitations in the locations in which trawl fishing can occur.

The most abundant species were consistent across the two methods, as well as being in line with historical site data. This indicates that the eDNA method provides data for the species captured by the traditional method as well as many other species that wouldn't typically be captured by trawl sampling alone.

Despite this, trawls appeared to capture some species not identified by concurrent eDNA samples, including three species of gurnard and a species of elasmobranch. In the case of the gurnard species, this was due to low taxonomic resolution of the assay (as the family level (*Triglidae*) was identified). Similarly, the cuckoo ray recorded in the trawl survey was identified as either the cuckoo or shagreen ray in the vertebrate assay eDNA data. As it was identified as one of two species of the of the same genus of skate, the decision was taken to include this in the final dataset as *Leucoraja* sp. As such it may be overly precautionary to remove taxa not identified beyond species level in the eDNA data and identification to genus can be seen as a suitable step for consideration in the data decontamination process. as with the traditional trawl method occasionally taxa can only be identified to genus level (e.g., as *Leucoraja* sp.). Low abundances of elasmobranchs and therefore the potentially the lower concentration of eDNA produced may also result in their eDNA not being detected. Fish identification in the field also can be subject to human error at times and identification of the juvenile forms of closely related species and/or species that interbreed can be difficult. eDNA would remove this potential source of error.

When read counts from the fish/vertebrate assay and trawl abundance were simplified to a presence/absence metric at each station there was a strong statistical difference in community composition found between the methods, likely due to greater numbers of species detected by eDNA.

Ordination plots indicate that the dissimilarity between the methods is primarily driven by flat fish species being more dominant in the trawls, whilst pelagic species including key forage fish (herring, sprat and Norway pout) were prevalent in the eDNA data. This aligns with known selectivity of the otter trawl gear. It also indicates the ability for eDNA sampling to detect both commercially and ecologically important species.

Seasonal & spatial trends

Both seasonal and spatial patterns in species occurrence and community composition were similar between the trawl and eDNA based sampling methodologies.

Seasonal trends noted in the 2022 trawl data were evident in the eDNA results, for example peak whiting catches and read counts (using the fish assay) were recorded in autumn and peak dab catches/read counts were recorded

in spring/summer (using both the fish and vertebrate assays). Historical pre- and post-construction monitoring at the site found whiting and dab have been recorded as contributing to seasonal differences over the entire monitoring period. Overall, there have generally been larger catches of; whiting in the summer and dab in autumn and spring. Although some slight seasonal variations from historic data were noted, these seasons are consecutive and could relate to specific survey timings within the seasonal sampling window. Sampling timings were often dictated by suitable weather windows for trawling, however the use of eDNA methods alone could alleviate such restrictions by reducing the health and safety limitations of standard survey equipment.

When comparing univariate analysis between the eDNA and trawl methods, station trends in diversity indices were broadly similar. However seasonal trends in the eDNA data at stations differed from both the 2022 and historic trawl data. For example, at station 8 this was driven by the greater species diversity in the eDNA data in spring (in both the fish and vertebrate assay). Multivariate analysis showed the same hierarchical clustering and similar ANOSIM results for the 2022 trawl data and eDNA fish assay (for stations sampled by both methods). These results indicate that eDNA methods not only pick up individual species trends but can also be used to calculate ecological diversity metrics and to track seasonal and between station differences in community composition.

Spatial trends: inside/outside the array area

Utilising eDNA methods allowed the area within the array to be surveyed which is not typically feasible using trawl methods and allowed for an assessment of the species composition around the turbines for the first time. The results indicate species which occur in greater relative abundance within the turbines (compared to outside) include bottom dwelling fish that prefer coarser rocky, reefy or sandy habitats. Given the predicted habitat type in the turbine area is mud, this finding supports the hypothesis made in the original Environmental Statement (NaREC, 2012) that the artificial hard substrate created by turbines may be providing sheltered feeding grounds for fish (such as mature cod and haddock).

Cod occurred in greater relative abundance within the turbines than outside for the vertebrate and fish assays, with the greatest relative abundance in winter, coinciding with their spawning period. Herring were found at greater relative abundance within the turbines in spring, whilst sprat and herring were found in greater abundances within the turbines in summer according to the fish assay. It may be the case that these species are utilising the shelter and food provided by the colonised artificial substrates as nursery and/or feeding grounds. In addition, the timing of the peak in haddock within the turbine area (summer) differing from the peak in cod (winter) could relate to cod being active hunters, preying on a range of species, including haddock (Durant *et al*. 2020).

Both Atlantic salmon and sea trout are migratory species and were both picked up in the eDNA data. These species are not typically captured using traditional trawl methods due to gear selectivity and nearshore migration routes which do not often interact with trawl fishing areas. Improving the understanding of their oceanic ecology and distribution has been identified as a current knowledge gap (Rikardsen *et al.* 2021).

There was also a group of pelagic species (from the fish assay) that occur in greater relative abundance inside the turbines including forage fish species. Herring and sprat are key forage fish species, playing an important role in the marine food chain (Englehard *et al*. 2014). These findings indicate that the eDNA methods can be used to evidence and potentially assess net positive impacts from offshore wind infrastructure due to the ability to obtain robust samples within the array area.

Other species groups

Four marine mammal species were identified by the vertebrate assay: minke whale, white-sided/white-beaked dolphin, bottlenose dolphin, and harbour porpoise. Information from visual boat-based surveys such as those conducted as part of Blyth OWF's monitoring programme confirms the presence of these species in the area. Minke whales are known to be seasonally present (late summer/autumn) while harbour porpoises, the most abundant species in the area, are known to be present year-round. The dolphin species also occur year-round but are observed in groups rather than singly (in contrast to harbour porpoise and minke whale).

The vertebrate assay detected the seasonal occurrence of minke whale and demonstrated that harbour porpoise are present all year round. More frequent sampling would be advised for capturing the dolphin species as they travel in groups and could be missed due to the timing of sampling.

Nonetheless, this work provides evidence for use of eDNA for describing the marine mammal fauna of the area.

eDNA data suitability and array performance

This study provides a proof of concept for use of eDNA to describe the fish ecology around offshore wind farms however, there are some limitations to the method. Firstly, the comparison of results between the fish and vertebrate assay, when looking at fish ecology alone, indicates the importance of selecting or designing the most effective assay to deliver the required information on receptor(s).

The vertebrate assay primers have good efficiency, in that they correspond well with sequences for fish, mammal and bird taxa. However, the region targeted can have the same sequences for multiple fish species, such as haddock, whiting and cod. The fish assay is designed to amplify and differentiate fishes which often results in identifying more fishes with more specific taxonomy assignment than assays designed to target a broader range of taxa such as the vertebrate assay. As such, the fish assay detected more fish species and more fish taxa identified to species level than the broader vertebrate assay.

A perceived limitation of the eDNA method was that read counts have to be used as a proxy for abundance. In this study forth root transformation of read counts was used as a proxy for abundance. Despite this initial concern, for the purposes of baseline setting or monitoring around offshore wind farms, this does not seem to be an issue as univariate and multivariate results between the trawls and eDNA were broadly consistent with each other, other than the greater number of species and therefore greater diversity captured in the eDNA data. Seasonal and spatial differences in community composition are captured effectively using the eDNA method with the same patterns in the data captured by both eDNA and trawl results.

Whilst these limitations exist, eDNA may provide a better tool for fish ecology assessments as it offers data on wider range of species (not just commercially important species) including migratory species and species of conservation importance, providing a robust baseline and informing better targeted mitigation. It also allows for sampling within turbines which cannot be surveyed by trawl. Furthermore, it is a non-destructive sampling method as opposed to trawling.

Survey costs and sampling effort

Replacing traditional survey methods for assessing fish populations around OWFs with eDNA sampling provides greater opportunities to collect the data required as a larger pool of vessels becomes accessible to undertake the survey work. The survey work can also be combined with other site-based activities (e.g., site investigation work). This greatly reduces the costs, resource consumption (e.g., fuel) and risks of delays to surveys and therefore to subsequent consents.

Furthermore, using eDNA also removes issues around uncertainty in the data from gear selectivity and human error in the misidentification of species. The adoption of the method has the potential for huge benefits to the industry with more efficient, affordable, and scalable consenting and site survey solutions which will speed up developments of OWFs and reduce costs for developers/operators, ultimately reducing the cost of overall energy production.

7. Recommendations

Given the findings of the Project, the authors believe eDNA sampling provides a viable alternative to traditional fish survey methods around OWFs. Regulator acceptance of eDNA for use in offshore baseline setting and monitoring will therefore be a key step towards accelerating and improving environmental monitoring for future offshore wind development. The findings from the Project will be shared with regulators to encourage discussion and provide evidence of the benefits demonstrated.

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Appendices

A. eDNA Laboratory Analysis

DNA Extraction

DNA was extracted from each filter using a DNeasy Blood and Tissue Kit (Qiagen) with the modified protocol for disc filters in buffer described in Spens et al., 2017. An extraction blank was processed with each batch of extractions to assess potential contamination in the extraction process. DNA was purified to remove polymerase chain reaction (PCR) inhibitors using a DNeasy PowerClean Pro Cleanup Kit (Qiagen). Purified DNA extracts were quantified using a Qubit 3.0 fluorometer (Thermo Scientific).

DNA Amplification

The 18S ribosomal Ribonucleic acid (RNA) (invertebrates) (Capra et al. 2016) and the 12S ribosomal RNA (teleost fish and vertebrates) (Miya et al. 2015; Riaz et al. 2011; Kelly et al. 2014) genes were amplified via a two-step PCR process. In the first step, multiple PCR replicates were performed on each water sample for each assay. PCR positive controls (i.e., a mock community with a known composition of proprietary synthetic sequences that do not match biological records) were included to verify sequence quality and PCR negative controls (i.e., PCR grade water) were included to detect potential cross-contamination. Amplification success was confirmed via gel electrophoresis. Successfully amplified first round PCR replicates were pooled per sample and purified using magnetic beads.

Library preparation and sequencing

A sequencing library was prepared from the purified amplicons using a combinational dual index approach, following Illumina's 16S Metagenomic Sequencing Library Preparation protocol. Indexed PCR products were subsequently purified using magnetic beads prior to being quantified, normalised, and pooled in equal volumes. The final pooled library was denatured, diluted and sequenced on an Illumina MiSeq using a V3 600 cycle reagent kit. A PhiX control library (illumina) was included on each sequencing run to provide a quality control for cluster generation, sequencing, and alignment, and a calibration control for cross-talk matrix generation, phasing, and prephasing.

Bioinformatics

All libraries were processed together for each of the three assays. Sequences were demultiplexed with bcl2fastq and processed via a custom NatureMetrics eDNA analysis pipeline. Paired-end FASTQ reads for each sample were merged with USEARCH (Edgar 2010). Forward and reverse primers were trimmed from the merged sequences using cutadapt (Martin 2011) with a length filter of 80-120 bp. Sequences were quality filtered with USEARCH to retain only those with an expected error rate per base of 0.01 or below and dereplicated by sample, retaining singletons to obtain zero-radius Operational Taxonomic Units (zOTUs). Unique sequences from all samples were denoised in a single analysis with UNOISE (Edgar, 2016).

Consensus taxonomic assignments were made for each zOTU using sequence similarity searches against the NCBI nucleotide (NCBI nt) reference. Searches against databases were made using blastn (Altschul et al. 1990; Camacho et al. 2009) and required hits to have a minimum e-score of 1e-20 and cover at least 90% of the query sequence. The taxonomic identification associated with all hits was converted to match the GBIF taxonomic backbone.

Assignments were made to the lowest possible taxonomic level where there was consistency in the matches, with minimum similarity thresholds of 98%, 95% and 92% for species, genus, and higher-level assignments respectively. Identifications were sense-checking against GBIF occurrence records for presence in the UK and elevated to higher taxonomic levels where required (rgbif; Chamberlain et al., 2022).

zOTUs were clustered at 97% similarity with USEARCH to obtain OTUs. An OTU-by-sample table was generated by mapping all dereplicated reads for each sample to the OTU representative sequences with USEARCH at an identity threshold of 97%.

All OTUs with species-level identifications were queried against the IUCN Red List (rredlist; Chamberlin 2018) to obtain global threat status and the Global Register of Introduced and Invasive Species (GRIIS) to obtain their invasive status in the UK. The OTU table was filtered to remove low abundance OTUs from each sample (<0.02% or <10 reads, whichever is the greater threshold for the sample). Unassigned OTUs, and OTUs identified to human and domesticated mammals, were removed from the dataset for subsequent analyses.

B. Vertebrate Assay Findings

Table B.1: Species unique to one sampling method or captured by both using the vertebrate assay and trawl data.

Common name	Latin name	Detected by eDNA	Identified in trawls	Captured by both methods
Pogge	*Agonus cataphractus*	TRUE	TRUE	TRUE
Atlantic wolffish	*Anarhichas lupus*	TRUE	FALSE	FALSE
Mediterranean scaldfish	*Arnoglossus laterna*	TRUE	FALSE	FALSE
Red gurnard	*Aspitriglia cuculus*	FALSE	TRUE	FALSE
Common dragonet	*Callionymus lyra*	TRUE	TRUE	TRUE
Tub gurnard	*Chelidonichthys lucerna*	FALSE	TRUE	FALSE
Yarrell's blenny	*Chirolophis ascanii*	TRUE	FALSE	FALSE
Five-bearded rockling	*Ciliata mustela*	TRUE	FALSE	FALSE
Crystal goby	*Crystallogobius linearis*	TRUE	FALSE	FALSE
Goldsinny	*Ctenolabrus rupestris*	TRUE	FALSE	FALSE
Lumpsucker	*Cyclopterus lumpus*	TRUE	FALSE	FALSE
Bass	*Dicentrarchus labrax*	TRUE	FALSE	FALSE
Lesser weever	*Echiichthys vipera*	TRUE	FALSE	FALSE
fourbeard rockling	*Enchelyopus cimbrius*	TRUE	FALSE	FALSE
Grey gurnard	*Eutrigla gurnardus*	FALSE	TRUE	FALSE
Cod	*Gadus morhua*	TRUE	TRUE	TRUE
Two spotted goby	*Gobiusculus flavescens*	TRUE	FALSE	FALSE
Smooth sandeel	*Gymnammodytes semisquamatus*	TRUE	FALSE	FALSE
Long rough dab	*Hippoglossoides platessoides*	TRUE	TRUE	TRUE
Atlantic halibut	*Hippoglossus hippoglossus*	TRUE	FALSE	FALSE
Corbin's sand eel	*Hyperoplus immaculatus*	TRUE	FALSE	FALSE
Rough skate	*Leucoraja sp.*	TRUE	FALSE	FALSE
Dab	*Limanda limanda*	TRUE	TRUE	TRUE
Sea snail	*Liparis liparis*	TRUE	FALSE	FALSE
Shanny	*Lipophrys pholis*	TRUE	FALSE	FALSE
Angler fish	*Lophius piscatorius*	TRUE	FALSE	FALSE
Angler fish	*Lophius sp.*	FALSE	TRUE	FALSE
Haddock	*Melanogrammus aeglefinus*	FALSE	TRUE	FALSE
Whiting	*Merlangius merlangus*	FALSE	TRUE	FALSE
Hake	*Merluccius merluccius*	TRUE	TRUE	TRUE
Norway bullhead	*Micrenophrys lilljeborgii*	TRUE	FALSE	FALSE
Lemon sole	*Microstomus kitt*	TRUE	TRUE	TRUE

Common name	Latin name	Detected by eDNA	Identified in trawls	Captured by both methods
Ling	*Molva molva*	FALSE	TRUE	FALSE
Red mullet	*Mullus surmuletus*	TRUE	TRUE	TRUE
European smelt	*Osmerus eperlanus*	TRUE	FALSE	FALSE
Butterfish	*Pholis gunnellus*	TRUE	FALSE	FALSE
Norwegian topknot	*Phrynorhombus norvegicus*	TRUE	FALSE	FALSE
Flounder	*Platichthys flesus*	FALSE	TRUE	FALSE
Plaice	*Pleuronectes platessa*	FALSE	TRUE	FALSE
Thornback ray	*Raja clavata*	FALSE	TRUE	FALSE
Cuckoo ray	*Raja naevus*	FALSE	TRUE	FALSE
Tadpole fish	*Raniceps raninus*	TRUE	FALSE	FALSE
Atlantic salmon	*Salmo salar*	TRUE	FALSE	FALSE
Sea trout	*Salmo trutta*	TRUE	FALSE	FALSE
European pilchard	*Sardina pilchardus*	TRUE	FALSE	FALSE
Atlantic mackerel	*Scomber scombrus*	TRUE	FALSE	FALSE
Turbot	*Scophthalmus maximus*	TRUE	TRUE	TRUE
Brill	*Scophthalmus rhombus*	TRUE	FALSE	FALSE
Small-spotted catshark	*Scyliorhinus canicula*	FALSE	TRUE	FALSE
Sole	*Solea solea*	TRUE	TRUE	TRUE
Sprat	*Sprattus sprattus*	FALSE	TRUE	FALSE
Norway pout	*Trisopterus esmarkii*	TRUE	TRUE	TRUE
Poor cod	*Trisopterus minutus*	TRUE	TRUE	TRUE
John Dory	*Zeus faber*	TRUE	TRUE	TRUE
Eelpout	*Zoarces viviparus*	TRUE	FALSE	FALSE

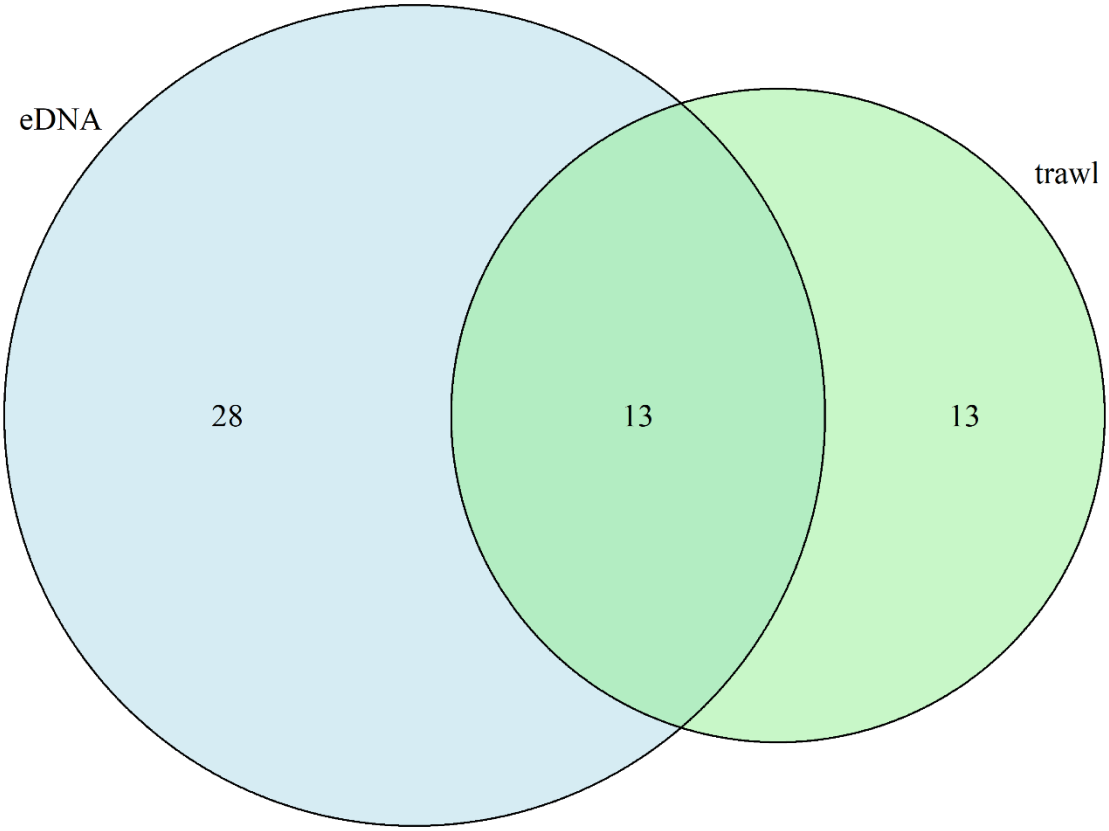

Figure B.1: Venn diagram showing the number of species detected by eDNA (using the vertebrate assay) and trawls.

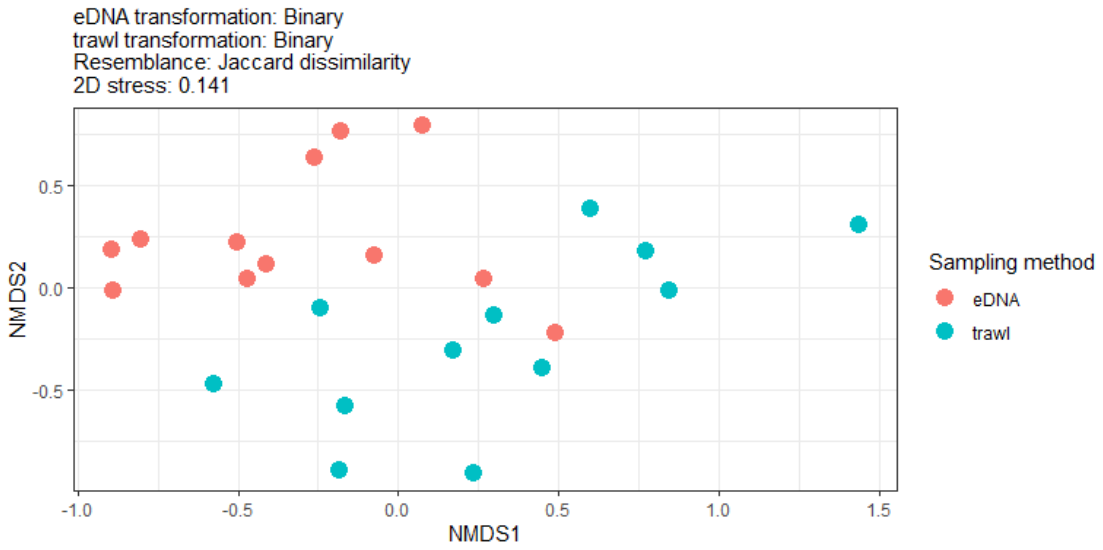

Figure B.2: NMDS plot showing Jaccard dissimilarity between samples from eDNA and trawl data after presence/absence transformation – using vertebrate assay.

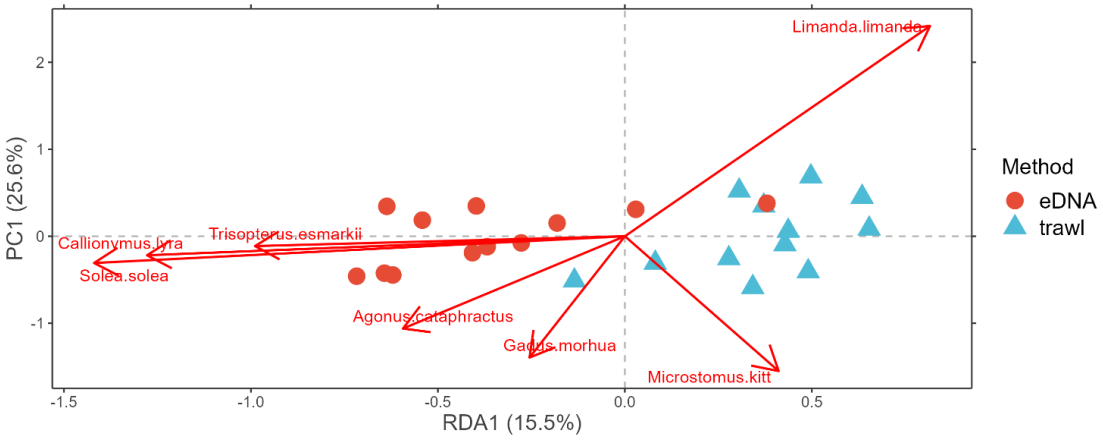

Figure B.3: Ordination showing species driving dissimilarity between sampling method – vertebrate assay.

Table B.2: eDNA occurrence of fish species identified using the Vertebrate Assay. Numbers presented are read counts from all stations except station 1 and 2.

Common name	Latin name	Winter	Spring	Summer	Autumn	Total
Cod	*Gadus morhua*	1090789	326799	0	0	1417588
Long rough dab	*Hippoglossoides platessoides*	109959	141043	19418	19818	290238
Lemon sole	*Microstomus kitt*	78373	60521	0	10382	149276
Hake	*Merluccius merluccius*	2218	0	94533	34038	130789

Common name	Latin name	Winter	Spring	Summer	Autumn	Total
Crystal goby	*Crystallogobius linearis*	807	91996	14944	26	107773
Dab	*Limanda limanda*	7779	0	14884	24726	47389
Norway pout	*Trisopterus esmarkii*	0	24631	21271	0	45902
Red mullet	*Mullus surmuletus*	28406	40	810	0	29256
Atlantic mackerel	*Scomber scombrus*	925	98	23941	0	24964
Sole	*Solea solea*	456	15925	3899	2064	22344
John Dory	*Zeus faber*	18242	255	0	0	18497
Turbot	*Scophthalmus maximus*	6766	79	8703	1169	16717
Pogge	*Agonus cataphractus*	12781	1637	171	92	14681
Butterfish	*Pholis gunnellus*	1582	6087	0	100	7769
Five-bearded rockling	*Ciliata mustela*	4385	1709	562	207	6863
Lumpsucker	*Cyclopterus lumpus*	5369	34	390	27	5820
Common dragonet	*Callionymus lyra*	589	4430	456	30	5505
fourbeard rockling	*Enchelyopus cimbrius*	968	2271	2203	0	5442
Angler fish	*Lophius piscatorius*	1309	235	3467	71	5082
Poor cod	*Trisopterus minutus*	1540	1183	1625	0	4348
European pilchard	*Sardina pilchardus*	0	1866	0	117	1983
Goldsinny	*Ctenolabrus rupestris*	114	376	512	44	1046
Yarrell's blenny	*Chirolophis ascanii*	61	137	768	0	966
Atlantic halibut	*Hippoglossus hippoglossus*	0	0	0	912	912
Shanny	*Lipophrys pholis*	216	0	689	0	905
Atlantic salmon	*Salmo salar*	518	323	0	0	841

Common name	Latin name	Winter	Spring	Summer	Autumn	Total
Bass	*Dicentrarchus labrax*	0	28	775	0	803
Two spotted goby	*Gobiusculus flavescens*	0	0	724	0	724
Eelpout	*Zoarces viviparus*	0	0	561	103	664
Tadpole fish	*Raniceps raninus*	437	126	50	0	613
Sea snail	*Liparis liparis*	0	568	0	0	568
Lesser weever	*Echiichthys vipera*	0	414	46	0	460
Atlantic wolffish	*Anarhichas lupus*	0	136	66	0	202
Brill	*Scophthalmus rhombus*	0	0	0	202	202
Sea trout	*Salmo trutta*	0	0	0	184	184
Mediterranean scaldfish	*Arnoglossus laterna*	0	57	68	0	125
European smelt	*Osmerus eperlanus*	29	0	0	73	102
Norway bullhead	*Micrenophrys lilljeborgii*	0	78	0	0	78
Norwegian topknot	*Phrynorhombus norvegicus*	0	31	0	0	31
Corbin's sand eel	*Hyperoplus immaculatus*	0	0	0	30	30
Rough skate	Leucoraja sp.	23	0	0	0	23

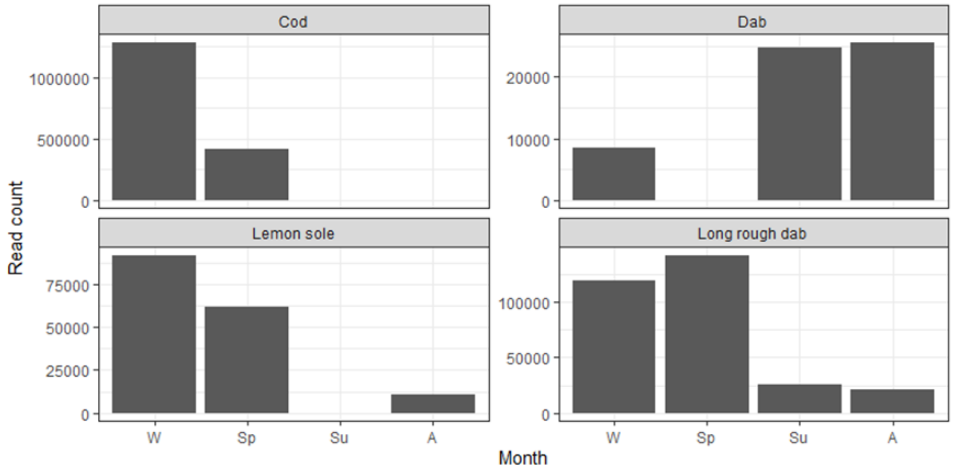

Figure B.4: Temporal variation in read counts from the Vertebrate Assay in species known to have a seasonal signal at the site.

Table B.3: Univariate measures of community structure, using the Vertebrate Assay.

Station	No. Taxa	No. Read Counts	Shannon-Wiener Diversity	Richness	Evenness	Effective Species Number
1	28	1852.92	1.34	3.59	0.40	3.83
2	27	2796.03	1.37	3.28	0.42	3.95
3	32	2795.10	1.37	3.91	0.40	3.94
5	26	4195.48	1.41	3.00	0.43	4.09
8	33	3432.25	1.63	3.93	0.47	5.09
9	30	3738.83	1.54	3.53	0.45	4.64

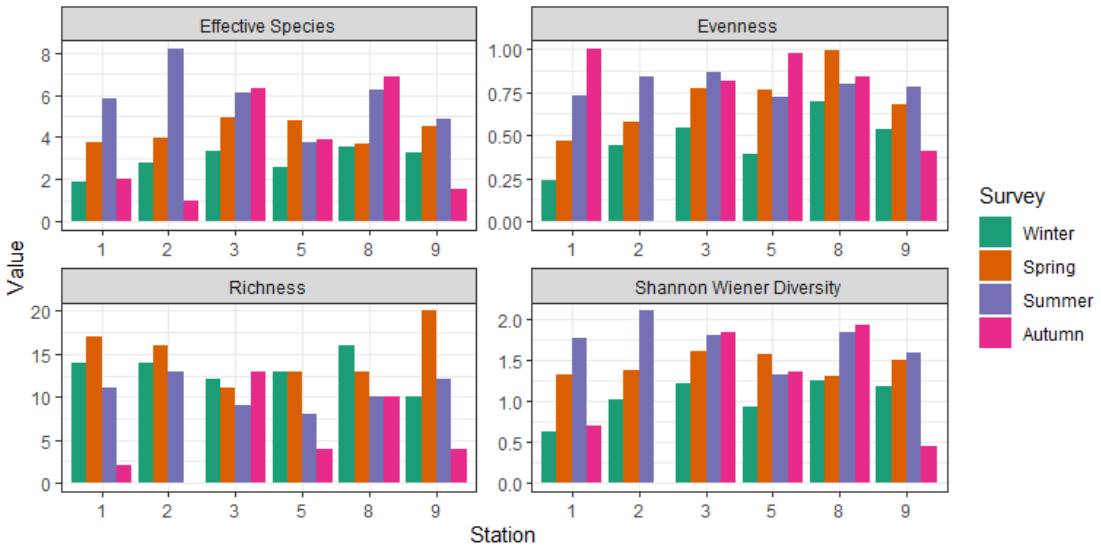

Figure B.5: Variation in univariate indices in eDNA data by survey and station, using the Vertebrate Assay.

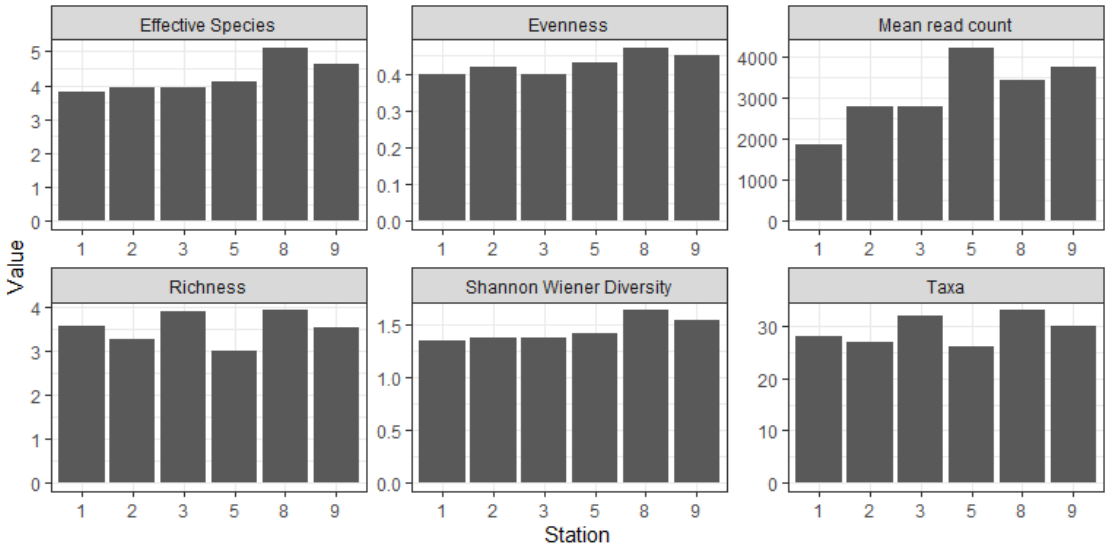

Figure B.6: Univariate indices of community composition in eDNA data, using the Vertebrate Assay.

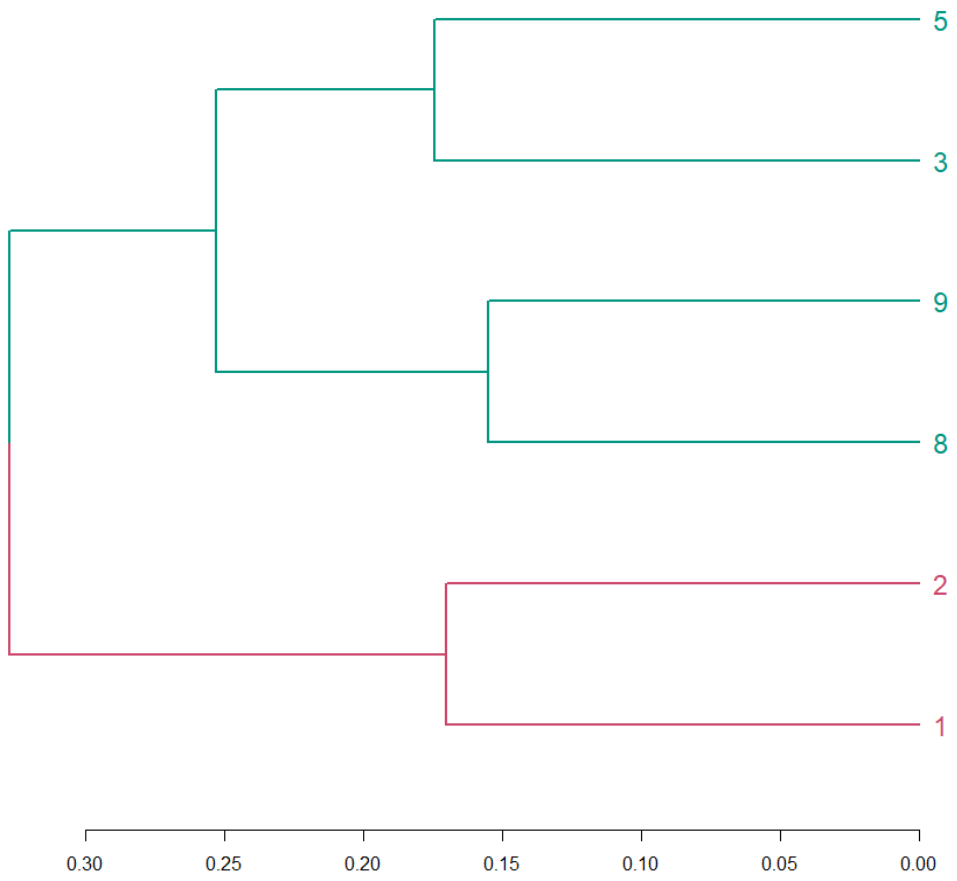

Figure B.7: Clusters from the vertebrate assay

Table B.4: The percentage of mean read counts from the Vertebrate Assay within the development area (stations 1 & 2) and those outside (stations 3,5, 8, and 9) for each species.

Common name	Latin name	Outside	Within
Pogge	*Agonus cataphractus*	0.79	0.77
Atlantic wolffish	*Anarhichas lupus*	0.07	0.54
Mediterranean scaldfish	*Arnoglossus laterna*	0.07	0.50
Common dragonet	*Callionymus lyra*	0.53	0.96
Yarrell's blenny	*Chirolophis ascanii*	0.42	0.73
Five-bearded rockling	*Ciliata mustela*	0.51	14.44
Crystal goby	*Crystallogobius linearis*	4.76	0.34
Goldsinny	*Ctenolabrus rupestris*	0.26	0.26
Lumpsucker	*Cyclopterus lumpus*	1.11	0.53

Common name	Latin name	Outside	Within
Bass	*Dicentrarchus labrax*	0.69	0.00
Lesser weever	*Echiichthys vipera*	0.20	0.45
fourbeard rockling	*Enchelyopus cimbrius*	0.49	0.56
Cod	*Gadus morhua*	50.86	60.11
Two spotted goby	*Gobiusculus flavescens*	0.62	0.04
Smooth sandeel	*Gymnammodytes semisquamatus*	0.00	0.10
Long rough dab	*Hippoglossoides platessoides*	6.25	2.30
Atlantic halibut	*Hippoglossus hippoglossus*	0.79	0.00
Corbin's sand eel	*Hyperoplus immaculatus*	0.05	0.00
Rough skate	*Leucoraja sp.*	0.04	0.00
Dab	*Limanda limanda*	1.63	2.23
Sea snail	*Liparis liparis*	0.49	0.00
Shanny	*Lipophrys pholis*	0.39	0.00
Angler fish	*Lophius piscatorius*	0.36	0.12
Hake	*Merluccius merluccius*	9.39	0.41
Norway bullhead	*Micrenophrys lilljeborgii*	0.13	0.43
Lemon sole	*Microstomus kitt*	4.67	3.53
Red mullet	*Mullus surmuletus*	2.52	0.20
European smelt	*Osmerus eperlanus*	0.09	0.07
Butterfish	*Pholis gunnellus*	0.64	0.17
Norwegian topknot	*Phrynorhombus norvegicus*	0.05	0.87
Tadpole fish	*Raniceps raninus*	0.26	0.90
Atlantic salmon	*Salmo salar*	0.72	0.00
Sea trout	*Salmo trutta*	0.11	1.11
European pilchard	*Sardina pilchardus*	1.14	0.00
Atlantic mackerel	*Scomber scombrus*	1.59	0.30
Turbot	*Scophthalmus maximus*	1.03	0.16
Brill	*Scophthalmus rhombus*	0.17	0.00
Sole	*Solea solea*	1.20	1.97
Norway pout	*Trisopterus esmarkii*	1.98	1.71
Poor cod	*Trisopterus minutus*	0.47	2.51
John Dory	*Zeus faber*	1.87	0.71
Eelpout	*Zoarces viviparus*	0.57	0.00

Table B.5: The percentage of mean read counts from the Vertebrate Assay within the development area (stations 1 & 2) and those outside (stations 3,5, 8, and 9) for each species by season.

Common name	Latin name	Winter Outside	Winter Within	Spring Outside	Spring Within	Summer Outside	Summer Within	Autumn Outside	Autumn Within
Pogge	*Agonus cataphractus*	1.02	1.34	0.76	0.50	0.27	0.91	0.28	0.00
Atlantic wolffish	*Anarhichas lupus*	0.00	0.55	0.09	0.69	0.31	0.00	0.00	0.00
Mediterranean scaldfish	*Arnoglossus laterna*	0.00	0.00	0.16	0.36	0.16	3.29	0.00	0.00
Common dragonet	*Callionymus lyra*	0.24	0.21	1.24	2.03	0.72	4.35	0.28	0.00
Yarrell's blenny	*Chirolophis ascanii*	0.10	0.18	0.19	1.99	3.62	0.00	0.00	0.00
Five-bearded rockling	*Ciliata mustela*	0.64	19.87	1.19	2.78	0.66	9.53	0.48	0.00
Crystal goby	*Crystallogobius linearis*	0.43	0.06	13.52	1.87	4.41	1.15	0.24	0.00
Goldsinny	*Ctenolabrus rupestris*	0.18	0.00	0.26	0.41	2.41	0.00	0.41	0.00
Lumpsucker	*Cyclopterus lumpus*	1.43	0.48	0.09	1.03	1.84	0.35	0.25	0.00
Bass	*Dicentrarchus labrax*	0.00	0.00	0.08	0.00	3.66	0.00	0.00	0.00
Lesser weever	*Echiichthys vipera*	0.00	0.00	0.39	0.80	0.22	2.49	0.00	0.00
fourbeard rockling	*Enchelyopus cimbrius*	0.31	0.06	0.91	1.40	1.48	1.52	0.00	0.00
Cod	*Gadus morhua*	72.54	64.55	38.02	67.84	0.00	0.00	0.00	0.00
Two spotted goby	*Gobiusculus flavescens*	0.00	0.00	0.00	0.00	1.71	0.23	0.00	0.00
Smooth sandeel	*Gymnammodytes semisquamatus*	0.00	0.00	0.00	0.00	0.00	0.57	0.00	0.00
Long rough dab	*Hippoglossoides platessoides*	7.31	3.10	17.12	0.94	4.58	14.46	14.12	49.07
Atlantic halibut	*Hippoglossus hippoglossus*	0.00	0.00	0.00	0.00	0.00	0.00	4.22	0.00
Corbin's sand eel	*Hyperoplus immaculatus*	0.00	0.00	0.00	0.00	0.00	0.00	0.28	0.00
Rough skate	*Leucoraja sp.*	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Common name	Latin name	Winter Outside	Winter Within	Spring Outside	Spring Within	Summer Outside	Summer Within	Autumn Outside	Autumn Within
Dab	*Limanda limanda*	0.65	0.31	0.00	0.00	3.51	22.04	20.82	50.93
Sea snail	*Liparis liparis*	0.00	0.00	0.79	0.00	0.00	0.00	0.00	0.00
Shanny	*Lipophrys pholis*	0.11	0.00	0.00	0.00	3.25	0.00	0.00	0.00
Angler fish	*Lophius piscatorius*	0.42	0.00	0.16	0.00	1.26	0.67	0.33	0.00
Hake	*Merluccius merluccius*	0.71	0.36	0.00	0.00	40.54	0.00	39.41	0.00
Norway bullhead	*Micrenophrys lilljeborgii*	0.00	0.00	0.22	0.68	0.00	0.00	0.00	0.00
Lemon sole	*Microstomus kitt*	5.21	4.63	8.45	1.27	0.00	0.00	8.74	0.00
Red mullet	*Mullus surmuletus*	2.52	0.28	0.11	0.25	3.82	0.00	0.00	0.00
European smelt	*Osmerus eperlanus*	0.05	0.06	0.00	0.00	0.00	0.00	0.68	0.00
Butterfish	*Pholis gunnellus*	0.32	0.15	1.55	0.26	0.00	0.00	0.46	0.00
Norwegian topknot	*Phrynorhombus norvegicus*	0.00	0.00	0.09	0.00	0.00	4.98	0.00	0.00
Tadpole fish	*Raniceps raninus*	0.70	0.40	0.18	1.92	0.24	0.53	0.00	0.00
Atlantic salmon	*Salmo salar*	0.83	0.00	0.90	0.00	0.00	0.00	0.00	0.00
Sea trout	*Salmo trutta*	0.00	0.96	0.00	0.00	0.00	0.00	0.57	0.00
European pilchard	*Sardina pilchardus*	0.00	0.00	5.21	0.00	0.00	0.00	0.54	0.00
Atlantic mackerel	*Scomber scombrus*	0.25	0.36	0.14	0.60	5.94	1.18	0.00	0.00
Turbot	*Scophthalmus maximus*	1.08	0.14	0.11	0.23	3.73	0.00	2.17	0.00
Brill	*Scophthalmus rhombus*	0.00	0.00	0.00	0.00	0.00	0.00	0.94	0.00
Sole	*Solea solea*	0.24	0.67	3.71	2.04	1.53	20.52	3.82	0.00
Norway pout	*Trisopterus esmarkii*	0.00	0.00	3.13	2.31	5.57	11.23	0.00	0.00
Poor cod	*Trisopterus minutus*	0.27	1.18	1.10	6.22	1.92	0.00	0.00	0.00

Common name	Latin name	Winter Outside	Winter Within	Spring Outside	Spring Within	Summer Outside	Summer Within	Autumn Outside	Autumn Within
John Dory	*Zeus faber*	2.43	0.11	0.14	1.58	0.00	0.00	0.00	0.00
Eelpout	*Zoarces viviparus*	0.00	0.00	0.00	0.00	2.65	0.00	0.95	0.00

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