MachairWind Offshore Windfarm

Appendix E – Environmental DNA Survey Interpretative Report

ScottishPower Renewables | MachairWind Offshore Windfarm

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MachairWind Phase 1 Geophysical and Environmental Survey

MachairWind Offshore Windfarm OAA
Environmental DNA Survey Interpretative Report

Survey Period: 24 August to 8 November 2023

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Complete

MachairWind Ltd

Document Control

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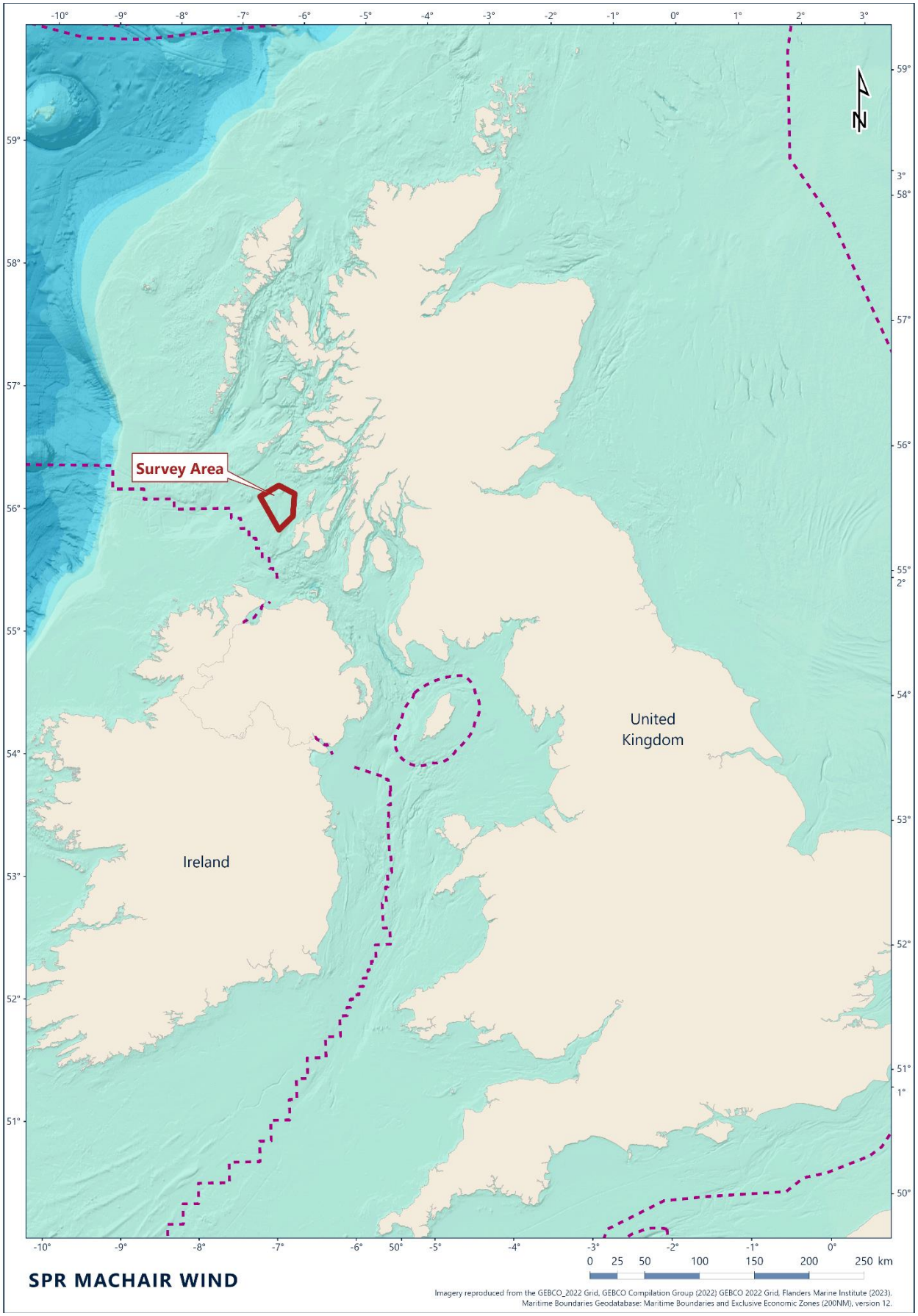

Executive Summary

Introduction

On the instruction of MachairWind Ltd, Fugro performed a geophysical and environmental characterisation site survey at the proposed MachairWind Offshore Wind Farm (OWF) Option Agreement Area (OAA). The survey area was located between the north-west of Islay and the west of Colonsay. Operations were conducted onboard the MV Fugro Galaxy during the survey period 24 August to 8 November 2023.

The environmental survey comprised a benthic sampling program to collect photographic data, using a drop-down video (DDV) system, grab samples for the analysis of benthic fauna, particle size distribution (PSD) and sediment contaminants. Environmental deoxyribonucleic acid (eDNA) in the water column were also collected. The environmental survey data are presented in the habitat report (Fugro 2024a Document No. 230633-MachairWind-V3) and the benthic interpretive report (Fugro 2024bDocument No. 230633-MachairWind-V5). This report details the results of the eDNA analysis.

The eDNA survey aimed at identifying mobile species (e.g. marine fish, excluding shark and rays), invertebrate and vertebrate taxa within the water column of the MachairWind Offshore Wind Farm OAA. Water samples were collected and analysed for evidence of these groups, with one sample taken near the surface (TOP) and near the seafloor (BOT) at each location. This report presented the eDNA analysis and interpretation of fish (excl. sharks and rays), invertebrate and vertebrate taxa.

The OAA was divided into four smaller blocks (A to D). Table S.1 presents the coordinates of each block within the MachairWind Offshore Wind Farm survey area.

Table S.1: Survey area extents

Geodetic Parameters: ETRS89, UTM Zone 29N, CM 3°W [m]				
Block A	**Easting**	**Northing**	**Latitude**	**Longitude**
A1	627 181.65	6 217 830.50	56° 05′ 19.20″ N	006° 57′ 21.36″ W
A2	644 309.15	6 227 853.71	56° 10′ 25.62″ N	006° 40′ 31.74″ W
A3	650 992.84	6 224 403.66	56° 08′ 26.70″ N	006° 34′ 11.58″ W
A4	632 344.82	6 208 795.63	56° 00′ 22.08″ N	006° 52′ 38.94″ W
Block B	**Easting**	**Northing**	**Latitude**	**Longitude**
B1	632 344.82	6 208 795.63	56° 00′ 22.08″ N	006° 52′ 38.94″ W
B2	650 992.84	6 224 403.66	56° 08′ 26.70″ N	006° 34′ 11.58″ W
B3	657 867.47	6 220 856.47	56° 06′ 24.06″ N	006° 27′ 41.22″ W
B4	635 950.55	6 202 487.08	55° 56′ 54.54″ N	006° 49′ 22.38″ W
Block C	**Easting**	**Northing**	**Latitude**	**Longitude**
C1	635 950.55	6 202 487.08	55° 56′ 54.54″ N	006° 49′ 22.38″ W
C2	657 867.47	6 220 856.47	56° 06′ 24.06″ N	006° 27′ 41.22″ W
C3	658 620.44	6 220 467.77	56° 06′ 10.56″ N	006° 26′ 58.56″ W

Geodetic Parameters: ETRS89, UTM Zone 29N, CM 3°W [m]				
C4	657 993.70	6 211 784.92	56° 01′ 30.78″ N	006° 27′ 53.22″ W
C5	639 483.03	6 196 307.37	55° 53′ 31.20″ N	006° 46′ 10.38″ W
Block D	**Easting**	**Northing**	**Latitude**	**Longitude**
D1	639 483.03	6 196 307.37	55° 53′ 31.20″ N	006° 46′ 10.38″ W
D2	657 993.70	6 211 784.92	56° 01′ 30.78″ N	006° 27′ 53.22″ W
D3	657 146.31	6 200 038.41	55° 55′ 12.18″ N	006° 29′ 06.78″ W
D4	644 440.30	6 187 629.57	55° 48′ 45.48″ N	006° 41′ 41.88″ W

Environmental Survey

The survey comprised 62 proposed environmental stations where photographic and grab samples were collected. At 30 stations 2 environmental DNA (eDNA) water samples were also collected.

Water samples were successfully acquired at 29 out of the 30 stations. Photographic data were successfully acquired at 59 of the 62 proposed stations. A full suite of grab samples was successfully acquired from 57 out of 62 proposed stations.

Stations MCW-D-ST90, MCW-D-ST96A and MCW-D-ST97A (Block D) were removed from the scope of work at the client's request. These also included one eDNA sample and three DDV transects.

Marine Water Fish

A total of 65 fish taxa was detected within the eDNA water samples. The most commonly detected fish taxa included Atlantic mackerel (*Scrombus scombus*), European sprat (*Sprattus sprattus*), Atlantic herring (*Clupea harengus*), Atlantic horse mackerel (*Trachurus trachurus*) and sandeels (Ammodytidae). These fish species are common within the survey area and may use the area and its surroundings as spawning or nursery grounds.

Marine Water Invertebrates

A total of 83 invertebrate taxa was detected within the eDNA samples. The most commonly detected invertebrate taxa were the copepods *Paracalanus parvus*, *Ditrichocorycaeus anglicus, Clausocalanus jobei, Pseudocalanus elongatus* and *Oithona similis*. Other taxa which were frequently detected included the polychaete order Spionida and the species *Sabellaria spinulosa*, the bivalve *Arctica islandica*, cnidarians of the family Campanulariidae, and the brittle star *Ophiothrix fragilis*. The invertebrate taxa detected are typical of the area, with copepod crustaceans being widely distributed and generally dominant within the aquatic zooplankton communities globally.

Marine Water Vertebrates

A total of 77 vertebrate taxa was detected within the eDNA samples. The most commonly detected vertebrate was the family of ray finned fishes (Clupeidae). Amongst the class Actinopterygii, other most commonly detected taxa included Atlantic mackerel (*S. scombrus*), sandeels (*Ammodytes*), poor cod (*T. minutus*) and cod (Gadidae), Atlantic horse mackerel (*T. trachurus*), flatfish (Pleuronectidae) and dragonet (*Callionymus lyra*). Amongst the class Aves, the most frequently detected taxa included common guillemot (*Uria aalge*), European shag (*Phalacrocorax aristotelis*), razorbill (*Alca torda*) and the northern gannet (*Morus bassanus*). Amongst the class Mammalia, detected taxa included common dolphin (*Delphinus delphis*), harbour porpoise (*Phocoena phocoena*), minke whale (*Balaenoptera acutorostrata*), Risso's dolphin (*Grampus griseus*) and fin whale (*Balaenoptera physalus*). The vertebrate taxa are typical of the survey area, with the most commonly detected taxa being widely distributed and abundant throughout waters off the west coast of Scotland.

Species of Conservation Importance

Among the species detected, Atlantic horse mackerel (*T. trachurus*), haddock (*Melanogrammus aeglefinus*), Atlantic cod (*Gadus morhua*) and fin whale (*Balaenoptera physalus*) are assessed as 'Vulnerable' under the International Union for the Conservation of Nature (IUCN) Red List in the UK. These species (excluding *M. aeglefinus*) are also listed under the Scottish Priority Marine Feature (PMF) list, along with Atlantic herring (*C. harengus*), Atlantic mackerel (*S. scombrus*), Atlantic salmon (*Salmo salar*), ling (*Molva molva*), Norway pout (*Trisopterus esmarkii*), saithe (*Pollachius virens*), sand goby (*Pomatoschistus minutus*), whiting (*Merlangius merlangus*), ocean quahog (*A. islandica*), harbour porpoise (*P. phocoena*), minke whale (*B. acutorostrata*), common dolphin (*D. delphis*) and risso's dolphin (*G. griseus*). Atlantic salmon (*S. salar*), cod (*G. morhua*), ocean quahog (*Arctica islandica*) and harbour porpoise (*P. phocoena*) are listed under the Oslo and Paris Commission (OSPAR) threatened and/or declining species list.

Atlantic cod (*G. morhua*), Atlantic herring (*C. harengus*), Atlantic horse mackerel (*T. trachurus*), Atlantic mackerel (*S. scombrus*), Atlantic salmon (*S. salar*), common sole (*Solea solea*), ling (*M. molva*), Norway pout (*T. esmarkii*), whiting (*M. merlangus*), common dolphin (*D. delphis*), fin whale (*B. physalus*), harbour porpoise (*P. phocoena*), minke whale (*B. acutorostrata*) and risso's dolphin (*G. griseus*) are all included in the Scottish Biodiversity list (NatScot, 2020).

Rainbow trout (*Oncorhynchus mykiss*) is non-indigenous to the UK and was detected within the survey area. This species has been observed to have undergone natural dissemination within European aquatic ecosystems subsequent to its introduction in western Europe, alongside instances of accidental aquaculture breaches and deliberate introductions for angling pursuits.

No other species listed as 'Near Threatened' to 'Critically Endangered' under the IUCN Red List, OSPAR threatened and/or declining habitats and species list, Scottish PMF list, or Scottish Biodiversity List were detected within the survey area.

Document Arrangement

Volume No.	Volume Title	Fugro Document No.	Client Document No.
Volume 1	Geophysical Field Operations Report	230633-MachairWind-V1	
Volume 2	Final Processing Report	230633-MachairWind-V2	
Volume 3	Final offshore wind farm geophysical habitat interpretative report	230633-MachairWind-V3	
Volume 4	Final offshore wind farm geophysical results report	230633-MachairWind-V4	
Volume 5	Final offshore windfarm benthic survey interpretive report	230633-MachairWind-V5	
Volume 6	Final offshore windfarm contaminant chemical analysis technical report	230633-MachairWind-V6	
Volume 7	**Final eDNA report with laboratory analysis**	**230633-MachairWind-V7**	

Contents

Appendices

Figures in the Main Text

Tables in the Main Text

Abbreviations

BOT	Near seafloor water sample
CM	Central meridian
DDV	Drop-down video
eDNA	Environmental deoxyribonucleic acid
excl.	Excluding
ETRS89	European Terrestrial Reference System 1989
FA	Faunal sample A
GNSS	Global Navigation Satellite System
GRIIS	Global Register of Introduced and Invasive Species
GRS	Geodetic Reference System
IUCN	International Union for the Conservation of Nature
JNCC	Joint Nature Conservation Committee
MV	Motor vessel
NCBI	National Centre for Biotechnology Information
NatScot	Nature Scotland
OAA	Option Agreement Area
OSPAR	Oslo and Paris Commission
OTU	Operational Taxonomic Unit
OWF	Offshore Wind Farm
PC	Physico-chemical sample
PCR	Polymerase chain reaction
PES	Polyethersulfone
PMF	Priority Marine Feature
PSD	Particle size distribution
SSS	Side scan sonar
TOP	Near surface water samples
USBL	Ultra -short baseline
UTM	Universal Transverse Mercator
zOTU	Zero-radius operational taxonomic units

1. Introduction

1.1 Background

On the instruction of MachairWind Ltd, Fugro performed a characterisation survey, including geophysical and environmental data acquisition at the MachairWind Offshore Wind Farm option agreement area (OAA). The survey area was located between the north-west of Islay and the west of Colonsay. Operations were conducted onboard the MV Fugro Galaxy during the survey period 24 August to 8 November 2023.

The OAA was divided into four smaller blocks (A to D) displayed in Figure 1.1. Table 1.1 presents the coordinates of each block within the MachairWind Offshore Wind Farm survey area.

The environmental survey aimed at characterising the benthic environment within the MachairWind OAA and comprised a benthic sampling programme to collect photographic data, using a drop-down video (DDV) system, grab samples for the analysis of benthic fauna, particle size distribution (PSD) and sediment contaminants (Fugro, 2024a and b) and acquisition of water samples for the eDNA analysis of invertebrate and vertebrate taxa, the latter being the focus of this report.

The aim of the eDNA survey was to identify mobile species (e.g. marine fish), invertebrate and vertebrate taxa within the water column of the MachairWind Offshore Wind Farm OAA. This was fulfilled through acquisition of water samples, which were subsequently analysed for eDNA taxonomic classification of fish (excluding sharks and rays), invertebrate and vertebrate taxa. At each location one sample was taken near the surface (TOP) and near the seafloor (BOT) and co-located with grab sample locations. The taxa detected by the eDNA water samples analysis were compared against those listed under the Priority Marine Features (PMFs) (JNCC, 2014) in Scotland's seas, on the Oslo and Paris Commission (OSPAR) list of threatened and/or declining species (OSPAR, 2008) or on the International Union for the Conservation of Nature (IUCN) Red List of Threatened Species (IUCN, 2023).

This report presented the eDNA analysis and interpretation of fish (excl. sharks and rays), invertebrate and vertebrate taxa.

Appendix A outlines the guidelines for use of this report.

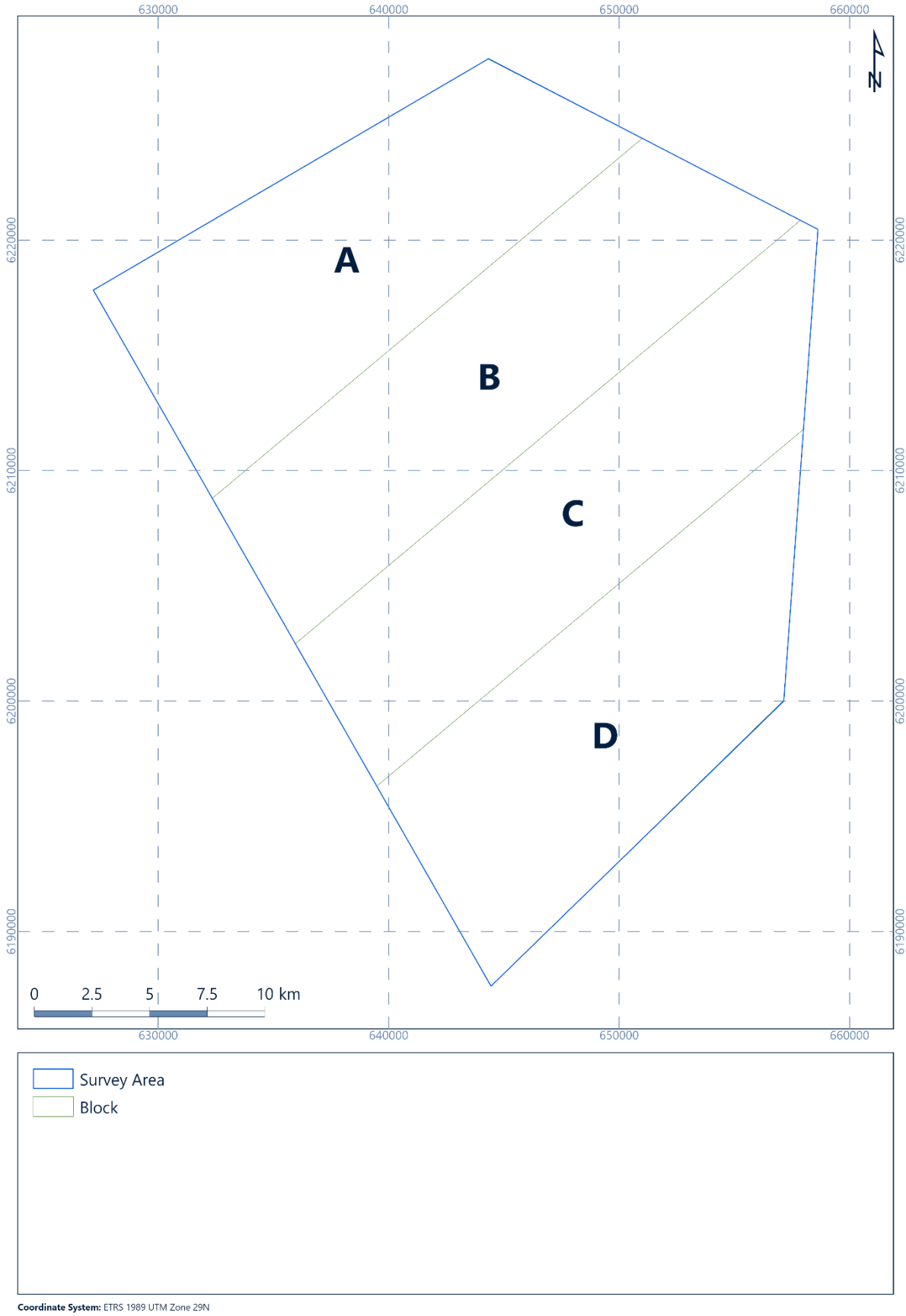

Figure 1.1: Blocks A to D in MachairWind Offshore Wind Farm option agreement area (OAA)

Table 1.1: Survey area extents

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D4	644 440.30	6 187 629.57	55° 48′ 45.48″ N	006° 41′ 41.88″ W

1.2 Coordinate Reference System

All coordinates detailed in this report are referenced to European Terrestrial Reference System 1989 (ETRS89) Universal Transverse Mercator (UTM) projection Zone 29N central meridian (CM) 9° West. Table 1.2 provides the detailed geodetic and projection parameters.

Table 1.2: Project geodetic and projection parameters

Global Navigation Satellite System (GNSS) Geodetic Parameters*			
Datum:		International Terrestrial Reference Frame 2014	ESPG: 1165
Spheroid:		GRS 1980	
Semi major axis:		a = 6 378 137.000 m	
Reciprocal flattening:		1/f = 298.257 222 101	
Local Geodetic Datum Parameters			
Datum:		European Terrestrial Reference System 1989	ESPG: 6258
Spheroid:		GRS 1980	
Semi major axis:		a = 6 378 137.000 m	
Reciprocal flattening:		1/f = 298.257 222 101	
Datum Transformation Parameters from ITRF2014 to ETRS89			
Shift dX:	+0.05608 m	Rotation rX: -0.0028148" arc sec	Scale Factor: 0.0036325 ppm
Shift dY:	+0.05358 m	Rotation rY: -0.0170275" arc sec	Coordinate Frame Rotation
Shift dZ:	-0.10023 m	Rotation rZ: +0.027522" arc sec	FUGRO: 41366
Local Projection Parameters†			
Map Projection:		Universal Transverse Mercator (TM)	
Grid System		UTM Zone 29N	ESPG: 16029
Central Meridian:		009° 00′ 00″ West	
Latitude of Origin:		00° 00′ 00″ North	
False Easting:		500 000 m	
False Northing:		0 m	
Scale factor on Central Meridian:		0.9996	
Units:		metre	

Notes
* = The geodetic datum of Fugro's global GNSS correction data is ITRF2014, epoch 2023.75 (01/10/2023 18:00:00)
† = This is the right-hand coordinate frame rotation used by the Fugro Starfix navigation software

2. Survey Strategy

Sixty-two environmental sampling stations were predetermined by the client. These stations were arranged to provide spatial coverage throughout the survey area and were aligned with the geophysical survey lines. At each environmental sampling station, video and stills were to be acquired prior to grab sampling. The total number of each sample acquired for each sample type is listed below:

- 43 macrofaunal (FA) samples;
- 32 particle size distribution (PSD) samples;
- 30 physico-chemical (PC) samples;
- 30 eDNA near surface (TOP) and 30 near seafloor (BOT) samples.

After geophysical data had been acquired, the side scan sonar (SSS) and bathymetric data were reviewed by the onboard environmental scientist in conjunction with the onboard geophysicist to confirm client predefined locations were suitable for grab sampling and camera investigations. Emphasis was placed on locating areas of potential conservation value (e.g. Annex I listed habitats), on boundaries between areas of differing sonic reflectivity, bathymetric highs and lows, and areas characteristic of the general background conditions of the survey area.

Figure 2.1 provides a spatial display of the proposed survey locations. Appendix B.1 provides the coordinates, data to be acquired and rationale for each location.

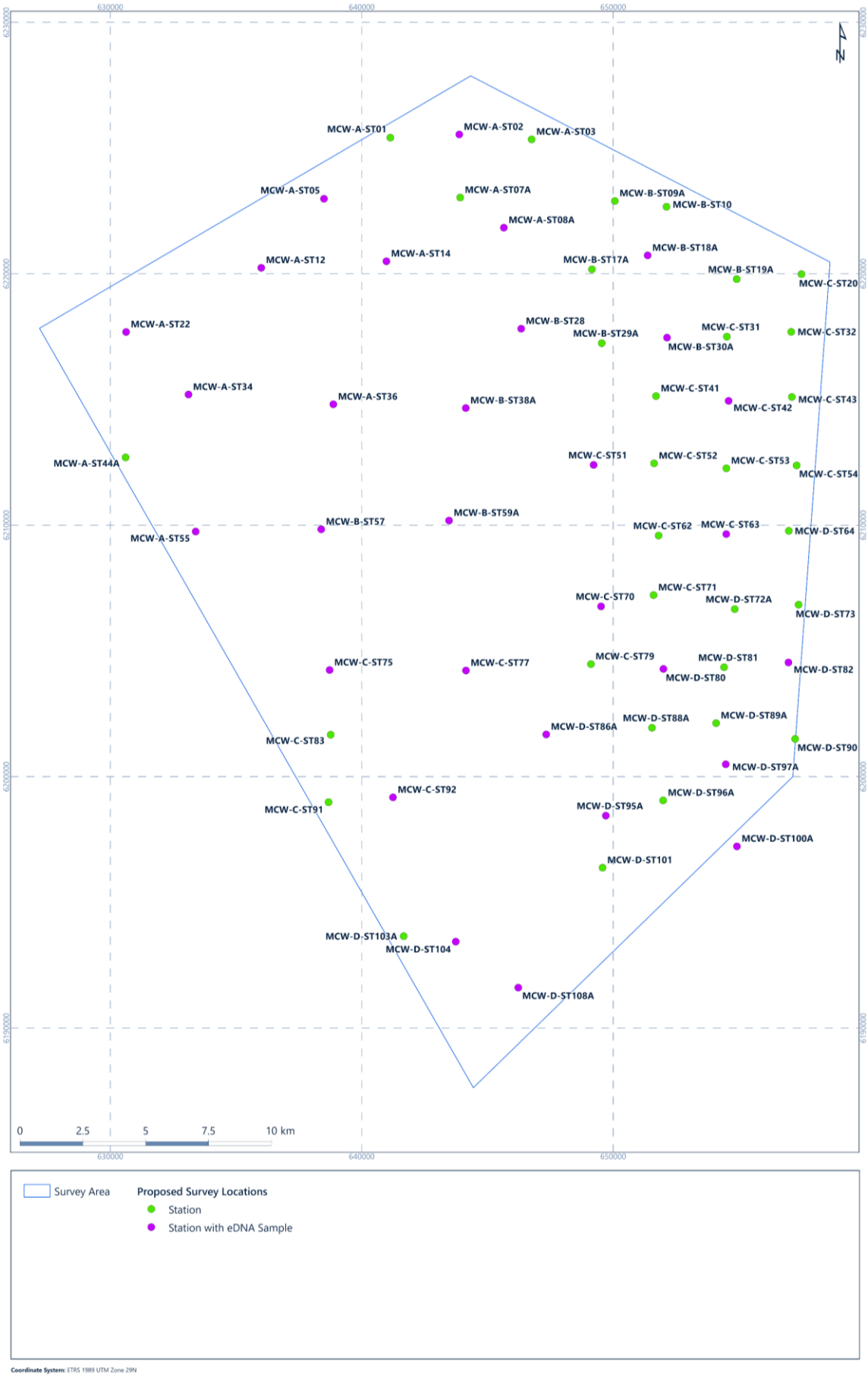


Figure 2.1: Proposed environmental survey locations

3. Methods

3.1 Water Sampling

Water samples were collected from the near surface water (TOP) and the near seafloor water (BOT) using a 5 L Niskin bottle.

Operational procedures for water sampling were as follows:

- The Niskin bottle was prepared for operations prior to arrival on station. An ultra-short baseline (USBL) beacon was attached to the wire below the bottle and a clump weight was attached to the end of the wire. The Bridge communicated to the deck via a VHF radio when the vessel was steady and on location, and the bottle was deployed from the stern A-frame;
- When the surveyor located the beacon and it reached the desired depth for sample acquisition (approximately 5 m above the seafloor for the BOT sample and 5 m below water surface for the TOP sample), the engineer operating the winch was informed (via VHF radio);
- The winch was stopped, and the survey engineer attached a messenger weight to the cable to trigger the Niskin bottle firing mechanism and collect the sample;
- When the survey engineer observed that the messenger had reached the bottle (evidenced through a vibration of the wire upon impact), the online surveyor was informed (via VHF radio) and a fix was taken;
- On recovery to the deck, the sample was inspected and judged acceptable if the bottle was full or otherwise rejected (e.g. if not triggered or only part full);
- Water samples were processed from the Niskin bottle using a NatureMetrics aquatic eDNA sampling kit and Vampire sampler. The samples preserved with the provided fixing agent, and stored in specimen bags provided at approximately −20 °C.

3.2 Water Extraction (Marine Standard)

Laboratory analysis was carried out by NatureMetrics.

Samples were processed in dedicated clean rooms, designed for the handling of eDNA samples, at NatureMetrics UK with all work undertaken in class II biosafety cabinets and all workstations decontaminated with a chemical disinfectant and UV irradiated before and after use.

Samples were collected with 0.8 µm Polyethersulfone (PES) filters with a modified Longmire's solution added to the filter housing to preserve DNA prior to extraction. DNA was extracted from the 0.8 µm PES filters using a DNeasy Blood and Tissue Kit (Qiagen) following Spens et al. (2016) method for disc filters in buffer, with proteinase K added directly to the filter housing to minimise the risk of contamination arising from handling of the filter. A negative control, consisting of molecular grade water, 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 (Thermo Fisher Scientific).

3.3 DNA Amplification

Replicate polymerase chain reactions (PCRs) for each sample and extraction blank were amplified via a two-step PCR process, with tails added to the 5'end of taxon specific primers to complement downstream adapter and index primer sequences.

Positive and negative controls, consisting of proprietary synthetic sequences (that do not match known biological records) and PCR-grade water, respectively, were included with every PCR plate to verify amplification performance. PCR amplification success was confirmed visually by gel electrophoresis.

3.4 Library Preparation and Sequencing

Successfully amplified first round PCR replicates were pooled per sample and purified using MagBind TotalPure NGS magnetic beads (Omega Biotek). A sequencing library was prepared from the purified amplicons using unique dual indexes, following illumina's 16S Metagenomic Sequencing Library Preparation protocol (16S Metagenomic Sequencing Library Preparation, n.d.). Indexed PCR products were subsequently purified, quantified, normalised, and pooled in equal volumes. The final pooled library was sequenced on an Illumina MiSeq system using a V3 600 cycle reagent kit (illumina, 2024).

3.5 Data Analysis

The original data analysis, as provided in the NatureMetrics report (Appendix B), was carried out by NatureMetrics. Additional data analysis and the interpretation was carried out by Fugro GB Limited. The following subsections presents the methods applied as per NatureMetrics (2024).

3.5.1 Data Rationalisation

Identifications were sense-checked against GBIF occurrence records for presence in the sampling country and elevated to higher taxonomic levels where required (rgbif; Chamberlain et al., 2023).

Zero-radius operational taxonomic units (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 %.

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. Any non-marine taxa were included in subsequent analyses due to their

genetic signature being detected, and the metrics being provided with the inclusion of all target taxa per each assay; however, these were not considered further for discussion as they are not part of the marine water community.

Individuals within a species can have slight differences in the target DNA sequences and small changes can occur during sequencing. Minimum similarity thresholds of 98 %, 95 % and 92 % were used for species, genus, and high-level assignments respectively, allowing for a 2 % to 8 % tolerance for dissimilarity between DNA sequences. Consequently, certain instances yielded multiple OTUs for the same taxa. To mitigate potential data inflation during statistical analyses, these redundant OTUs were consolidated by merging the respective taxa.

3.5.2 Bioinformatics

Sequences were demultiplexed with bcl2fastq and processed via a custom NatureMetrics eDNA analysis pipeline. Demultiplexing involves reorganizing the FASTQ files based on the index information and generating the statistics and reporting files. FASTQ format is a text-based format for storing both a biological sequence (usually nucleotide sequence) and its corresponding quality scores. 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). 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 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 National Centre for Biotechnology Information nucleotide (NCBI nt). 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 99 %, 97 % and 95 % for species, genus, and higher-level assignments respectively.

The eDNA analysis aims at displaying species level or the lowest taxonomic level confidently detected. The eDNA signal, which indicates the proportion of DNA sequences within a sample, is represented using a bubble plot, with a bubble indicating the presence of that species in that sample. A larger bubble size potentially indicates a stronger eDNA signal.

Table 3.1 summarises the community statistics used for the analysis.

Table 3.1: Community Statistics

Statistic	Definition
Tree of life	This analysis provides a view of the species detected in the samples and their taxonomic relationship with taxa on the same branch indicating more similar taxa than those on different branches. The resulting chart is structured with the highest taxonomic rank at the centre (e.g., kingdom, phylum, class), moving through the ranks of order, family, genus, species as you move to the outer edge. The centre and outer ranks will change depending on the test applied and the number of species detected. The legend in the bottom right of the chart indicates how to relate the colour in the branches to the number of species, ranging from grey (indicating very few species) to blue (indicating a lot of species).
Species Richness	A biodiversity metric that is consistently reported for biodiversity monitoring, for eDNA analysis refers to the total count of OTUs detected in each sample and it is reported both for each sample (alpha diversity) and for the total number of OTUs from all samples taken (gamma diversity). It is called 'Species Richness' because an OTU is a hypothesised species based upon clusters of similar DNA sequences. This metric is not the sum of OTUs identified to species-level. Although some OTUs cannot be assigned to a species, these are approximately equivalent to species. Higher 'Species Richness' is a broad indication of a healthier functioning ecosystem
Evolutionary Diversity	A biodiversity metric calculated for each sample, is a measure of the variety of the diversity of species detected, based on how distantly-related those species are. Evolutionary Diversity is a strong complementary indicator of biodiversity status alongside Species Richness. An increase in Evolutionary Diversity indicates a more varied species assemblage, which is generally associated with a better functioning ecosystem and more ecological niches available. We calculate the Evolutionary Diversity of samples by arranging all OTUs in a family tree based on the similarity of the DNA sequences. The overall size of the family tree (including lengths of all family tree branches) gives the value for Evolutionary Diversity. The metric used is 'Faith's Phylogenetic Diversity' which is commonly used in ecological science and biodiversity monitoring

3.5.3 eDNA Comparative Analysis

As the eDNA analysis targeting vertebrates also target fish taxa (Actinopterygii), using a different genetic primer, data were analysed by means of in-house data analysis (within R v. 2023.12.1 environment) to generate a Venn Diagram. Venn Diagrams are used to investigate the relationships between two or more datasets highlighting their similarities and/or differences (Joyce, 2008). The proportion of overlapping taxa, those detected by both eDNA analyses, is shown at the intersection of the Venn Diagram circles.

3.5.4 Species of Conservation Importance

All OTUs with species-level identifications were queried against the IUCN Red List to obtain global threat status (IUCN, 2023). Species were also assessed for their conservation status using the OSPAR list of threatened and/or declining species and habitats (OSPAR, 2008), the Scottish PMF list (JNCC, 2014) and the Scottish Biodiversity list (NatureScotland [NatScot], 2020).

3.5.5 Non-Indigenous Species

All OTUs with species-level identifications were queried against the Global Register of Introduced and Invasive Species (GRIIS) to obtain their invasive status in the sampling country.

4. Results

4.1 Field Operations

4.1.1 Water Sampling

The eDNA samples were successfully collected at 29 out of 30 proposed stations at near surface (approximately 5 m below water surface) and near seafloor (approximately 5 m above seafloor) depths (Table 4.1) generating a total of 58 water samples.

One eDNA sampling station in Block D, MCW-D-ST97A was removed from the scope as per the client's request.

Table 4.1: Completed water sampling stations

Geodetic Parameters: ETRS89, UTM Zone 29N, CM 3°W [m]					
Station	Easting	Northing	Sample Depth [m LAT]		Sample Acquisition
			TOP	BOT	
Block A					
MCW-A-ST02	643 878.7	6225 537.1	2	62	eDNA
MCW-A-ST05	638 497.5	6222 980.8	6	58	eDNA
MCW-A-ST08A	645 653.0	6221 831.0	4	55	eDNA
MCW-A-ST12	636 003.8	6220 235.4	4	61	eDNA
MCW-A-ST14	640 980.0	6220 496.2	3	47	eDNA
MCW-A-ST22	630 628.1	6217 682.7	5	69	eDNA
MCW-A-ST34	633 109.9	6215 197.5	3	58	eDNA
MCW-A-ST36	638 869.7	6214 808.1	3	45	eDNA
MCW-A-ST55	633 394.1	6209 747.7	3	50	eDNA
Block B					
MCW-B-ST18A	651 370.9	6220 729.2	5	47	eDNA
MCW-B-ST28	646 340.8	6217 811.7	5	57	eDNA
MCW-B-ST30A	652 112.3	6217 501.2	5	46	eDNA
MCW-B-ST38A	644 140.2	6214 661.0	5	55	eDNA
MCW-B-ST57	638 391.2	6209 834.4	5	49	eDNA
MCW-B-ST59A	643 472.9	6210 184.3	5	59	eDNA
Block C					
MCW-C-ST42	654 589.5	6214 943.2	5	42	eDNA
MCW-C-ST51	649 221.4	6212 397.9	5	52	eDNA
MCW-C-ST63	654 496.0	6209 644.7	5	45	eDNA
MCW-C-ST70	649 516.5	6206 770.4	7	47	eDNA

Geodetic Parameters: ETRS89, UTM Zone 29N, CM 3°W [m]					
Station	Easting	Northing	Sample Depth [m LAT]		Sample Acquisition
			TOP	BOT	
MCW-C-ST75	638 718.8	6204 237.0	5	52	eDNA
MCW-C-ST77	644 144.7	6204 221.6	5	60	eDNA
MCW-C-ST92	641 243.9	6199 176.3	5	50	eDNA
Block D					
MCW-D-ST80	651 997.7	6204 285.1	5	50	eDNA
MCW-D-ST82	656 968.7	6204 540.2	5	52	eDNA
MCW-D-ST86A	647 337.3	6201 679.9	5	48	eDNA
MCW-D-ST95A	649 708.5	6198 445.3	5	47	eDNA
MCW-D-ST100A	645 921.0	6197 227.0	5	54	eDNA
MCW-D-ST104	643 736.8	6193 438.6	5	55	eDNA
MCW-D-ST108A	646 228.2	6191 609.5	5	44	eDNA

Notes
LAT = Lowest Astronomical Tide
eDNA = Environmental deoxyribonucleic acid
TOP = Near surface water sample
BOT = Near seafloor water sample

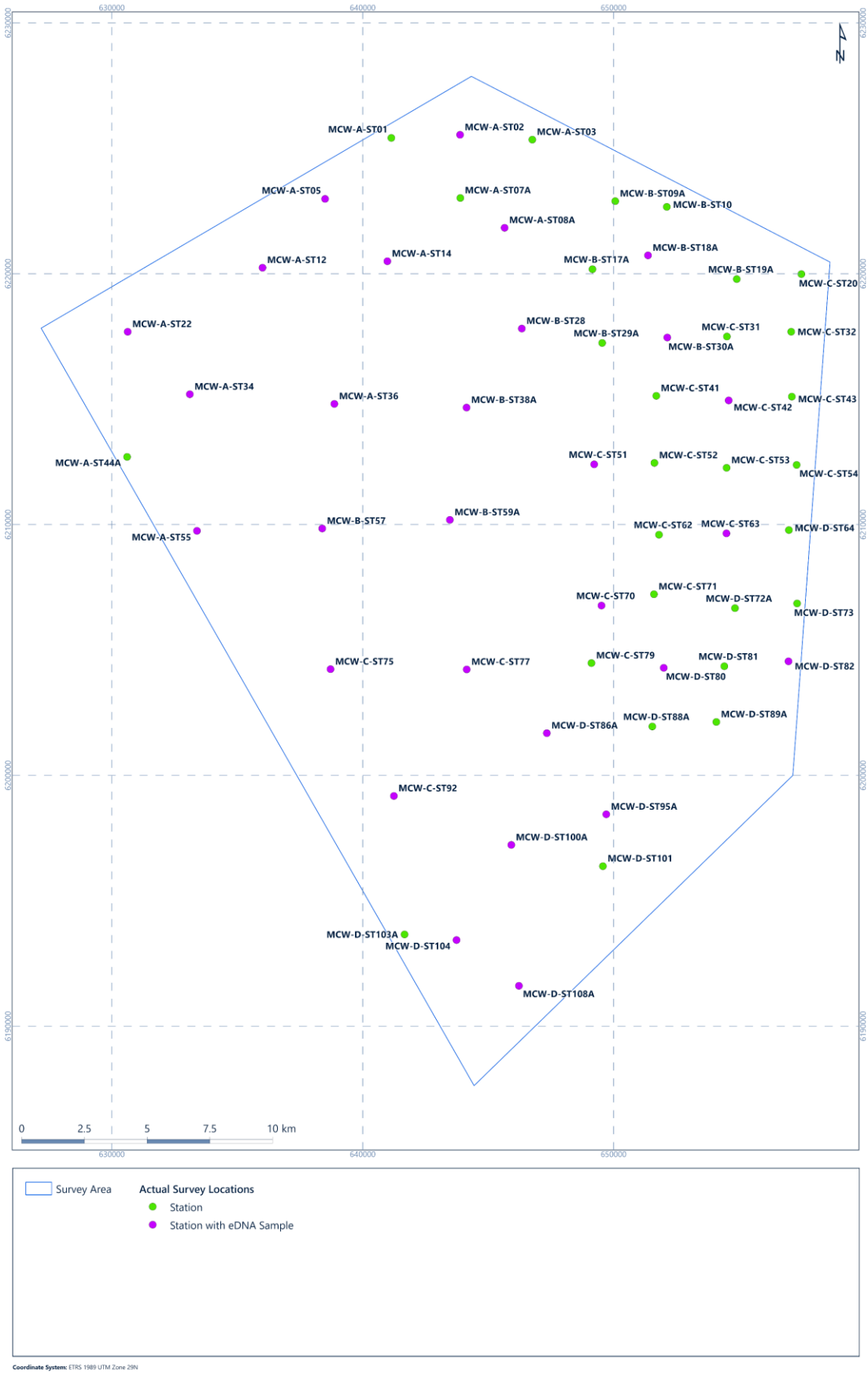


Figure 4.1: Actual environmental survey locations

4.2 Water Column eDNA

Both samples, TOP and BOT, were successfully collected at 29 sampling locations, generating a total of 58 samples, which were analysed for eDNA taxonomic classification of fish (excluding sharks and rays), invertebrate and vertebrate taxa.

4.2.1 Marine Water Fish (excluding Sharks and Rays)

High quality fish data were successfully obtained from 55 eDNA samples. Amplification of DNA sequences could not be obtained in TOP samples MCW-A-ST12, MCW-C-ST51 and MCW-A-ST05, owing to insufficient DNA concentration or PCR inhibition and therefore no results were reported for these stations.

Table 4.2 provides the number of OTUs detected and the percentage of OTUs identified to each taxonomic level.

Table 4.2: Number of OTUs detected and the percentage of OTUs identified to each taxonomic level

Number of OTUs	Phylum [%]	Class [%]	Order [%]	Family [%]	Genus [%]	Species [%]
66	100	100	100	98.48	89.39	78.79
Notes OTU = Operational Taxonomic Unit						

Gamma diversity, calculated as the total number of OTUs from all samples taken (Table 3.1), was reported as 66 (Table 4.2). Following rationalisation of data, a total of 65 fish taxa were detected and 80.0 % (52 taxa) were at least 98 % similar to a species in the global reference database. The remaining taxa were identified to genus (7 taxa; 10.8 %), family (5 taxa; 7.7 %) and to order (1 taxa; 1.5 %) level (Table 2 within Appendix C.2).

Following the colour gradient, the Tree-of-Life (Table 3.1; Figure 4.2) indicates that the taxonomic composition of the fish water column community within the MachairWind Offshore Wind Farm OAA was mainly formed by ray-finned fish (Perciformes), followed by cod-like fishes (Gadiformes) and flatfish (Pleuronectiformes).

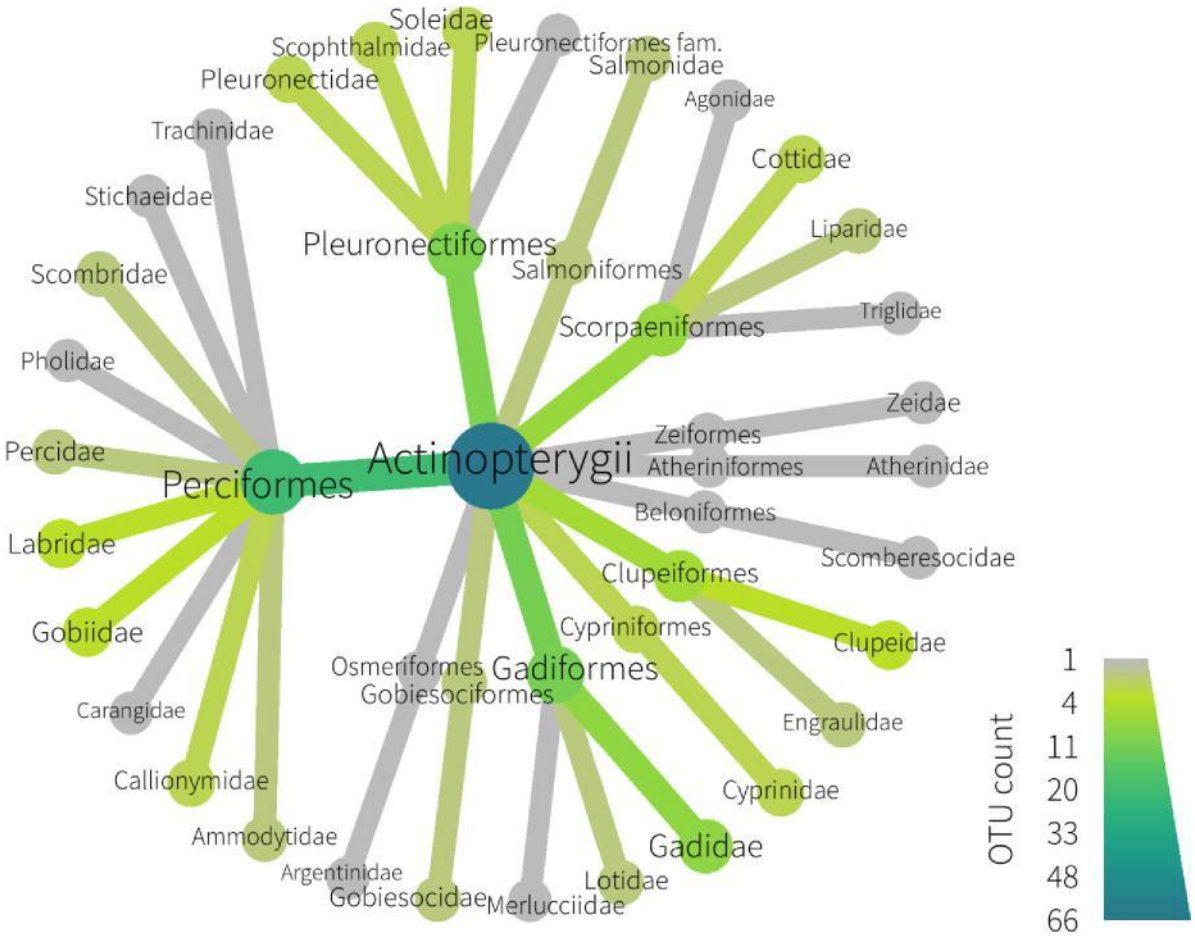
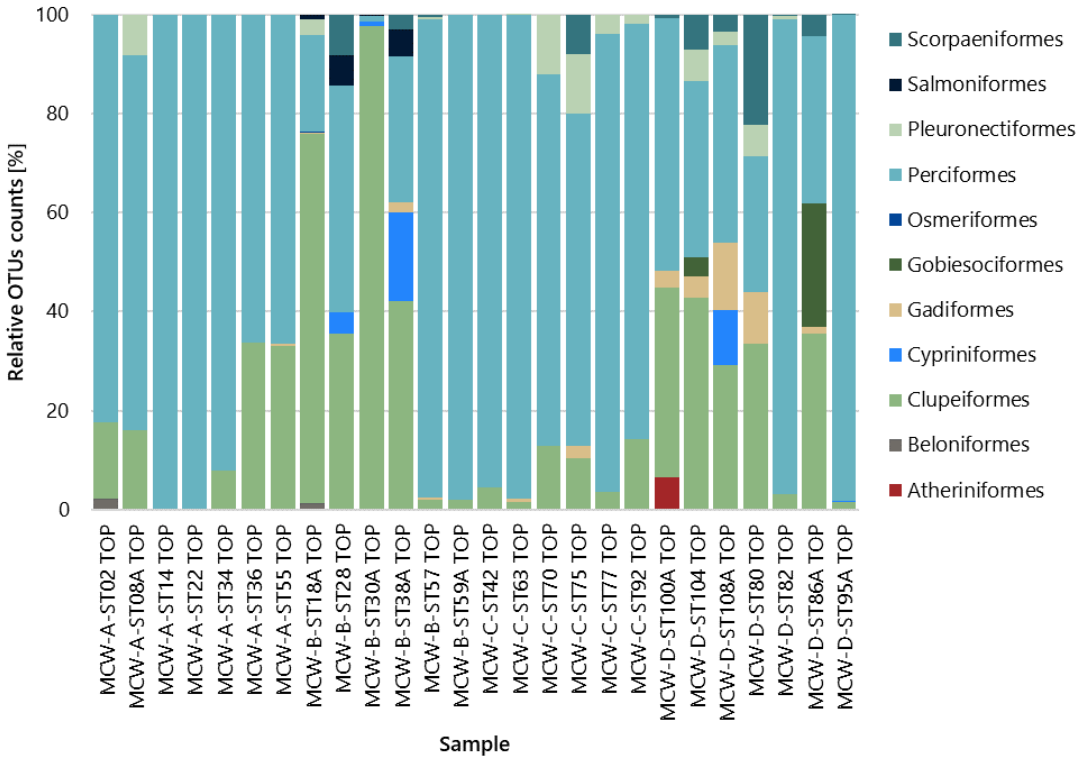


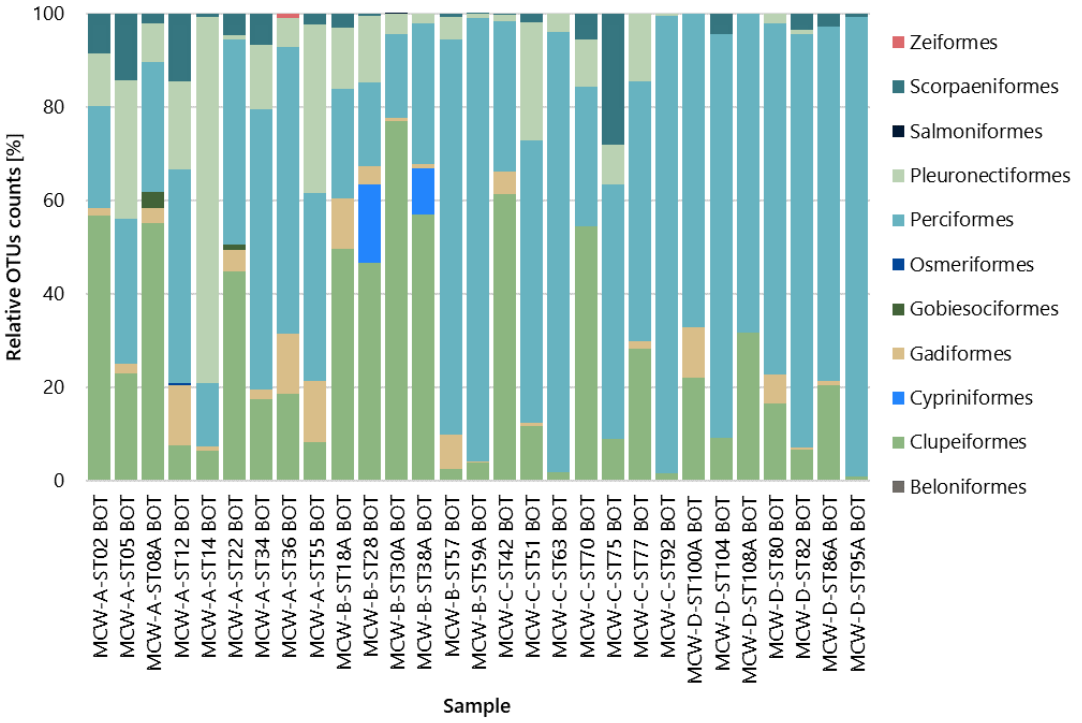
Figure 4.2: Taxonomic composition of eDNA fish samples

Figure 4.3 presents the bar plots of the relative OTU counts of the fish taxa detected by eDNA sampling rationalised to 'order' taxonomic level for TOP (A) and BOT (B) samples.

Taxa recorded in the TOP and BOT samples were mostly comparable, with a higher proportion of bottom-dwelling taxa such as flatfish (Pleuronectiformes) within the BOT samples and higher proportion of ray-finned fish (Perciformes) within the TOP samples. The order Scorpaeniformes (which are now accepted as a sub-order under Perciformes; WoRMS, 2024) was detected within TOP and BOT samples, but it was more frequently detected within the latter ones. The order Atheriniformes was detected within the TOP samples, but it was absent from the BOT samples. Similarly, the order Zeiformes was detected within BOT samples, but it was absent from the TOP samples.



A



B

Notes

Non-target taxa were excluded from the plot

OTU = Operation taxonomic unit

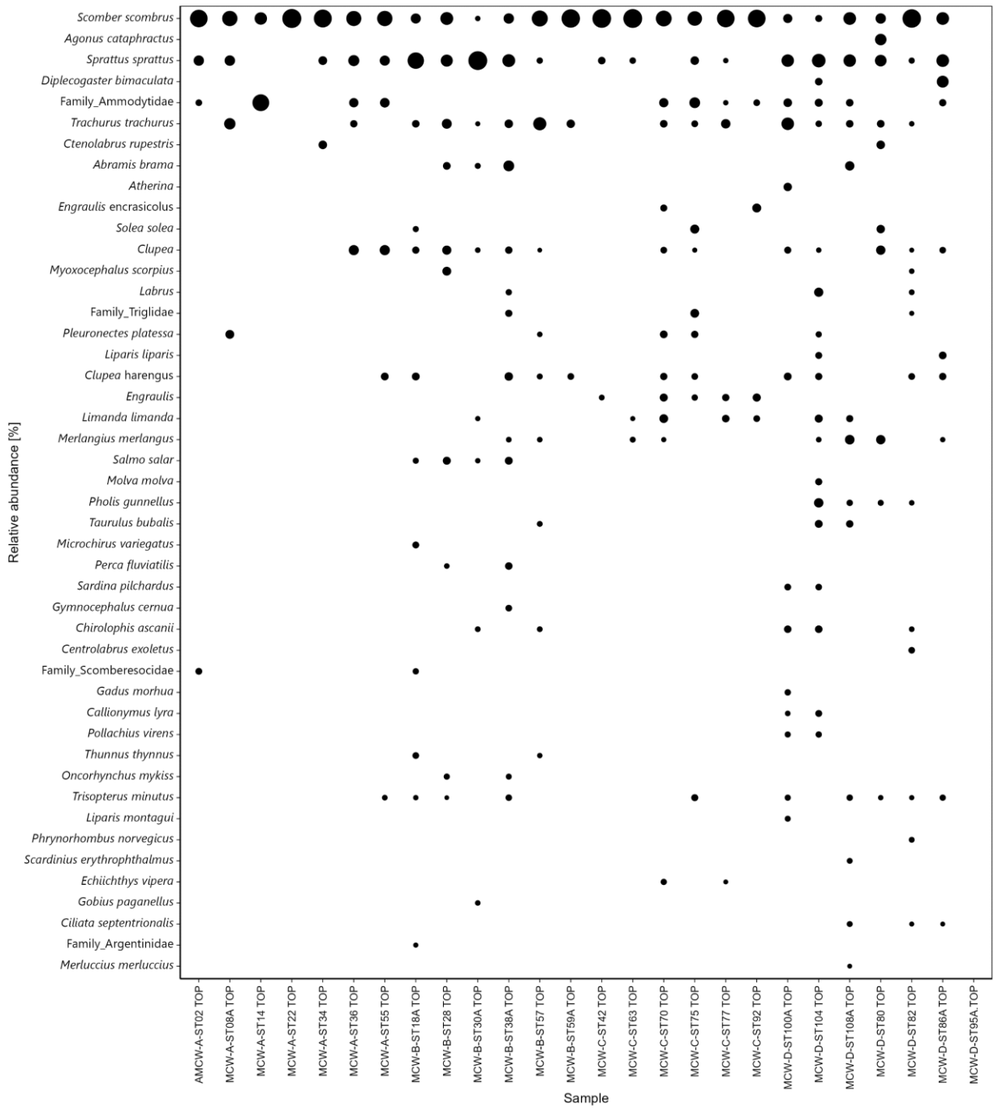
TOP = Near surface water samples

BOT = Near seafloor water samples

Figure 4.3: Relative OTU counts of target fish taxa detected to order level in TOP (A) and BOT (B)

Figure 4.4 presents the community composition of fish taxa detected and relative proportion of DNA sequences within each sample for TOP (A) and BOT (B). Bubble size corresponds to the proportion of DNA within a sample, with larger bubbles indicating a more pronounced eDNA signal. The taxon with the highest detection rate was Atlantic mackerel (*Scomber scombrus*), which was recorded in all samples.

Other most commonly detected taxa included the European sprat (*Sprattus sprattus*) (44 samples; 80.0 %), Atlantic horse mackerel (*Trachurus trachurus*) (40 samples; 72.7 %), Atlantic herring (*Clupea harengus*) (37 samples; 67.3 %), herring (*Clupea* sp.) (34 samples; 61.8 %) and sandeels (Ammodytidae) (34 samples; 61.8 %).



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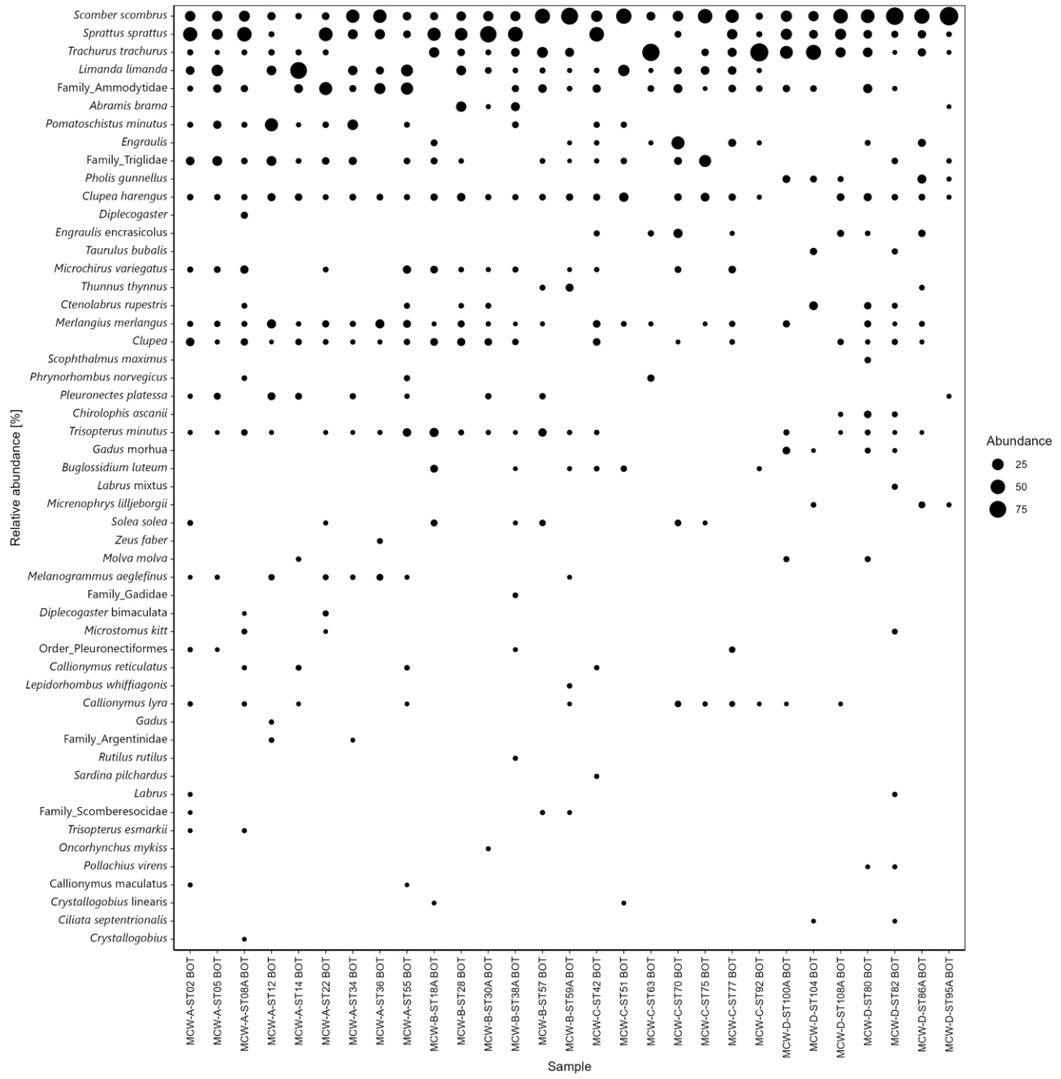


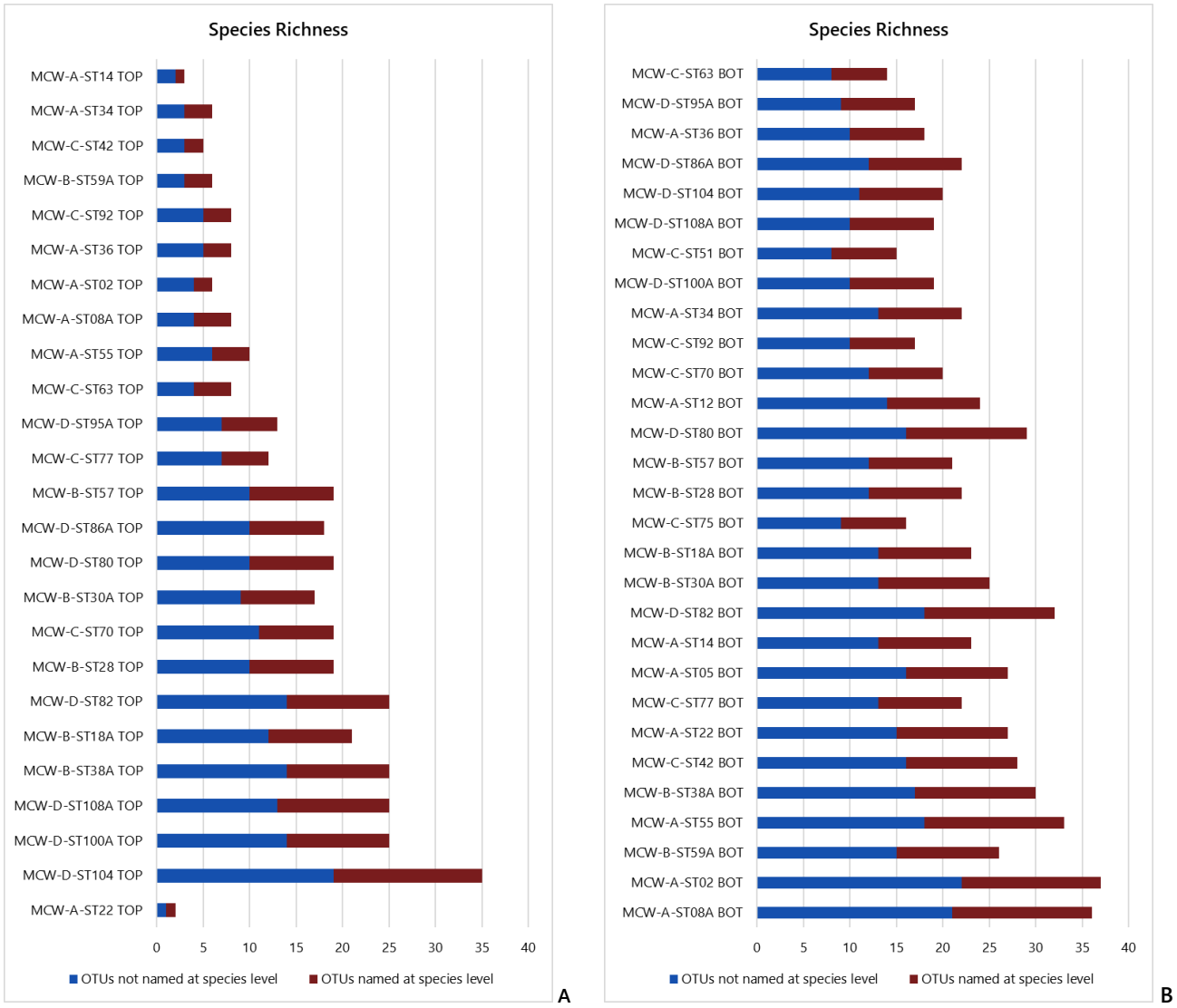
B

Notes
Non-target taxa were excluded from the plot
OTU = Operation taxonomic unit
TOP = Near surface water samples
BOT = Near seafloor water samples

Figure 4.4: Bubble plot of community composition of fish taxa detected in eDNA samples TOP (A) and BOT (B)

Figure 4.5 presents the total count of OTUs detected in each sample for TOP (A) and BOT (B)., represented as Species Richness. The blue portion of each bar indicates the number of OTUs identified to species level, whilst the red portion of each bar indicates the number of OTUs identified to a higher taxonomic level.

Species Richness is defined by the total number of OTUs detected in each sample (i.e. alpha diversity). Alpha diversity ranged from 1 (sample MCW-A-ST22 TOP) to 22 (sample MCW-A-ST02 BOT).



Notes

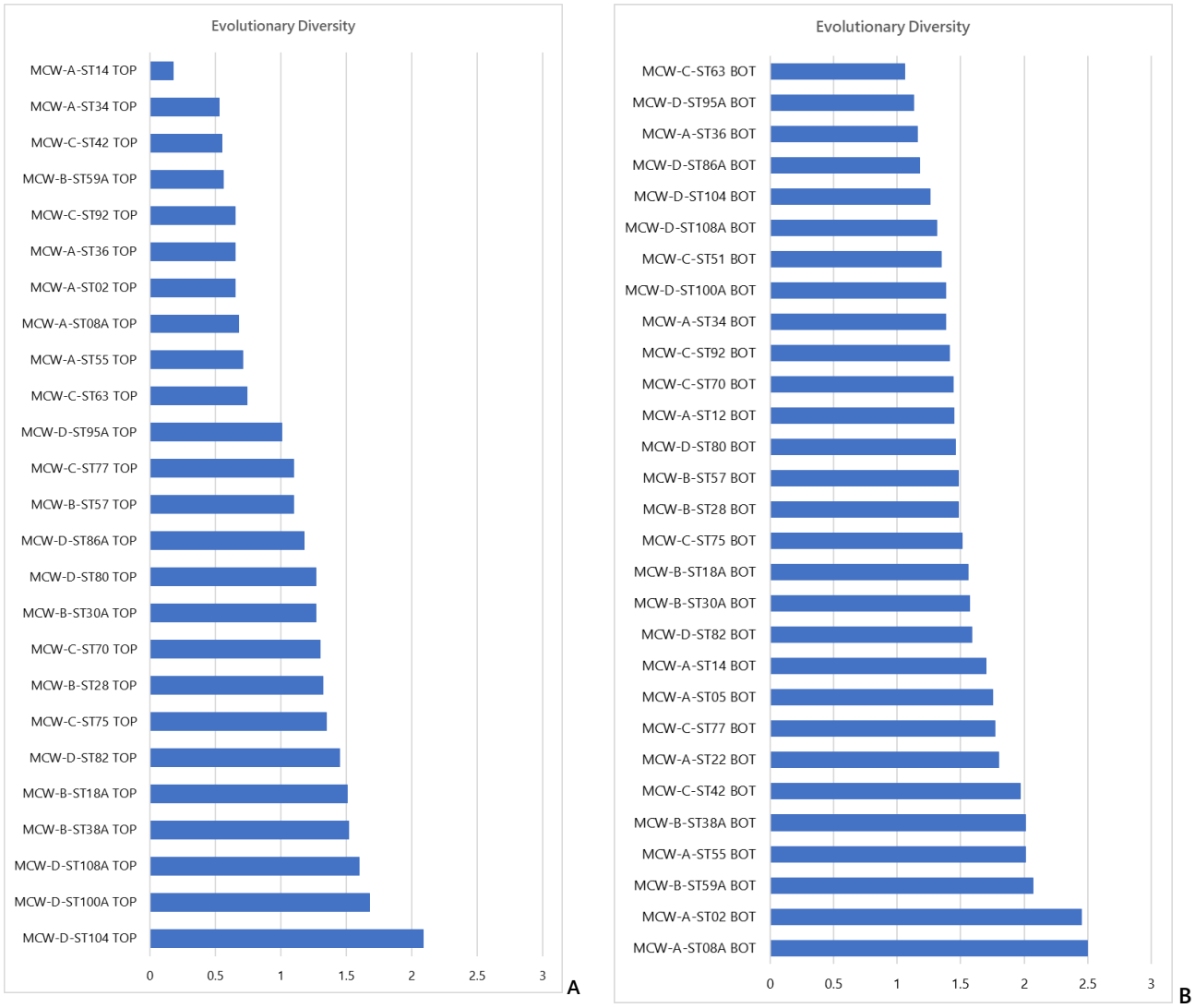
OTU = Operation taxonomic unit

TOP = Near surface water samples

BOT = Near seafloor water samples

Figure 4.5: Fish Species Richness detected in eDNA samples TOP (A) and BOT (B)

Figure 4.6 displays the Evolutionary Diversity calculated for each water eDNA sample for TOP (A) and BOT (B). Evolutionary Diversity, a key metric in assessing biodiversity, measures the diversity of species detected and their genetic relationships. It compliments Species Richness by providing insights into ecosystem health and available ecological niches (Table 3.1). The Evolutionary Diversity ranged from 0.18 (sample MCW-A-ST14 TOP) to 2.5 (MCW-A-ST08A BOT) (Table 3 within Appendix C.1). The TOP sample MCW-A-ST22 comprised only one taxon, resulting in the inability to resolve Evolutionary Diversity for this sample.



Notes

Non-target taxa detected were excluded from the plot

OTU = Operation taxonomic unit

TOP = Near surface water samples

BOT = Near seafloor water samples

Figure 4.6: Evolutionary Diversity of fish calculated for each eDNA sample TOP (A) and BOT (B)

4.2.2 Marine Water Invertebrates

High quality invertebrate data were obtained for all 58 eDNA samples analysed. Table 4.3 provides the number of OTUs detected and the percentage of OTUs identified to each taxonomic level.

Table 4.3: Number of OTUs detected and the percentage of OTUs identified to each taxonomic level

Number of OTUs	Phylum [%]	Class [%]	Order [%]	Family [%]	Genus [%]	Species [%]
89	98.88	98.88	95.51	92.13	82.02	69.66
Notes OTU = Operational Taxonomic Unit						

Gamma diversity, calculated as the total number of OTUs from all samples taken (Table 3.1), was reported as 89 (Table 4.3). Following rationalisation of data, a total of 83 invertebrate taxa were detected and 68.7 % (57 taxa) were at least 98 % similar to a species in the global reference database. The remaining taxa were identified to genus (11 taxa; 13.3 %), family (9 taxa; 10.1 %), order (2 taxa; 2.4 %), class (3 taxa; 3.6 %) and to kingdom (1 taxa; 1.2 %) level (Table 2 within Appendix C.2).

Following the colour gradient, the Tree-of-Life (Table 3.1; Figure 4.7) indicates that the taxonomic composition of the invertebrate water column community within the MachairWind Offshore Wind Farm OAA was mainly formed by Arthropoda, followed by Mollusca, Annelida, Cnidaria and Echinodermata.

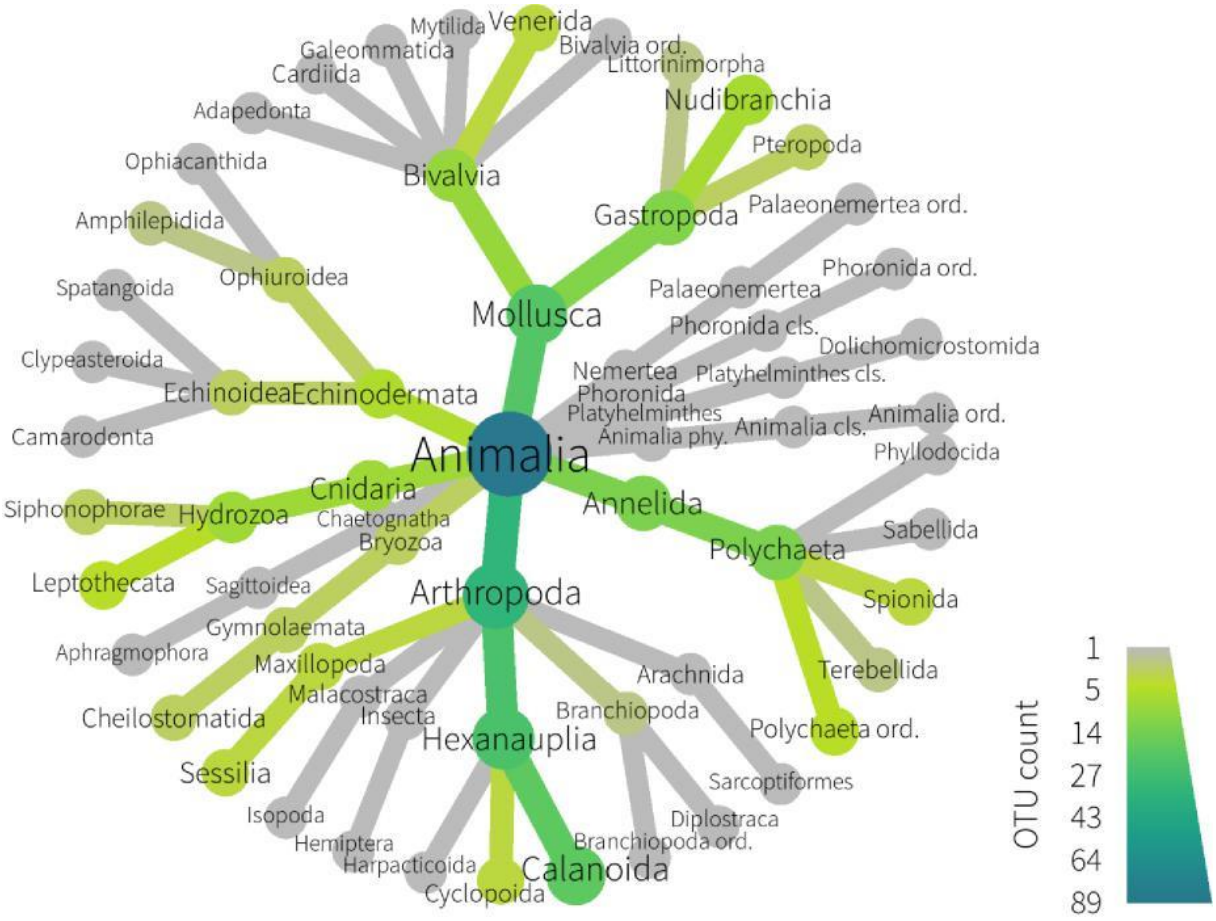
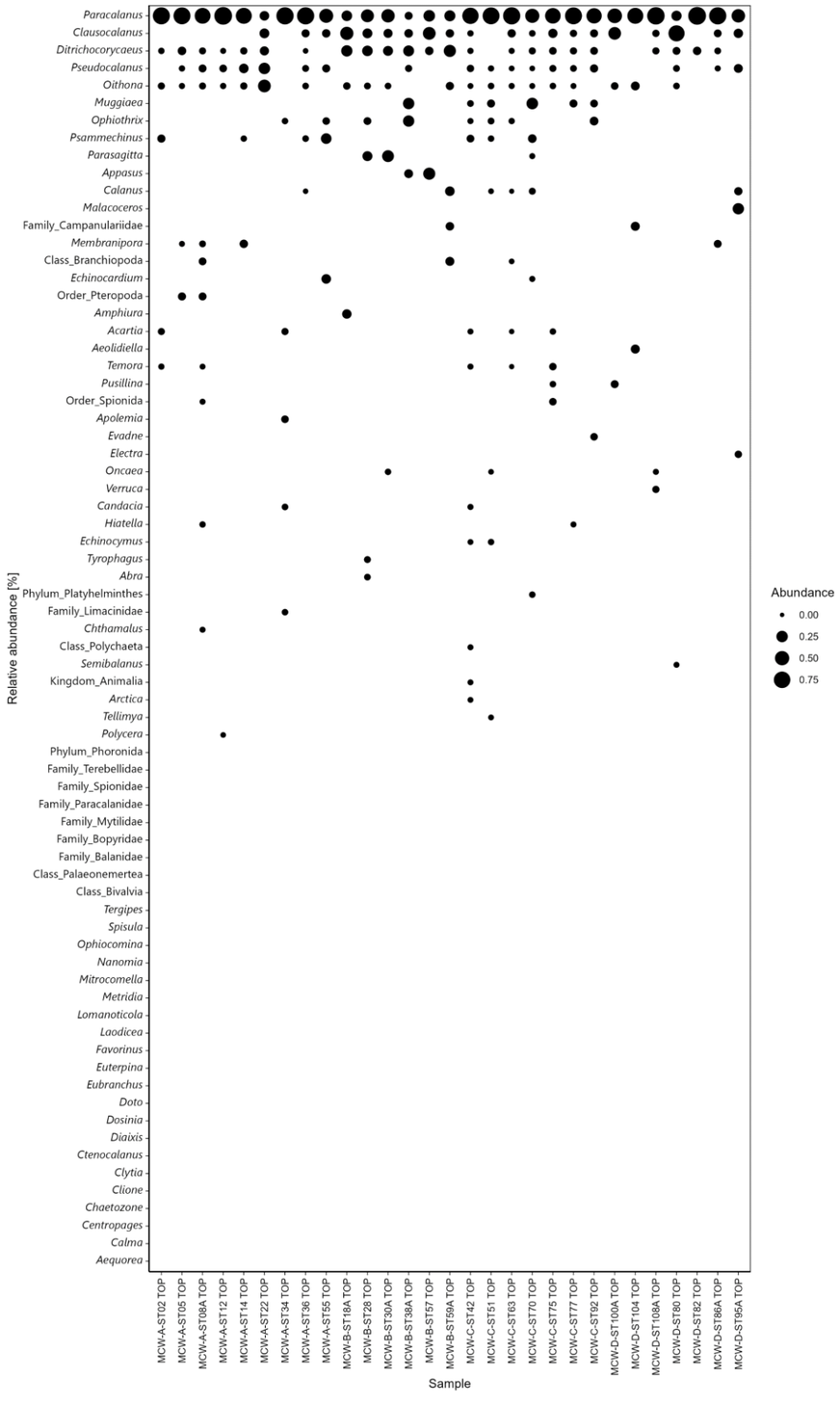


Figure 4.7: Taxonomic composition of eDNA invertebrate samples

Figure 4.8 presents the community composition of invertebrate taxa recorded and relative proportion of DNA sequences within each sample for TOP (A) and BOT (B). Bubble size corresponds to the proportion of DNA within a sample, with larger bubbles indicating a more pronounced eDNA signal. The taxon with the highest detection rate was the copepod *Paracalanus parvus*, which was recorded in all samples (100 %). Other most commonly detected taxa included the copepods *Ditrichocorycaeus anglicus* (50 samples; 86.2 %), *Clausocalanus jobei* (43 samples; 74.1 %), *Pseudocalanus elongatus* (38 samples; 65.5 %) and *Oithona similis* (36 samples; 62.1 %).



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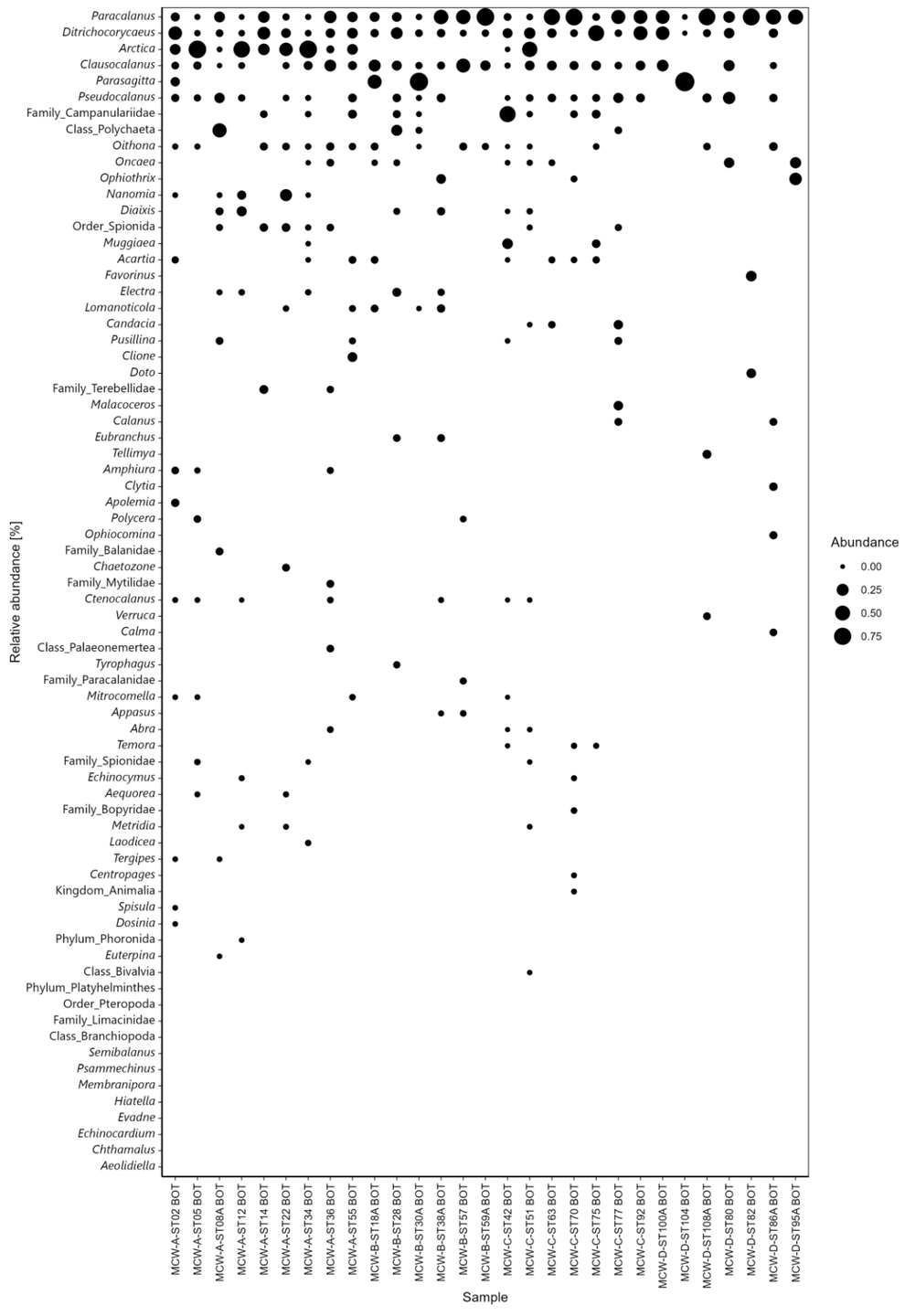


B

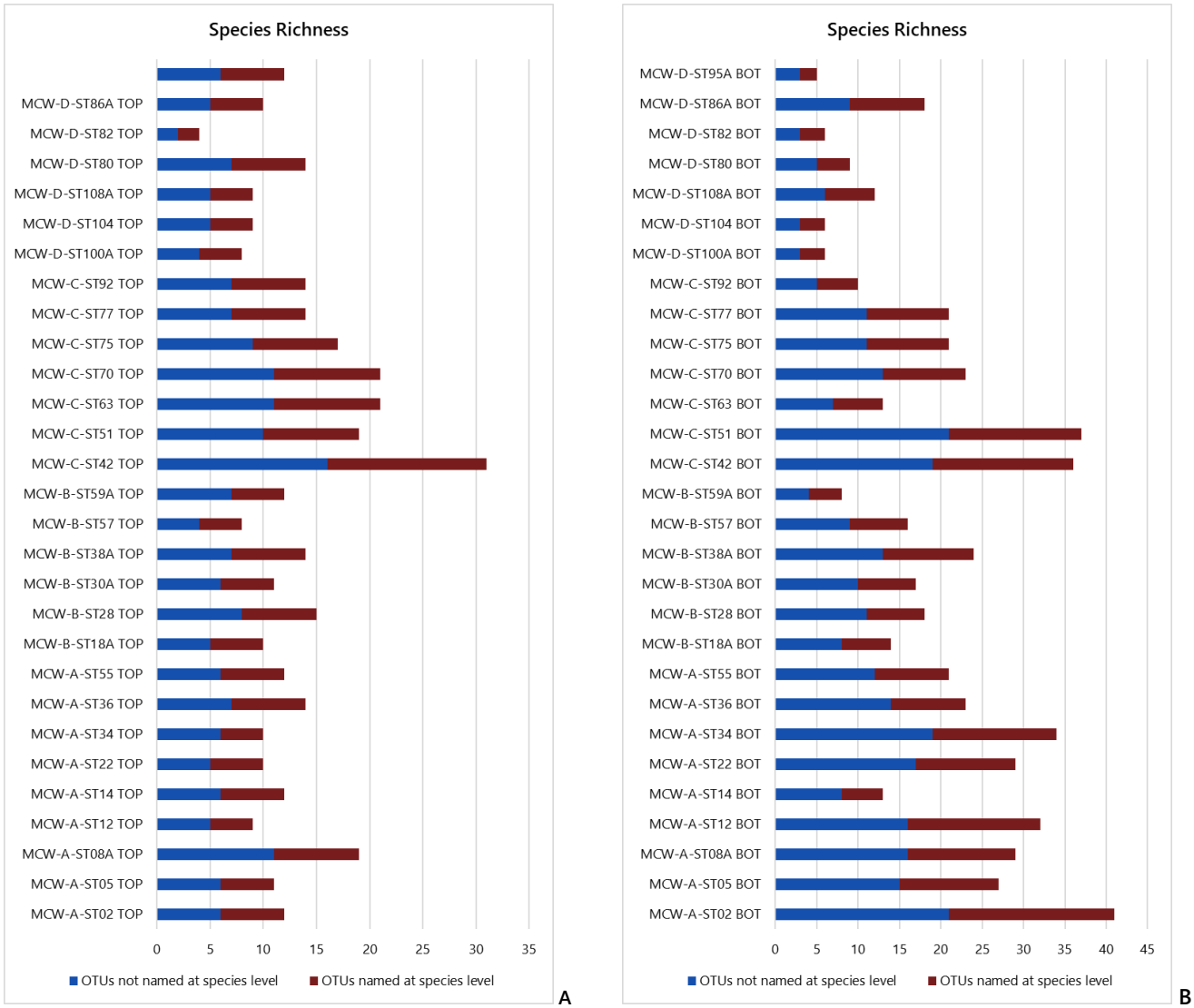
Notes

Non-target taxa were excluded from the plot OTU = Operation taxonomic unit TOP = Near surface water samples BOT = Near seafloor water samples

Figure 4.8: Bubble plot of community composition of invertebrate taxa detected and relative proportion of DNA sequences in eDNA samples TOP (A) and BOT (B)

Figure 4.9 presents the total count of OTUs detected in each sample for TOP (A) and BOT (B), represented as Species Richness. The blue portion of each bar indicates the number of OTUs identified to species level, whilst the red portion of each bar indicates the number of OTUs identified to a higher taxonomic level.

Species Richness is defined by the total number of OTUs detected in each sample (i.e. alpha diversity). Alpha diversity ranged from 2 (sample MCW-D-ST82 TOP) to 21 (samples MCW-A-ST02 BOT and MCW-C-ST51 BOT).



Notes

Non-target taxa were excluded from the plot

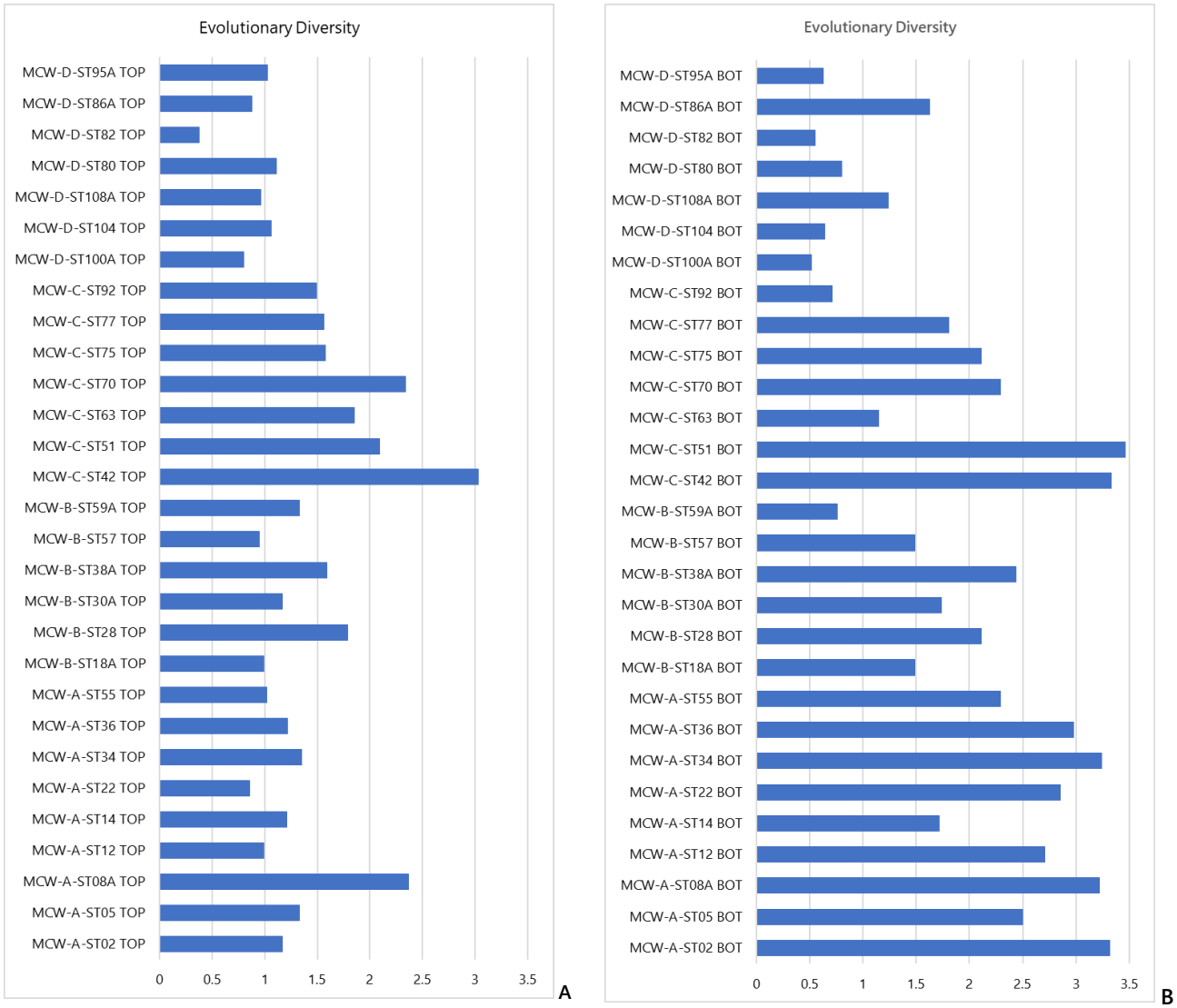
OTU = Operation taxonomic unit

TOP = Near surface water samples

BOT = Near seafloor water samples

Figure 4.9: Fish Species Richness detected in invertebrate eDNA samples TOP (A) and BOT (B)

Figure 4.10 displays the Evolutionary Diversity calculated for each water eDNA sample for TOP (A) and BOT (B). Evolutionary Diversity, a key metric in assessing biodiversity, measures the diversity of species detected and their genetic relationships. It compliments Species Richness, offering insight into ecosystem health and available ecological niches (Table 3.1). The Evolutionary Diversity ranged from 0.38 (sample MCW-D-ST82 TOP) to 3.46 (MCW-C-ST51 BOT).



Notes

Non-target taxa detected were excluded from the plot

OTU = Operation taxonomic unit

TOP = Near surface water samples

BOT = Near seafloor water samples

Figure 4.10: Evolutionary Diversity of invertebrates calculated for each eDNA sample TOP (A) and BOT (B)

4.2.3 Marine Water Vertebrates

High quality vertebrate data were successfully analysed from 51 of the 58 eDNA samples. However, in TOP samples MCW-A-ST55, MCWAST34, MCWCST75, MCW-B-ST18A and MCW-D-ST100A, and BOT samples MCW-C-ST75 and MCW-D-ST100A, there was insufficient target DNA in the sample to obtain positive identification and therefore no results are reported for these samples.

Table 4.4 provides the number of OTUs detected and the percentage of OTUs identified to each taxonomic level.

Table 4.4: Number of OTUs detected and the percentage of OTUs identified to each taxonomic level

Number of OTUs	Phylum [%]	Class [%]	Order [%]	Family [%]	Genus [%]	Species [%]
77	100.00	100.00	100.00	97.40	80.52	67.53

Notes
OTU = Operational Taxonomic Unit

Gamma diversity, calculated as the total number of OTUs from all samples taken (Table 3.1), was reported as 77 (Table 4.4). A total of 77 vertebrate taxa were detected and 67.5 % (52 taxa) were at least 98 % similar to a species in the global reference database. The remaining taxa were identified to genus (10 taxa; 13.0 %), family (13 taxa; 16.9 %) and order (2 taxa; 2.6 %) level (Table 2 within Appendix B.3).

Following the colour gradient, the Tree-of-Life (Table 3.1; Figure 4.11) indicates that the taxonomic composition of the vertebrate water column community within the MachairWind Offshore Wind Farm OAA was mainly formed by 57 fish taxa OTUs (Actinopterygii), 11 bird taxa OTUs (Aves) and 9 mammal taxa OTUs (Mammalia).

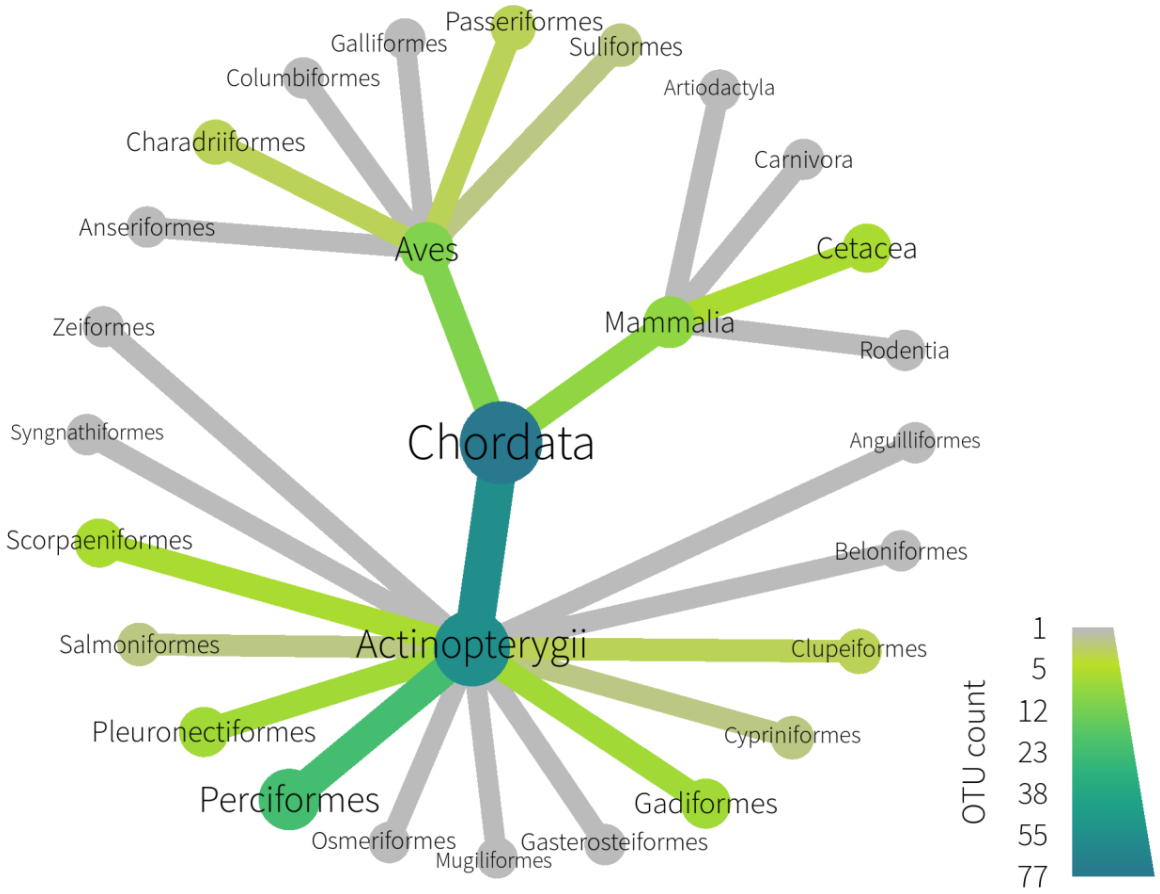
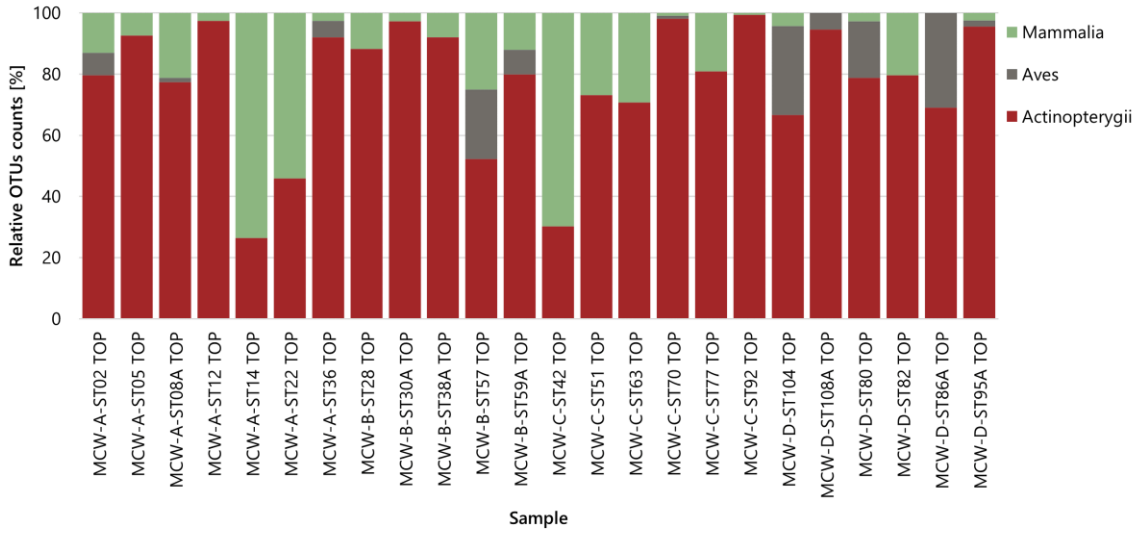


Figure 4.11: Taxonomic composition of eDNA vertebrate samples

Figure 4.12 presents the bar plots of the relative OTU counts of the vertebrate taxa detected by eDNA sampling for TOP (A) and BOT (B), rationalised to 'class' taxonomic level for TOP (A) and BOT (B) samples.

Taxa recorded in the TOP and BOT samples were mostly comparable, with a higher proportion of ray finned fish (Actinopterygii) in BOT samples and a higher proportion of both mammals (Mammalia), which included cetaceans (Cetacea), and birds (Aves) in TOP samples.



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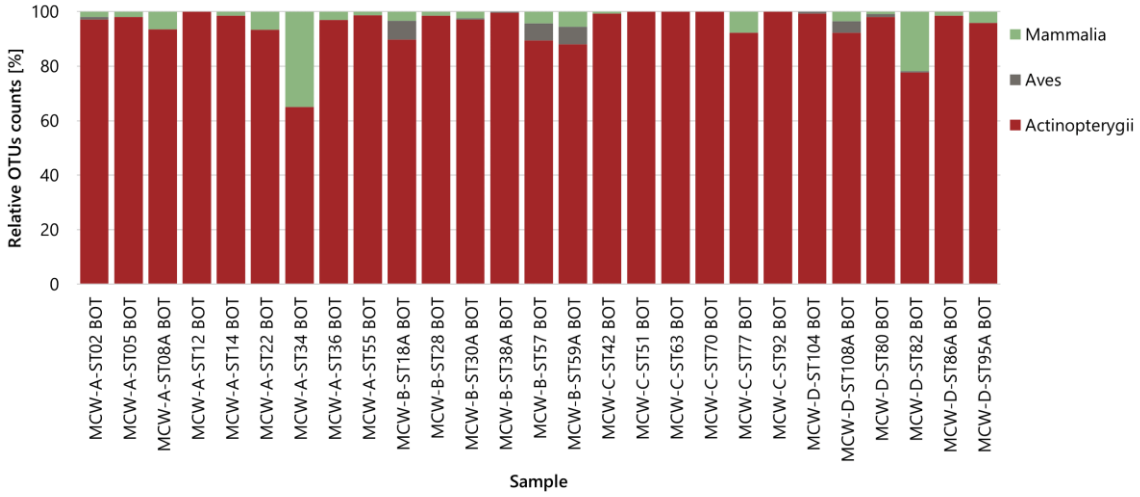


B

Notes
OTU = Operation taxonomic unit
TOP = Near surface water samples
BOT = Near seafloor water samples

Figure 4.12: Relative OTU counts of target vertebrate taxa detected to class level in TOP (A) and BOT (B)

Table 4.5 shows the taxonomic composition of the OTUs of vertebrata (subphylum Chordata).

Table 4.5: Taxonomy composition of Vertebrata OTU, phylum Chordata

Class	Order	Family	Genus	Species
Actinopterygii	Anguilliformes	Congridae	–	–
	Beloniformes	Scomberesocidae	–	–
	Clupeiformes	Engraulidae	*Engraulis*	*Engraulis encrasicolus*
		Clupeidae	*Sardina*	*Sardina pilchardus*
			–	–
	Cypriniformes	Cyprinidae	*Abramis*	*Abramis brama*
			Rutilus	*Rutilus rutilus*

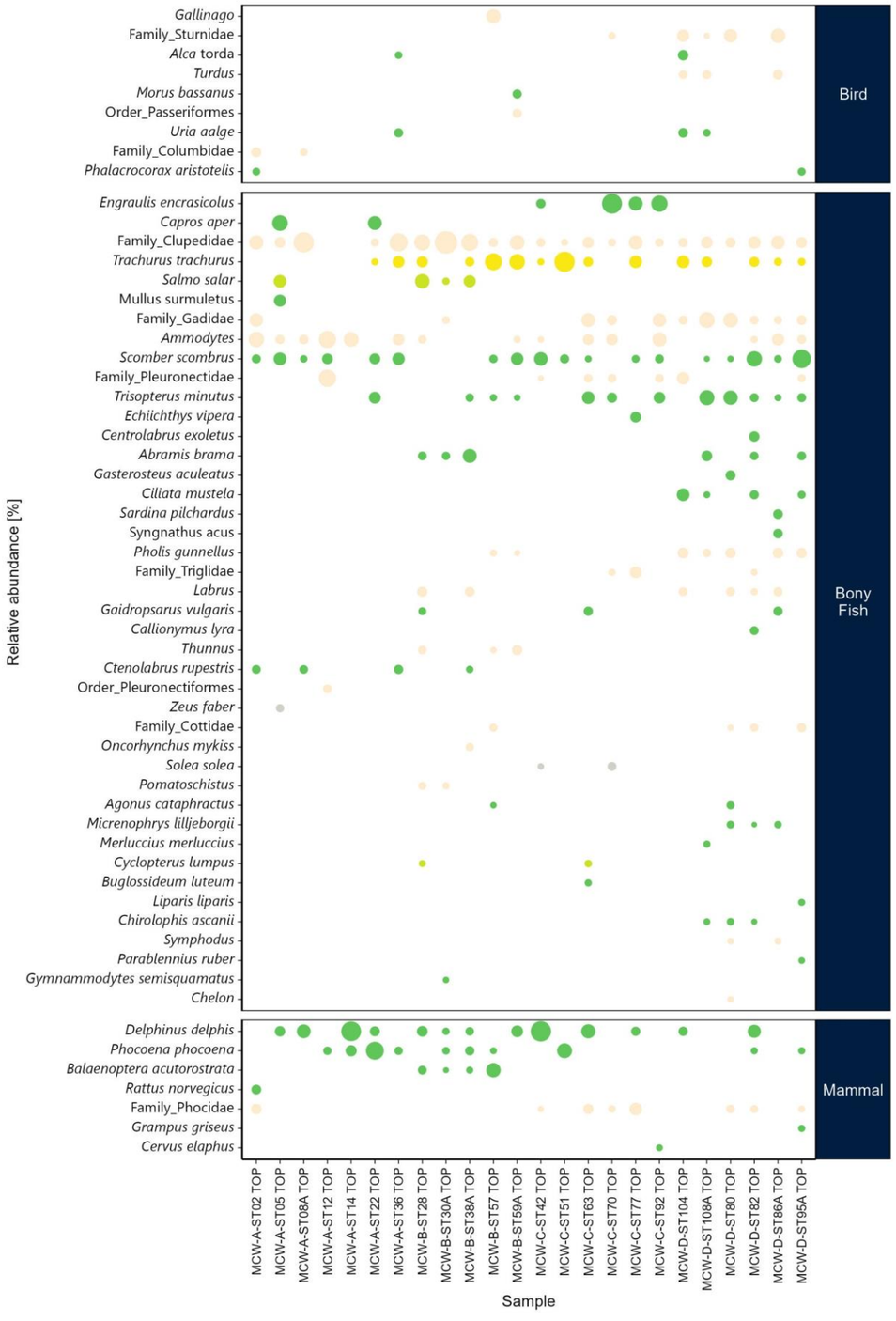
Class	Order	Family	Genus	Species
	Gadiformes	Gadidae	*Trisopterus*	*Trisopterus esmarkii*
				Trisopterus minutus
			–	–
		Lotidae	*Ciliata*	*Ciliata mustela*
			Gaidropsarus	*Gaidropsarus vulgaris*
			–	–
		Merlucciidae	*Merluccius*	*Merluccius merluccius*
	Gasterosteiformes	Gasterosteidae	*Gasterosteus*	*Gasterosteus aculeatus*
	Mugiliformes	Mugilidae	*Chelon*	–
	Osmeriformes	Argentinidae	*Argentina*	*Argentina sphyraena*
	Perciformes	Ammodytidae	*Ammodytes*	–
			Gymnammodytes	*Gymnammodytes semisquamatus*
			Hyperoplus	*Hyperoplus immaculatus*
		Blenniidae	*Parablennius*	*Parablennius ruber*
		Callionymidae	*Callionymus*	*Callionymus lyra*
				–
		Caproidae	*Capros*	*Capros aper*
		Carangidae	*Trachurus*	*Trachurus trachurus*
		Gobiidae	*Buenia*	–
			Crystallogobius	*Crystallogobius linearis*
			Gobiusculus	*Gobiusculus flavescens*
			Pomatoschistus	–
		Labridae	*Centrolabrus*	*Centrolabrus exoletus*
				Ctenolabrus rupestris
			Labrus	*Labrus mixtus*
				–
			Symphodus	–
		Mullidae	*Mullus*	*Mullus surmuletus*
		Pholidae	*Pholis*	*Pholis gunnellus*
		Scombridae	*Scomber*	*Scomber scombrus*
			Thunnus	–
		Stichaeidae	*Chirolophis*	*Chirolophis ascanii*
		Trachinidae	*Echiichthys*	*Echiichthys vipera*
	Pleuronectiformes	Bothidae	*Arnoglossus*	*Arnoglossus imperialis*
		Pleuronectidae	–	–
		Scophthalmidae	*Phrynorhombus*	*Phrynorhombus norvegicus*
			Zeugopterus	*Zeugopterus punctatus*

Class	Order	Family	Genus	Species
		Soleidae	*Buglossidium*	*Buglossidium luteum*
			Solea	*Solea solea*
		–	–	–
	Salmoniformes	Salmonidae	*Oncorhynchus*	*Oncorhynchus mykiss*
			Salmo	*Salmo salar*
	Scorpaeniformes	Agonidae	*Agonus*	*Agonus cataphractus*
		Cottidae	*Micrenophrys*	*Micrenophrys lilljeborgii*
			–	–
		Cyclopteridae	*Cyclopterus*	*Cyclopterus lumpus*
		Liparidae	*Liparis*	*Liparis liparis*
		Triglidae	–	–
	Syngnathiformes	Syngnathidae	*Syngnathus*	*Syngnathus acus*
	Zeiformes	Zeidae	*Zeus*	*Zeus faber*
Aves	Anseriformes	Anatidae	–	–
	Charadriiformes	Alcidae	*Alca*	*Alca torda*
			Uria	*Uria aalge*
		Scolopacidae	*Gallinago*	–
	Columbiformes	Columbidae	–	–
	Galliformes	Phasianidae	*Perdix*	*Perdix perdix*
	Passeriformes	Sturnidae	–	–
		Turdidae	*Turdus*	–
		–	–	–
	Suliformes	Phalacrocoracidae	*Phalacrocorax*	*Phalacrocorax aristotelis*
		Sulidae	*Morus*	*Morus bassanus*
Mammalia	Artiodactyla	Cervidae	*Cervus*	*Cervus elaphus*
	Carnivora	Phocidae	–	–
	Cetacea	Balaenopteridae	*Balaenoptera*	*Balaenoptera acutorostrata*
				Balaenoptera physalus
		Delphinidae	*Delphinus*	*Delphinus delphis*
			Grampus	*Grampus griseus*
			–	–
		Phocoenidae	*Phocoena*	*Phocoena phocoena*
	Rodentia	Muridae	*Rattus*	*Rattus norvegicus*

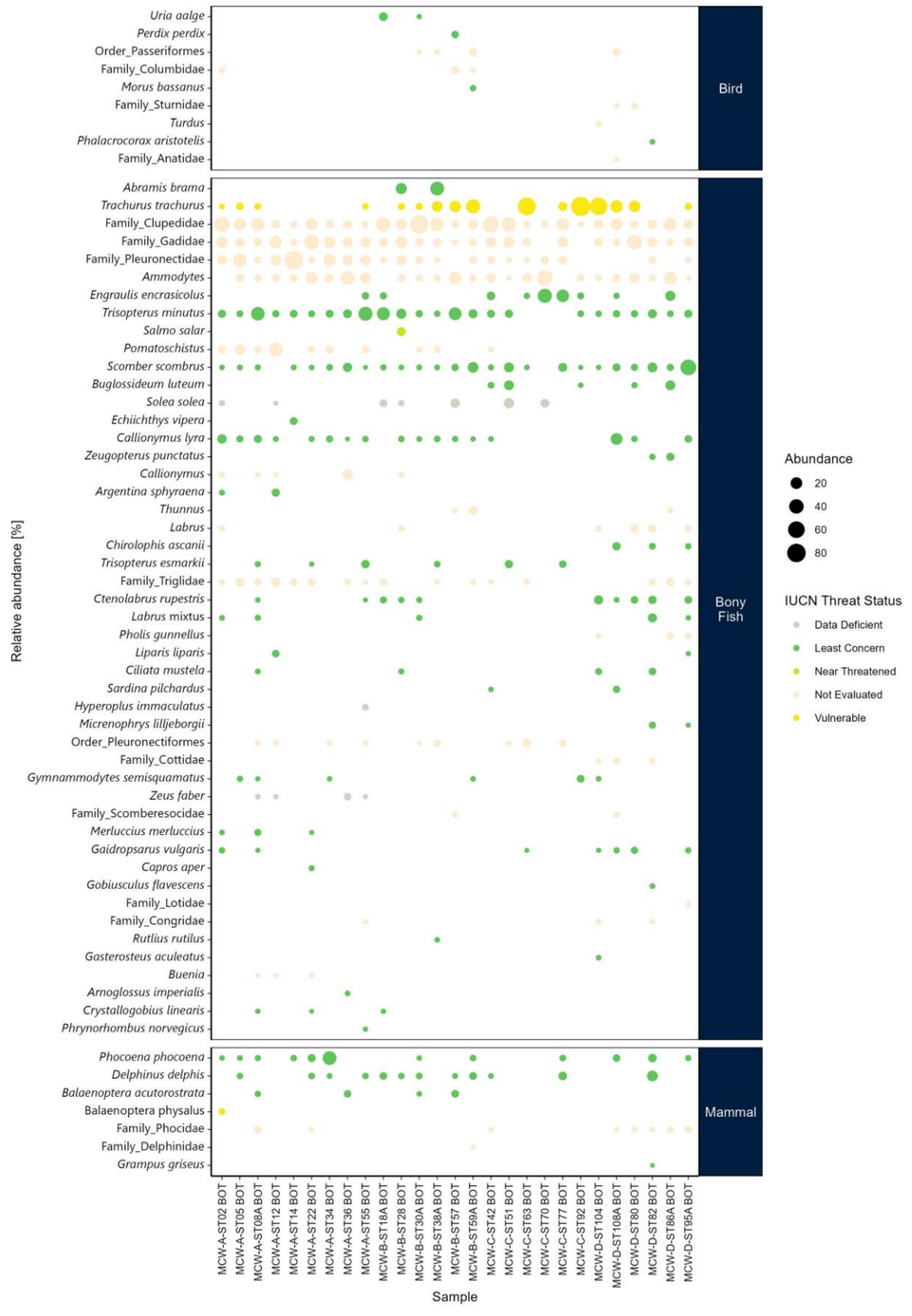
Figure 4.13 presents the community composition of vertebrate taxa recorded and relative proportion of DNA sequences within each sample for TOP (A) and BOT (B). Bubble size corresponds to the proportion of DNA within a sample, with larger bubbles indicating a more pronounced eDNA signal. The IUCN Red list assessment is also displayed. The taxon with the highest detection rate was the family of ray finned fishes (Clupeidae), which was recorded in 49 samples (96.1 %). Amongst the class Actinopterygii, other most commonly detected taxa included Atlantic mackerel (*Scomber scombrus*) (43 samples; 84.3 %), sandeels (*Ammodytes*) (39 samples; 76.5 %), poor cod (*Trisopterus minutus*) and cod (Gadidae) (both 36 samples; 70.6 %), Atlantic horse mackerel (*Trachurus trachurus*) (31 samples; 60.8 %), flatfish (Pleuronectidae) (28 samples; 54.9 %) and dragonet (*Callionymus lyra*) (18 samples; 35.3 %).

Within the class Aves, the most frequently detected taxa included common guillemot (*Uria aalge*) (5 samples; 9.8 %), European shag (*Phalacrocorax aristotelis*) (3 samples; 5.9 %), razorbill (*Alca torda*) and the northern gannet (*Morus bassanus*) (both 2 samples; 3.9 %).

Amongst the class Mammalia, detected taxa included common dolphin (*Delphinus delphis*) (25 samples; 49.0 %), harbour porpoise (*Phocoena phocoena*) (22 samples; 43.1 %), minke whale (*Balaenoptera acutorostrata*) (8 samples; 15.7 %), risso's dolphin (*Grampus griseus*) (2 samples; 3.9 %) and fin whale (*Balaenoptera physalus*) (1 sample; 2.0 %).



A



B

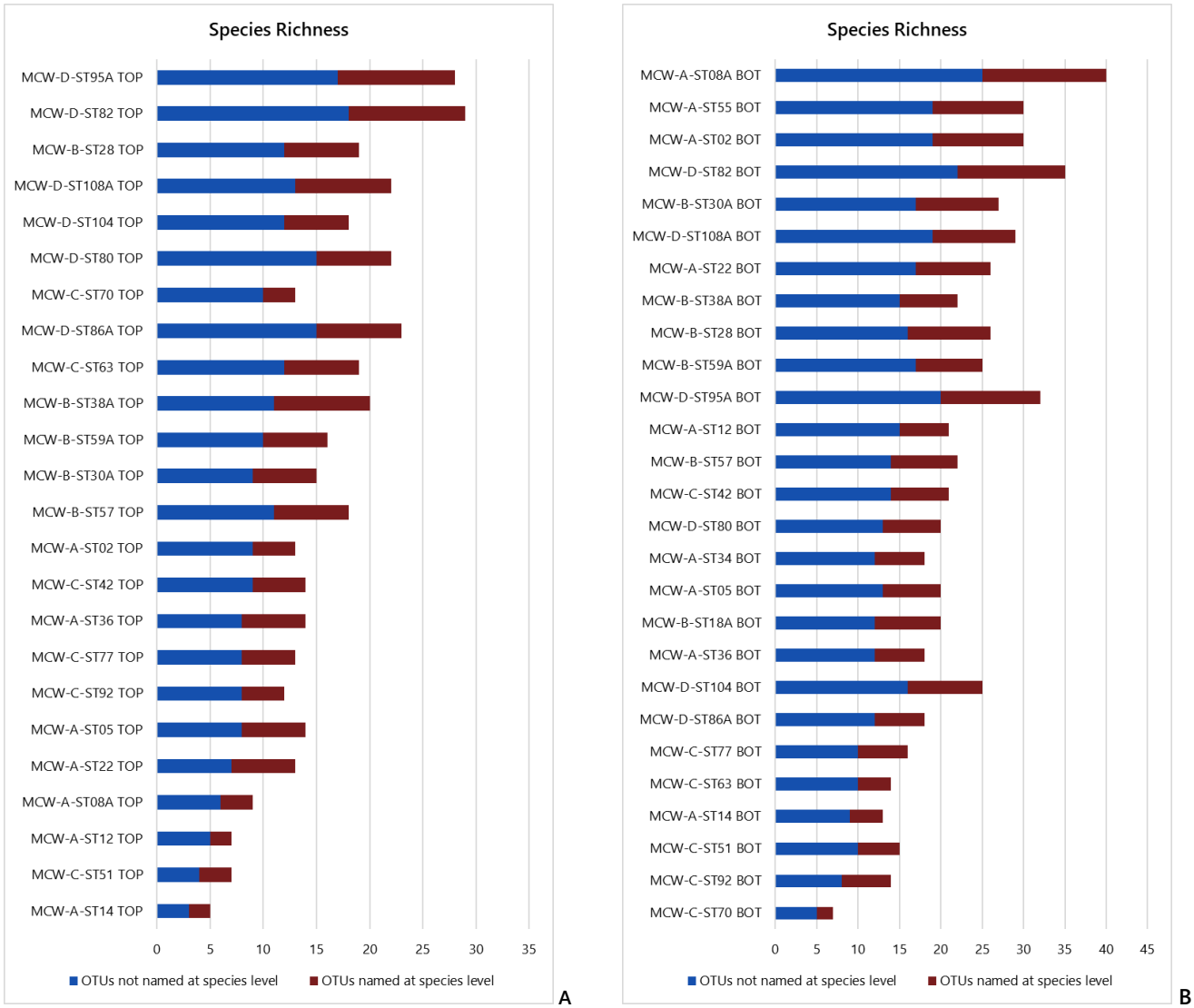
Notes

Non-target taxa were excluded from the plot OTU = Operation taxonomic unit TOP = Near surface water samples BOT = Near seafloor water samples

Figure 4.13: Bubble plot of community composition of vertebrate taxa detected and relative proportion of DNA sequences in eDNA samples TOP (A) and BOT (B)

Figure 4.14 presents the total count of OTUs detected in each sample, for TOP (A) and BOT (B), represented as Species Richness. The blue portion of each bar indicates the number of OTUs identified to species level, whilst the red portion of each bar indicates the number of OTUs identified to a higher taxonomic level.

Species Richness is defined by the total number of OTUs detected in each sample (i.e. alpha diversity). Alpha diversity ranged from 3 (sample MCW-A-ST14 TOP) to 25 (samples MCW-A-ST08A BOT).



Notes

Non-target taxa were excluded from the plot

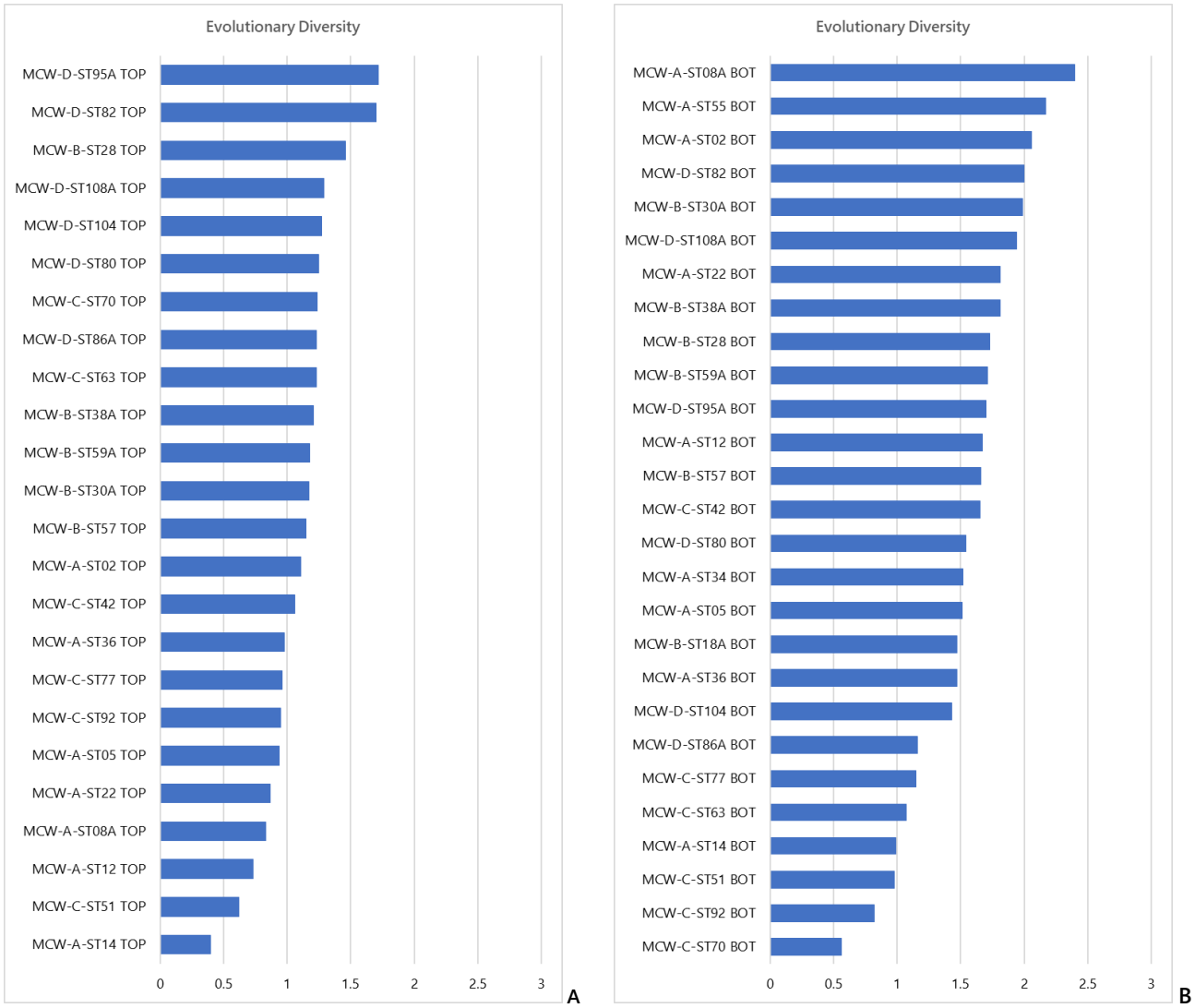
OTU = Operation taxonomic unit

TOP = Near surface water samples

BOT = Near seafloor water samples

Figure 4.14: Fish Species Richness detected in vertebrate eDNA samples TOP (A) and BOT (B)

Figure 4.15 displays the Evolutionary Diversity calculated for each water eDNA sample for TOP (A) and BOT (B). Evolutionary Diversity, a key metric in assessing biodiversity, measures the diversity of species detected and their genetic relationships. It compliments Species Richness, offering insight into ecosystem health and available ecological niches (Table 3.1). The Evolutionary Diversity ranged from 0.4 (sample MCW-A-ST14 TOP) to 2.4 (MCW-A-ST08A BOT).



Notes

Non-target taxa detected were excluded from the plot

OTU = Operation taxonomic unit

TOP = Near surface water samples

BOT = Near seafloor water samples

Figure 4.15: Evolutionary Diversity of vertebrates calculated for each eDNA sample TOP (A) and BOT (B)

4.2.4 eDNA Comparative Analysis: Fish vs. Vertebrate data

As the eDNA analysis targeting vertebrates also target fish taxa (Actinopterygii), a Venn Diagram was used to investigate the proportion of overlapping fish taxa, detected by both the vertebrate and fish eDNA analyses, which is shown at the intersection of the Venn Diagram circles. Figure 4.16 illustrates the overlap between fish and vertebrate taxa identified in each eDNA analysis. Taxa were grouped to genera or higher taxonomic level for comparability.

Taxa detected by the eDNA vertebrate analysis was 73, 54 of which were fish taxa. The total number of fish taxa identified by fish eDNA analysis was 55. The overall number of fish taxa identified for the survey area was 77, with 32 taxa (41.6 %) being identified by both methods and a further 23 taxa (29.9 %) being identified by the eDNA fish analysis and a further 22 (28.6 %) taxa by the eDNA vertebrate analysis.

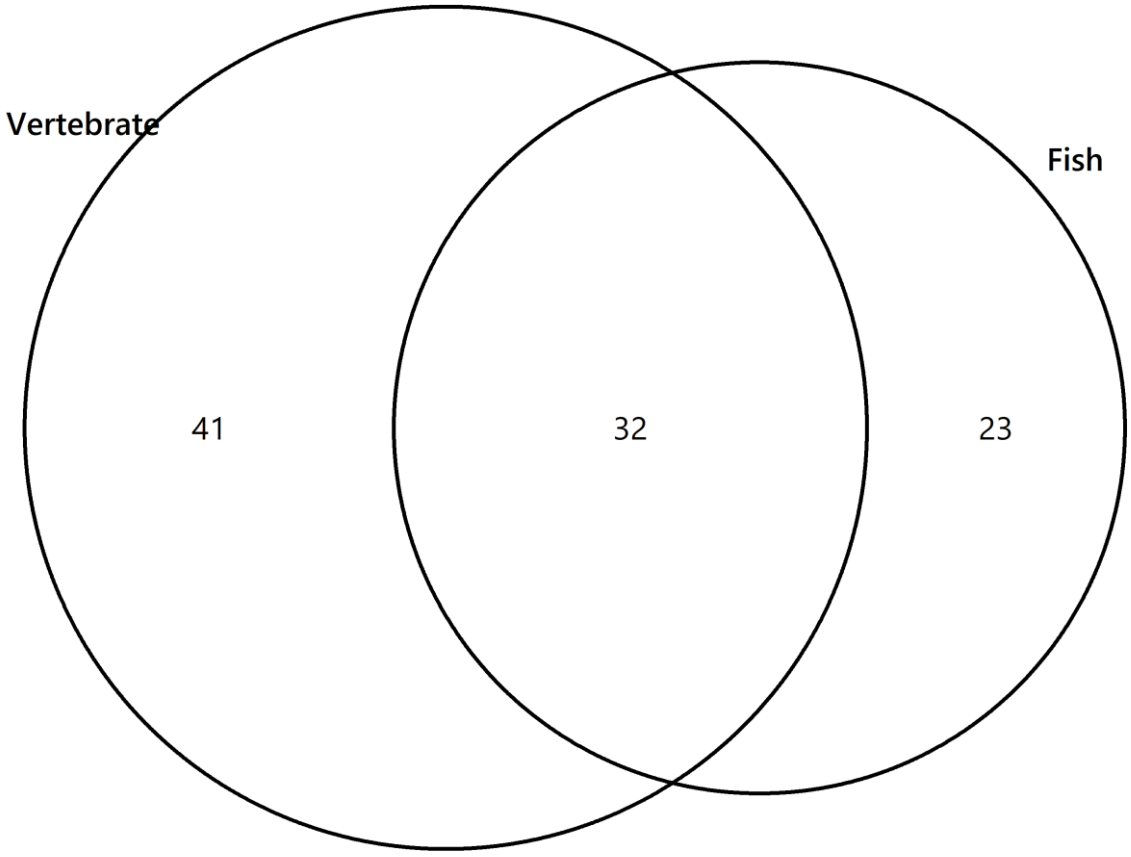


Figure 4.16: Venn diagram comparing fish and vertebrate genera or higher taxonomic level identified by eDNA data analysis across the survey area

4.2.5 Species of Conservation Importance

Table 4.6 lists the species of conservation importance detected in the eDNA water samples. Atlantic horse mackerel (*T. trachurus*), haddock (*Melanogrammus aeglefinus*), Atlantic cod (*Gadus morhua*) and fin whale (*B. physalus*) are assessed as 'Vulnerable' in the IUCN Red List (IUCN, 2023). The remaining taxa detected were assessed as 'Least Concern' or 'Data Deficient' on the IUCN Red List. These species (excluding *M. aeglefinus*) are also listed under the Scottish PMF list, along with Atlantic herring (*C. harengus*), Atlantic mackerel (*S. scombrus*), Atlantic salmon (*Salmo salar*), ling (*Molva molva*), Norway pout (*Trisopterus esmarkii*), saithe (*Pollachius virens*), sand goby (*Pomatoschistus minutus*), whiting (*Merlangius merlangus*), ocean quahog (*Arctica islandica*), harbour porpoise (*P. phocoena*), minke whale (*B. acutorostrata*) common dolphin (*D. delphis*) and risso's dolphin (*Grampus griseus*).

Atlantic cod (*G. morhua*), Atlantic salmon (*S. salar*), ocean quahog (*A. islandica*) and harbour porpoise (*P. phocoena*) are also included on the OSPAR list of threatened and/or declining habitats and species (Table 4.6; OSPAR, 2008).

Atlantic cod (*G. morhua*), Atlantic herring (*C. harengus*), Atlantic horse mackerel (*T. trachurus*), Atlantic mackerel (*S. scombrus*), Atlantic salmon (*S. salar*), common sole (*Solea solea*), ling (*M. molva*), Norway pout (*T. esmarkii*), whiting (*M. merlangus*), common dolphin (*D. delphis*), fin whale (*B. physalus*), harbour porpoise (*P. phocoena*), minke whale (*B. acutorostrata*) and risso's dolphin (*G. griseus*) are all included in the Scottish Biodiversity List (Table 4.6; NatScot, 2020).

Table 4.6: Species of conservation importance recorded within the eDNA water samples

Detected species	Conservation Status
Fish (excl. sharks and rays)	
Common sole (*Solea solea*)*	Scottish Biodiversity List
Saithe (*Pollachius virens*)	Scottish PMF
Sand goby (*Pomatoschistus minutus*)	Scottish PMF
Whiting (*Merlangius merlangus*)	Scottish PMF Scottish Biodiversity List
Atlantic cod (*Gadus morhua*)	Scottish PMF IUCN Red List designated as 'Vulnerable' in the UK OSPAR list of threatened and/or declining habitats and species Scottish Biodiversity List
Atlantic herring (*Clupea harengus*)	Scottish PMF – the focus is on juveniles and spawning adults Scottish Biodiversity List
Atlantic horse mackerel (*Trachurus trachurus*)	Scottish PMF IUCN Red List designated as 'Vulnerable' in the UK Scottish Biodiversity List

Detected species	Conservation Status
Atlantic mackerel (*Scomber scombrus*)	Scottish PMF Scottish Biodiversity List
Haddock (*Melanogrammus aeglefinus*)	IUCN Red List designated as 'Vulnerable' in the UK
Ling (*Molva molva*)	Scottish PMF Scottish Biodiversity List
Norway pout (*Trisopterus esmarkii*)	Scottish PMF Scottish Biodiversity List
Atlantic salmon (*Salmo salar*)	Scottish PMF OSPAR list of threatened and/or declining habitats and species Scottish Biodiversity List
Invertebrates	
Ocean quahog (*Arctica islandica*)	Scottish PMF OSPAR list of threatened and/or declining habitats and species
Vertebrates - Marine Mammals	
Common dolphin (*Delphinus delphis*)	Scottish PMF Scottish Biodiversity List
Fin whale (*Balaenoptera physalus*)	Scottish PMF IUCN Red List designated as 'Vulnerable' in the UK Scottish Biodiversity List
Risso's dolphin (*Grampus griseus*)	Scottish PMF Scottish Biodiversity List
Harbour porpoise (*Phocoena phocoena*)	Scottish PMF OSPAR list of threatened and/or declining habitats and species Scottish Biodiversity List
Minke whale (*Balaenoptera acutorostrata*)	Scottish PMF Scottish Biodiversity List
Notes IUCN = International Union for the Conservation of Nature, PMF = Priority Marine Feature OSPAR = Oslo and Paris Commission * = Detected in vertebrate analysis	

No other species listed as 'Near Threatened' to 'Critically Endangered' under the IUCN Red List, OSPAR threatened and/or declining habitats and species list, Scottish PMF list, or Scottish Biodiversity list were detected within the survey area.

4.2.6 Non-Indigenous Species

The eDNA analysis detected the presence of the non-indigenous rainbow trout (*Oncorhynchus mykiss*) in TOP samples MCW-B-ST28 and MCWBST38A and BOT sample MCW-B-ST30A (Block B).

5. Discussion

5.1 Water Column eDNA

Environmental DNA comprises DNA fragments shed from any living form into the environment, including the water environment. eDNA in water can be sampled by filtration and it can be analysed to determine the taxa composition of water body at the time of sampling. For the current survey, eDNA sampling was used to determine the fish, invertebrate and vertebrate composition in the pelagic habitat within the survey area.

5.1.1 Marine Water Fish

Both fish and vertebrate eDNA analyses had fish taxa as target. The eDNA amplification methods employ different primers to target different components of the marine communities, potentially causing an overlap in detected taxa. The fish analysis identified 55 taxa, whilst the number of fish taxa identified by the vertebrate data analysis was 54. The Venn Diagram (Figure 4.16) showed the fish eDNA analysis detected a comparable number of taxa to the fish taxa identified in the vertebrate eDNA analysis, with almost half of the overall fish taxa detected by both methods. Fish taxa detected by the vertebrate eDNA analysis included 17 genera and 5 families, with the most commonly detected taxa including Clupeidae, *Ammodytes* sp., Pleuronectidae, *Gaidropsarus* sp., Cottidae and *Gymnammodytes* sp. The fish eDNA analysis detected a further 21 genera and 2 families, with the most commonly detected taxa being *Clupea* sp., *Sprattus* sp., Ammodytidae, *Merlangius* sp., *Limanda* sp., *Microchirus* sp., and *Pleuronectes* sp. The two methods used to detect bony fish populations complemented each other, providing a more comprehensive view of the fish community.

Overall, the most commonly detected fish taxa included the Atlantic mackerel (*S. scombrus*). The genus *Scomber* sp., which includes *S. scombrus,* was also detected by the video analysis of the habitat survey throughout the survey area (Fugro, 2024). Mackerel is one of the most abundant, widespread and commercially important fish stocks in the North Atlantic (ICES, 2011). Despite the known migration towards the North of Scotland for spawning in July, a large proportion of stock reside on the west coast of Scotland and may use areas in the vicinity of the survey area as spawning or nursery grounds (Jansen and Gislason, 2013; Ellis et al., 2012). The diet of mackerel can vary depending on the region and time of year, with crustaceans (shrimps), sandeels, herring and Norway pout comprising the majority of their food sources (Barreto and Bailey, 2014). Shrimps (Caridea) were recorded in the habitat survey (Fugro, 2024), while herring (*C. harengus*) and sandeels (*Ammodytes* sp.) were detected in the eDNA water samples, indicating suitable food availability for this species within the survey area.

Other mostly detected fish taxa within the survey area included sprat (*S. sprattus*), horse mackerel (*T. trachurus*), herring (*C. harengus*) and sandeels (*Ammodytes* sp.). Herring (*C. harengus*) is widely distributed and spawns in late August off the west and north of the Outer Hebrides (Barreto and Bailey, 2014). Sprat (*S. sprattus*) also occurs off the west coast of

Scotland and in nearby estuaries (Lawrence and Fernandes, 2021; De Silva, 1973). These species, along with horse mackerel (*T. trachurus*), primarily feed on crustaceans such as shrimps and copepods (Möllmann et al., 2004; Macer, 1977). Herring (*C. haregnus*) also includes sandeels (Ammodytidae) in its diet (Barreto and Bailey, 2014). As discussed in section 5.1.2, copepods were prevalent within the survey area, with shrimps (Caridea) and sandeels also present (Fugro, 2024), suggesting that the survey area may be visited by these species for feeding.

Overall results indicated mostly comparable eDNA taxa composition within the TOP and BOT samples. As expected, the proportion of OTU counts of bottom-dweller taxa (e.g. Pleuronectiformes), was higher in the BOT samples.

5.1.2 Marine Water Invertebrates

Copepoda was the most common invertebrate taxon throughout the survey area, with *P. parvus* being the most commonly detected species, followed by *D. anglicus, C. jobei, P. elongatus* and *O. similis*. Copepod crustaceans are widely distributed and generally dominant within the aquatic zooplankton communities globally. Copepods are generally considered herbivorous, foraging phytoplankton (principally diatoms) in the water column, although many species are omnivorous (supplementing their diet of phytoplankton with microzooplankton, eggs and larvae), with some more nutritionally reliant on animal than plant food sources (Kleppel, 1993).

Other taxa commonly detected within the invertebrate community included polychaetes of the order Spionida and cnidarians of the family Campanulariidae. Taxa detected in the eDNA samples, and also observed during the photographic data analysis (Fugro, 2024), included the brittle stars *O. filiformis, O. fragilis*, the bivalve *A. islandica,* and the polychaete *S. spinulosa*.

5.1.3 Marine Water Vertebrates (Birds and Mammals)

A total of 11 bird taxa OTUs (Aves) and 9 mammal taxa OTUs (Mammalia) were identified in the vertebrate analysis. As expected, birds (Aves) and mammals (Mammalia) had a higher proportion of relative OTU counts in the TOP samples. The most commonly detected vertebrates included taxa from the class Mammalia, with the common dolphin (*Delphinus delphis*) identified in the largest number of samples throughout the survey area. This species is abundant and widespread throughout north-east Atlantic and densities in the west coast of Scotland generally increases in autumn, corresponding to the time of year at which this survey was conducted. Such aggregations have been reported to be related to the distribution of their preferred prey species such as sardine (*Sardina pilchardus*), mackerel (*S. scombrus*), horse mackerel (*T. trachurus*) and Norway pout (*T. esmarkii*) (Murphy et al., 2013). These fish species were detected in the eDNA water samples across the survey area, suggesting *D. delphis* are using areas in the vicinity of the survey area as feeding grounds.

Other most commonly detected mammals included harbour porpoise (*P. phocoena*), minke whale (*B. acutorostrata*), risso's dolphin (*G.* griseus), fin whale (*B.* physalus), seals of the family Phocidae and dolphins of the family Delphinidae. These taxa are frequently found in the waters around the west coast of Scotland, serving as year-round feeding and breeding

grounds, as well as a migration route for large whales (Weir et al., 2019; Dolman et al., 2013; Santos et al., 2004; Macleod et al., 2003).

Amongst the birds (Aves) identified, the most frequently detected taxa included common guillemot (*U. aalge*), European shag (*P. aristotelis*), razorbill (*A. torda*) and the northern gannet (*M. bassanus*). These taxa are common in inshore waters off the west coast of Scotland and use the area as breeding and feeding grounds (Reid et al., 2001; Halley et al., 1995).

5.1.4 Species of Conservation Interest

Four species detected following eDNA analysis and assessed as 'Vulnerable' in the UK under the IUCN Red List, included Atlantic horse mackerel (*T. trachurus*), haddock (*M. aeglefinus*), Atlantic cod (*G. morhua*) and fin whale (*B. physalus*). With the exception of haddock (*M. aeglefinus*), these species, together with Atlantic herring (*C.* harengus), Atlantic mackerel (*S. scombrus*), Atlantic salmon (*S. salar*), ling (*M.* molva), Norway pout (*T. esmarkii*), saithe (*P. virens*), sand goby (*P. minutus*), whiting (*M. merlangus*), ocean quahog (*A. islandica*), harbour porpoise (*P. phocoena*), minke whale (*B. acutorostrata*) and risso's dolphin (*Grampus griseus*) are listed as PMFs.

Species listed under the OSPAR threatened and/or declining species list included Atlantic salmon (*S. salar*) (for regions I, II, III and IV), Atlantic cod (*G. morhua*) (for regions II and III), ocean quahog (*A. islandica*) (for regions II and III) and harbour porpoise (*P. phocoena*) (for regions I, II, III, IV, and V) (OSPAR, 2008). The survey area is located within OSPAR region III. Ocean quahog (*A. islandica*) was detected predominantly in BOT eDNA water samples, with adult and juveniles of this species also recorded in the grab data and recorded by the photographic data analysis.

Atlantic cod (*G. morhua*), Atlantic herring (*C. harengus*), Atlantic horse mackerel (*T. trachurus*), Atlantic mackerel (*S. scombrus*), Atlantic salmon (*S. salar*), common sole (*Solea solea*), ling (*M. molva*), Norway pout (*T. esmarkii*), whiting (*M. merlangus*), common dolphin (*D. delphis*), fin whale (*B. physalus*), harbour porpoise (*P. phocoena*), minke whale (*B. acutorostrata*) and risso's dolphin (*G. griseus*) are all included in the Scottish Biodiversity list (NatScot, 2020).

No other species under the IUCN Red List, OSPAR threatened and/or declining species and habitats list, Scottish PMFs, or Scottish Biodiversity List were observed within the survey areas.

5.1.5 Non-Indigenous Species

The non-indigenous species rainbow trout (*O. mykiss*) was detected within the survey area. The species is considered an invasive species in Europe under the GRIIS list. Native to western North America, this species is known to have spread naturally in European waters after their introduction in western Europe and accidental escapes from aquaculture and deliberate releases for recreational fishing. However, the establishment of self-sustaining populations of *O. mykiss* has been limited, with only 130 confirmed or potential self-sustaining populations being recorded across 16 European countries (Stanković et al., 2015). Although *O. mykiss* can be found throughout the UK, they have generally failed to establish reproducing populations in Scotland (Stanković et al., 2015).

6. Conclusions

The aim of this report has been to evaluate the existing biological components in the water column within the survey area. Based on the overall assessment of the survey area, the following key conclusions can be stated:

- The eDNA analysis of fish taxa detected a fish community typical of the area including the Atlantic mackerel (*S. scombrus*), the European sprat (*S. sprattus*), Atlantic herring (*C. harengus*), Atlantic horse mackerel (*T. trachurus*) and sandeels (Ammodytidae);
- The eDNA analysis of invertebrate taxa detected an invertebrate community typical of the area, with the main taxa reported being copepods;
- The eDNA analysis of the vertebrate taxa detected marine mammals, birds and fish typical of the area, with most frequently detected taxa identified as the family of ray finned fishes (Clupeidae), mackerel (*S. scombrus*) and common dolphin (*D. delphis*);
- Fish taxa identified in the vertebrate analysis included an additional 17 genera and 5 families in comparison to the fish analyses, with the most commonly detected being Clupeidae, *Ammodytes* sp., Pleuronectidae, *Gaidropsarus* sp., Cottidae and *Gymnammodytes* sp.
- The OSPAR threatened and/or declining species Atlantic salmon (*S. salar*), Atlantic cod (*G. morhua*), ocean quahog (*A. islandica*) and harbour porpoise (*P. phocoena*) were detected within the survey area;
- Of the fish taxa detected within the survey area the Atlantic horse mackerel (*T. trachurus*), haddock (*M. aeglefinus*) and Atlantic cod (*G. morhua*) are assessed as 'Vulnerable' under the IUCN Red List in the UK;
- Sixteen species detected are listed under the Scottish PMF list (*G. morhua, C. harengus, T. trachurus, S. scombrus, S. salar, M. molva, T. esmarkii, P. virens, P. minutus* and *M. merlangus*, *B. physalus, A. islandica, P. phocoena*, *B. acutorostrata, G. griseus and D. delphis*;
- Fourteen species detected are listed under the Scottish Biodiversity List (*G. morhua*, *C. harengus*, *T. trachurus*, *S. scombrus*, *S. salar*, *S. solea*, *M. molva*, *T. esmarkii*, *M. merlangus*, *D. delphis*, *B. physalus*, *P. phocoena*, *B. acutorostrata* and *G. griseus*);
- The non-indigenous rainbow trout (*O. mykiss*) was identified within the survey area. This species is known to have spread naturally in European waters after their introduction in NW Europe and accidental escapes from aquaculture and deliberate releases for recreational fishing.

Overall, the taxonomic communities identified were common for the survey area.

7. Limitations of the Method

The eDNA-based techniques have a great potential for deriving biodiversity information and complementing traditional sampling methods. They are a promising tool particularly with regards to marine biosecurity programmes worldwide. The qualitative information provided by this technique can be effectively used for identifying new arrivals at different temporal and geographic scales (Danziger et al., 2022), guiding possible interventions.

Due to the current reference databases available to match the genetic sequences, taxa identified at species level often are caveated by '*There is lower support for this taxonomic identification as it is based on fewer than three matches to sequences in the reference database, and/or limited geographic occurrence records for the taxon*'. This affects the taxonomic resolution that can be used with confidence during data analysis, such as comparisons.

A limitation of eDNA techniques in general is that they usually cannot be effectively related to measures of abundance and/or biomass information, but only relative proportions of taxa in the community can be inferred from the percentage of sequence reads obtained through metabarcoding (Zaiko et al., 2018). Moreover, the eDNA signal can be impacted by biological (e.g., biomass, life stage, activity, body condition), environmental (e.g. temperature, pH, salinity, conductivity), and technical factors (e.g. primer bias, PCR stochasticity) (NatureMetrics, 2024) as shown by some studies (e.g. Danziger et al., 2022).

All tests are compared against the GBIF taxonomy for taxa nomenclature and currently a match with the WoRMS (2024) database taxonomy is not provided.

Metrics are provided, including all target taxa. For the Vertebrate group, this means that taxa which are not exclusively part of the marine communities are considered when calculating the metrics. This needs to be considered for future comparative studies.

8. References

16S Metagenomic Sequencing Library Preparation. (n.d.). Illumina. https://support.illumina.com/documents/documentation/chemistry_documentation/16s/16s-metagenomic-library-prep-guide-15044223-b.pdf

Altschul, S.F., Gish, W., Miller, W., Myers, E.W., & Lipman, D.J. (1990). Basic local alignment search tool. *Journal of Molecular Biology*, *215*(3), 403–410. https://www.biostat.wisc.edu/bmi576/papers/blast.pdf

Barreto, E., & Bailey, N. (2014). *Fish and shellfish stocks 2014 edition*. https://www.gov.scot/binaries/content/documents/govscot/publications/statistics/2014/09/revised-editon-fish-shellfish-stocks-2014-edition/documents/00458803-pdf/00458803-pdf/govscot%3Adocument/00458803.pdf

Calvignac-Spencer, S., Merkel, K., Kutzner, N., Kühl, H., Boesch, C., Kappeler, P.M., Metzger, S., Schubert, G., & Leendertz, F.H. (2013). Carrion fly-derived DNA as a tool for comprehensive and cost-effective assessment of mammalian biodiversity. *Molecular ecology*, *22*(4), 915-924. https://api.semanticscholar.org/CorpusID:206179613

Camacho, C., Coulouris, G., Avagyan, V., Ma, N., Papadopoulos, J. S., Bealer, K., & Madden, T. (2009). BLAST+: architecture and applications. *BMC Bioinformatics, 10*(1). https://doi.org/10.1186/1471-2105-10-421

Chamberlain, S., Barve, V., Mcglinn, D., Oldoni, D., Desmet, P., Geffert, L., & Ram, K. (2023). rgbif: Interface to the Global Biodiversity Information Facility API. https://CRAN.R-project.org/package=rgbif

Danziger, A. M., Olson, Z. H., & Frederich, M. (2022). Limitations of eDNA analysis for *Carcinus maenas* abundance estimations. *BMC Ecology and Evolution*, *22*(1). https://doi.org/10.1186/s12862-022-01969-z

De Silva, S. (1973). Aspects of the reproductive biology of the sprat, Sprattus sprattus (L.) in inshore waters of the west coast of Scotland. *Journal of Fish Biology*, *5*(6), 689–705. https://doi.org/10.1111/j.1095-8649.1973.tb04505.x

Dolman, S. J., Hodgins, N. K., MacLeod, C. D., Pierce, G. J., & Weir, C. R. (2013). Harbour porpoises (*Phocoena phocoena*) and minke whales (*Balaenoptera acutorostrata*) observed during land-based surveys in The Minch, north-west Scotland. *Journal of the Marine Biological Association of the United Kingdom, 94*(6), 1185–1194. https://doi.org/10.1017/s0025315413000507

Edgar, R.C. (2010). Search and clustering orders of magnitude faster than BLAST. *Bioinformatics*, *26*, 2460–2461. https://doi.org/10.1093/bioinformatics/btq461

Edgar, R.C. (2016). *UNOISE2: improved error-correction for Illumina 16S and ITS amplicon sequencing*. bioRxiv. https://doi.org/10.1101/081257

Ellis, J.R., Milligan, S.P., Readdy, L., Taylor, N. & Brown, M.J. (2012). *Spawning and nursery grounds of selected fish species in UK waters* (Report No. 147). Sci. Ser. Tech. Rep., Cefas.

Fugro. (2024a). *Phase 1 Geophysical and environmental survey - Geophysical results and habitat assessment site survey report* (Fugro report No. 230633-MachairWind-V3). Fugro GB Limited.

Fugro. (2024b). *MachairWind Phase 1 Geophysical and Environmental Survey. report* (Fugro report No. 230633-MachairWind-V5). Fugro GB Limited.

GRIIS. (2006). *Global Register of Introduced and Invasive Species*. https://griis.org/download

Jansen, T., & Gislason, H. (2013). Population Structure of Atlantic Mackerel (*Scomber scombrus*). *PLOS ONE*, *8*(5), e64744. https://doi.org/10.1371/journal.pone.0064744

Joint Nature Conservation Committee [JNCC] (2014). *Priority Marine Features in Scotland's seas.* https://hub.jncc.gov.uk/assets/151356a8-06cc-43fc-b724-c8212089a2da

Halley, D. J., Harrison, N., Webb, A., & Thompson, D. R. (1995). Seasonal and geographical variations in the diet of common guillemots *Uria aalge* off a western Scotland. In *Seabird*, *17*, 12–20. http://www.researchgate.net/publication/287490220

International Council for the Exploration of the Sea [ICES]. (2011). *Report of the Working Group on Widely Distributed Stocks (WGWIDE)*. (ICES CM 2011/ACOM: 15). ICES.

illumina (2024). *16S Metagenomic Sequencing Library Preparation*. https://support.illumina.com/documents/documentation/chemistry_documentation/16s/16s-metagenomic-library-prep-guide-15044223-b.pdf

International Union for Conservation of Nature [IUCN]. (2023). *The IUCN Red List of Threatened Species*. Version 2023-1. https://www.iucnredlist.org. Accessed on 12 February 2024.

Joyce, C. (2008). *Venn diagrams*. https://arbs.nzcer.org.nz/venn-diagrams

Kelly, R.P., Port, J.A., Yamahara, K.M., & Crowder, L.B. (2014). Using environmental DNA to census marine fishes in a large mesocosm. *PLOS ONE*, *9*(1), p.e86175. https://doi.org/10.1371/journal.pone.0086175

Kleppel, G. S. (1993). On the diets of calanoid copepods. *Marine Ecology Progress Series, 99*, 183–195. https://doi.org/10.3354/meps099183

Lawrence, J. M., & Fernandes, P. G. (2021). A switch in species dominance of a recovering pelagic ecosystem. *Current Biology*, *31*(19), 4354-4360.e3. https://doi.org/10.1016/j.cub.2021.07.0200

Leray, M., Yang, J.Y., Meyer, C.P., Mills, S.C., Agudelo, N., Ranwez, V., Boehm, J.T., & Machida, R.J. (2013). A new versatile primer set targeting a short fragment of the mitochondrial COI region for metabarcoding metazoan diversity: application for characterizing coral reef fish gut contents. *Frontiers in zoology*, *10*(1), 34. https://doi.org/10.1186/1742-9994-10-34

Macer, C. T. (1977). Some aspects of the biology of the horse mackerel *Trachurus trachurus* (L.) in waters around Britain. *Journal of Fish Biology*, *10*(1), 51–62. https://doi.org/10.1111/j.1095-8649.1977.tb04041.x

Macleod, K., Simmonds, M., & Murray, E. (2003). Summer distribution and relative abundance of cetacean populations off north-west Scotland. *Journal of the Marine Biological Association of the United Kingdom, 83*(5), 1187–1192. https://doi.org/10.1017/s0025315403008476h

Martin, M. (2011). Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet.journal, 17*(1). https://doi.org/10.14806/ej.17.1.200

Möllmann, C., Kornilovs, G., Fetter, M., & Köster, F. (2004). Feeding ecology of central Baltic Sea herring and sprat. *Journal of Fish Biology*, *65*(6), 1563–1581. https://doi.org/10.1111/j.0022-1112.2004.00566.x

Murphy, S. M., Pinn, E. H., & Jepson, P. D. (2013). The short-beaked common dolphin (*Delphinus delphis*) in the North-eastern Atlantic: distribution, ecology, management and conservation status. *Oceanography and Marine Biology*, *51*, 193–280. https://www.ascobans.org/sites/default/files/document/Murphy_CD-Review_OMBAR-2013.pdf

NatureMetrics (2024). Report Interpretation Guide. https://www.naturemetrics.com/report-interpretation-guide

NatureScotland [NatScot]. (2020). *Scottish Biodiversity List*. https://www.nature.scot/scottish-biodiversity-list

Oslo and Paris Commission [OSPAR]. (2008). *OSPAR List of threatened and/or declining species and habitats*. Reference Number: 2008-06. https://www.ospar.org/documents?v=32794

Reid, J. B., Pollock, C. M., & Mavor, R. (2001). Seabirds of the Atlantic Frontier, north and west of Scotland. *Continental Shelf Research, 21*(8–10), 1029–1045. https://doi.org/10.1016/s0278-4343(00)00123-0

Santos, M., Pierce, G. J., Learmonth, J., Reid, R. J., Ross, H., Patterson, I. A. P., Reid, D. G., & Beare, D. J. (2004). Variability in the diet of harbour porpoises (*Phocoena phocoena*) in Scottish waters 1992–2003. *Marine Mammal Science, 20*(1), 1–27. https://doi.org/10.1111/j.1748-7692.2004.tb01138.x

Spens, J., Evans, A.R., Halfmaerten, D., Knudsen, S.W., Sengupta, M.E., Mak, S.S.T., Sigsgaard, E.E., & Hellström, M. (2016). Comparison of capture and storage methods for aqueous macrobial eDNA using an optimized extraction protocol: advantage of enclosed filter. *Methods in Ecology and Evolution 8*(5), 635–645. https://doi.org/10.1111/2041-210X.12683

Stanković, D., Crivellì, A. J., & Snoj, A. (2015). Rainbow trout in Europe: introduction, naturalization, and impacts. *Reviews in Fisheries Science & Aquaculture*, *23*(1), 39–71. https://doi.org/10.1080/23308249.2015.1024825

Weir, C. R., Hodgins, N. K., Dolman, S. J., & Walters, A. E. M. (2019). Risso's dolphins (*Grampus griseus*) in a proposed Marine Protected Area off east Lewis (Scotland, UK), 2010–2017. *Journal of the Marine Biological Association of the United Kingdom, 99*(3), 703–714. https://doi.org/10.1017/s0025315418000516

Wood, S. A., Biessy, L., Latchford, J. L., Zaiko, A., Von Ammon, U., Audrézet, F., Cristescu, M. E., & Pochon, X. (2020). Release and degradation of environmental DNA and RNA in a marine system. *Science of the Total Environment*, *704*, 135314. https://doi.org/10.1016/j.scitotenv.2019.135314

WoRMS Editorial Board [WoRMS]. (2024). *World Register of Marine Species*. https://www.marinespecies.org

Zaiko, A., Pochon, X., García-Vázquez, E., Olenin, S., & Wood, S. A. (2018). Advantages and Limitations of environmental DNA/RNA tools for marine biosecurity: Management and surveillance of non-indigenous species. *Frontiers in Marine Science*, *5*. https://doi.org/10.3389/fmars.2018.00322

Appendix A

Guidelines on Use of Report

This report (the "Report") was prepared as part of the services (the "Services") provided by Fugro GB Limited ("Fugro") for its client (the "Client") under terms of the relevant contract between the two parties (the "Contract"). The Services were performed by Fugro based on requirements of the Client set out in the Contract or otherwise made known by the Client to Fugro at the time.

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Appendix B

Survey Strategy

B.1 Proposed sampling stations

Geodetic Parameters: ETRS89, UTM Zone 29N, CM 3°W [m]				
Station	Easting	Northing	Rationale	Data and Sample Acquisition
Block A				
MCW-A-ST01	641 137.9	6 225 410.2	Client predefined	Video, stills, PSD, FA
MCW-A-ST02	643 878.0	6 225 536.8	Client predefined	Video, stills, PC, FA, eDNA
MCW-A-ST03	646 757.3	6 225 342.1	Client predefined	Video, stills, PSD, FA
MCW-A-ST05	638 497.8	6 222 980.4	Client predefined	Video, stills, PC, eDNA
MCW-A-ST07A	643 915.1	6 223 028.5	Original station location moved to investigate an area of high SSS reflectivity	Video, stills, PSD, FA
MCW-A-ST08A	645 652.5	6 221 830.4	Relocated from original position on a rocky island to investigate area of changeable seafloor with sediment ripples	Video, stills, PC, FA, eDNA
MCW-A-ST12	636 003.8	6 220 235.0	Client predefined	Video, stills, PC, eDNA
MCW-A-ST14	640 980.1	6 220 494.4	Client predefined	Video, stills, PC, eDNA
MCW-A-ST22	630 628.1	6 217 682.3	Client predefined	Video, stills, PC, eDNA
MCW-A-ST34	633 107.6	6 215 194.0	Client predefined	Video, stills, PC, eDNA
MCW-A-ST36	638 870.0	6 214 807.6	Client predefined	Video, stills, PC, eDNA
MCW-A-ST44A	630 608.2	6 212 696.0	Station moved to investigate area of high SSS reflectivity and potential rippled sediment	Video, stills, PSD, FA
MCW-A-ST55	633 395.3	6 209 745.9	Client predefined	Video, stills, PC, eDNA
Block B				
MCW-B-ST09A	650 065.9	6 222 892.3	Station moved 1096 m to the east from the original*	Video, stills, PSD, FA
MCW-B-ST10	652 120.3	6 222 662.4	Client predefined	Video, stills, PSD, FA
MCW-B-ST17A	649 155.4	6 220 174.6	Station moved 500 m to the north-west from the original location*	Video, stills, PSD, FA
MCW-B-ST18A	651 370.4	6 220 727.7	Station moved 500 m to the south-east. Transect extended to investigate a patch of high SSS reflectivity*	Video, stills, PC, FA, eDNA
MCW-B-ST19A	654 912.3	6 219 783.6	Station moved 500 m to the south-east from the original location*	Video, stills, PSD, FA
MCW-B-ST28	646 339.9	6 217 812.1	Client predefined	Video, stills, PC, eDNA
MCW-B-ST29A	649 544.8	6 217 237.8	Station moved 500 m to the south-east from the original location*	Video, stills, PSD, FA

Geodetic Parameters: ETRS89, UTM Zone 29N, CM 3°W [m]				
Station	Easting	Northing	Rationale	Data and Sample Acquisition
MCW-B-ST30A	652 141.6	6 217 458.6	Station moved 500 m to the south-east from the original location*	Video, stills, PC, FA, eDNA
MCW-B-ST38A	644 136.5	6 214 657.6	Station moved 500 m to the south-east from the original location*	Video, stills, PC, eDNA
MCW-B-ST57	638 388.4	6 209 834.5	Client predefined	Video, stills, PC, eDNA
MCW-B-ST59A	643 471.4	6 210 183.5	Station moved 755 m to the north-west from the original location*	Video, stills, PC, eDNA
Block C				
MCW-C-ST20	657 485.3	6 219 984.4	Client predefined	Video, stills, PSD, FA
MCW-C-ST31	654 519.6	6 217 495.9	Client predefined	Video, stills, PSD, FA
MCW-C-ST32	657 080.4	6 217 686.5	Client predefined	Video, stills, PSD, FA
MCW-C-ST41	651 703.6	6 215 133.0	Client predefined	Video, stills, PSD, FA
MCW-C-ST42	654 589.7	6 214 943.9	Client predefined	Video, stills, PC, FA, eDNA
MCW-C-ST43	657 107.2	6 215 098.2	Client predefined	Video, stills, PSD, FA
MCW-C-ST51	649 221.2	6 212 397.3	Client predefined	Video, stills, PC, eDNA
MCW-C-ST52	651 625.9	6 212 457.0	Client predefined	Video, stills, PSD, FA
MCW-C-ST53	654 502.8	6 212 260.2	Client predefined	Video, stills, PSD, FA
MCW-C-ST54	657 296.2	6 212 376.3	Client predefined	Video, stills, PSD, FA
MCW-C-ST62	651 805.5	6 209 585.5	Client predefined	Video, stills, PSD, FA
MCW-C-ST63	654 497.1	6 209 644.6	Client predefined	Video, stills, PC, FA, eDNA
MCW-C-ST70	649 517.0	6 206 771.2	Client predefined	Video, stills, PC, FA, eDNA
MCW-C-ST71	651 606.3	6 207 218.9	Client predefined	Video, stills, PSD, FA
MCW-C-ST75	638 721.0	6 204 239.3	Client predefined	Video, stills, PC, eDNA
MCW-C-ST77	644 143.5	6 204 220.4	Client predefined	Video, stills, PC, eDNA
MCW-C-ST79	649 114.1	6 204 475.0	Client predefined	Video, stills, PSD, FA
MCW-C-ST83	638 764.7	6 201 665.2	Client predefined	Video, stills, PSD, FA
MCW-C-ST91	638 680.2	6 198 983.5	Client predefined	Video, stills, PSD, FA
MCW-C-ST92	641 244.2	6 199 176.8	Client predefined	Video, stills, PC, eDNA
Block D				
MCW-D-ST64	656 984.8	6 209 773.9	Client predefined	Video, stills, PSD, FA
MCW-D-ST72A	654 833.7	6 206 663.5	The proposed grab location was on rocky reef. Grab location moved to area of soft sediment 501 m away to the south-east	Video, stills, PSD, FA
MCW-D-ST73	657 373.9	6 206 836.9	Client predefined	Video, stills, PSD, FA
MCW-D-ST80	651 997.4	6 204 283.6	Client predefined	Video, stills, PC, FA, eDNA

Geodetic Parameters: ETRS89, UTM Zone 29N, CM 3°W [m]				
Station	Easting	Northing	Rationale	Data and Sample Acquisition
MCW-D-ST81	654 411.2	6 204 350.8	Client predefined	Video, stills, PSD, FA
MCW-D-ST82	656 969.8	6 204 539.7	Client predefined	Video, stills, PC, FA, eDNA
MCW-D-ST86A	647 336.7	6 201 678.2	Station moved 716 m to the east from the original location	Video, stills, PC, eDNA
MCW-D-ST88A	651 542.8	6 201 944.0	Station moved 504 m to the north-west from the original location	Video, stills, PSD, FA
MCW-D-ST89A	654 093.0	6 202 125.7	Station moved 507 m to the north-west from the original location	Video, stills, PSD, FA
MCW-D-ST90	657 236.5	6 201 500.0	Client predefined	Video, stills, PSD, FA
MCW-D-ST95A	649 709.0	6 198 447.1	Station moved 1001 m to the south-east from the original location	Video, stills, PC, eDNA
MCW-D-ST96A	651 988.0	6 199 054.1	Station moved 814 m to the east from the original location	Video, stills, PSD, FA
MCW-D-ST97A	654 477.5	6 200 490.3	Station moved 1396 m to the north from the original location	Video, stills, PC, FA, eDNA
MCW-D-ST100A	654 921.0	6 197 226.7	Station moved 1000 m to the north-west to an area of soft sediment	Video, stills, PC, FA, eDNA
MCW-D-ST101	649 576.3	6 196 377.7	Client predefined	Video, stills, PSD, FA
MCW-D-ST103A	641 665.6	6 193 656.0	Station moved 242 m to the south-east to an area of soft sediment	Video, stills, PSD, FA
MCW-D-ST104	643 738.1	6 193 436.9	Client predefined	Video, stills, PC, eDNA
MCW-D-ST108A	646 225.7	6 191 608.1	Station moved 501 m to the north-west to an area of soft sediment	Video, stills, PC, eDNA

Notes
SSS = Side scan sonar
PC = Physical chemical
PSD = Particle size distribution
FA = Faunal sample A
eDNA = Environmental deoxyribonucleic acid
* = Stations relocated to coincide with priority geophysical survey lines
Station names with the suffix 'A' were moved from original client defined positions

Appendix C

Results

C.1 Marine Water Fish (excluding sharks and rays)

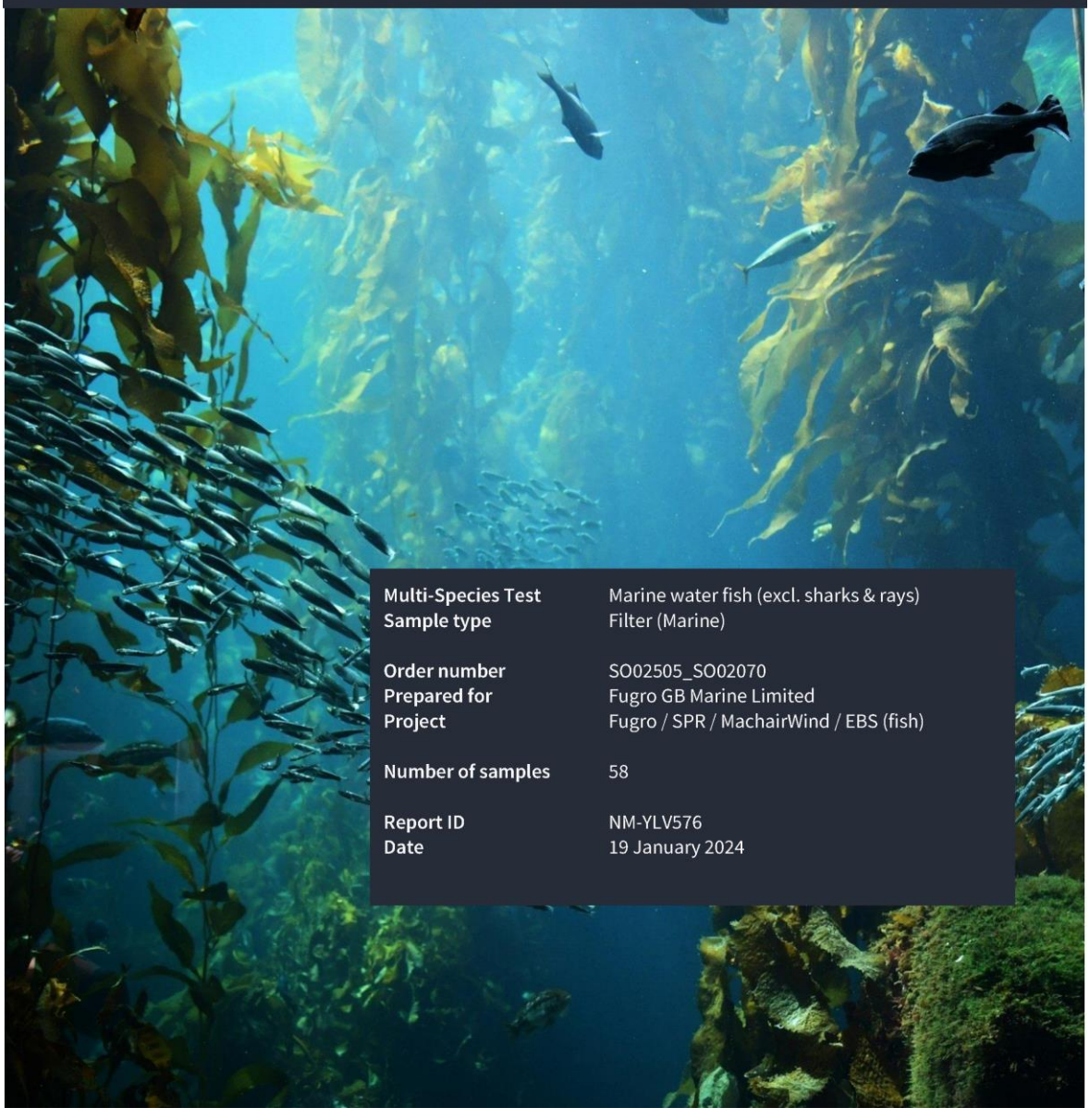
NatureMetrics eDNA Metabarcoding Results lab report

C.1.1 Report

NATURE METRICS
DNA-BASED MONITORING

Environmental DNA Report

Marine water fish (excl. sharks & rays)



Multi-Species Test	Marine water fish (excl. sharks & rays)
Sample type	Filter (Marine)
Order number	SO02505_SO02070
Prepared for	Fugro GB Marine Limited
Project	Fugro / SPR / MachairWind / EBS (fish)
Number of samples	58
Report ID	NM-YLV576
Date	19 January 2024

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Data Tables: Accompanying spreadsheet with results at the individual sample level: species detected, metrics and quality control: NM-YLV576.SO02505_SO02070.Fish.Results.xlsx

- Data Description
- Species Data Table: Percentages
- Species Data Table: Read Counts
- Metrics by Sample Table
- Quality Control Table

Throughout the report you'll see reference to 'OTU'. This stands for Operational Taxonomic Unit; an OTU is broadly equivalent to a species in most cases.

Executive Summary

Field Samples submitted:	58
Field Samples reported:	55
Field Blanks submitted:	0
Species Richness:	66
Average Species Richness per sample:	11
Total number of IUCN Red List Species:	3
Total number of Invasive Species:	1

Reported samples are those that passed Quality Control and are included in the Species Data Table

Please be careful when sharing this report, it contains biodiversity information that may be sensitive, particularly with respect to endangered or protected species. Please share responsibly. If the report is shared, we kindly ask that the report is shared in its entirety - to limit the possibility of any information being taken out of context.

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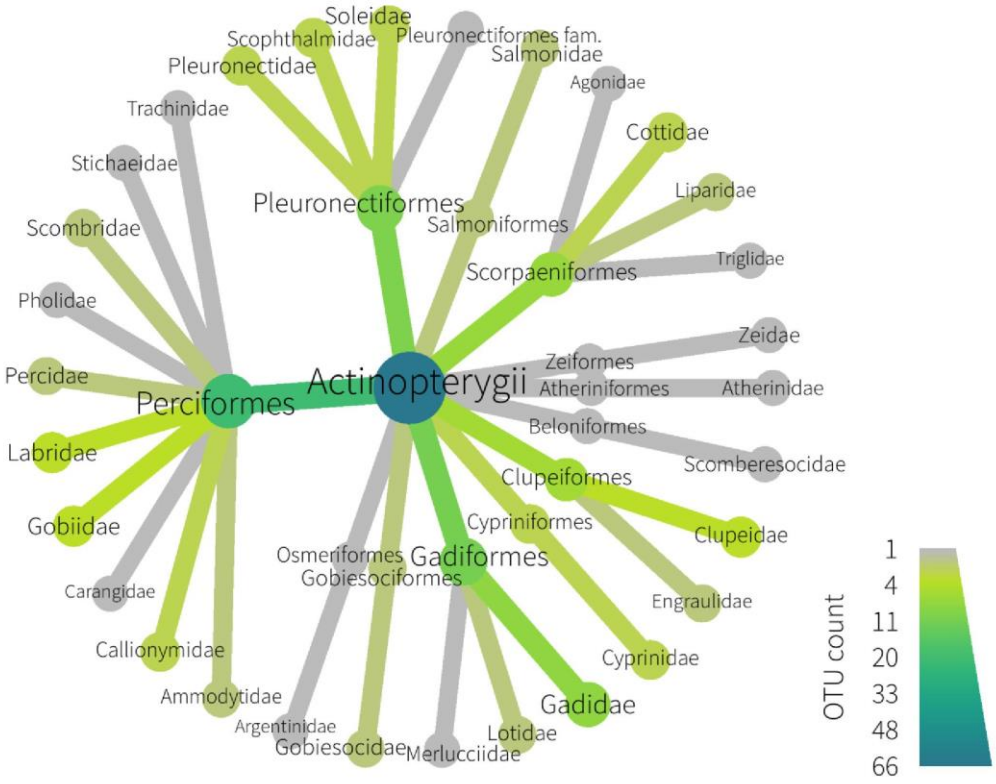
Something exciting or unexpected that you'd like to discuss further, our team of experts are looking forward to speaking with you: www.naturemetrics.com/contact

NATURE METRICS
DNA-BASED MONITORING

REPORT

Taxonomic Composition

This chart provides a view of the species detected in your samples and their taxonomic relationship, (names on the same branch are more similar than those on different branches). The chart is structured with the highest taxonomic rank at the centre (e.g., kingdom, phylum, class), moving through the ranks of order, family, genus, species as you move to the outer edge. Note that the centre and outer ranks will change depending on the test applied and the number of species detected. The legend in the bottom right of the chart indicates how to relate the colour in the branches to the number of species. The colour scale goes from grey - indicating very few species, to blue - indicating a lot of species.



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2

FUGRO

Taxonomic Resolution

This table provides the number of OTUs detected and the percentage of OTUs identified to each taxonomic level.

Depending on completeness of reference databases for the region where you sampled, some OTUs may not match to a reference at species level. Global DNA reference databases contain millions of barcodes, but gaps remain, particularly in regions and taxonomic groups that are more diverse and less studied. Coverage is expected to improve over time and data tables can be updated to include new information at a future date.

Number of OTUs	Phylum	Class	Order	Family	Genus	Species
66	100%	100%	100%	98.48%	89.39%	78.79%

Want to increase the number of species named to species level? If you have specimens of species you have identified, we can sequence the DNA and add the species to our reference databases. We will then be able to enhance the reference library and report if the species is detected. Please contact us about this service and we can send you our barcoding kits, but note that we only offer these kits for fish and amphibians.

IUCN Red List Species

These are the IUCN (International Union for Conservation of Nature) Red List species detected in your samples. These are detected species that are designated as one of the IUCN Red List Threatened Categories (Vulnerable, Endangered and Critically Endangered). An increase in the number of threatened species is generally associated with a positive trend in biodiversity or habitat condition.

Species	Common name	Threat Status
Gadus morhua	Atlantic Cod	Vulnerable
Melanogrammus aeglefinus	Haddock	Vulnerable
Trachurus trachurus	Atlantic Horse Mackerel	Vulnerable
Number of species		3

The Data Tables contain further information for all species, including their designations as Least Concern or Near Threatened status.

Invasive Species

These are the Invasive species detected in your samples. These species are invasive according to the Global Register of Introduced and Invasive Species (GRIIS) in the country where sampling occurred. GRIIS is an IUCN Invasive Species Specialist Group initiative. The Convention on Biological Diversity defines an invasive species as one whose introduction and/or spread threatens biological diversity. An increase in the number of invasive species is generally associated with enhanced pressures at your site and reduced resilience of the native community. Please note: this label is only available for animals; and GRIIS lists marine species as invasive for a country, even if the species is known to be invasive in only one marine area bordering the country.

Species	Common name
Oncorhynchus mykiss	Rainbow Trout
Number of species	1

Community Composition

This chart lists the species found in each sample. The presence of a bubble means a species was detected in that sample. The chart displays at species level, unless the number of species detected is too great to display clearly in the document. In these cases, the chart displays at a higher taxonomic level. The full species level chart is provided as an appendix.

The size of the bubbles represents the proportion of DNA sequences within a sample. A larger bubble size can indicate a stronger eDNA signal. This signal may be linked to abundance of species in the environment but should be interpreted only as a coarse measure because the signal is also impacted by biological (e.g., biomass, life stage, activity, body condition), environmental (e.g., temperature, pH, salinity, conductivity), and technical factors (e.g., primer bias, PCR stochasticity).

Chart attached separately.

Species Richness

This is the total count of OTUs detected in each sample. The blue portion of each bar indicates the number of OTUs identified to a species.

Chart attached separately.

High Species Richness generally indicates a healthier and functioning ecosystem and is the simplest biodiversity metric that is consistently reported in biodiversity monitoring.

Evolutionary Diversity

Evolutionary Diversity calculated for each sample. This is a measure of the variety of species types that occurred in your samples.

Chart attached separately.

Evolutionary Diversity is a strong complementary indicator of biodiversity progress alongside Species Richness. Increasing Evolutionary Diversity can indicate an increasing resilience of the community.

Looking for something more?

We also offer comparative reporting. This includes statistical comparison of metrics and communities according to categories that you define. For instance, these might include waterbody, Site, Management Regime, or anything else that is a focus of your project. Please contact us for further details.

END OF REPORT

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C.1.2 Tables

Click the icon to open the associated eDNA spreadsheet:

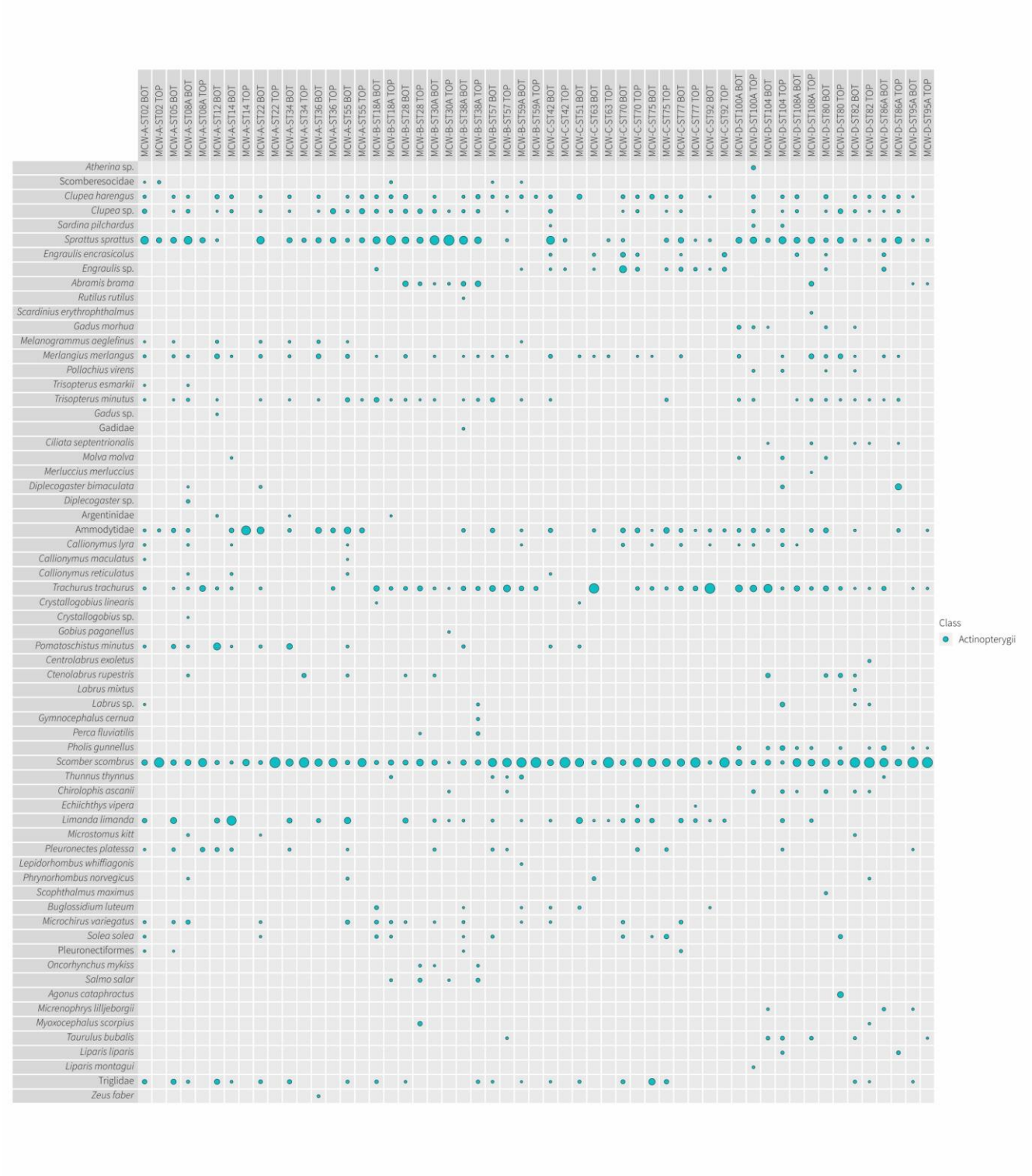
- *Table 1. Species data percentages table. Percentages correspond to the percentage of DNA sequences assigned to the species detected in each sample;*
- *Table 2. Species data read counts table. Read counts correspond to the number of DNA sequences assigned to a species;*
- *Table 3. Metrics by sample table;*
- *Table 4. Quality control table.*

210836_eDNA_Fish_Results

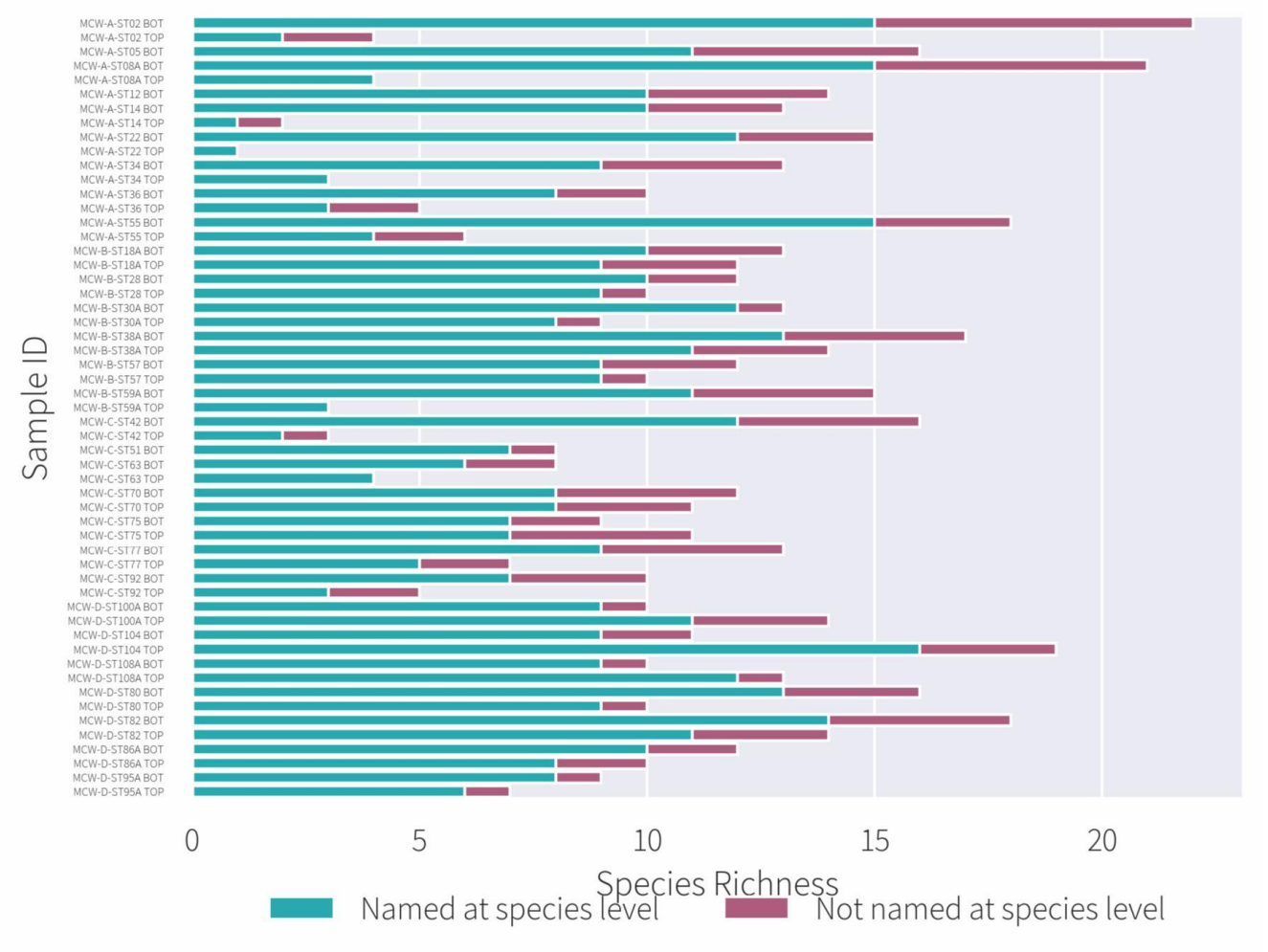
C.1.3 Figures

Figures supplied by NatureMetrics:

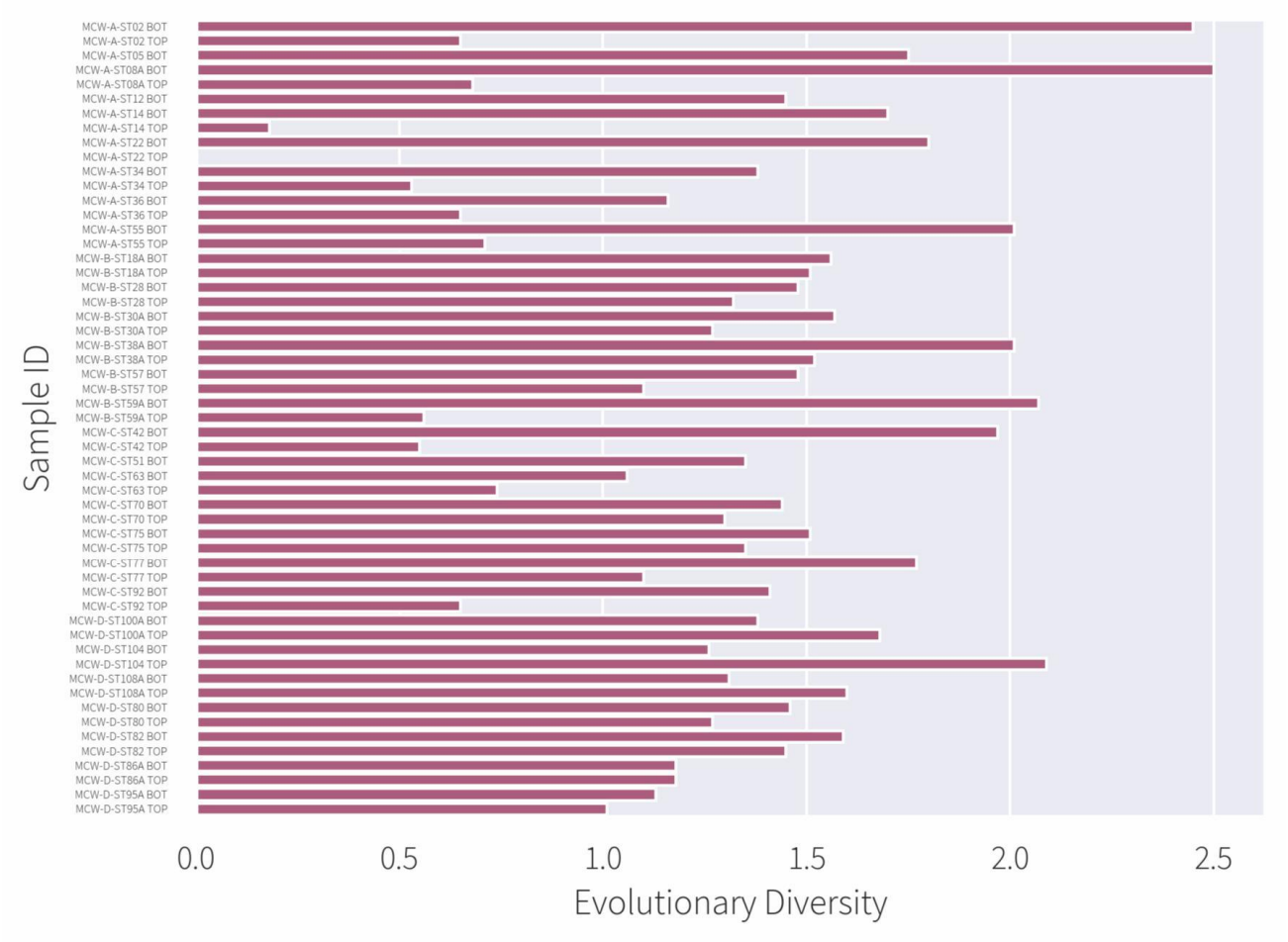
- *Figure 1. The proportion of the sequencing output allocated to the different taxa (rows) within each sample (columns). Each bubble per sample represents the proportion of DNA for each taxon for that sample. The size of the bubble is relative to the number of sequences from all taxa detected in that sample.*



- *Figure 2. The total count of OTUs detected in each sample, represented as Species Richness.*



- *Figure 3. Evolutionary diversity for each sample. This is calculated by arranging all OTUs in a family tree based on the similarity of DNA sequences and summing the overall size of the family tree (including lengths of all family tree branches).*



C.2 Marine Water Invertebrates

NatureMetrics eDNA Metabarcoding Results lab report

C.2.1 Report

Thank you for choosing NatureMetrics

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Your report consists of:

This document: Providing you with our world class insights and metrics.

Data Tables: Accompanying spreadsheet with results at the individual sample level: species detected, metrics and quality control: NM-USP253.SO02505_SO02070.Invertebrates.Results.xlsx

- Data Description
- Species Data Table: Percentages
- Species Data Table: Read Counts
- Metrics by Sample Table
- Quality Control Table

Throughout the report you'll see reference to 'OTU'. This stands for Operational Taxonomic Unit; an OTU is broadly equivalent to a species in most cases.

Executive Summary

Field Samples submitted:	58
Field Samples reported:	58
Field Blanks submitted:	0
Species Richness:	89
Average Species Richness per sample:	9
Total number of IUCN Red List Species:	0
Total number of Invasive Species:	0

Reported samples are those that passed Quality Control and are included in the Species Data Table

Please be careful when sharing this report, it contains biodiversity information that may be sensitive, particularly with respect to endangered or protected species. Please share responsibly. If the report is shared, we kindly ask that the report is shared in its entirety - to limit the possibility of any information being taken out of context.

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NATURE METRICS
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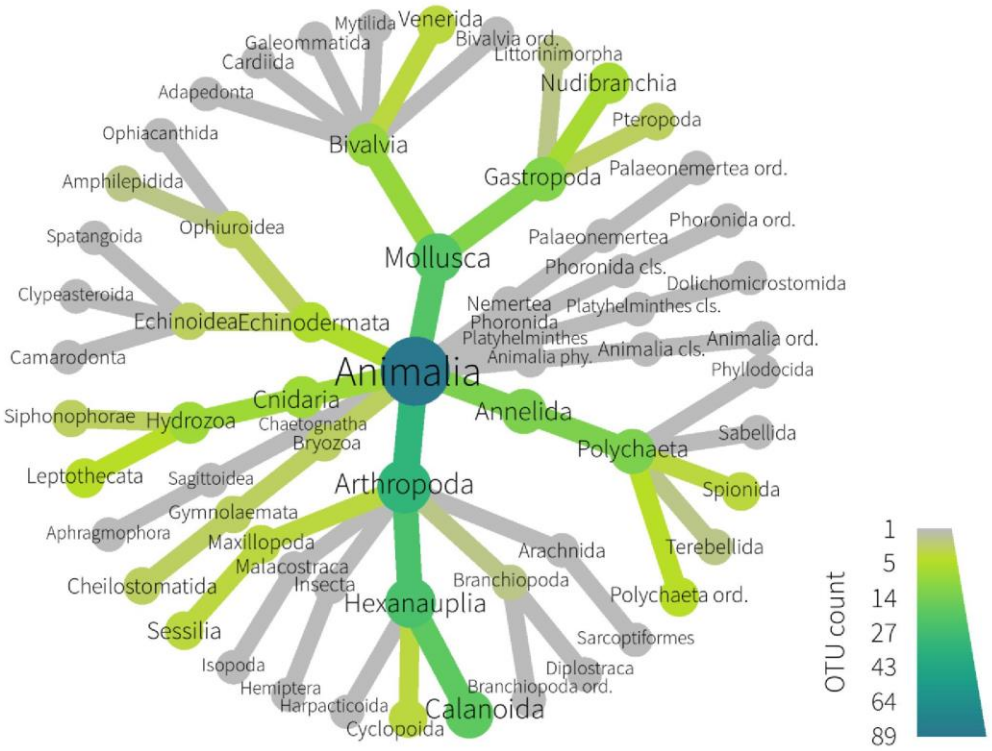
REPORT

Taxonomic Composition

This chart provides a view of the species detected in your samples and their taxonomic relationship, (names on the same branch are more similar than those on different branches). The chart is structured with the highest taxonomic rank at the centre (e.g., kingdom, phylum, class), moving through the ranks of order, family, genus, species as you move to the outer edge. Note that the centre and outer ranks will change depending on the test applied and the number of species detected. The legend in the bottom right of the chart indicates how to relate the colour in the branches to the number of species. The colour scale goes from grey - indicating very few species, to blue - indicating a lot of species.



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Taxonomic Resolution

This table provides the number of OTUs detected and the percentage of OTUs identified to each taxonomic level.

Depending on completeness of reference databases for the region where you sampled, some OTUs may not match to a reference at species level. Global DNA reference databases contain millions of barcodes, but gaps remain, particularly in regions and taxonomic groups that are more diverse and less studied. Coverage is expected to improve over time and data tables can be updated to include new information at a future date.

Number of OTUs	Phylum	Class	Order	Family	Genus	Species
89	98.88%	98.88%	95.51%	92.13%	82.02%	69.66%

Want to increase the number of species named to species level? If you have specimens of species you have identified, we can sequence the DNA and add the species to our reference databases. We will then be able to enhance the reference library and report if the species is detected. Please contact us about this service and we can send you our barcoding kits, but note that we only offer these kits for fish and amphibians.

IUCN Red List Species

These are the IUCN (International Union for Conservation of Nature) Red List species detected in your samples. These are detected species that are designated as one of the IUCN Red List Threatened Categories (Vulnerable, Endangered and Critically Endangered). An increase in the number of threatened species is generally associated with a positive trend in biodiversity or habitat condition.

No species designated Vulnerable, Endangered or Critically Endangered were detected in the samples.

The Data Tables contain further information for all species, including their designations as Least Concern or Near Threatened status.

Invasive Species

These are the Invasive species detected in your samples. These species are invasive according to the Global Register of Introduced and Invasive Species (GRIIS) in the country where sampling occurred. GRIIS is an IUCN Invasive Species Specialist Group initiative. The Convention on Biological Diversity defines an invasive species as one whose introduction and/or spread threatens biological diversity. An increase in the number of invasive species is generally associated with enhanced pressures at your site and reduced resilience of the native community. Please note: this label is only available for animals; and GRIIS lists marine species as invasive for a country, even if the species is known to be invasive in only one marine area bordering the country.

No invasive species were detected in the samples.

Community Composition

This chart lists the species found in each sample. The presence of a bubble means a species was detected in that sample. The chart displays at species level, unless the number of species detected is too great to display clearly in the document. In these cases, the chart displays at a higher taxonomic level. The full species level chart is provided as an appendix.

The size of the bubbles represents the proportion of DNA sequences within a sample. A larger bubble size can indicate a stronger eDNA signal. This signal may be linked to abundance of species in the environment but should be interpreted only as a coarse measure because the signal is also impacted by biological (e.g., biomass, life stage, activity, body condition), environmental (e.g., temperature, pH, salinity, conductivity), and technical factors (e.g., primer bias, PCR stochasticity).

Chart attached separately.

Species Richness

This is the total count of OTUs detected in each sample. The blue portion of each bar indicates the number of OTUs identified to a species.

Chart attached separately.

High Species Richness generally indicates a healthier and functioning ecosystem and is the simplest biodiversity metric that is consistently reported in biodiversity monitoring.

Evolutionary Diversity

Evolutionary Diversity calculated for each sample. This is a measure of the variety of species types that occurred in your samples.

Chart attached separately.

Evolutionary Diversity is a strong complementary indicator of biodiversity progress alongside Species Richness. Increasing Evolutionary Diversity can indicate an increasing resilience of the community.

Looking for something more?

We also offer comparative reporting. This includes statistical comparison of metrics and communities according to categories that you define. For instance, these might include waterbody, Site, Management Regime, or anything else that is a focus of your project. Please contact us for further details.

END OF REPORT

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C.2.2 Tables

Click the icon to open the associated eDNA spreadsheet:

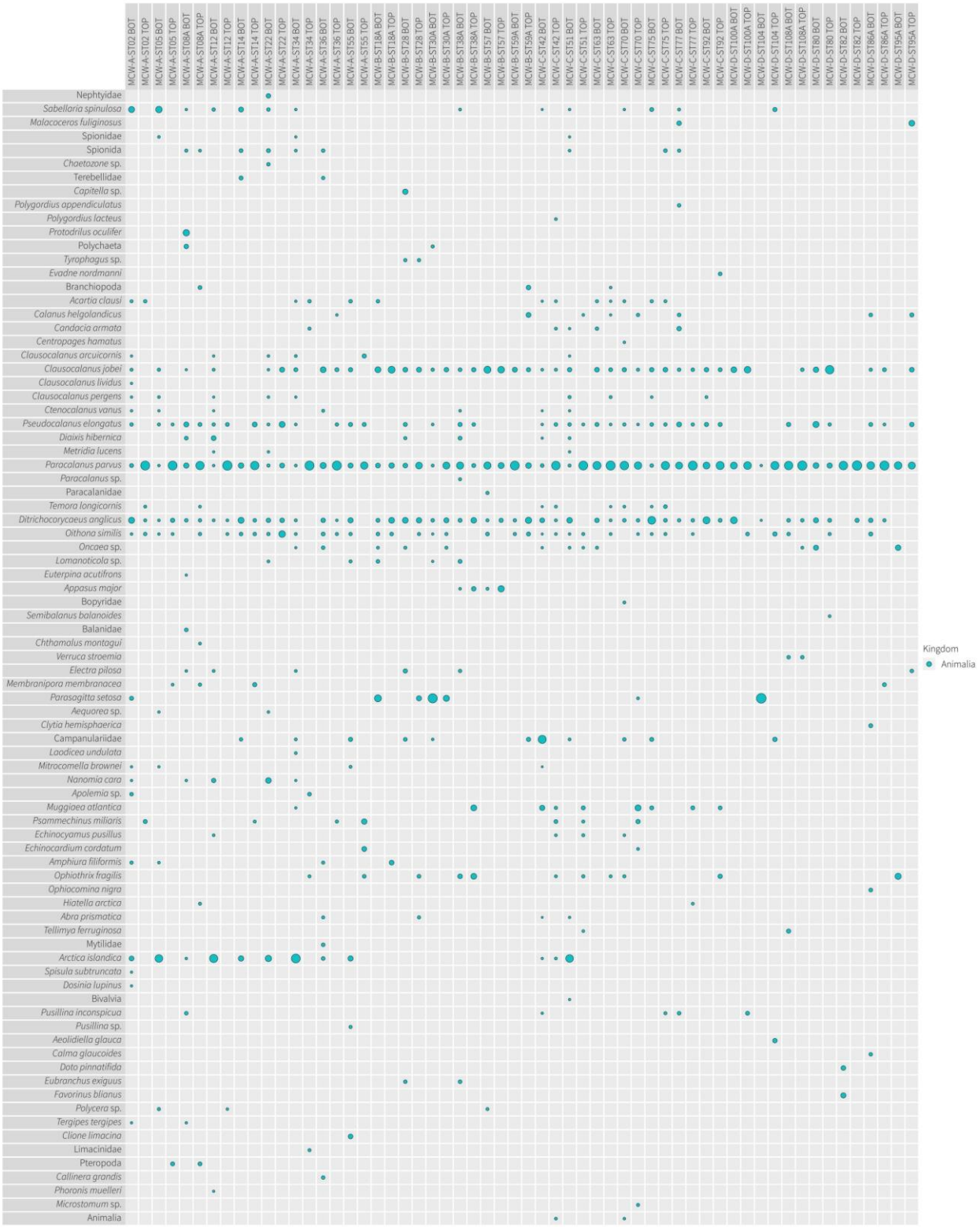
- *Table 1. Species data percentages table. Percentages correspond to the percentage of DNA sequences assigned to the species detected in each sample;*
- *Table 2. Species data read counts table. Read counts correspond to the number of DNA sequences assigned to a species;*
- *Table 3. Metrics by sample table;*
- *Table 4. Quality control table.*

210836_eDNA_Invertebrates_Results

C.2.3 Figures

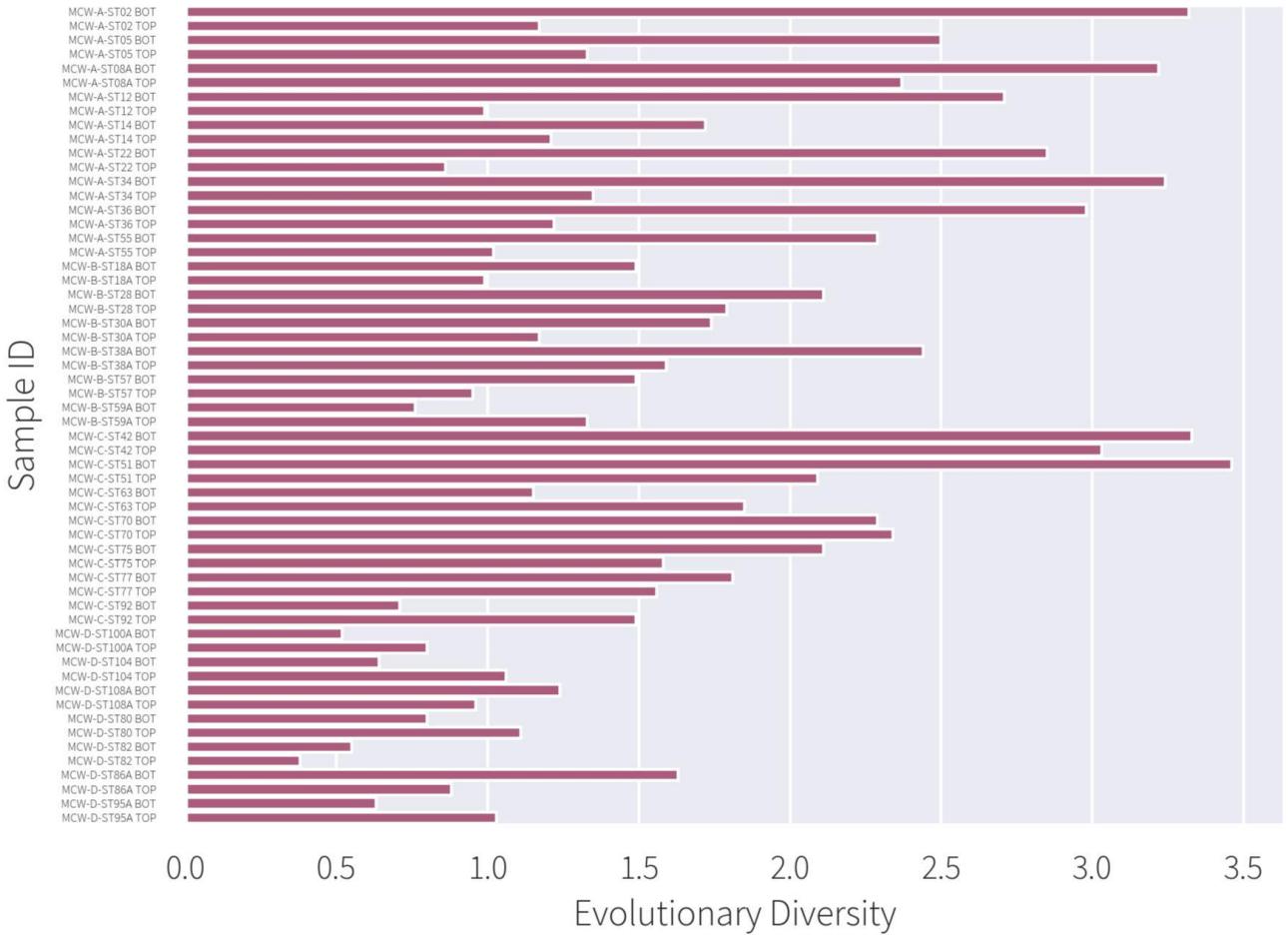
Figures supplied by NatureMetrics:

- *Figure 1. The proportion of the sequencing output allocated to the different taxa (rows) within each sample (columns). Each bubble per sample represents the proportion of DNA for each taxon for that sample. The size of the bubble is relative to the number of sequences from all taxa detected in that sample.*



- *Figure 2. The total count of OTUs detected in each sample, represented as Species Richness.*

- *Figure 3. Evolutionary diversity for each sample. This is calculated by arranging all OTUs in a family tree based on the similarity of DNA sequences and summing the overall size of the family tree (including lengths of all family tree branches).*



C.3 Marine Water Vertebrates

NatureMetrics eDNA Metabarcoding Results lab report

C.3.1 Report

Thank you for choosing NatureMetrics

Your Nature Intelligence Partner

Welcome to your report

Your report consists of:

This document: Providing you with our world class insights and metrics.

Data Tables: Accompanying spreadsheet with results at the individual sample level: species detected, metrics and quality control: NM-OZX641.SO02505_SO02070.Vertebrates.Results.xlsx

- Data Description
- Species Data Table: Percentages
- Species Data Table: Read Counts
- Metrics by Sample Table
- Quality Control Table

Throughout the report you'll see reference to 'OTU'. This stands for Operational Taxonomic Unit; an OTU is broadly equivalent to a species in most cases.

Executive Summary

Field Samples submitted:	58
Field Samples reported:	51
Field Blanks submitted:	0
Species Richness:	77
Average Species Richness per sample:	12
Total number of IUCN Red List Species:	2
Total number of Invasive Species:	2

Reported samples are those that passed Quality Control and are included in the Species Data Table

Please be careful when sharing this report, it contains biodiversity information that may be sensitive, particularly with respect to endangered or protected species. Please share responsibly. If the report is shared, we kindly ask that the report is shared in its entirety - to limit the possibility of any information being taken out of context.

New to our reports? Our Report Interpretation Guide is here to help:
www.naturemetrics.co.uk/report-interpretation-guide

Something exciting or unexpected that you'd like to discuss further, our team of experts are looking forward to speaking with you: www.naturemetrics.com/contact

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REPORT

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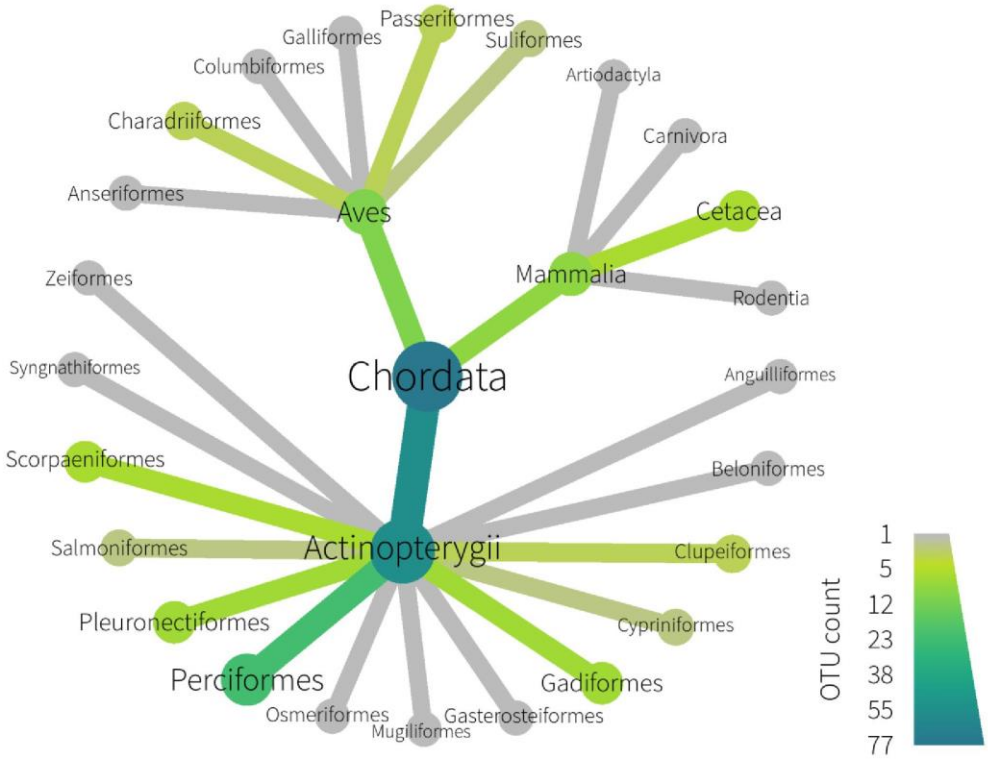

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210836_eDNA_Vertebrate_Results

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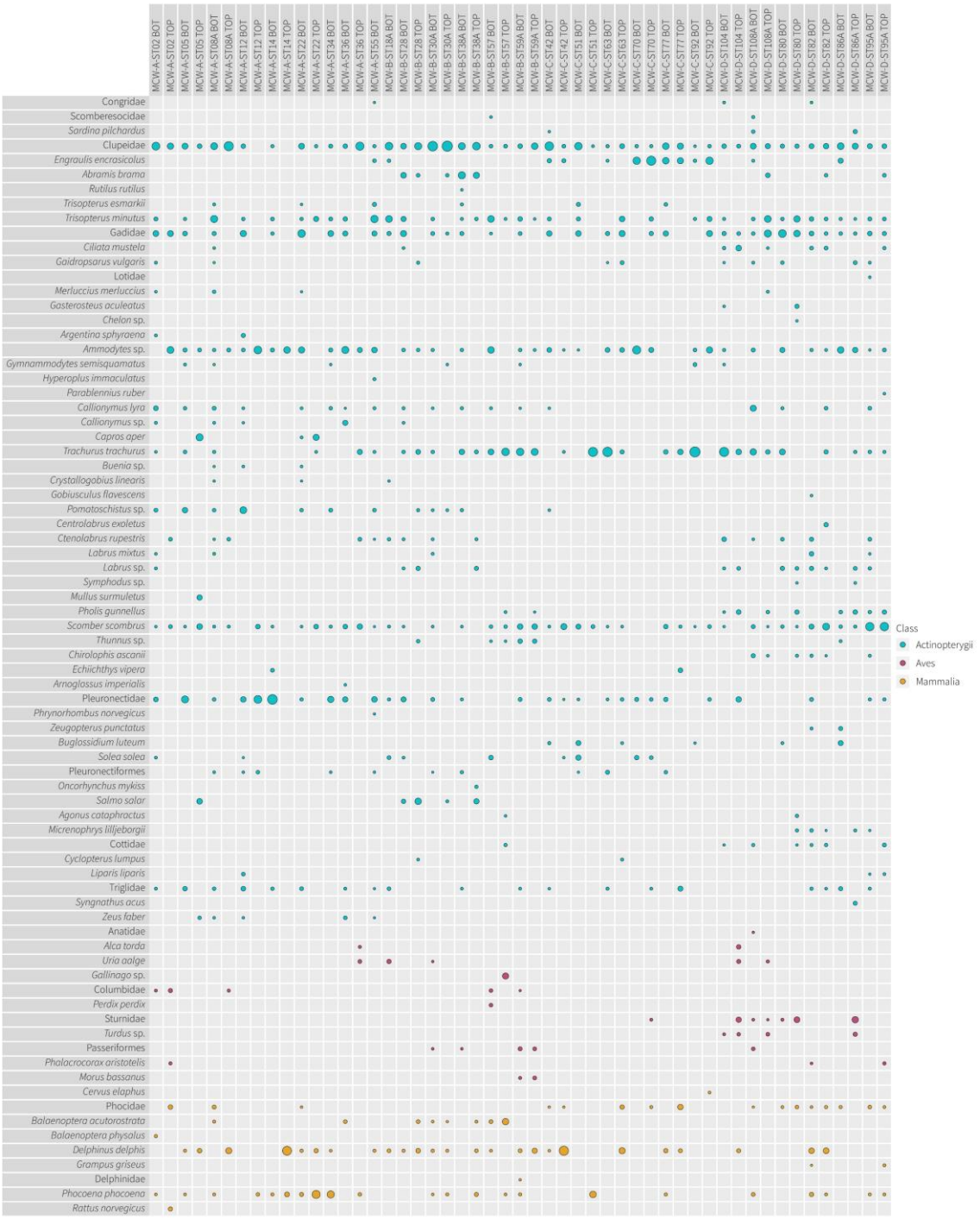

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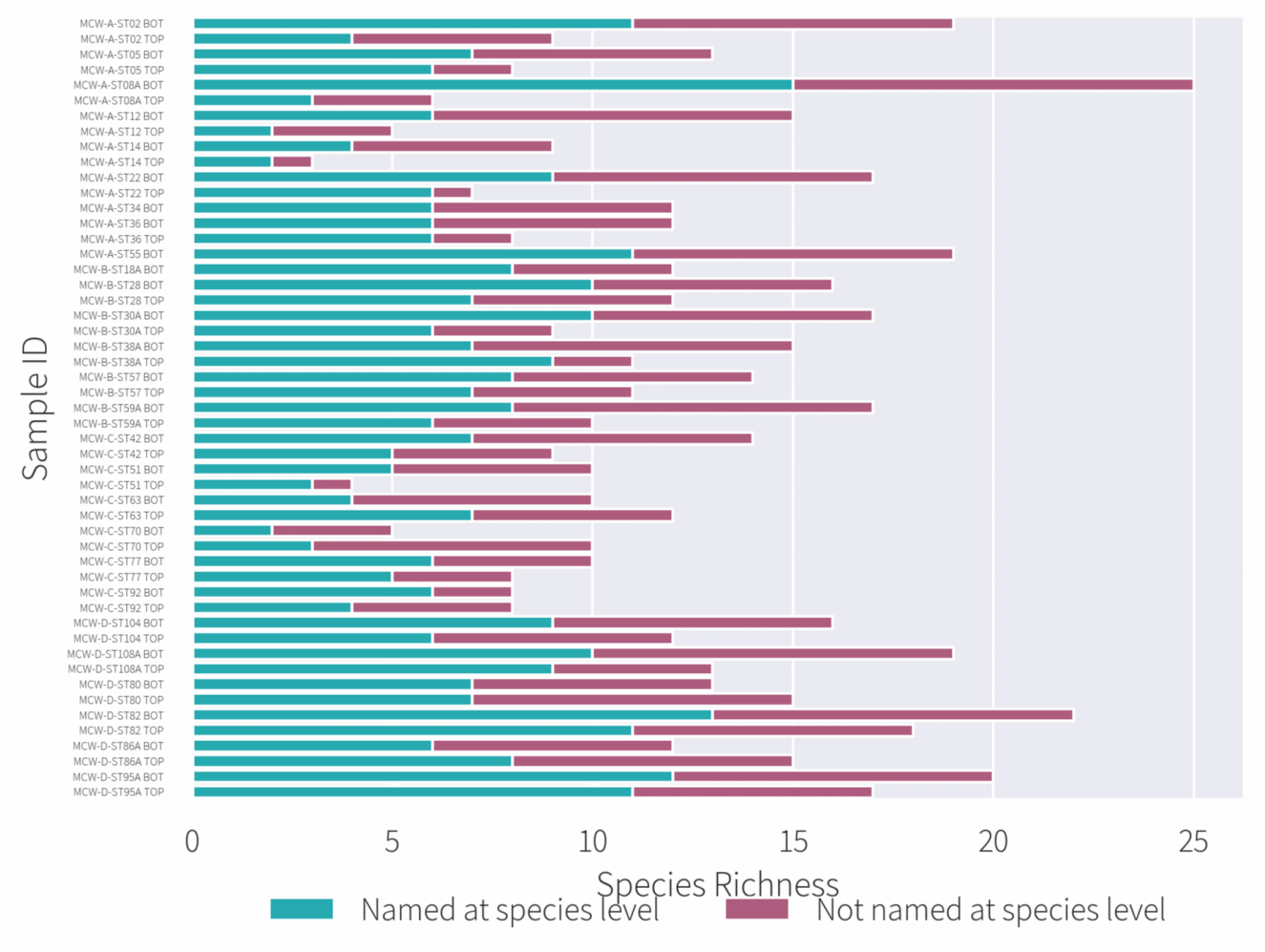

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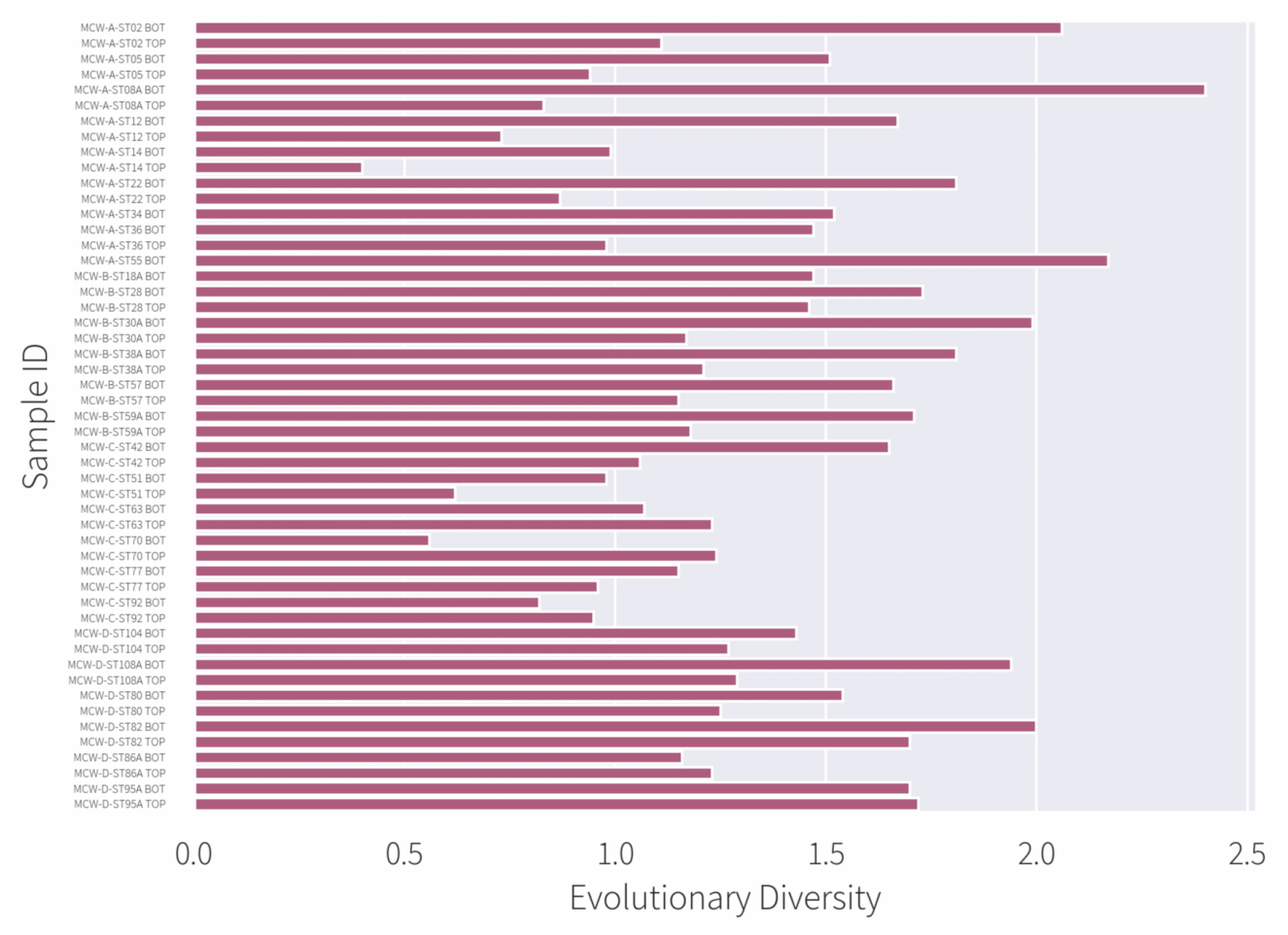


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