



# **Sporad na Mara Offshore Wind Farm**

## **Offshore Project**

### **Environmental Impact Assessment Report**

#### **Annex 12.1.2: eDNA Report, Volume 2c**

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## Spiorad na Mara OWF Subtidal Environmental Baseline Survey: eDNA Report

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## 1. Introduction

### 1.1. Project Overview

The Spiorad na Mara 'N4' Offshore Wind Farm (OWF) is a proposed fixed bottom OWF located in the North Atlantic Ocean between 5 km – 13 km off the west coast of the Isle of Lewis in the Outer Hebrides. The Spiorad na Mara OWF site, 'The Project Area', has an expected nominal capacity of 900 megawatts (MW) and is owned by Northland Power Inc (NLP) (75.5%) and ESB (24.5%) with NLP leading the development, operation, and construction of the project.

Spiorad na Mara Limited (The Project) contracted Ocean Ecology Limited (OEL) to undertake a subtidal environmental characterisation of the project area in October 2023. The survey had two key objectives: (1) to describe the biological and physico-chemical characteristics of the seabed across the proposed project area by collecting seabed imagery, sediment samples and water samples; and (2) to ground-truth geophysical data collected by The Project during the summer and autumn of 2023. This baseline data will help to understand potential impacts of the project on the marine environment

This report provides a summary of sediment and water environmental DNA (eDNA) analysis and complements the wider technical report (Ocean Ecology Limited, 2024) in setting out a detailed description of the biological community across the project area.

### 1.2. Aims and Objectives

The primary aim of the eDNA assessment was to provide additional information to enhance that which was collected as part of the subtidal environmental baseline survey report and provide a comprehensive understanding of the subtidal biodiversity of the site and its environs .

The key objective of the eDNA report is to develop understanding of biodiversity in the project area and identification of potential indicators of ecosystem health following Marine Directorate Science Evidence Data Digital (MD-SEDD) advice by introducing water eDNA sampling.

## 2. Survey Design

### 2.1. Rationale

The environmental characterisation sampling plan was developed to provide maximum geographic coverage of the proposed project area, whilst also ensuring that all key habitats and communities likely to be encountered across the project area were adequately targeted across a range of depths. This environmental characterisation sampling plan was presented to and approved by NatureScot ahead of the survey mobilising.

Prior to commencement of the survey, The Project collected near-full coverage geophysical datasets including Side-Scan Sonar (SSS), Multi-Beam Echo Sounder (MBES) and magnetometer data. The environmental characterisation sampling plan was based on a stratified sampling approach following a detailed review and interpretation of the geophysical datasets and in consideration of the recommendations of best practice guidance where relevant (Scottish Natural Heritage 2011; Natural England 2021) whilst also accounting for all surface, subsurface, and subsea hazards, and their respective exclusion/ buffer zones.

The geophysical datasets were reviewed manually to identify areas of differing substrate type and seabed elevation. Substrate type was inferred primarily from SSS based on the reflectivity (coarser sediments providing greater reflectivity) and seabed elevation which was determined by review of the MBES bathymetry dataset.

The full catalogue of information that was assessed in the development of the sampling plan includes:

- 2023 geophysical campaign preliminary processed MBES bathymetry, SSS, and magnetometer data in mosaiced geotiff format.
- All previous mapping of key features from historic data including all project specific surveys as set out in the BMP (sources include: [EUSeaMap 2021](#), [EMODnet Bathymetry data](#)).
- All available GIS shapefiles and rasters in ESRI format including: the array and OCAS areas, planned and existing infrastructure to include all oil and gas surface and subsurface infrastructure within the project boundary or within close proximity to it, the latest relevant MPA boundaries, admiralty charts for the project area (if available) (sources include: Project shapefiles, [EMODnet](#), [NatureScot Open Data](#)).

## 2.2. Sampling Approach

To fully characterise the subtidal environment across the project area, a suite of sampling approaches was employed. This included grab sampling, DDC, baited remote underwater video (BRUV), sediment and water eDNA sampling. This report focuses on the eDNA sampling.

Prior to the collection of grab samples, high-resolution seabed imagery (stills and video) was collected via DDC at each sampling station to i) determine the suitability of the station for grab sampling (i.e., no hazards or sensitive habitat) and ii) provide an indication of the epibiota present at each location. Grab sample locations were designated based on a stratified approach providing broad coverage of the survey area whilst targeting all interpreted sediment types and depths (using both geophysical data and EUSeaMap 2021 data). Sediment eDNA samples were collected from all successfully sampled chemical contaminant grab stations where sufficient sediment volume allowed for the additional eDNA sampling. This equated to five stations which were targeted for eDNA analysis.

Water eDNA samples were collected from 10 stations across the survey area. These 10 locations were positioned at the centroid of 10 of the 12 DDC transects run as part of the environmental characterisation survey (Ocean Ecology Limited, 2024), as DDC transects were positioned specifically to target potential sensitive habitats. Collecting water eDNA from these locations targeted detection of potentially rare or cryptic species (Figure 1). Water eDNA samples were collected from three water depths: surface, middle, and bottom. These samples were stored frozen with a preservative after collection.

## 2.3. Timing

The sampling was undertaken aboard the *MV Situla* during periods of favourable weather between the 17<sup>th</sup> and the 27<sup>th</sup> of October 2023.

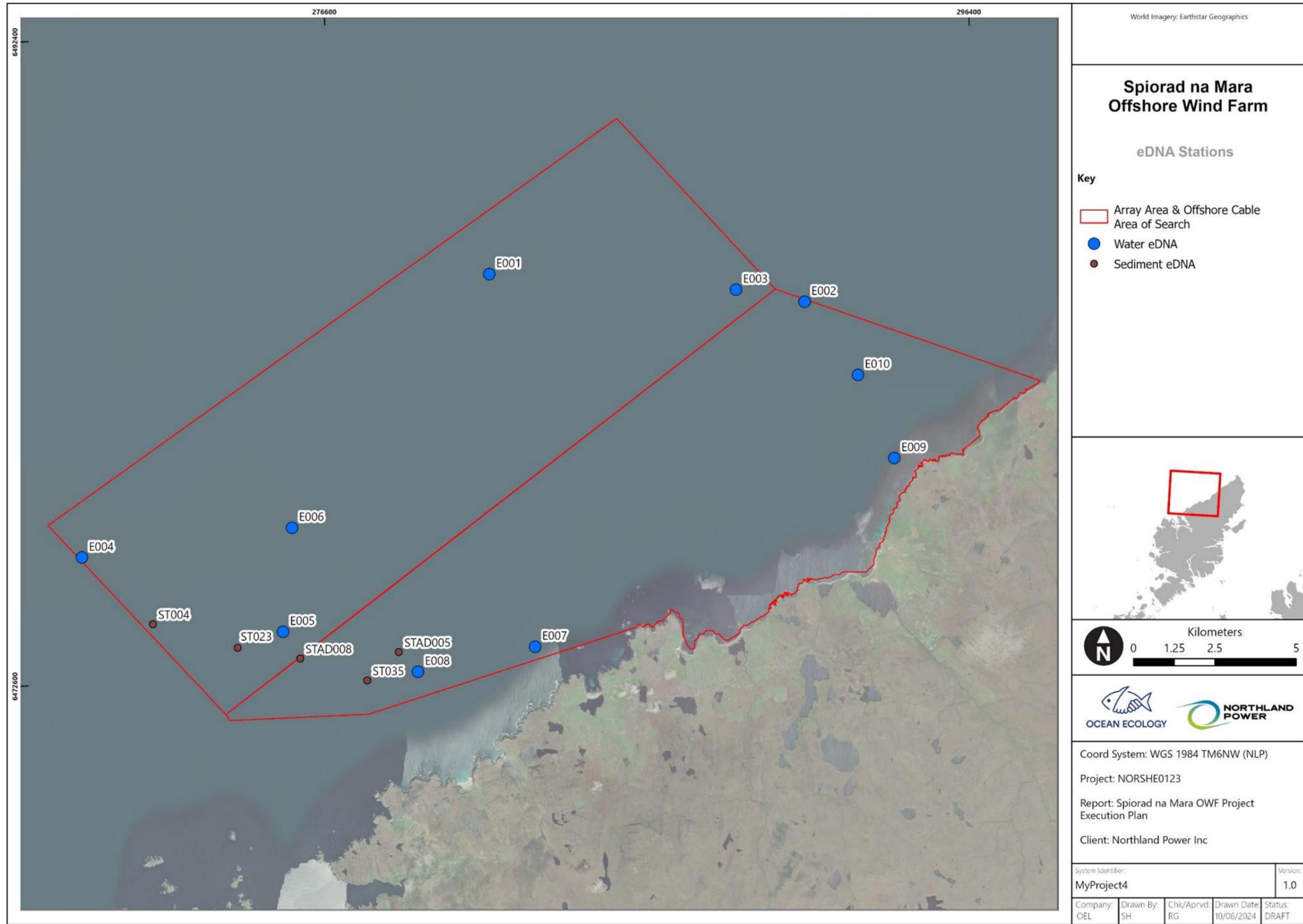


Figure 1 Overview of eDNA sampling stations across the project area.

### 3. Field Methods

#### 3.1. Survey Vessel

Sampling was conducted between the 17<sup>th</sup> and 27<sup>th</sup> of October 2023 aboard the 38.1 m vessel *MV Situla* (Plate 1). The vessel was mobilised from Galway, Ireland, and used Ullapool as a port of opportunity during periods of poor weather. Operations were performed on a 24-hour basis.

**Table 1** Vessel details.

<b>Vessel Name</b>	<i>MV Situla</i>
<b>Area of operation</b>	Offshore
<b>Call Sign</b>	HO8727
<b>IMO Number</b>	9246188
<b>Mobilisation Port</b>	Galway
<b>Length</b>	38.1 m
<b>Beam</b>	9.5 m
<b>Draft</b>	2.9 m



**Plate 1** Survey vessel *MV Situla*.

## 3.2. Geodetic Parameters

The following geodetic parameters, as provided by The Project, were used throughout the survey.

### 3.2.1. Horizontal Datum

**Table 2** Geodetic parameters

Parameter	Details
Name	World Geodetic System 1984 (WGS84)
Ellipsoid	WGS 84
Semi-Major Axis (a)	6378137.000 m
Semi-Minor Axis (b)	6356752.314 m
Inverse Flattening	298.257 223 563
Geodetic parameters EPSG Code	4326

**Table 3** Projection parameters.

Projection	Transverse Mercator 6 NW
Name	TM 6 NW
Longitude of Natural Origin	6° West
Latitude of Natural Origin	0°
False Easting	1 500 000.00 m
False Northing	0.00 m
Scale Factor at Natural Origin	1
Units	metres

### 3.2.2. Datum Transformation Parameters

All data provided in this report is referenced to WGS84, TM 6 NW, with no datum transformation need. No conversion or test coordinate was provided by the Client.

### 3.2.3. Vertical Datum

All altitude and depth data above seabed are referenced to LAT. All depth data below the seabed is referenced to LAT where available, depths are reported as derived from ultra-short baseline (USBL) beacon.

### 3.2.4. Unit Format and Conversions

The following have been used throughout this project and are expressed using the following conventions.

**Table 4** Project unit format and convention details.

Unit Formats and Conventions	
Geographical Coordinates	Latitude N DD° MM.mmmmmm' to 6 decimal places. Longitude E/W DD° MM.mmmmmm' to 6 decimal places.
Grid Coordinates	Metres in the following format: Easting EEE EEE.eee m to 3 decimal places. Northing NNN NNN.nnn m to 3 decimal places.
Linear distances	Meters to 1 decimal places.
Offset measurement sign conventions	Metres in the following format: 'Y' is positive forward. 'X' is positive to starboard. 'Z' values are positives upwards from the waterline.
Time	UTC (GMT).

### 3.3. Survey Navigation

#### 3.3.1. Surface Positioning

The *MV Situla* was equipped with a Hemisphere V104s Global Positioning System (GPS) compass system. The Hemisphere V104s internal GPS receiver utilises a minimum of 4 GPS satellites, managing the navigation information required to obtain a position within 3 m at 95 % accuracy. The V104s automatically tracks Satellite-Based Augmentation System (SBAS) differential correction to improve position accuracy to > 1 m at 95 % accuracy. The V104s includes an integrated gyro and two tilt sensors to provide an accurate heading for navigation software.

#### 3.3.2. Subsea Positioning

The vessel was equipped with an Easytrak Nexus 2 Lite USBL system and 1329A Omni-directional +/- 90 ° Micro Beacons for subsea positioning of the camera and grab. The Easytrak Nexus 2 Lite is an advanced USBL positioning and tracking system that determines the position of dynamic subsea targets through the transmission and reception of acoustic signals between the submerged transceiver and a target beacon. The USBL was fully calibrated prior to survey operations and a Valeport SWiFT Sound Velocity Profiler (SVP) was used for taking sound of speed measurements throughout the survey. Readings were obtained daily from both the up-cast and down-cast. All USBL operations were conducted with strict adherence to the Marine Mammal Mitigation Plan (MMMP). The MMMP and the related measures undertaken are outlined in the Marine Mammal Mitigation Report (Ocean Ecology Limited, 2023).

### 3.3.3. Navigation Software

A vessel-based positioning system was employed utilizing EIVA NaviPac V4.6 software to ensure the accurate positioning of the vessel and subsea positioning of the sampling equipment via the USBL system as well as recording continuous track plots of the sampling equipment and recording sampling fixes. A navigation screen, displaying EIVA Helmsman Display was provided at the helm position of the vessel for the Officer on Watch.

### 3.3.4. Positional Checks & Calibrations

The GPS has an internal precision calculation which outputs a graphical representation of horizontal accuracy, displaying numerical precision as easting and northing. The accuracy of vessel heading, and reference systems was verified during mobilisation using agreed reference points.

A USBL calibration was undertaken using the inbuilt Easytrak Nexus calibration software package to eliminate any alignment errors of the installation. Offsets were measured dynamically between the Easytrak Nexus transceiver head and the external sensors interfaced. This enabled accurate operation of the Easytrak Nexus tracking system when pole-mounted onto a vessel with external VRU and gyro.

## 3.4. Survey Equipment and Sampling

### 3.4.1. Sediment eDNA sampling

Sediment samples were collected from within 50 m of the target sampling location using OEL's 0.1 m<sup>2</sup> Day grab sampler. The grab system was deployed and retrieved from the hydraulic 'A' frame on the aft deck of the *MV Situla* using the deck mounted coaxial winch. To ensure consistency in sampling, grab samples were screened by the lead Environmental Scientist and considered unacceptable if:

- The sample was less than 5 L. i.e., the sample represented less than half the 10 L capacity of the grab used.
- The jaws failed to close completely or were jammed open by an obstruction, allowing fines to pass through (washout or partial washout).
- The sample was taken at an unacceptable distance from the target location (> 50 m).
- There was obvious contamination of the sample from survey equipment, paint chips etc.

Initial processing of the sediment samples from eDNA was undertaken onboard the survey vessel in line with the following methodology:

- Inspection cover lifted and general assessment of sample size and acceptability made ensuring sediment surface was undisturbed and no obvious sign of contamination.

NB ensure no grease, oils or lubes entered the sample once the inspection cover was open.

- A complete sterile sampling kit was provided by the subcontracted laboratory consisting of a sampling container, sample collection scoop, sample preservative (preservation buffer), and nitrile gloves. A sub-sample of sediment, approximately 50 ml, was decanted using the sampling scoop into the dedicated sampling container. A preservative was added to the sample before it was frozen at -20°C in an onboard freezer. The volume of sediment decanted was of suitable quantity to fill the container completely (i.e. no head space).

### 3.4.2. Water eDNA Sampling

At each station water eDNA samples were taken at 2 m above the seabed, mid-water depth and 2 m below the surface using a 5 L Niskin bottle attached to the deployment cable using bulldog clips and friction tape. Water depth was determined using the live depth measurements received from the vessel echosounder. When the Niskin sampler was at the required depth, a water sample representative of that depth was collected by sending a messenger weight down the deployment wire to trigger the sampling mechanism. The required depth was determined by the use of 5 m incremental markings on the deployment wire that were counted by the winch operator. Where the winch wire was determined to be deploying at an angle, for example due to vessel movement or currents, the following formula can be used to calculate the length of winch wire required to sample the desired water depth:

$$a = \sqrt{((\tan(\theta)/b)^2 + b^2)}$$

Where a = length of winch wire, b = desired sample depth,  $\theta$  = angle of separation from 90° aft of the A-Frame winch wire block.

Sufficient time was allowed for this to travel to the sampler, depending on water depth.

When the equipment reached the surface, it was recovered to deck and the sampler removed. A single water eDNA sample was collected from each sampled water depth.

For each sample, a Vampire Pump was attached to the outlet of the Niskin bottle and eDNA sample processed as follows:

- Pump run slowly by pressing the drive unit trigger slowly to fill the hose with water.
- When the hose was filled, filter inlet attached to hose adaptor.
- Pump run slowly to begin with.
- When the flow of water leaving the filter outlet (wide end) slowed, pump speed decreased to reduce the build-up of pressure.
- Once all water had passed through the filter, or the filter was fully clogged, hose removed and all water drained from hose. Pump continued to run until no more water exited from the filter. Filter detached from hose.

Preservative solution was applied to the filter and the filter then placed into the specimen bag and sample frozen immediately at -20°C in an onboard freezer and subsequently transferred to an ultra-low temperature freezer (maintained at -80°C or below) upon return to the laboratory.

## 4. Laboratory and Analytical Methods

### 4.1. Environmental DNA

eDNA extraction and analysis was conducted by Nature Metrics.

#### 4.1.1. Metabarcoding

##### **Sediment eDNA**

Two metabarcoding assays for sediment samples were employed: eukaryotes and invertebrates.

Following removal of the preservation buffer, sediments were rinsed with 10X phosphate buffered saline (PBS), homogenised, and DNA was extracted from approximately 10g of the resulting homogenate. A negative control was processed with each batch of samples to monitor for exogenous DNA contamination. Extraction yields were checked by measuring DNA concentration using a Qubit fluorometer with the Qubit dsDNA broad range assay kit.

Replicate PCRs for each sample and extraction blank were amplified via a two-step polymerase chain reaction (PCR) process, amplification was performed with a commercially available Hot Start DNA polymerase targeting the mitochondrially encoded Cytochrome c oxidase subunit I (mt-COI) gene for invertebrates and the 18S ribosomal RNA (18S rRNA) gene for eukaryotes.

##### **Water eDNA**

Two metabarcoding assays for the water samples were employed and aimed at detecting the full breadth of marine vertebrates present across the survey area.

Marine Fish (excluding sharks and rays) were targeted using the MiFish-U-F MiLamprey\_F MiCatfish\_F 5'- GCCGGTAAAACCTCGTGCCAGC GCTGGTAAACCTCGTGCCAGC GTCGGTAAAATTCGTGCCAGC-3' and MiFish-U-R MiLamprey\_R 5'- CATAGTGGGGTATCTAATCCCAGTTTG CATAGCGGGTATCTAATCCCGGTTTG-3' primers (Alfaro-Cordova et al., 2022) to amplify a region of the 12S ribosomal RNA gene. Marine vertebrates were targeted using the F1 5'- ACTGGGATTAGATACCCC-3' and R1 5'- TAGAACAGGCTCCTCTAG-3' primers (Kelly et al., 2014).

Primers are short stretches of DNA which are complementary to a certain region in the genome. They are designed to target a region which is significantly variable to distinguish taxa. Some primers can target a large coverage of species while others target certain taxa. Although these primers may not amplify all the DNA present in the water column, they provide a broad coverage of taxa focussed on the most important groups. There are many considerations to consider when designing and selecting which primer to use, one of which is to consider the sequences available on the reference database when selecting primers.

DNA from each filter was extracted using a commercial DNA extraction kit with a protocol modified to increase DNA yields. An extraction blank was also processed for the extraction batch. DNA was purified to remove PCR inhibitors using a commercial purification kit. Purified DNAs were amplified with PCR for a hypervariable region of the 12S rRNA gene to target fish species.

A standard analysis, including 12 replicate PCRs per sample was performed. All PCRs were performed in the presence of both a negative control and a positive control sample (a mock community with a known composition). Amplification success was determined by gel electrophoresis. PCR replicates were pooled and purified, and sequencing adapters were added. Success was determined by gel electrophoresis. Amplicons were then purified and checked again by gel electrophoresis; these were then quantified using a Qubit high sensitivity kit according to the manufacturer's protocol.

All purified index PCRs were pooled into a final library with equal concentrations. The final library was sequenced using an Illumina MiSeq V3 kit at 10.5 pM with a 20% PhiX spike inside. Sequence data was processed using a custom bioinformatics pipeline for quality filtering, Operational Taxonomic Units (OUT) clustering, and taxonomic assignment.

#### 4.1.2. Bioinformatics

Sequence data was processed using a custom bioinformatics protocol for quality filtering, Operational Taxonomic Unit (OTU)<sup>1</sup> clustering (97 %) and taxonomic assignment. Similar sequences were clustered into an OTU at a defined similarity threshold and these units were approximately equivalent to species and treated as such in analyses. Taxonomic assignments were not always possible, as this depends on the availability of reference sequences and the similarity between closely related species in the amplified marker.

The Global Biodiversity Information Facility (GBIF) taxonomic backbone<sup>2</sup> was used for consistency between databases. Results from both searches were combined and assignments made to the lowest possible taxonomic level where there was consistency in the matches. Conflicts between the results of both searches were flagged and resolved manually. Minimum similarity thresholds (the lowest percentage value to which the DNA sequence must match to a known reference sequence to make a confident identification) of 98 %, 95 %, and 92 % were required for species, genus, and higher-level assignments respectively.

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<sup>1</sup> Operational Taxonomic Unit (OTU) is an operational definition used to classify groups of closely related individuals. Sequences are clustered according to their similarity to one another and OTUs are defined based on the similarity threshold of 97%. OTUs may refer to an individual, species, genus or class.

<sup>2</sup> GBIF is an international network and data infrastructure aimed at providing open access to data about all types of life on Earth. The GBIF taxonomic backbone is a single, synthetic management classification. It allows GBIF to integrate name based information from different resources such as GenBank, Encyclopedia of Life, IUCN etc and allow for consistent searching and reporting across all resources.

- 98% similarity for species means that the DNA sequence we have generated must match at least 98% of the genetic material of a known species to be confidently identified as that species.
- 95% similarity for genus means that if the sample is only 95% similar to known references, it's close enough to confidently identify it as belonging to the same genus, but not necessarily to species level.
- 92% similarity for higher-level assignments means that the match is good enough to place into a family or order but not to a specific genus or species.

The lower the percentage, the less specific the identification, and the thresholds set the minimum level of similarity needed to trust the taxonomic match.

Any identifications that were based on fewer than three reference matches were also flagged.

## 5. Results

Sediment eDNA was used as a complementary monitoring tool alongside grab sampling to further assess for the presence of cryptic, rare and invasive, non-native species which may elude grab sampling. Similarly, water eDNA was used to compliment DDC, BRUV data and MMMO sightings as it provides insights into the epifaunal and mobile species communities.

### 5.1. Sediment eDNA

eDNA was extracted from five grab samples collected across the survey area. The full data is provided in Appendix I while an overview of the main findings is included below.

Notable taxa include taxa of conservation and economical interest, as well as invasive non-native species (INNS). Sediment eDNA was used as a monitoring tool to compliment the results of the grab sampling as the results are comparable, while water eDNA was used to compliment DDC, BRUV data and MMMO sightings. None of the notable taxa recorded in the macrobenthic grab samples were recorded in the sediment eDNA samples; however, the INNS of red algae *Bonnemaisonia hamifera* was recorded at station ST023. Of the top ten taxa that mostly contributed to total abundance based on the macrobenthic grab samples (for details on calculation methodology, refer to (Ocean Ecology Limited, 2024)) the polychaetes *Pisione remota* and *Polygordius appendiculatus* were also recorded in the sediment eDNA invertebrate array. For *P. remota* however there was low support in the identification as the sequences matched fewer than three identified reference sequences in the database. The most frequently occurring taxon found in all sediment eDNA samples was Paramesochridae, a copepods family, while the species with the strongest eDNA signals were the polychaetes *Hesionura elongata* and *Protodrilus oculifer* and the gastropod *Microhedyle glandulifera*. Of these, only *H. elongata* was recorded in the grab samples.

### 5.2. Water eDNA

eDNA was extracted from water samples at 10 stations from the surface, middle and bottom of the water column at each station. The full data is provided in Appendix II while an overview of the main findings is included below.

In this study, we analysed the eDNA results from three distinct vertical zones of the water column: the surface, middle, and bottom layers. By focusing on the vertical distribution, rather than comparing eDNA samples spatially across the study area, we aimed to better understand how organisms utilize different depths of the environment. Given the relatively small size of the study area and the potential for eDNA transport between locations due to hydrodynamic processes, a vertical approach provides a clearer representation of habitat usage.

This method also accounts for the influence of stratification and vertical gradients in temperature, salinity, and density, which may restrict the presence of eDNA to specific depths. Furthermore, eDNA persistence in the water column can range from 1 day to 1 month, so this

approach is particularly valuable in environments where rapid DNA transport could blur spatial distinctions.

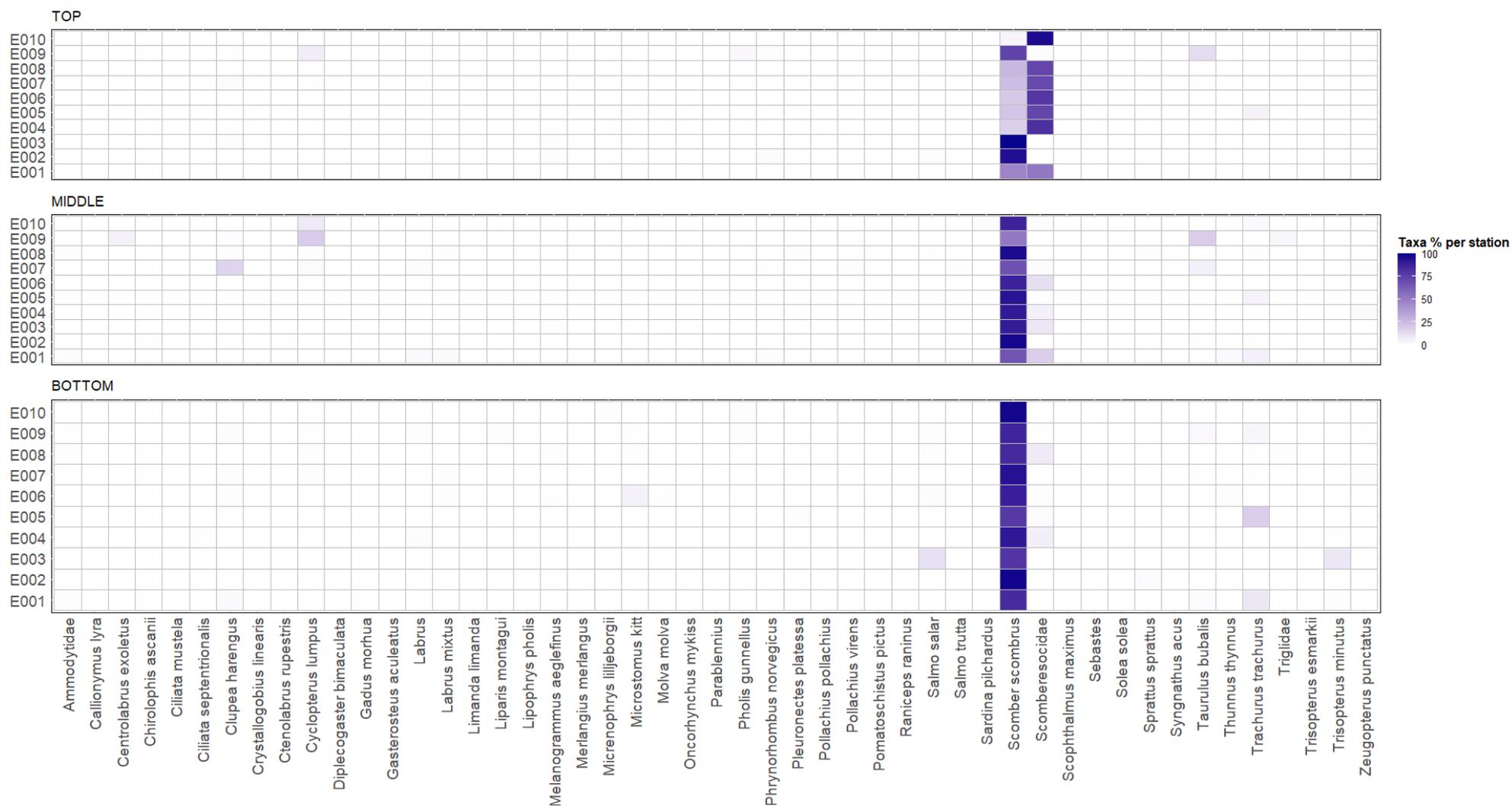
### 5.2.1. Fish Assay

An examination of the eDNA results led to the identification of a diverse fish community derived from 50 OTUs representative of fish taxa. The most prevalent fish species among these OTUs were the Atlantic Mackerel, Atlantic Salmon, and Atlantic Herring (Table 5). Of the fish identified, 11 were Priority Marine Feature (PMF) species protected in Scottish waters, while a further 23 species were also listed as species of commercial importance in Scotland, and one species was classified as invasive (Table 5). It should be noted that for 15 of the most common fish taxa there was low confidence in their identification as it was based on fewer than three matches to sequences in the reference database, and/or limited geographic occurrence records for the taxon (Table 5). A strong eDNA signal for the Atlantic Mackerel was detected in each sample (surface, middle, and bottom). For all other fish species, their presence was less than 20% and no clear pattern was observed with water depths (Figure 2).

**Table 5** Most relevant fish taxa identified across the survey area based on eDNA analysis. Asterisk (\*) identifies taxa with low confidence in the identification of their OTUs, as it was based on fewer than three matches to sequences in the reference database, and/or limited geographic occurrence records for the taxon.

Fish	Common Name	Status	% of samples in which the taxa occurred	% Breakdown by sample depth		
				Surface	Middle	Bottom
<i>Scomber scombrus</i>	Atlantic Mackerel	PMF/Commercial	100%	100%	100%	100%
<i>Salmo salar</i>	Atlantic Salmon	PMF/Commercial	67%	80%	60%	60%
<i>Clupea harengus</i>	Atlantic Herring	PMF/Commercial	60%	60%	50%	70%
<i>Trachurus trachurus</i>	Atlantic Horse Mackerel	PMF/Commercial	53%	40%	60%	60%
<i>Melanogrammus aeglefinus</i>	Haddock	Commercial	37%	20%	40%	50%
<i>Trisopterus minutus</i>	Poor Cod	PMF	33%	10%	30%	60%
<i>Labrus mixtus</i>	Cuckoo Wrasse		33%	20%	30%	50%
<i>Cyclopterus lumpus</i>	Lumpsucker	Commercial	33%	20%	40%	40%
<i>Taurulus bubalis</i>	Long-Spined Bullhead		30%	20%	30%	40%
<i>Chirolophis ascanii</i> *	Yarrell's Blenny		23%	30%	10%	30%
<i>Zeugopterus punctatus</i> *	Common Topknot		20%	10%	30%	20%
<i>Pollachius virens</i>	Saithe	PMF/Commercial	17%	0%	20%	30%
<i>Molva molva</i> *	Common Ling	PMF/Commercial	17%	0%	0%	50%
<i>Pholis gunnellus</i>	Rock Gunnel		17%	20%	0%	30%
<i>Microstomus kitt</i>	Lemon Sole	Commercial	17%	0%	10%	40%
<i>Pleuronectes platessa</i>	European Plaice	Commercial	17%	10%	20%	20%
<i>Phrynorhombus norvegicus</i> *	Norwegian Topknot		17%	30%	20%	0%
<i>Sprattus sprattus</i>	European Sprat	Commercial	10%	0%	20%	10%
<i>Gadus morhua</i>	Atlantic Cod	PMF/Commercial	10%	0%	10%	20%
<i>Ciliata septentrionalis</i> *	Northern Rockling	Commercial	10%	0%	10%	20%
<i>Thunnus thynnus</i>	Atlantic Bluefin Tuna	Commercial	10%	10%	10%	10%
<i>Micrenophrys lilljeborgii</i> *	Norway Bullhead		10%	10%	0%	20%

Fish	Common Name	Status	% of samples in which the taxa occurred	% Breakdown by sample depth		
				Surface	Middle	Bottom
<i>Liparis montagui</i> *	Montagu's Seasnail		10%	10%	10%	10%
<i>Sardina pilchardus</i>	European Pilchard	Commercial	7%	0%	20%	0%
<i>Pollachius pollachius</i>	Pollack	Commercial	7%	0%	0%	20%
<i>Ciliata mustela</i>	Fivebeard Rockling	Commercial	7%	0%	20%	0%
<i>Diplecogaster bimaculata</i> *	Two-Spotted Clingfish		7%	0%	10%	10%
<i>Merlangius merlangus</i>	Whiting	PMF/Commercial	3%	0%	10%	0%
<i>Raniceps raninus</i> *	Tadpole Fish		3%	0%	10%	0%
<i>Trisopterus esmarkii</i> *	Norway Pout	PMF	3%	0%	0%	10%
<i>Gasterosteus aculeatus</i>	Three-Spined Stickleback		3%	10%	0%	0%
<i>Lipophrys pholis</i>	Smooth Blenny		3%	0%	10%	0%
<i>Callionymus lyra</i>	Common Dragonet		3%	0%	0%	10%
<i>Crystallogobius linearis</i> *	Crystal Goby		3%	0%	0%	10%
<i>Pomatoschistus pictus</i>	Painted Goby		3%	0%	10%	0%
<i>Centrolabrus exoletus</i> *	Rock Cook		3%	0%	10%	0%
<i>Ctenolabrus rupestris</i>	Goldsinny Wrasse		3%	0%	10%	0%
<i>Limanda limanda</i>	Common Dab	Commercial	3%	0%	0%	10%
<i>Scophthalmus maximus</i> *	Turbot	Commercial	3%	10%	0%	0%
<i>Solea solea</i>	Common Sole	Commercial	3%	0%	10%	0%
<i>Oncorhynchus mykiss</i>	Rainbow Trout	Alien/Commercial	3%	0%	0%	10%
<i>Salmo trutta</i>	Brown Trout	PMF/Commercial	3%	0%	10%	0%
<i>Syngnathus acus</i>	Greater Pipefish		3%	0%	10%	0%



**Figure 2** Fish percentage abundance heat map: Analysis of surface, middle, and bottom depths at each station. Colour intensity indicates the percentage of sequences per sample based on all DNA sequences within an individual sample (the sum of one station (row) is 100 %).

### 5.2.2. Vertebrate Assay

eDNA was also analysed on a vertebrate assay which yielded results for bony fish, birds, and marine mammals, as well as terrestrial animals such as brown rat and red deer. At four stations, E003 (bottom and middle samples), E004 (middle sample), E008 (middle sample), and E010 (middle sample) the water samples yield no amplifiable DNA and therefore no species were reported for those five samples (Appendix III).

#### **Fish**

Of the 43 fish taxa identified in this analysis, 5 species were not recorded in the above fish assessment but recorded in the vertebrate array, namely the Smooth Sandeel *Gymnammodytes semisquamatus*, the Portuguese Blenny *Parablennius ruber*, the Boarfish *Capros aper*, the Nile Tilapia *Oreochromis niloticus*, and the Common Seasnail *Liparis liparis*<sup>3</sup> (Figure 3).

Of these, the Smooth Sandeel and the Nile Tilapia are of commercial value. However, it is highly unlikely that the presence of Nile Tilapia was a true detection as this species is a freshwater fish known to occur in parts of Africa and the Levant and the identification was of low confidence. Note that 15 of these OTUs were of low confidence in their identification as it was based on fewer than three matches to sequences in the reference database, and/or limited geographic occurrence records for the taxon (Appendix III).

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<sup>3</sup> Although the common name 'seasnail' might suggest otherwise, *Liparis liparis* is a species of fish. *Liparis liparis* is a tautonym with *Liparis* meaning 'sleek-skinned' in Greek. The name derives from its smooth skinned, scaleless appearance.



## Marine Mammals

Six marine mammal taxa were identified across the survey area: seals from the family Phocidae, the Common Minke Whale *Balaenoptera acutorostrata*, the common dolphin *Delphinus delphis*, dolphins from the genus *Lagenorhynchus*, and the Harbour Porpoise *Phocoena phocoena* (Table 6 and Figure 4A). All of these are protected under the EU Habitats Directive which was transposed into UK law by The Conservation of Habitats and Species Regulations 2017 within 12 nautical miles (nm).

**Table 6** Marine mammal taxa identified across the survey area based on eDNA analysis.

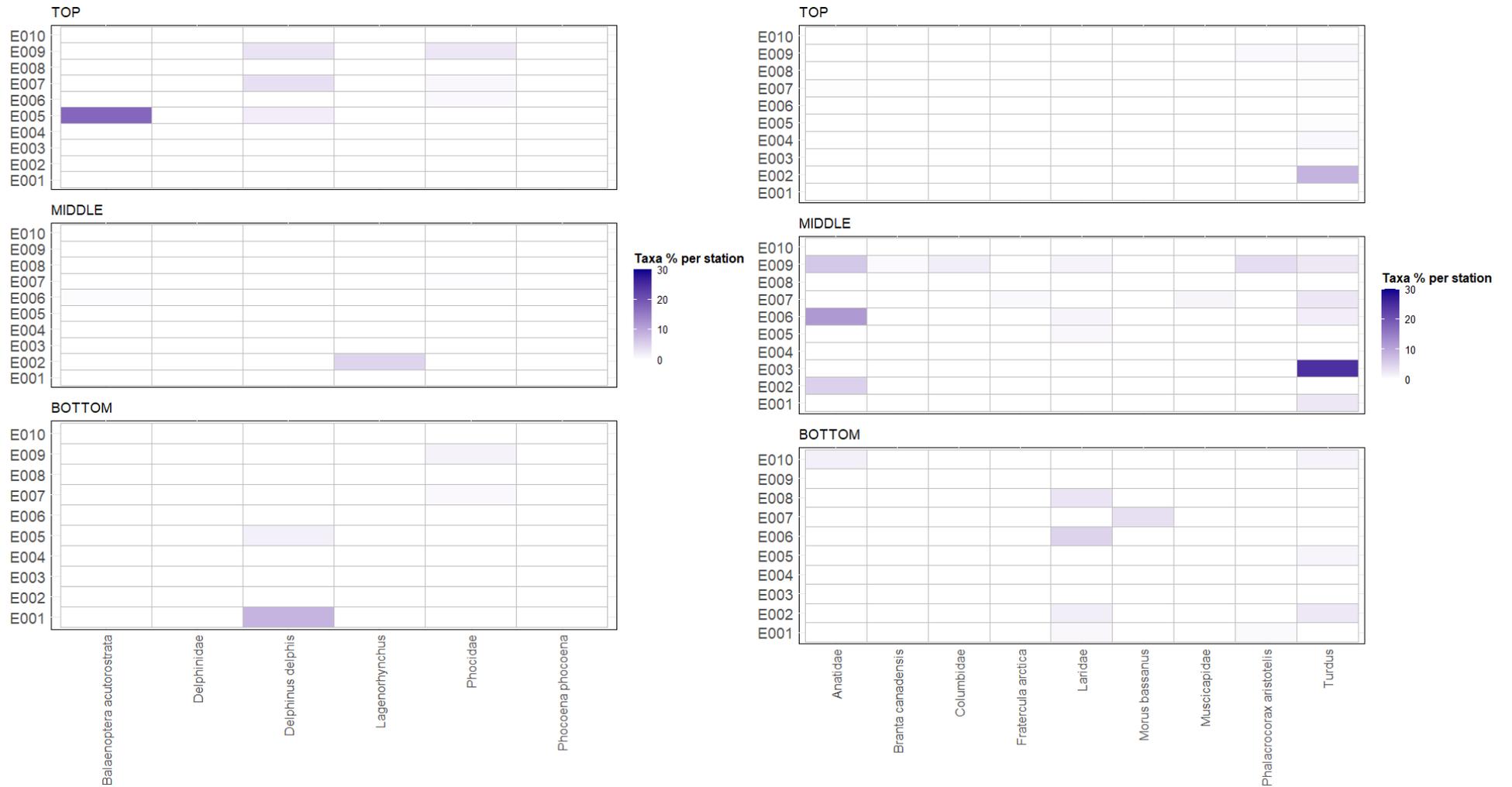
Taxa	Common Name	N of samples in which taxa occurred
<i>Phocidae</i>		6
<i>Balaenoptera acutorostrata</i>	Common Minke Whale	3
<i>Delphinus delphis</i>	Common Dolphin	5
<i>Lagenorhynchus</i>		2
<i>Delphinidae</i>		1
<i>Phocoena phocoena</i>	Harbour Porpoise	1

## Birds

Nine bird taxa were identified across the survey area (Table 7 and Figure 4B) with the Canadian Goose, the European Shag, and the Northern Gannet protected by the Wildlife and Countryside Act 1981, and the Atlantic Puffin classified in the UK as Red Listed under the Birds of Conservation Concern and Vulnerable on the global IUCN Red List of Threatened Species.

**Table 7** Bird taxa identified across the survey area based on eDNA analysis.

Taxa	Common Name	N of samples in which taxa occurred
<i>Branta canadensis</i>	Canada Goose	1
<i>Anatidae</i>		6
<i>Fratercula arctica</i>	Atlantic Puffin	1
<i>Laridae</i>		9
<i>Columbidae</i>		1
<i>Muscicapidae</i>		1
<i>Turdus</i>		14
<i>Phalacrocorax aristotelis</i>	European Shag	3
<i>Morus bassanus</i>	Northern Gannet	1



**Figure 4** Percentage abundance heat map for marine mammals (A) and birds (B). eDNA vertebrate analysis of surface, middle, and bottom depths at each station. Colour intensity indicates the percentage of sequences per sample based on all DNA sequences within an individual sample.

### 5.2.3. Invertebrate Assay

Thirty-three marine macrofaunal taxa were identified across the survey area among the 10 bottom water samples collected at the 10 eDNA stations. For 8 of these taxa there was low confidence in their identification as it was based on fewer than three matches to sequences in the reference database, and/or limited geographic occurrence records for the taxon (Appendix IV).

The copepods (zooplankton) *Clausocalanus jobei* and *Ditrichocorycaeus anglicus* were present in all samples, with the latter having some of the strongest eDNA signatures across the survey area. Other taxa with strong eDNA signatures included the coastal arrow worm (zooplankton) *Parasagitta setosa* at station E001, the hydrozoan (zooplankton) *Muggiaea atlantica* at station E003, the polychaete *Protodrilus oculifer* at station E007 and the copepod *Paracalanus parvus* at stations E009 and E010. However, it should be noted that low confidence was given to the identification of *P. oculifer*.

Other organisms recorded in the invertebrate array included 27 taxa of Chromist, 10 taxa of green algae Chlorophyta and six Fungi of the phylum Ascomycota known as sac fungi (Appendix IV).

## 6. Discussion

The use of eDNA has become increasingly popular as a non-invasive and effective method for surveying and monitoring of species in their natural habitats as organisms shed their DNA into their environments as shed cells, waste matter, blood, gametes and decaying material (JNCC, 2022). eDNA metabarcoding methods allow the rapid and cost-efficient collection of information on species diversity and composition of fish assemblages in aquatic habitats, which is of particular importance given the current increase in anthropogenic disturbance and associated declines in aquatic biodiversity in these ecosystems. To note that the eDNA analysis presented here was targeted to invertebrates, vertebrates and bony fish meaning that elasmobranchs (rays and skates) might not be as readily detected.

Given the relatively recent development of eDNA as a monitoring tool, there remain gaps in our understanding of several key factors that can influence the interpretation of results. These include the rates at which different species/groups release DNA into their environment, for example, in general elasmobranchs are often difficult to detect using eDNA as they do not shed large amounts of DNA compared to other taxa. Another factor to consider is the rate of eDNA degradation, with reported degradation times ranging from as little as one day to one month (Rees et al., 2014) depending on environmental conditions such as temperature, UV exposure and microbial activity. The other main factors to consider is the mechanisms by which eDNA is transported through aquatic systems and the reliability of reference databases, including correct identification of sequences and taxonomic gaps in the databases. Ongoing research is necessary to refine our knowledge in these areas, which will ultimately improve the accuracy and reliability of eDNA based assessments. The advantages and limitations of using eDNA techniques underscore the importance of employing them as a complementary monitoring tool at present.

The results of the sediment eDNA invertebrate array analysis were used to assess whether grab sampling overlooked any rare/cryptic macrobenthic species or species of conservation importance. None of the notable taxa recorded in the macrobenthic grab samples were recorded in the sediment eDNA samples; however, the INNS of red algae *B. hamifera* was recorded at station ST023. This may be due to some of the limitations previously mentioned, for example the rates at which different species/groups release DNA into the environment. Derycke et al. (2021) found that species with no sclerotization and large body size showed a higher probability of being detected in sediment eDNA samples. eDNA sampling is best used to ground truth and compliment other sampling methods as it can detect a broader range of organisms including microscopic or cryptic species.

The persistence of DNA in the water column depends on a multitude of factors, including environmental conditions, water movement, and the specific type of DNA present. Generally, DNA can remain detectable for varying durations, which can range from a few hours to several weeks or even months.

The degradation rates of DNA are contingent upon elements such as ultraviolet (UV) radiation, water temperature, and the presence of nucleases and other enzymatic activities in the water. Additionally, exposure to sunlight and high temperatures can accelerate the degradation process. Conversely, in colder and darker environments, the degradation of DNA may decelerate, allowing it to persist for longer periods (Littlefair et al. 2021, Monuki et al. 2021).

The results of the water eDNA analysis indicated the presence of a diverse fish community including 11 PMF species and 23 species of commercial importance. Additionally, four of the detected fish species are listed on the IUCN Red List as vulnerable; these were the Atlantic Horse Mackerel, the Haddock, the Atlantic Cod. Conducting eDNA sampling at multiple depths can yield valuable insights into the distribution and dynamics of genetic material in the water column. For instance, the Atlantic Mackerel, a PMF species with high commercial value, was detected in high concentrations at all three depths, indicating their use of the entire water column. Bathymetry data suggested that seabed depth for the Spiorad na Mara OWF site ranged from approximately 40 to 60 m Lowest Astronomical Tide (LAT) (Ocean Ecology Limited, 2024) which falls well within the typical depth range of Atlantic Mackerel, known to inhabit depths between 15-200 m (ICES-FishMap, 2014). Mackerel are highly active, with vertical distribution influenced by factors such as food availability and water temperature (Olafsdottir et al., 2019), which could explain the presence of DNA in all three depths.. Conversely, the Atlantic Salmon was detected across all three depths, yet it exhibited a stronger eDNA signal in the bottom layer of the water column.

The strong presence of Atlantic Mackerel may exert significant predation pressure on other species, potentially contributing to their dominance in the results.(Trenkel et al. 2014). Sampling at various depths provides a more complete picture of the area, taking into account things such as different species habitat preferences, environmental conditions (i.e. water properties such as temperature and salinity vary with depth influencing the species that occur), ocean currents etc. Additionally, it aids in the identification of the sources and sinks of genetic material, offering insights into the behaviour and ecological interactions of organisms within the marine environment. Furthermore, the finding of the Atlantic Salmon underscores the need to better understand the interaction of migratory fish species of ecological and cultural significance with offshore wind farms.

The occurrence of species like the Boarfish outside their typical geographical ranges could be indicative of range shifts possibly influenced by changing oceanic conditions, including temperature and currents. However, these detections should be treated with some caution given they relied on fewer than three matches within the reference database and may not be entirely dependable without repeat sampling. Two freshwater fish were detected, rainbow trout and Nile Tilapia, however it is important to note that the identification of Nile Tilapia is of low confidence as it was based on fewer than three matches to sequences in the reference database so may be an erroneous detection, misidentification of sequences in the reference database or transport of DNA. The presence of rainbow trout DNA could be attributed to

escapees from loch aquaculture or the transport of their DNA into marine environments via water flow.

The family Scomberesocidae was detected at all depths, with a strong presence in the surface section of the water column. We could not identify the DNA sequences to species level as they did not meet the minimum similarity threshold of 98%. This could be due to several reasons such as DNA degradation, differences in population genetics or misidentifications in the reference database due to misidentification of specimens or the presence of synonyms. The most likely species that these DNA sequences could represent is *Scomberesox saurus* as the sampling location is within the species range, however we cannot rule out other species within the family as the minimum similarity threshold for species ID was not met. Additionally, other species within this family are known to occur in the Atlantic, and their range may have shifted. Both BRUV analysis (Ocean Ecology Limited, 2024) and eDNA analysis reported the presence of Blackbelly rosefish, Cuckoo wrasse, Atlantic cod, Common dab, Sandeel and Painted goby across the survey area. However, eight of the fish taxa recorded in the BRUV footage were not recorded in the eDNA data while 44 of the taxa recorded via eDNA were not recorded in the BRUV footage. Additionally, three elasmobranchs were recorded in the BRUV footage but their eDNA was not captured in the analysis.

Marine mammals and birds were also identified as part of the eDNA analysis. The analysis confirmed the presence of seals from the family *Phocidae*, the Common Minke Whale, the common dolphin, dolphins from the family *Lagenorhynchus*, and the Harbour Porpoise. This was consistent with the BRUV footage which captured two grey seals as well as with observations made by marine mammal observers (MMOs) as part of the marine mammal mitigation undertaken during the geophysical survey of the area (Ocean Ecology Limited, 2023). Notably, both the eDNA analysis and the marine mammal mitigation report (Ocean Ecology Limited, 2023) highlighted the common dolphin as one of the most abundant species in the survey area. While the marine mammal mitigation report specifically identified the Risso's dolphin, the White-beaked dolphin, and the Harbour seal, the eDNA results only identified them to family or genus level. The prevalence of the common dolphin in the area is a strong indicator of its substantial presence within the local marine environment. In terms of birds, the species identified through the eDNA analysis are common to the entire UK, and to Scotland in particular. Among the taxa identified to a species level, the Atlantic Puffin, European Shag, and Northern Gannet are commonly observed in coastal settings. However, the presence of the Canada Goose in northern Scotland is unusual, as it is mainly absent from this region. However, it has been detected on some occasions during the winter season in the survey area. ([British Trust for Ornithology](#)).

It was also noteworthy that three groups of largely terrestrial birds were detected, the genus *Turdus* and the families *Columbidae* and *Muscicapidae* in the water samples as it raises questions over the reliability of the results. It is difficult to identify the specific vectors for DNA of terrestrial species being present across the site although possible explanations include

migration, wind and airborne transport of DNA, interactions with the marine environment such as feeding in coastal areas or river and runoff transport in which DNA can be transported into the sea, the presence of DNA in waste matter of predators that might have fed on prey or decaying material from terrestrial sources and/or vessels navigating across the survey area. Predatory birds are known to serve as significant agents in transporting terrestrial material to marine ecosystems through their droppings. These droppings can contain the DNA of the organisms consumed by the birds, thereby facilitating the transfer of terrestrial genetic material into the marine environment (Leempoel et al. 2020, Polanco. et al. 2021). This idea is further supported by the observation that terrestrial mammal species were primarily detected in the surface of the water column rather than at greater depths.

Economically important species, as well as those included in the IUCN Red List of Threatened Species, such as the Atlantic Cod, Poor Cod, Common Ling, and Cuckoo Wrasse, were detected through both eDNA analysis and BRUV footage (Ocean Ecology Limited, 2024). This consistency between the two methods enhances the confidence of the findings.

Bottom water samples were additionally targeted for invertebrate eDNA to assess whether DDC and BRUV sampling overlooked any rare/cryptic epibenthic species or species of conservation importance. No INNS or protected species (e.g. PMF species) were recorded in the invertebrate eDNA array from bottom water samples. Most samples captured the presence of zooplankton such as copepods and hydrozoan (Siphonophorae) rather than epibenthic species, which were typically found in only one sample with the most frequently occurring, the wart barnacle *Verruca stroemia*, being recorded in three bottom water samples.

Data presented in this report demonstrate that eDNA metabarcoding can be used in compliment with other techniques to provide a non-destructive means of collecting insightful fish community information, for example 44 of the taxa recorded via eDNA were not recorded in the BRUV footage (Ocean Ecology Limited, 2024). There are however limitations to the use of this technique which should be considered when interpreting the findings, namely that the resulting data can only provide a qualitative understanding of the community diversity with true abundance not quantified and only represented as a 'strong/weak' DNA signal.

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