TotalEnergies E&P North Sea UK Ltd

Culzean - Floating Offshore Wind Turbine Pilot Project

Appendix D: Environmental Baseline Survey Report (2023)

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Environmental Baseline Report

TotalEnergies PWT Site Survey

Geophysical, Geotechnical and Environmental Survey

Culzean Field, Central North Sea

CLIENT
TotalEnergies E&P North Sea UK Ltd

DATE
1st of September 2023

CREATED BY
Iris Duranović

OI DOC NO.
104728-TOT-OI-SUR-REP-ENVBASRE

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Table of Contents

Appendices

List of Figures

List of Tables

Abbreviations and Definitions

BGS ..... British Geological Society
CNS ..... Central North Sea
CCME ..... Canadian Council of Ministers of the Environment
CEFAS ..... Centre for Environment, Fisheries and Aquaculture Science
CPF ..... Central Processing Platform
CPI ..... Carbon Preference Index
CPT ..... Cone Penetration Test
CTD ..... Conductivity, Temperature and Depth
DBT ..... Dibutyltin
DDV ..... Drop Down Video
DNA ..... Deoxyribonucleic Acid
DPR ..... Daily Progress Report
DP2 ..... Dynamic Positioning 2
DTM ..... Digital Terrain Model
DVV ..... Dual van Veen grab
EAC ..... Environment Assessment Criteria
EC ..... European Commission
ED50 ..... European Datum 1950
eDNA ..... Environmental DNA
EAC ..... Environmental Assessment Criteria
EPA ..... Environmental Protection Agency
EQS ..... Environment Quality Standards
ERL ..... Effective Range Low
EUNIS ..... European Nature Information System
GIS ..... Geographic Information System
GNSS ..... Global Navigation Satellite System

HG	Hamon Grab
IMR	Inspection. Maintenance, and Report
ISQG	Interim Sediment Quality Guidelines
IUCN	International Union for Conservation of Nature
JNCC	Joint Nature Conservation Committee
KP	Kilometer Post
LAT	Lowest Astronomical Tide (vertical datum)
LoD	Limit of Detection
MAC	Mobilisation and Calibration
MAG	Magnetometer
MBT	Monobutyltin
MBES	Multibeam Echo Sounder
nMDS	Non-metric Multi-Dimensional Scaling
MDS	Multi-Dimensional Scaling
M/V	Motor Vessel
NEA	Norwegian Environment Agency
NMBAQC	National Marine Biological Analytical Quality Control
OCP	Organochlorine Pesticides
OI	Ocean Infinity Group Holding (Sweden) AB
OTU	Operational Taxonomic Units
OSPAR	The Oslo and Paris Conventions for the protection of the marine environment of the North-East Atlantic
PAH	Polycyclic Aromatic Hydrocarbons
PBDEs	Polybrominated Diphenyl Ethers
PCA	Principal Component Analysis
PCB	Polychlorinated Biphenyls
PCR	Polymerase Chain Reaction
PEL	Probably Effect Level
PMF	Priority Marine Feature
PPS	Pulser Per Second
PSA	Particle Size Analysis
PSD	Particle Size Distribution
ROV	Remotely Operated Vehicle
SBET	Smoothed Best Estimated Trajectory
SBL	Scottish Biodiversity List
SBP	Sub-bottom Profiler
SIMPER	Similarity Percentage
SIMPROF	Similarity Profiling Algorithm
SSIV	Sub-Sea Underwater Intervention Valve
SSS	Side Scan Sonar
TBT	Tributyltin

THC.....Total Hydrocarbons
TOC.....Total Organic Carbon
TOM .....Total Organic Matter
TSS.....Total Suspended Solids
UCM .....Unresolved Complex Mixture
UK.....United Kingdom
UKOOA .....United Kingdom Offshore Operators Association
ULQ .....Utilities and Living Quarters
UTC.....Coordinated Universal Time
UTM .....Universal Transverse Mercator
VC.....Vibrocore
VORF.....Vertical Offshore Reference Frame
WFD.....Water Frame Directive
WGS84 .....World Geodetic System 1984

Executive Summary

This report details the results of the Environmental Baseline Survey for the proposed pilot floating wind turbine in the Culzean field, located approximately 230 kilometres off the coast of Aberdeen, Scotland in the Central North Sea.

The benthic and environmental survey data acquisition included sediment sampling and imagery, with continuous video, and water sampling for eDNA profiling to establish a baseline for the habitats and faunal communities within the survey area. The benthic and environmental survey was carried out from the survey vessel M/V Deep Helder between the 3rd and 8th of April 2023.

Seabed imagery and grab samples were acquired at all of the 8 planned grab sample sites. All of the 8 planned water sample sites were also completed. Three of the sampling sites (E13, E32 and E7) were selected in order to provide a comparison with the corresponding 2013 sampling sites (ENV13, ENV32 and ENV7).

Geophysical data were used to determine water depths, surficial geology, seabed features, shallow geology, and objects present within the survey area. Equipment used during the geophysical survey included Multibeam Echo Sounder, Side Scan Sonar, Sub-Bottom Profiler, Sparker and a single Magnetometer.

The geophysical interpretation combined with the environmental data was used as the basis for the EUNIS habitat classifications and assessments of potential areas and species of conservation importance.

A total of one EUNIS habitat, three habitat complexes, as well as one artificial habitat, were identified within the survey area.

The OSPAR habitat Sea-pens & burrowing megafauna was identified both within the site survey area and the cable route corridor. During the 2013 survey, no areas were assessed as OSPAR habitat Sea-pens and burrowing megafauna. The Sea-pens & burrowing megafauna habitat is a component of the Priority Marine Feature Burrowed Mud. Sandy Ray, *Leucoraja circularis*, listed as a Priority Marine Feature and in the Scottish Biodiversity List, and was identified within the site survey area.

No habitats listed in the Annex I of the Habitats Directive were identified within the site survey area or within the route cable corridor.

EUNIS is a hierarchal classification of habitats, a catalogue, based on physical features, depth, topography, and substrate as well as species recorded as present. OSPAR and Annex I are regulatory frameworks based on assessments by governing bodies aiming to identify vulnerable habitats and species.

The most abundant non-colonial phyla in still photographs, from the visual data analysis, were echinoderms followed by Cnidaria and Arthropoda. The Ophiurida was the overall most frequently occurring taxa per site and still photo.

The sediment composition had limited variation throughout the survey area. Fine sand/V Fine sand was the dominant sediment fraction. The PCA plot mainly grouped the sites based on the silt and clay content and to a lesser extent on sand to gravel ratio.

Metal concentrations in sediment samples were generally low, with all levels within background ranges for the Central North Sea. Hydrocarbon levels were equally low across the site, with all levels remaining within what is expected for the area. Results for Prisatne, Phytane and Carbon Preference index were all consistent with that of a relatively uncontaminated area and suggested there was a slight dominance of biogenic compounds. Polychlorinated biphenyl, organotin, pesticide and brominated flame retardant concentrations were below the limit of detection for all analysed samples.

When comparing the metal concentrations obtained in three of the grab samples to samples taken in the same locations during the 2013 Gardline survey, a general decrease was revealed. Total organic matter, total organic carbon and hydrocarbon concentrations showed minimal changes between datasets.

Metal concentrations in water samples were low across the survey area for the majority of analytes. Zinc was the only metal to exceed any of the Water Framework Directive Environmental Quality standards, although this metal is considered highly variable within the marine environment.

Total Sulphate was within normal levels for seawater at all but one site, where levels were elevated in the sample taken close to the seabed. Hydrocarbon concentrations were below limits of detection.

The faunal analyses of the grab sample showed that the phyletic composition was dominated by annelids. The two most abundant taxa were the annelids *Paramphinome jeffreysii* and *Galathowenia oculata*.

Pielou's Evenness index and Simpson's Index of Dominance had a limited variation, whereas Margalef's Richness Index and Shannon-Wiener index presented slightly higher variation across the grab samples, with the Similarity Profile Routine test identifying three faunal groups. Echinodermata comprised most of the biomass. The colonial fauna was dominated by Cnidaria.

When comparing the species composition of the grab samples between the 2013 and 2023 surveys, 2013 samples presented higher values both regarding the total number of taxa and abundance of species. The annelid *P. jeffreysii* was the most abundant taxon both years. The compared multivariate statistical faunal analyses, presented three statistically distinct SIMPROF groups.

1. Introduction

1.1 Project Information

Ocean Infinity (OI) has been contracted by TotalEnergies E&P North Sea UK Ltd (TotalEnergies) to perform geophysical, environmental, and shallow geotechnical investigations for a floating wind turbine in the Culzean Field (UKCS 22/25a).

The Culzean field is located approximately 230 kilometres off the coast of Aberdeen, Scotland in the Central North Sea (Figure 1).

The site survey area covers a 2 km by 2 km area and encompasses the proposed location for a floating wind turbine and its associated moorings. The centre of the main survey area is 2.1 km west of the Culzean ULQ platform. A 2.3 km long power cable will connect the floating wind turbine to the Culzean CPF platform.

Project details are stated in Table 1.

Table 1 Project details.

Client:	TotalEnergies
Project	Total Energies PWT Site Survey
Ocean Infinity (OI) Project Number	104728
Survey Type	Geophysical, Geotechnical and Environmental Survey
Area	Central North Sea
Survey period	March/April 2023
Survey Vessels	M/V Deep Helder
OI Sweden Project Manager	Edward Lloyd Rich
Client Project Manager	Mark Grove Smith

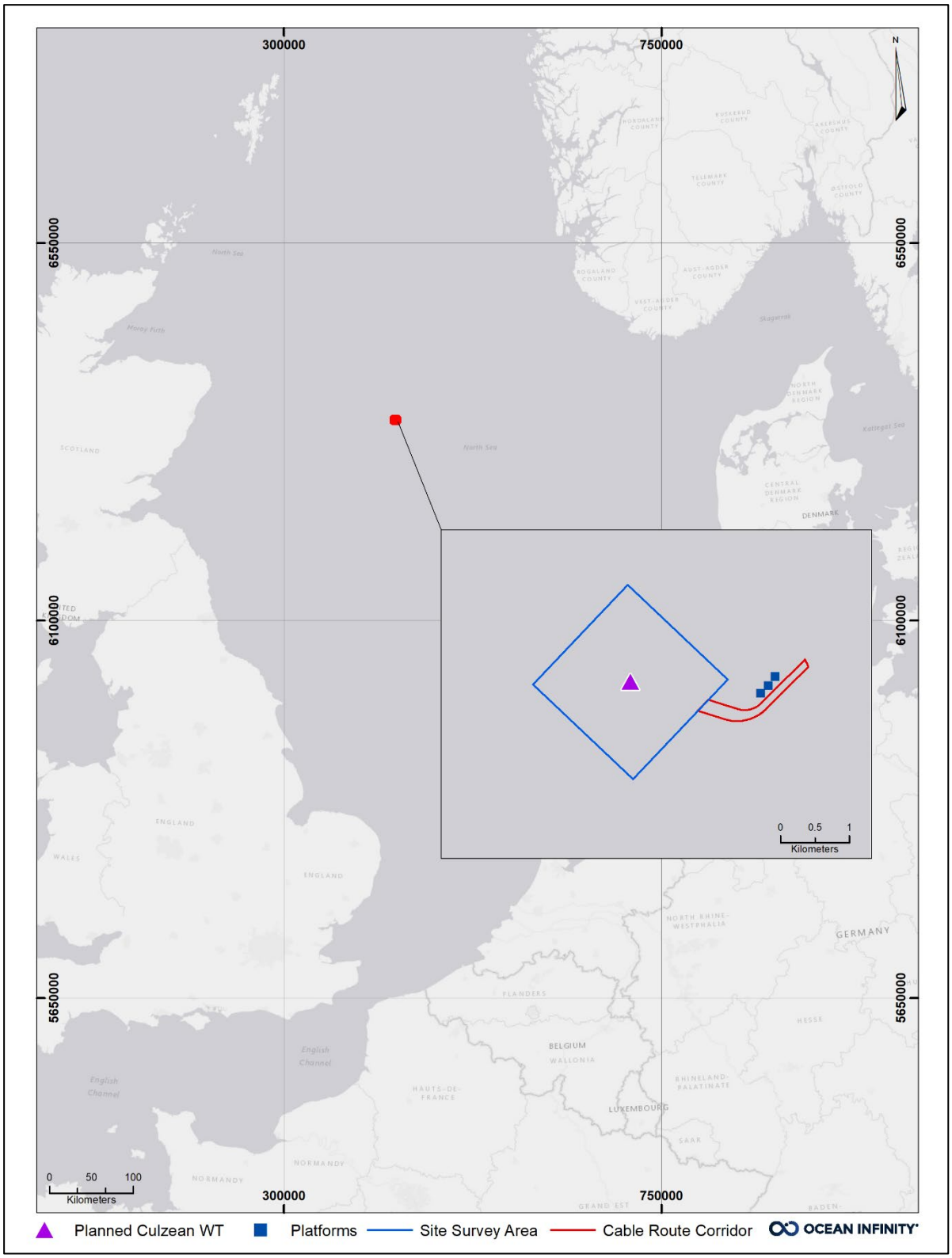


Figure 1 Overview of the survey area.

1.2 Scope of Work – Benthic and Environmental Survey

The aim of the Benthic and Environmental Survey was to collect data for Habitat Assessment and to provide an Environmental Baseline to allow for future determination of possible environmental impacts as a result of site developments.

The objectives of the Benthic and Environmental sampling and photography were to characterise the area and obtain baseline data that will:

- Support environmental applications
- Recognise any contamination or sensitive habitats already present in the area
- Provide a baseline set of observations that will allow any cumulative impact to be monitored by future surveys

The following summarises the Environmental Survey Scope of Work:

- Drop down video (DDV) for identifying epifauna and habitat
- Grab sampling for faunal taxonomy, biomass, particle size analysis (PSA) and contaminants
- Grab sampling for Environmental DNA (eDNA)
- Water sampling for contaminants and eDNA

1.2.1 Scope of Work – Geophysical Survey

The aim of the Geophysical Survey was to acquire data to evaluate the seabed and sub-seabed conditions, including potential associated hazards (geohazards or man-made hazards), affecting the future installation of a floating wind turbine and subsea cable.

The Geophysical Survey scope included the acquisition of multibeam echo sounder (MBES), side scan sonar (SSS), magnetometer (MAG), sub-bottom profiler (SBP) and Sparker data. The SBP was used to map variations in the top 3 to 5 m of sediment and the lower frequency Sparker system was used for detailed geological mapping of the uppermost 50 m of the seabed sediments.

1.2.2 Scope of Work – Geotechnical Survey

The shallow geotechnical survey included vibrocore (VC) and CPT investigations at the 3 planned mooring locations and at 500 m intervals along the proposed cable route to the Culzean CPF platform.

1.3 Purpose of Document

The purpose of this report is to present the Environmental Baseline Survey Methodology and results for the survey, including the results of the laboratory analyses of sediment and water samples. This report, together with overview charts and Geographic Information System (GIS) database, presents the environmental conditions at the TotalEnergies PWT site. The EBS survey aims to describe characteristics and conditions of a set of measurable parameters to provide a baseline for impact evaluation and mitigation.

All existing OI data from the Geophysical and Benthic and Environmental Survey are correlated to each other and compared against the existing background information and the publicly available environmental data, to strengthen the accuracy of the interpretations.

This EBS Report incorporates the habitat assessment information from the Habitat Assessment Report (104728-TOT-O1-SUR-REP-HABASRE, Rev B, 21/07/2023). The results of the subsequent laboratory analyses have not led to any changes to the habitat assessment for the PWT site.

2. Survey Parameters

2.1 Geodetic Datum and Grid Coordinate System

2.1.1 Geodetic Datum

Acquisition

Details of the geodetic datum used during acquisition are presented in Table 2. The survey data acquisition software QINSy had transformation parameters (Table 4) implemented to transform the online positions from WGS84 to the survey datum ED50.

The projection parameters will also be used in QINSy (Table 6).

Table 2 Geodetic datum parameters used during acquisition.

Horizontal Datum: WGS 84 (EPSG: 4326)	
Datum	World Geodetic System 1984 (6326)
Ellipsoid	World Geodetic System 1984 (7030)
Prime Meridian	Greenwich (8901)
Semi-major axis	6 378 137.000 m
Semi-minor axis	6 356 752.3142 m
Inverse Flattening (1/f)	298.257223563
Unit	International metre

Processing

The geodetic datum used during processing and reporting is presented in Table 3.

Table 3 Geodetic parameters used during processing.

Horizontal Datum: ED50	
Datum	ED50 (6230)
Ellipsoid	International 1924 (7022)
Prime Meridian	Greenwich (8901)
Semi-major Axis	6 378 388.000 m
Semi-minor Axis	6 356 911.946 m
Inverse Flattening (1/f)	297
Unit	International metre

2.1.2 Transformation Parameters

The transformation parameters used during the project are presented in Table 4. The transformation was used in the survey data acquisition software QINSy, although raw outputs from QINSy are in the WGS84 datum. Test coordinates for the transformation are presented in Table 5.

Table 4 Transformation parameters.

Datum Shift Parameters: From WGS84 To ED50 (Reversed EPSG 1311)	
Shift dX (m)	+89.5 m
Shift dY (m)	+93.8 m
Shift dZ (m)	+123.1 m
Rotation rX (“)	0 sec
Rotation rY (“)	0 sec
Rotation rZ (“)	0.156 sec
Scale Factor (ppm)	-1.2 ppm

Table 5 Test coordinate for datum shift.

UTM Zone	Datum	Easting (M)	Northing (M)	Latitude	Longitude
31N	WGS 84			55° 43' 17.274” N	004° 48' 06.789” E
	ED50	613272.04	6176763.30	55° 43' 19.677” N	004° 48' 11.870” E

2.1.3 Projection Parameters

The projection parameters used during survey are presented in Table 6.

Table 6 Projection parameters.

Projection Parameters	
Projection	UTM
Zone	31 N
Central Meridian	03° 00’ 00’’ E
Latitude origin	0
False Northing	0 m
False Easting	500 000 m
Central Scale Factor	0.9996
Units	metres

2.2 Vertical Datum

The vertical reference parameters used during survey are presented in Table 7.

Table 7 Vertical reference parameters.

Vertical Reference Parameters	
Vertical Reference	LAT
Height Model	VORF

2.3 Time Datum

Coordinated universal time (UTC) is used on all survey systems on board the vessel. The synchronisation of the vessel's onboard system is governed by the pulse per second (PPS) issued by the primary positioning system. All displays, overlays, logs and the daily progress reports (DPRs) are annotated in UTC.

3. Survey Vessel and Equipment

3.1 Survey Vessel

M/V Deep Helder

The M/V Deep Helder (Figure 2) is a Multi-Purpose Survey, Inspection, Maintenance and Repair (IMR) and Intervention Vessel, built in 2014. The vessel is equipped with a Dynamic Positioning 2 (DP2) system, an offshore crane, survey and Remotely Operated Vehicle (ROV) systems. Deployment of equipment can be done via a moon pool or an A-frame.

Figure 2 M/V Deep Helder.

3.2 Environmental Sampling Equipment

The Environmental Survey work at the Total Energies PWT site was carried out between the 3rd and 8th of April 2023 using the environmental sampling equipment listed in Table 8.

Table 8 M/V Deep Helder Benthic survey equipment.

Equipment	Name
Benthic Grabs	Dual Van Veen (2*0.1 m^2), Hamon Grab (0.1 m^2)
Drop Down Video (DDV) System	STR SeaSpyder
Sieve Table	0.5 mm and 5 mm Sieves and Sampling Table
Water Sampler	Rosette with Niskin Bottles (5*5L)
eDNA Sampler (water)	Vampire Sampler

Further information about the vessel, equipment set-up and performance can be found in the Operations Report 104728-TOT-OI-SUR-REP-FIELDOPS. Detailed information about the equipment calibrations and verifications can be found in the Mobilisation and Calibration (MAC) Report 104728-TOT-OI-MAC-REP-DEEPHELD.

4. Methodology

4.1 Offshore Field Methods

4.1.1 Survey Design

The number and locations of environmental sample sites were provided to OI by TotalEnergies prior to the start of the survey (Figure 3).

A Senior Benthic Ecologist reviewed the pre-selected sites based on the acquired geophysical data and preliminary geological interpretations, ensuring that the different habitats as interpreted from the Side Scan Sonar (SSS), Multibeam Echo Sounder (MBES), including normalised backscatter values, were ground-truthed. Final sampling sites were agreed upon in consultation with the Client prior to the commencement of the sample collection.

Before conducting grab sampling the Drop Down Video camera system (DDV) was deployed at each grab sample site. A minimum of 5 still images, with continuous video, were acquired at each grab sample site to collate information on epifaunal and faunal assemblage.

Grab sampling was planned at a total of eight (8) sites. At each of the eight (8) sites, three (3) replicate samples were to be allocated for taxonomic and biomass analyses, one (1) sample for Particle Size Analysis (PSA) and contaminants analyses. Additionally, a sub-sample for eDNA was to be collected from the third faunal replicate at each site.

Water sampling was planned at a total of eight (8) sites and was to be co-located with the planned grab sample sites. Water samples were to be collected at two (2) depths, close to the seabed and close to the surface, at each site. Samples were collected for both contaminants and eDNA analyses. All water samples were to be acquired on the up cast of the water sampler rosette.

A detailed account of selected sites and positions is presented in Appendix A.

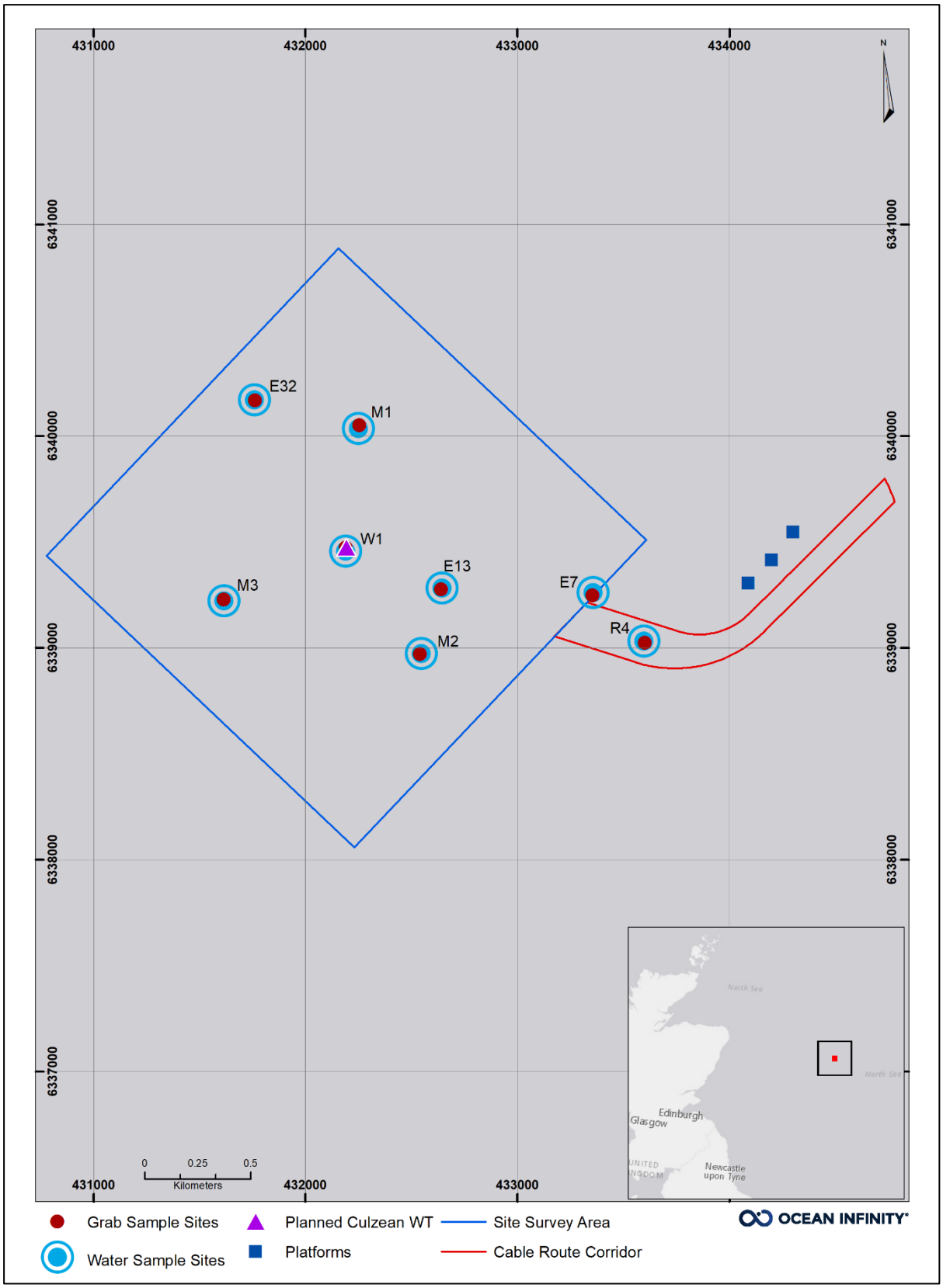


Figure 3 Overview of the proposed sampling design.

4.1.2 Drop Down Video

A SeaSpyder DDV system from STR (Figure 4 and Figure 5) was used to acquire still and video imagery at each sample site.

Figure 4 SeaSpyder DDV System.

Figure 5 SeaSpyder DDV example still photo.

Video transects of length 100 m were planned at each sampling site. These covered the centre location of the proposed grab sample site. Still photos were acquired every 25 m along the 100 m transects at positions +50 m, +25 m, 0 m, -25 m, and -50 m from the centre of the grab sample site. In total, a minimum of five (5) still photos were taken and more frequently if the seabed exhibited features of interest i.e., reefs and/or evidence of increased diversity.

The camera was positioned as close as possible to the pre-selected starting point using the vessel's dynamic positioning system during the survey. The camera frame was lowered onto the seabed to adjust the camera focus. When the camera focus was set, an initial photo was taken, before the video recording was initiated.

The camera frame was eased off the seabed and towed slowly at approximately 0.2 - 0.5 knots. It was positioned as close to the seabed as possible with an approximate altitude of 0.5 - 1 m. Altitude was determined by seabed topography and weather conditions.

A field log was maintained during photo and video collection at each site to provide each grab sample site with a preliminary description of findings. This included the drop number, position in relation to the proposed location, duration and a summary of the sediment type and conspicuous fauna observed. Anthropogenic impacts that were visible were also recorded including evidence of fishing activity, existing infrastructure, and marine debris.

Prior to grab sampling, an experienced Benthic Ecologist reviewed all video transect data onboard to confirm the presence/absence of any potentially sensitive habitats or features of conservation interest.

4.1.3 Faunal Grab Sampling and Sample Preservation

At each grab sample site, four (4) grab samples were acquired: three (3) samples for benthic faunal analyses and one (1) sample that was subsampled for Particle Size Analysis (PSA) and contaminant analyses. A sub-sample for eDNA was collected from the third faunal sample at each grab site.

Upon retrieval, samples were checked for adequate sample volume and samples covering less than 0.1 m^2 of bottom surface sediment were deemed unacceptable. No samples of less than 5 cm (7 cm in fine sediments) for the Dual Van Veen (DVV) or 7 litres for the Hamon grab (HG) were considered acceptable samples (Worsfold, Hall, & O'Reilly, 2010; Davies, et al., 2001). Samples that were not accepted were not included in any statistical analyses. During survey, only the DVV was deployed due to the nature of the seabed and lack of coarse substrates.

Sediment samples for eDNA were sampled according to the guidance specifications and materials provided by NatureMetrics.

A minimum of 40 g of sediment was collected at each site. Extreme care was taken to minimise any contamination of the samples. Each sample was stored in a sealed bag, in which the sediment was mixed to homogenise the sample.

All samples were photo-documented in-situ. Approved faunal samples were carefully sieved using seawater in a 5 mm over a 0.5 mm mesh sieve using gentle hose pressure. Sieve fractions were preserved with 96 % ethanol in separate jars, that were labelled with a unique label containing grab sample site ID and replicate number. A field log of sample positions including time, sediment type, and water depth was kept for later reference.

For further information regarding sample volume and the number of attempts see Appendix B.

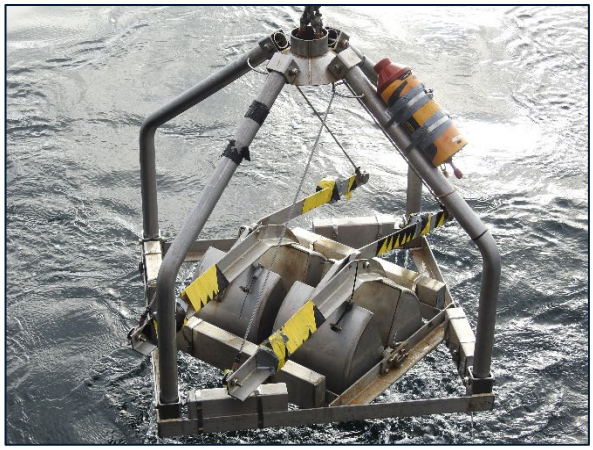


Figure 6 Dual Van Veen sampler.

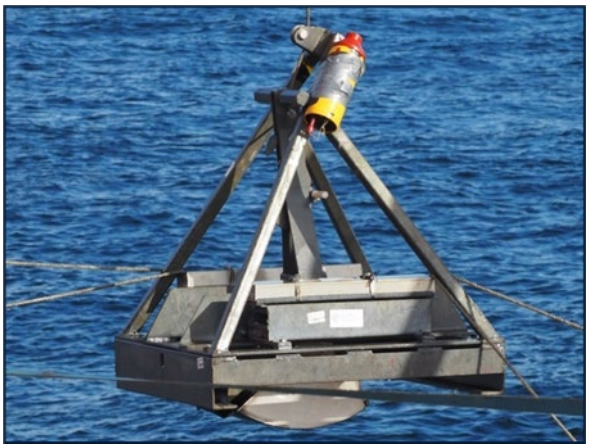


Figure 7 Hamon grab sampler.

4.1.4 Particle Size and Contaminants Grab Sampling

The primary grab sampler utilised for PSA and contaminants sampling was the Dual Van Veen (DVV) (Figure 6). The Hamon Grab (HG) was mobilised as a secondary grab to sample PSA and fauna in areas of coarse sediment should it be required. However, the Hamon Grab could not be used for contaminant samples (Figure 7).

Upon retrieval, samples were checked for adequate sample volume and samples covering less than 0.1 m^2 of bottom surface sediment were deemed unacceptable. No samples of less than 5 cm (7 cm in fine sediments) for the DVV or 2.7 litres for HG were considered acceptable PSA samples (Worsfold, Hall, & O'Reilly, 2010; Davies, et al., 2001). During survey, only the DVV was deployed due to the nature of the seabed and lack of coarse substrates.

Samples for metals, organics (Total Organic Matter (TOM) and Total Organic Carbon (TOC)), hydrocarbons (Total Hydrocarbons (THC) and Polycyclic Aromatic Hydrocarbon (PAH)), Polychlorinated Biphenyls (PCB), organotins (Monobutyltin (MBT), Dibutyltin (DBT) and Tributyltin (TBT)), pesticides and flame-retardants were taken from an undisturbed surface. The sediments were collected with a plastic spoon for metals and a metal spoon for organics, hydrocarbons, PCB, organotins, pesticides and flame-retardant to ensure minimal contamination risk. The grab sampler was cleaned between samples and sample sites.

A one (1) litre plastic container was used for the metal samples as well as PSA samples. For the contaminant analysis of organics, hydrocarbons, PCBs, organotins, pesticides and flame-retardants, a 250 ml tin container was used for storage. The different containers ensured that there was no outside contamination of the samples.

The sample containers were labelled with a unique sample site ID. The contaminants samples were stored frozen (-21°C) according to the analysing lab's recommendations before and during shipment.

A field log of sample positions including time, sediment type, and water depth was kept for later reference. Samples were photo-documented in situ. For further information regarding sample volume and the number of attempts, see Appendix B.

4.1.5 Contaminants and eDNA Water Sampling

Water sampling was performed using 5 L Niskin bottles attached to a Rosette sampler (Figure 8). The open bottles were lowered into the water and closed at pre-assigned depths. A CTD sensor was fitted to the Rosette sampler. There were five (5) Niskin bottles attached to the Rosette, two (2) for bottom water samples, and two (2) for top water samples with one bottle collected as a redundancy. The bottles were labelled according to the depth they triggered (Bottom and Top). Samples from the two (2) depths were collected from a single cast.

As the Rosette sampler was winched down to the bottom, the CTD sensor recorded depth, temperature, conductivity/salinity in the water column. When close to the seabed, a position fix was taken, and the rosette sampler was then winched upwards for recovery. During the upcast, the bottles closed at their pre-assigned depths to collect the water samples.

Once the sampler was recovered to the vessel, water for metals, THC, PAH and Total Suspended Solids (TSS) analyses was collected from the Bottom and Top bottles into pre-labelled 1 L amber glass jars and stored in the onboard freezer at -21°C.

Retrieved samples were assigned a sample number and their UTM coordinates, date and time of collection, and water depth were documented.

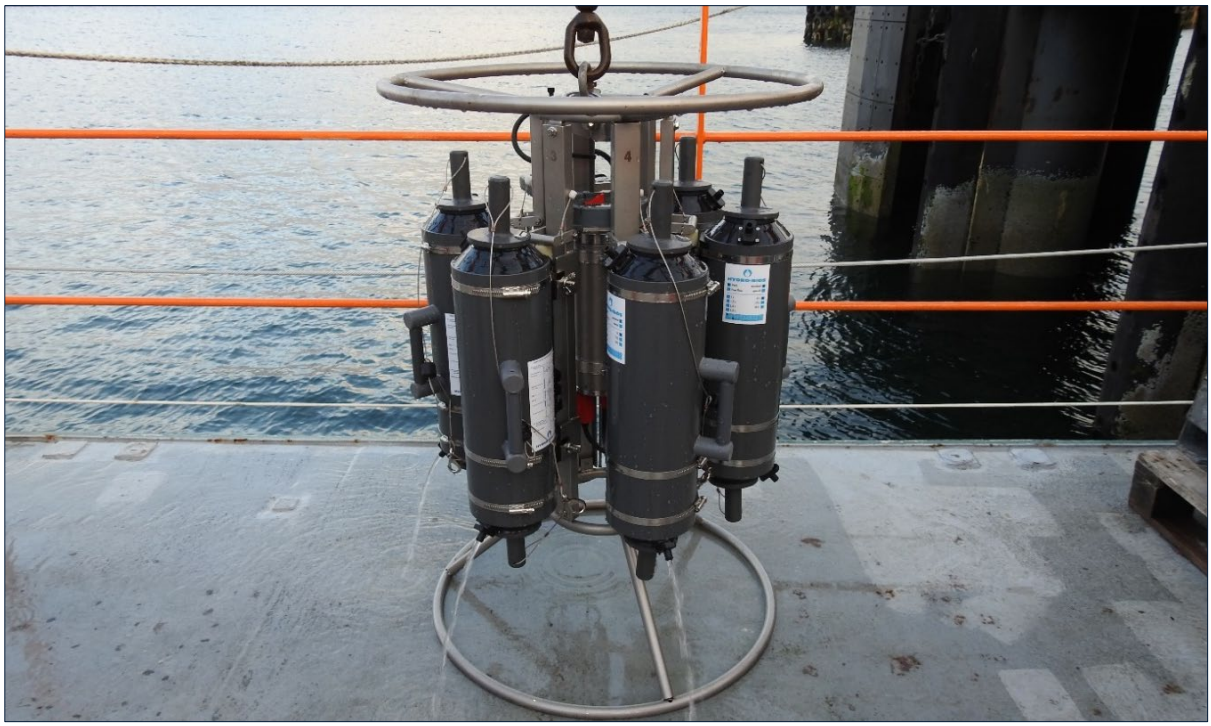


Figure 8 Rosette sampler equipped with Niskin bottles and CTD sensors.

Top and Bottom water were also filtered for eDNA using the Vampire sampling pump and following the guidance specifications provided by NatureMetrics. Water sampling for eDNA was carried out to determine the presence of fish, vertebrates, marine mammals, invertebrates and eukaryotes.

Care was taken to minimize contamination by performing the eDNA sampling in a dedicated area on the back deck. Separate eDNA sampling kits, consisting of Nitrile gloves, enclosed filters, a syringe filled with preservative solution, silicone hose, specimen bag, datasheet and disinfectant wipe were used for each sample/water body to avoid cross-contamination.

The silicone hose was inserted at the top of the Niskin with an enclosed filter (0.8 µm pore size, polyethersulfone) attached to the hose adapter. Once the entire 5 L Niskin bottle was filtered through, the filter was carefully detached from the hose.

A syringe filled with 1.5 mL DNA preservative solution was twisted onto the filter and the entire preservative solution was slowly added into the filter. The filter was then detached from the syringe and sealed with a separate Luer Lock cap.

Filters were stored in a resealable specimen bag, which was in turn placed into the eDNA kit bags alongside the datasheet noting the Sample ID and depth from which it was taken. Samples were then stored in the onboard freezer at -21°C.

4.2 Laboratory Methods

4.2.1 Particle Size Analysis

The Particle Size Analysis (PSA) was conducted by UK-based company Kenneth Pye Associates Limited. (KPAL).

Up to one litre of sediment from each sample site was analysed to detail the different particle fraction components with a combination of sieving and sedimentation methods.

PSA samples were analysed in accordance with the National Marine Biological Analytical Quality Control (NMBAQC) Guidelines for Particle Size Analysis (PSA) for Supporting Biological Analysis (Mason, 2022) to provide data over the complete particle size range allowing determination of the gravel to sand plus mud ratio. KPAL also hold MMO accreditation for particle size analysis.

Samples were wet separated at 2.0 mm. The >2.0 mm fraction, where present, was analysed using nested British Standard sieves at 'half' phi intervals. The sub-2.0 mm fraction was analysed via laser diffraction (size range 0.04 µm to 2.0 mm). The laser and sieve data were mathematically merged and calculations of particle size summary parameters (percentages of mud, sand, and gravel, silt/clay ratio, sand/mud ratio, median, mean, d10, d90, etc.) were calculated using GRADISTAT software (Blott & Pye, 2001).

The particle sizes were grouped into five large textural groups for description purposes (Table 9). The samples were described according to British standard 1377 (British Standard, 2010) and BGS-modified Folk classification (Long, 2006).

Table 9 British standard (2010) sieve sizes.

Classification	Particle Size Intervals (Diameter mm)	Grouped Classification
Boulder	>75	Boulders/cobbles
Cobble	75 – 64	
Coarse Gravel	64 – 20	Gravel
Medium Gravel	20 – 6	
Fine Gravel	6 – 2	
Coarse Sand	2 – 0.6	Sand
Medium Sand	0.6 – 0.2	
Fine Sand	0.2 – 0.063	
Coarse Silt	0.063 – 0.02	Silt
Medium Silt	0.02 – 0.006	
Fine Silt	0.006 – 0.002	
Clay	<0.002	Clay

4.2.2 Sediment Chemical and Contaminant Analyses

The sediment chemical and contaminant analyses were conducted by the UK-based company SOCOTEC. The different compounds that were analysed along with detection limits are stated in Table 10. The analyses included concentrations/contents of Total Organic Carbon (TOC), Total Organic Matter (TOM), metals, organotins (MBT, DBT, TBT), Total Hydrocarbon Content (THC), Polyaromatic Hydrocarbons (PAH), Polychlorinated Biphenyls (PCB), Organochlorine Pesticides (OCP), and Brominated Flame Retardants (PBDE).

Table 10 Marine sediment chemical and contaminant analyses.

Test Marine Sediment Contaminant Analyses	Method	Accreditation	Method Reporting Limit, PPM Unless Stated Otherwise
Particles Size Analysis and Distribution (PSA, PSD)	NMBAQC	NMBAQC	N/A
Total Organic Carbon	Sulphurous acid/combustion at 1600°`C/NDIR	UKAS 17025	0.02 %
Total Organic Matter by LOI	Combustion at 450°C	Not accredited	0.20 %
Moisture content	Oven drying @ 120°C	UKAS 17025	0.2 %
Metals suite: As(0.5), Cd(0.04), Cr(0.5), Co(0.5), Cu(0.5), Pb(0.5), Hg(0.01), Mn(0.5), Mo(0.5), Ni(0.5), Se(1), Sb(0.1), Sn(0.5), V(0.5), Zn(2)	Aqua Regia extraction and ICPMS	UKAS 17025 except Mo, Sb, Se, Sn, V	Limits of detection in mg/kg within parentheses
Metals suite: Al(10), Ba(0.5), Be(0.1), Fe(36), P(4), Ti(6)	Aqua Regia extraction and ICPOES	UKAS 17025 except Mo, Sb, Se, Sn, V	Limits of detection in mg/kg within parentheses
Organotins: MBT(0.001), DBT(0.001), TBT(0.001)	ASC/SOP/301	UKAS/MMO	Limits of detection within parentheses.
THC (inc. saturates)	Solvent extraction & GC-FID	Not accredited	100 µg/kg (Total) 1 µg/kg (Individual alkanes)
PAH	Solvent extraction & GC-MS	UKAS 17025	1 µg/kg
PCB	Solvent extraction & GC-MS	UKAS/MMO	0.00008 mg/kg
OCP	Solvent extraction & GC-MS	UKAS/MMO	0.0001 mg/kg
PBDE	Solvent extraction & GC-MS	Not accredited	0.05 µg/kg (BDE209 0.1 µg/kg)

4.2.3 Water Chemical and Contaminant Analyses

The water chemical and contaminant analyses were conducted by the UK-based company SOCOTEC. The different compounds that were analysed along with detection limits are stated in Table 11. The analyses included concentrations/contents of Total Suspended Solids (TSS), metals, Total Hydrocarbon Content (THC), and Polyaromatic Hydrocarbons (PAH).

Table 11 Water sample analyses.

Test Marine Sediment Contaminant Analyses	Method	Accreditation	Method Reporting Limit, PPM Unless Stated Otherwise
Total Suspended Solids	Determination by gravimetry	Not accredited, WSLM10	5 mg/l
THC (inc. saturates)	Solvent extraction & GC-FID	Not accredited, ASC/SOP/306	100 µg/kg (Total) 1 µg/kg (Individual alkanes)
PAH (DTI 2-6 ring aromatics + EPA 16)	Solvent extraction & GC-MS	Not accredited, ASC/SOP/304	1 µg/l
Metal suite: As(0.001), Cd(0.00002), Cr(0.001), Co(0.001), Cu(0.001), Pb(0.001), Mn(0.002), Hg(0.00003), Mo(0.001), Ni(0.001), Sb(0.001), Se(0.001), Sn(0.001), V(0.001), Zn(0.002)	ICPMS	Not accredited	mg/l
Metal suite: Al(0.01), Ba(0.01), Be(0.01), Fe(0.01), SO_4 as Sulphate(3), Ti(0.01)	ICPOES	Not accredited	mg/l

4.2.4 Biological Analyses

The macrofaunal analyses were conducted by the UK-based company APEM Ltd. Analyses were conducted in accordance with the NMBAQC scheme (Worsfold, Hall, & O'Reilly, 2010), and all the samples were quality controlled.

The macrofaunal grab samples were sorted from sediment residue, and the fauna was identified to the lowest taxonomic level possible, mainly species, counted and weighted. When the species could not be identified, the specimen was grouped into the nearest identifiable taxon of a higher rank (i.e. genus, family, or order etc). If the species remained unknown but clearly separated from any other found specimen within the same genus, it was assigned a “Type” denomination, i.e. Type A or Type B. Juveniles were marked with the qualifier “juvenile”, and included in the statistical analyses. Biomass was measured for each taxon within each sample, to the nearest 0.1 mg. All macrofaunal analyses followed the NMBAQC scheme.

4.2.5 Environmental DNA Analyses

The environmental DNA anlyses were conducted by the UK-based company NatureMetrics Ltd. A total of (5) assays were targeted for the water samples; Marine Water Vertebrates (12S gene), Marine Water Eukaryotes (18S gene), Marine Water Invertebrates (CO1 gene), Marine Water Fish (12S gene) and the Mammals (12S gene). A total of two (2) assays were targeted for the sediment samples; Marine Invertebrates (18S gene) and Bacteria (16S gene).

DNA from each sample was extracted using an extraction kit with a protocol modified to maximise the DNA yields. An extraction blank was also processed alongside each batch of samples. The DNA extracts were amplified with the Polymerase Chain Reaction (PCR) using primers that target a specific region of a barcode gene (i.e., test assay). The PCR reaction was repeated many times on each sample to maximise the detection of target species.

PCR replicates for each sample were pooled and purified, and sequencing adapters were added that uniquely identify DNA sequences from each sample.

DNA sequence data were processed using a custom bioinformatics channel for quality filtering, Operational Taxonomic Unit (OUT) clustering, and taxonomic assignment. Taxonomic assignments were made for each DNA sequence by similarity matching with reference databases relevant for the gene being targeted.

NatureMetrics uses NCBI nucleotide (GenBank), the Barcode of Life Database (BOLD), SILVA and the NatureMetrics Database of Life. Results from all searches are combined and assignments made to the lowest possible taxonomic level where there are consistent matches.

Taxa identified from eDNA samples are referred to as an OTU, standing for Operational Taxonomic Unit. Thus, OTU is a unique DNA sequence found in a sample and is roughly equivalent to a species. If an OTU could not be assigned to a species, the sequence was assigned to the lowest taxonomic level possible (i.e., genus of family). For defining different OTUs, a threshold was applied to determine maximum dissimilarity allowed for clustering OTUs as the same taxa (Juhel, et al., 2020).

4.3 Data Analysis

4.3.1 Visual Data Analysis

The stills were analysed to identify species and species densities, including seabed substrate. The video recordings were used to aid in the assessment of features and extent of habitats. Particular attention was paid to the elevation of habitats above ambient seabed level, together with their spatial extent, percentage biogenic cover, and patchiness, as these are key criteria for evaluating areas of conservation interest.

Quantitative methods were used for the identification of biota in still photographs, with all the data presented as individuals per square metre and percentage cover of colonial species. Stills were analysed in AutoCAD Map 3D, where visual epibenthic fauna was counted, and results were summarised in a log containing scientific name, position, date, time, and stills ID. Qualitative methods were used for the identification of biota in the video recordings.

4.3.2 Acoustic Data Analysis

Multibeam echo sounder (MBES) and side scan sonar (SSS) data from the geophysical survey together with the epifaunal composition from visual ground truthing data was used to determine the extent of habitats (Figure 9 and Figure 10). Video recordings, as well as field descriptions of grab samples, were also used to assign habitat classifications and delineate habitat boundaries.

The acoustic character of the SSS data has been correlated to the general seabed morphology (MBES DTM and backscatter) and used to help classify the seabed sediments. The SSS data is presented as a greyscale image mosaic where the darker grey to black colours indicate higher intensity sonar returns corresponding to coarser sediments, and lighter grey colours indicate lower intensity sonar returns corresponding to finer sediments.

Once all data has been reviewed, the different data sets are aligned, and habitat classifications are extrapolated based on textural similarity, reflectivity, and topographical features. The different datasets are combined to strengthen the accuracy of interpretations.

Extrapolating a large area based on a low number of samples may lead to a lower hierarchic biotope level for that area, compared to the actual biotope level for the samples within the habitat. If two different habitats are classified at two different sites/transects in what appears to be a similar habitat, based on the geophysical interpretation, this may lead to the assignment of a matrix of the two habitats. These compromises are reviewed individually. A smaller homogenous and distinctive area can be assigned to a higher hierarchic level compared to a larger and more variable area containing several different biotopes. The result of the habitat classification is presented in the results section and GIS charts.

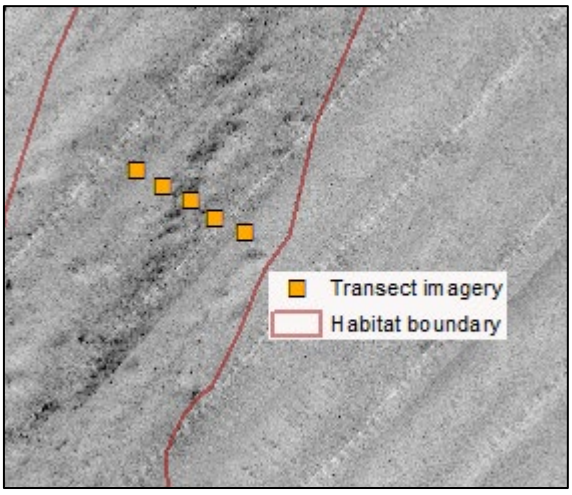


Figure 9 Example of side scan sonar image of furrows and areas of finer sediments.

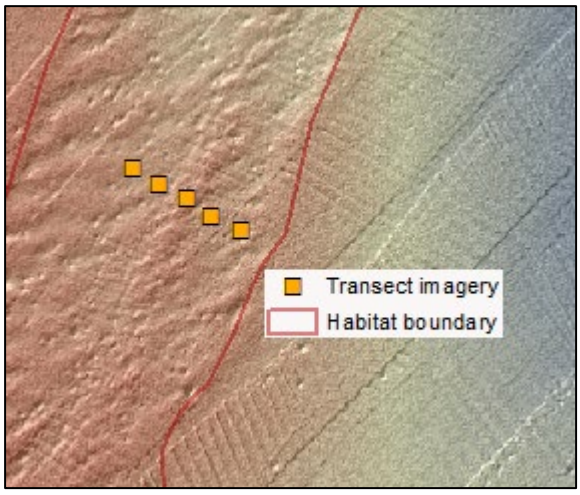


Figure 10 Corresponding bathymetric image of furrows and areas of finer sediments.

4.3.3 Backscatter

The use of backscatter data to assist habitat interpretations and mapping is a methodology under development, increasingly used in these types of analyses (Lurton and Lamarche, 2015). Backscatter Normalised Values are a measurement of the MBES echo that is scattered in the direction of the transducer. This data records the intensity, in decibels (dB), of the echo that returns to the transducer after the emitted pulse interacts with the seabed. The backscatter amplitude varies with several factors such as frequency, beam pattern, range and losses due to absorption and spreading, angle with the seabed as well as sediment type and other factors.

The raw data were processed with the Fledermaus (FMGT) software, which applied various standard normalisations to the data to compensate for how the intensity varied across the swath, producing a grayscale floating-point raster image gridded at 1 m, where each gridded cell contains a measured intensity value.

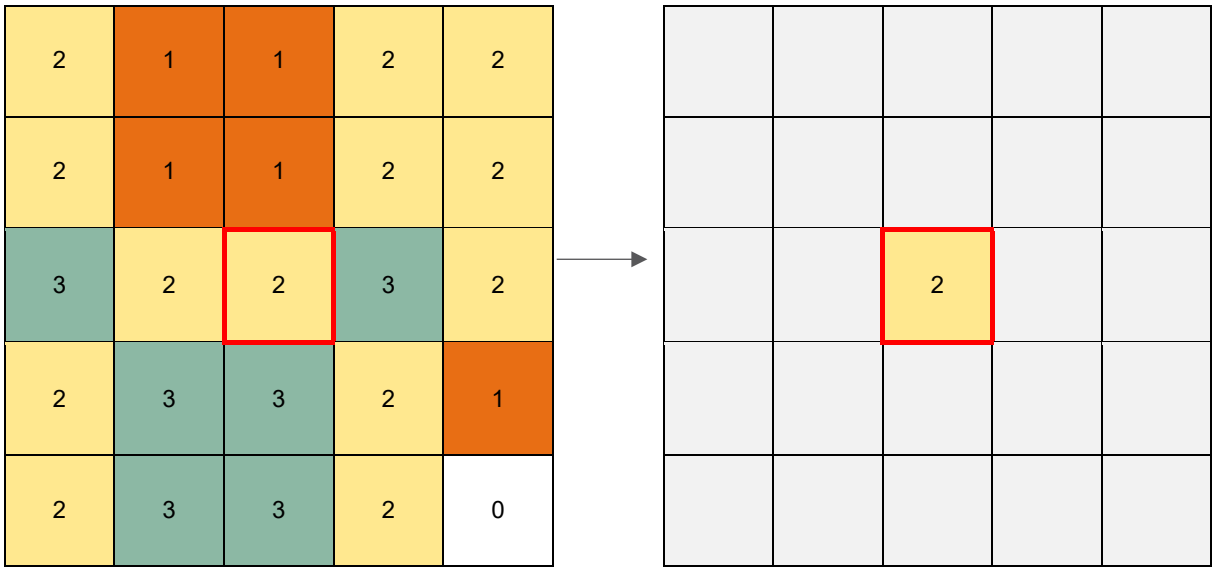
The values ranged from 9.7 to -61.5 dB, with the higher values (9.7) indicating harder/coarser seabed, and the lower values (-61.5) indicating softer/finer seabed.

The raster image extent was further clipped to align with the survey boundaries to remove outlier values often associated with line turn and/ or end of the survey line. The values post-clipping ranged from -2.6 to -55.8 dB.

Backscatter values varied across a small spatial scale, making interpretations on a larger scale challenging due to the small-scale variation. To mitigate this, the Focal Statistics tool in ArcGIS was used to reduce the variation in the values. The backscatter raster data was imported into ArcGIS and a raster image was created based on the measured intensity values for each cell and plotted.

Within ArcGIS, a secondary raster image was created through the calculation of the cell value with the Focal Statistics tool. The tool calculates a new value for each input cell based on the neighbouring cell values. The new value output was based on the average value of the neighbouring cells in a 10 x 10 m (10 x 10 cells) square area with the target cell included (Table 12). The new cells maintained the original cell size of 1 x 1 m.

Table 12 Focal Statistics settings.



Ground-truthing data (imagery) together with geophysical data were used to align the backscatter reflectivity intervals based on the trends interpreted, with regards to substrate and habitats (Lurton and Lamarche, 2015). However, some limitations in interpretation should be considered as the directionality of the survey lines varied and the changes in elevation and angle of the seabed affect the amount of reflected sound, resulting in the fact that overlapping lines could show different noise signatures. This was partially mitigated by using the Focal Statistics tool in ArcGIS, as the interpolation used in the tool averages out the overestimated and underestimated values from the backscatter.

Outlier values from the outermost ranges from the data sets were naturally excluded as the grouping of the intervals was set and these are detailed in Table 13.

Table 13 Backscatter Intensity colour schema for each area (intensity is presented in dB).

Datasets	Colour Bars and Classes (dB)	Outliers (dB)
RAW	-61.5 to -29.96 -29.95 to -27.45 -27.44 to -25.21 -25.2 to -22.7 -22.69 to 9.68	9.68 to - 2.6; -61.5 to -55.8
Site Survey Area and Cable Route Corridor	-29 to -25.6 -25.59 to -24.7 -24.69 to -20.8	-40 to -29; -20 to -9

4.3.4 Particle Size Analyses

Sediment particle size distribution statistics for each sample were calculated from the raw data by the laboratory. Main sediment fractions and percentages were plotted to examine sediment composition changes across the survey area and used to aid the habitat assessment. Multivariate analyses were undertaken on the PSA data set, to identify patterns in the sediment distribution.

Analyses included hierarchical clustering employing the Euclidean distance resemblance matrix, SIMPROF analysis and principal component analysis (PCA). The dataset was normalised prior to the analyses being performed.

PSA results were analysed using the Plymouth Routines in Multivariate Ecological Research (PRIMER) v7.0 statistical package (Clarke & Gorley, PRIMER v7: User Manual/Tutorial. Plymouth: PRIMER-E., 2015) and normalised before being included in any statistical analysis. Data for the percentage composition was analysed in a cluster analysis using the Euclidean distance. A Principal Component Analysis (PCA) was undertaken on the sediment data set to identify spatial patterns and relationships between variables.

Detailed results for each grab sample site are provided in Appendix E.

4.3.5 Sediment Chemical and Contaminant Analyses

Environmental Quality Standards (EQS) for metals and hydrocarbons in sediments are not yet developed for UK waters. However, in 2001, the United Kingdom Offshore Operators Association (UKOOA) published a report which established a series of sediment quality guidelines based on the compilation and analysis of datasets from benthic surveys carried out in the British North Sea (UKOOA, 2001). The UKOOA report used data collected between 1975-95 at least 5km from any oil and gas platform, setting out a series of "background" levels for a variety of parameters. Although this data is relatively old, it is still considered relevant for comparing current data with area specific "background" values. For the current environmental survey, the 50th and 95th percentiles for the Central North Sea (CNS), as well as the mean values for sediments dominated by fine sands in the CNS were available for comparison of metals, Total Organic Matter (TOM) and hydrocarbons.

The data for the current survey has been further compared to other background data available. OSPAR's Environmental Assessment Criteria (EACs) are under development, and OSPAR uses "Effect range-low" (ERL) values for sediment assessment of metals and PAH, where EACs are not available. The ERL value indicates a concentration below which adverse effects on organisms are rarely observed (OSPAR, 2011).

Assessment criteria developed by the Canadian Council of Ministers of the Environment (CCME) together with the Centre for Environment, Fisheries and Aquaculture Science (CEFAS) guideline action levels for disposal of dredged material have also been considered common practice to use.

The Canadian sediment quality guidelines include two values as assessment criteria, the Interim Sediment Quality Guidelines (ISQG) and Probable Effect Level (PEL). The ISQG are threshold levels that are set to protect all aquatic life during an indefinite period of exposure, and for values above PEL, adverse effects are expected to occur frequently (CCME, 1995; CCME, 2001). For concentrations between the ISQG and PEL, adverse effects occur occasionally.

CEFAS Action Levels are used as a part of assessing the contamination status in dredged material, where material below Action Level 1 (AL1) generally indicates that contaminant levels are of no concern, while contaminant levels above Action Level 2 (AL2) generally are considered unsuitable for disposal in the sea (CEFAS, 2020)

Condition classes established by the Norwegian Environmental Agency (NEA) for contamination in coastal sediments (NEA, 2016, revised 2020) were used in the absence of UKOOA background data. The NEA reference levels are to be used with caution, as they are intended to fine sediment and adapted to Norwegian conditions. This system uses five classes, class 1 – Background levels, class 2 – Good, with no known toxic effects, class 3 – Moderate, with chronic effects at long-term exposure, class 4 – Poor, with acute toxic effects at short-term exposure and class 5 – Very Poor, with extensive toxic effects.

Dutch intervention levels for aquatic sediments can also offer a useful comparison. Concentrations above the Dutch intervention values represent a serious level of contamination, where functional properties of the sediment are seriously impaired or threatened (Hin, Osté, & Schmidt, 2010).

4.3.6 Water Chemical and Contaminant Analyses

The Scottish Environment Protection Agency (SEPA) has produced Environmental Quality Standards (EQS) and Standards for Discharges to Surface Waters based on the latest scientific understanding of the UK Technical Advisory Group (UKTAG) for the Water Framework Directive (WFD) (SEPA, 2018). The guidelines include both UK and EU standards for a variety of water pollutants. Concentrations of pollutants below the environmental quality standards are considered not to have adverse effects on ecosystems.

EU standards include an Annual Average value (AA), as well as a Maximum Allowable Concentration (MAC), whereas the UK standards include an AA and a 95th percentile value. However, not all four reference levels are available for every contaminant analysed in the current survey.

Some caution must be taken when using these standards for comparison, as they are based on point data which is likely to show certain geographical and temporal variability which is not reflected on the single proposed EQS value. It is also worth noting that the standards refer to marine surface water from transitional and coastal waters, which presents some limitations when comparing to samples taken in open water. The EQS have been used in the current survey to provide context to the levels of heavy and trace metal contamination present.

4.3.7 Univariate Statistical Analyses

Univariate analyses were undertaken using PRIMER v7.0 statistical package (Clarke & Gorley, PRIMER v7: User Manual/Tutorial. Plymouth: PRIMER-E., 2015). Univariate analyses included the primary variables, the number of taxa (S) and abundance (N) together with Margalef's index of Richness (D), Pielou's index of Evenness (J), Shannon- Wiener index of Diversity (H') and the Simpson's index of Dominance (1-λ) which are summarised in Table 14.

Table 14 Univariate statistical analyses.

Analyses	Parameters	Formula	Description
No. of Taxa (S)	Species richness	S	The number of species (taxa) in each sample.
No. of Individuals (N)	Abundance	N	The number of individuals in each sample.
Margalef's Index of Richness (D)	Richness	D = (S-1) / ln(N)	A measure of the number of species present for a given number of individuals
Shannon-Wiener Index of Diversity (H')	Diversity	H' = Σi Pi ln(Pi)	The diversity index incorporates both species richness and equitability, where Pi is the proportion of the total count arising from the/th species. A lower value equals a high chance that all abundance is concentrated to one species.
Pielou's Index of Evenness (J)	Evenness	J = H' / ln (s)	Measures how evenly individuals are distributed between species. Gives a value between 0 to 1, where a higher value equals a more even community.
Simpson's Index of Dominance (1-λ)	Dominance	λ = (Σ pi2)	Dominance index between 0 – 1 where 0 corresponds to assemblages whose total abundance is dominated by one or very few of the species present and 1 represents a more evenly species distribution.

4.3.8 Multivariate Statistical Analyses

Multivariate analysis was undertaken using PRIMER v7.0 statistical package (Clarke & Gorley, PRIMER v7: User Manual/Tutorial. Plymouth: PRIMER-E., 2015). The statistical analyses were based on macrofaunal data derived from the taxonomic analyses of a single replicate from each grab sample site (only one (1) replicate was analysed if two (2) replicates were collected at a given site). Grab samples with insufficient sample volume were excluded from the statistical analyses. Abundances were expressed as the number of individuals per 0.1 m^2.

The macrofaunal organisms were separated into non-colonial and sessile colonial fauna. Colonial fauna refers to sessile (attached) epifauna which are not counted as individuals or number of colonies but are annotated qualitatively as Present (P). The Taxonomic Discrimination Protocol (TDP) produced by NMBAQC provides guidelines for processing marine macrobenthic invertebrate samples and gives guidance, for the purposes of standardising the identification within the industry, on how major taxa should be treated.

Colonial fauna was not quantified in the laboratory analysis and was treated separately in the statistical analyses. All colonial fauna was also considered epifauna. Juvenile (JUV) taxa were included and foraminiferans were excluded from the datasets. The faunal composition was linked to physical variables such as depth and sediment composition.

Square root transformation was applied to the non-colonial enumerated fauna datasets before calculating the Bray-Curtis similarity measures. This transformation was made to prevent abundant species from influencing the Bray-Curtis similarity index measures, excessively and to take the rarer species into account (Clarke & Warwick, 2001).

The macrofaunal laboratory results were compared for faunal composition between sampling sites. Site-related differences in community structure were examined in a clustering analysis using Euclidean distance and the Bray-Curtis similarity coefficient. This method is common when measuring ecological distance in biological sample data.

Multi-Dimensional Scaling (MDS) analysis was undertaken in conjunction with the cluster analysis. The MDS analysis is based on the same similarity matrix as that of the cluster analysis and produces a multidimensional ordination of samples.

The number of restarts was set to 999 with a minimum stress of 0.01. The MDS plot visualises the relative (dis)similarities between samples; the closer they are the more similar the species composition between the samples. The degree to which these relations can be satisfactorily represented is expressed as the stress coefficient statistic, low values (<0.1) indicate a good ordination with low probabilities of misleading interpretation. Generally, the higher the stress, the greater the likelihood of non-optimal solutions (Clarke & Warwick, 2001).

A Similarity profiling algorithm (SIMPROF) test was run in conjunction with the cluster analysis, which was used to identify significantly different naturally occurring groups among grab samples.

The results are presented in the cluster dendrogram as black lines indicating significant statistical differences. Red lines represent samples that are not statistically different. The SIMPROF is based on taxa, and the abundance of each taxon in each sample, thus different SIMPROF groups may host similar fauna which differ in abundance.

A Similarity Percentage Analysis (SIMPER) was undertaken following the cluster analysis. SIMPER examines variable relations to each other and presents the species' contributions and similarities within and among groups.

PSA data was analysed in PRIMER and normalised before being included in any statistical analysis. Data for the percentage composition was analysed in a cluster analysis using the Euclidean distance.

The relationship between the physical and biological data was tested using the BIOENV method, with Spearman rank correlations, in the BEST procedure in PRIMER v.7. This analysis identifies variables that exert the greatest influence on the spatial distribution of the input datasets. Prior to the BEST analyses species abundance data were square root transformed and the physical variables were normalised.

4.4 Habitat Classification

Habitats were classified to the lowest hierarchic level possible and based on interpretations that combine biotope descriptions of species abundance, diversity, depth and seabed features from grab samples, video and photos acquired at each sample site.

The classification of the communities of the different habitat types was based on physical characteristics such as benthic geology, wave exposure, tidal currents, temperature, and salinity together with key species present in the area. In addition, normalized backscatter data from MBES was used to delineate habitats in areas of homogenous sediments.

4.4.1 EUNIS

Habitats within this report were classified to the lowest hierarchic level possible and based on interpretations of the combined geophysical data and ground truthing imagery. The EUNIS classification (EEA, 2022) is divided into six hierarchic levels, Figure 11.

At Level 1, the habitats are divided into marine, coastal and terrestrial habitats. The marine habitats are further divided into three separate categories: benthic, pelagic and ice-associated habitats.

At Level 2, the biological zone and presence/ absence of rock are classification criteria, and at Level 3, the classifications are separated into marine regions.

Level 4 gives references to specific taxa. For rocky substrates, the major epifauna is used, and for softer substrates, the classification relies on both zonation and physical attributes. Further, at Level 5, the classification is based on both the physical and biological characteristics of the habitats and classes are defined with both infauna and epifauna on different substrates. At the highest level, level 6, the different characterising taxa are associated with different environmental characteristics of the habitat.

If two different habitat classifications within what appears to be a similar habitat are identified, without any apparent differences in the interpreted geophysical data, a low number of samples/ transects may lead to the assignment of a matrix of two habitats. Extrapolating a large area based on a low number of samples may lead to a lower hierarchic biotope level for that area, than the actual biotope level for a singular sample within the habitat.

These compromises are reviewed individually. A smaller homogenous and distinctive area can be assigned to a higher hierarchic level compared to a larger and more variable area containing several different biotopes. The result of the habitat classification is presented in the results section and GIS charts.

L1	(M) Marine Habitats
L2	(MC4) Circalittoral mixed sediment
L3	(MC42) Atlantic circalittoral mixed sediment
L4	(MC421) Faunal communities of Atlantic circalittoral mixed sediment
L5	(MC4211) *Cerianthus lloydii* and other burrowing anemones in circalittoral muddy mixed sediment
L6	(MC42111) *Cerianthus lloydii* with *Nemertesia* spp. and other hydroids in circalittoral muddy mixed sediment

Figure 11 Example of 2022 EUNIS Hierarchy.

4.5 Habitats and Species Assessments Criteria

For the assessment and classification of potential areas and/or species of conservation importance, the following legislation and guidelines have been applied when relevant.

The European Commission (EC) Habitat Directive specifies the European nature conservation policy (EUR 28, 2013). Species and habitats of special interest for conservation are specified in the different annexes to the directive. Annex I states the habitats of special conservation interest and Annex II states the species of special conservation interest. Among the habitats specified in Annex I are the “Reefs” (code 1170). Reefs can be of biogenic, e.g. mussel beds or corals, or geogenic origin, e.g. stony areas with epifauna.

The Oslo and Paris Conventions for the protection of the marine environment of the North-East Atlantic (OSPAR), list protected species and habitats, as well as sensitive habitats and species in need of protection in the North-East Atlantic (OSPAR, 2008). This serves also as a complement to the EC Habitats Directive.

The species and habitats found in this survey were compared to the list of Scottish Priority Marine Features (PMF) (Tyler-Walters, et al., 2016) that further defines the habitats and species which are considered to be marine nature conservation priorities in Scottish waters.

In addition to the above-mentioned policies and guidelines the Scottish Biodiversity List (SBL) identifying the species and habitats which are the highest priority for biodiversity conservation in Scotland was also consulted (Scottish Biodiversity Forum, 2012).

In the Habitat Directive's interpretation manual (EUR 28, 2013) reefs are explained as follows:

"Reefs can be either biogenic concretions or of geogenic origin. They are hard compact substrates on solid and soft bottoms, which arise from the sea floor in the sublittoral and littoral zone. Reefs may support a zonation of benthic communities of algae and animal species as well as concretions and corallogenic concretions."

The distinction between what *is* and what *is not* a "reef" is not so precise and is generally referred to as "reefiness". This is particularly relevant in the case of the tube-building polychaete, *Sabellaria spinulosa* and areas of cobbles and boulders (stony reef).

If for example *S. spinulosa* or the horse mussel, *Modiolus modiolus,* is found in an area it does not automatically qualify as a "reef", Annex I habitat or a potential Annex I habitat. Therefore, a scoring/assessment system based on a series of physical, biological and spatial characteristics is used to assess the degree of "reefiness".

A method to assess 'reefiness' was presented by Gubbay (2007) and involves the quantification of three separate criteria: elevation (average tube height in cm), Area (m^2) and patchiness (percentage cover) as presented in Table 15. A similar assessment matrix for stony reefs by Irving (2009) is presented in Table 16.

Table 15 Proposed chart for Sabellaria spinulosa reef identification (Gubbay, 2007).

Characteristic	Not A Reef	"Reefiness"		
		Low	Medium	High
Elevation (cm) (average tube height)	<2	2 – 5	5 – 10	>10
Extent (m^2)	<25	25 – 10,000	10,000 – 1,000,000	>1,000,000
Patchiness (% cover)	<10	10 – 20	20 – 30	>30

Table 16 Guidelines used to categorise 'reefiness' for stony reefs (Irving, 2009).

Measure of 'reefiness'	Not a stony reef	Low	Medium	High
Composition	<10 %	10 - 40 % Matrix supported	40 - 95 %	>95 % Clast supported
Notes: Diameter of cobbles / boulders being greater than 64 mm. Percentage cover relates to a minimum area of 25 m^2. This 'composition' characteristic also includes 'patchiness'.				
Elevation	Flat Seabed	<0.064 m	0.064 m - 5 m	>5 m
Notes: Minimum height (64 mm) relates to minimum size of constituent cobbles. This characteristic could also include 'distinctness' from the surrounding seabed.				
Extent	<25 m^2	>25 m^2		
Biota	Dominated by infaunal species			>80 % of species present composed of epifaunal species.

This scoring system indicates that stony reefs should be elevated by at least 0.064 m and with a composition of at least 10 % stones, covering an area of at least 25 m^2 and having an associated community of largely epifaunal species. For "Bedrock Reefs" no similar scoring system exists. In areas where the geophysical data cannot provide information on the degree of exposure, on bedrock, these areas will be delineated as "Potential Bedrock Reefs". The qualifying criteria for the classification "Bedrock Reefs" is the presence of bedrock that could support an epifaunal community.

5. Results

5.1 Field Operations

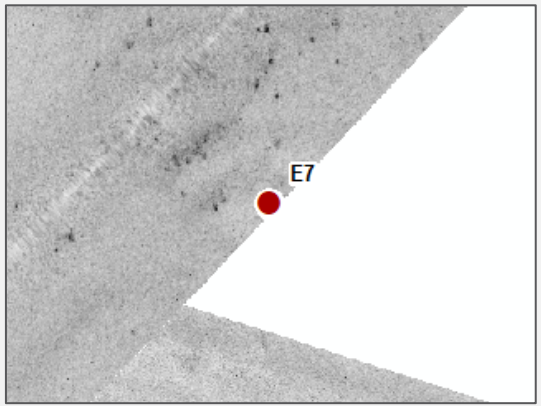
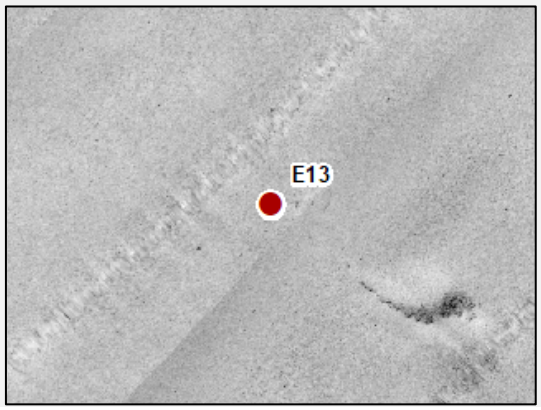
DDV transect and grab sampling as well as water sampling was undertaken at the eight (8) pre-selected sites (Table 17, Figure 12). Samples for particle size analyses, contaminants and fauna, including eDNA, were taken at all grab sample sites. Samples sites RD, M1 – M3 were offset 100 m from their planned location due to coinciding with vibrocore locations.

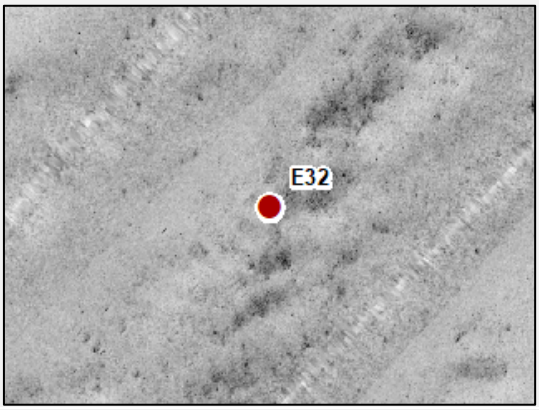
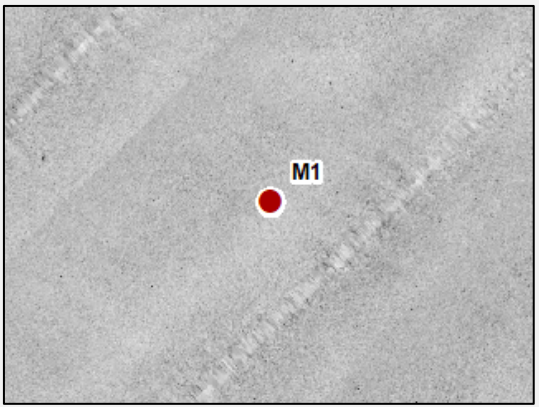
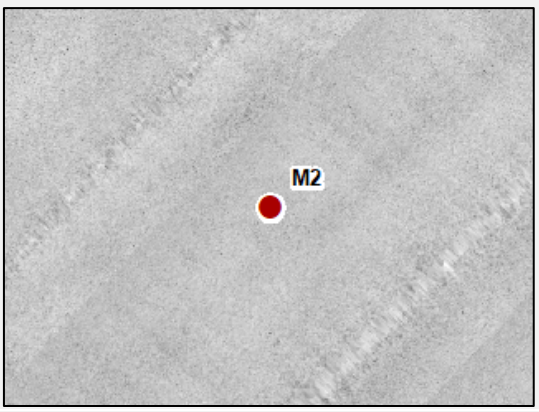
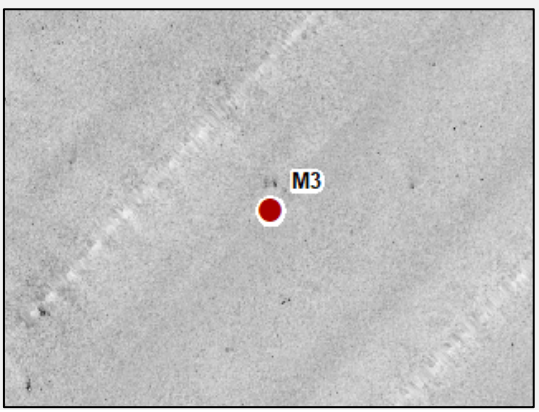
A geophysical data example of each planned grab sample site is presented in Table 17. Further information regarding sample sites is given in Appendix A.

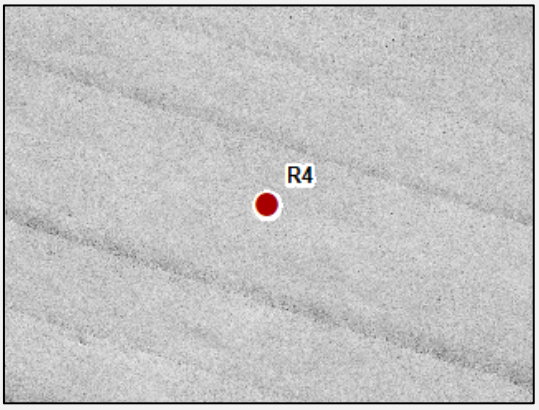
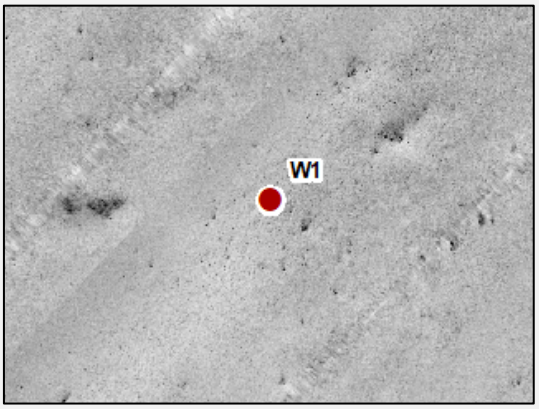
Table 17 Number of sample sites and transects.

No. of Sample Sites	Photo Transect Sites	Grab Sample Sites	PSA/Chem Sample Sites	Water/ eDNA Sample Sites
	8	8	8	8

Table 18 List of proposed grab sample sites.

Site ID	Easting	Northing	Geophysical Data Overview
E7	433355	6339248	
E13	432652	6339277	

Site ID	Easting	Northing	Geophysical Data Overview
E32	431761	6340167	
M1	432357.6	6340049.2	
M2	432609.9	6339041.5	
M3	431611	6339326.8	

Site ID	Easting	Northing	Geophysical Data Overview
R4	433682.9	6339006.7	
W1 (at Total Energies PWT)	432192.8	6339472.5	

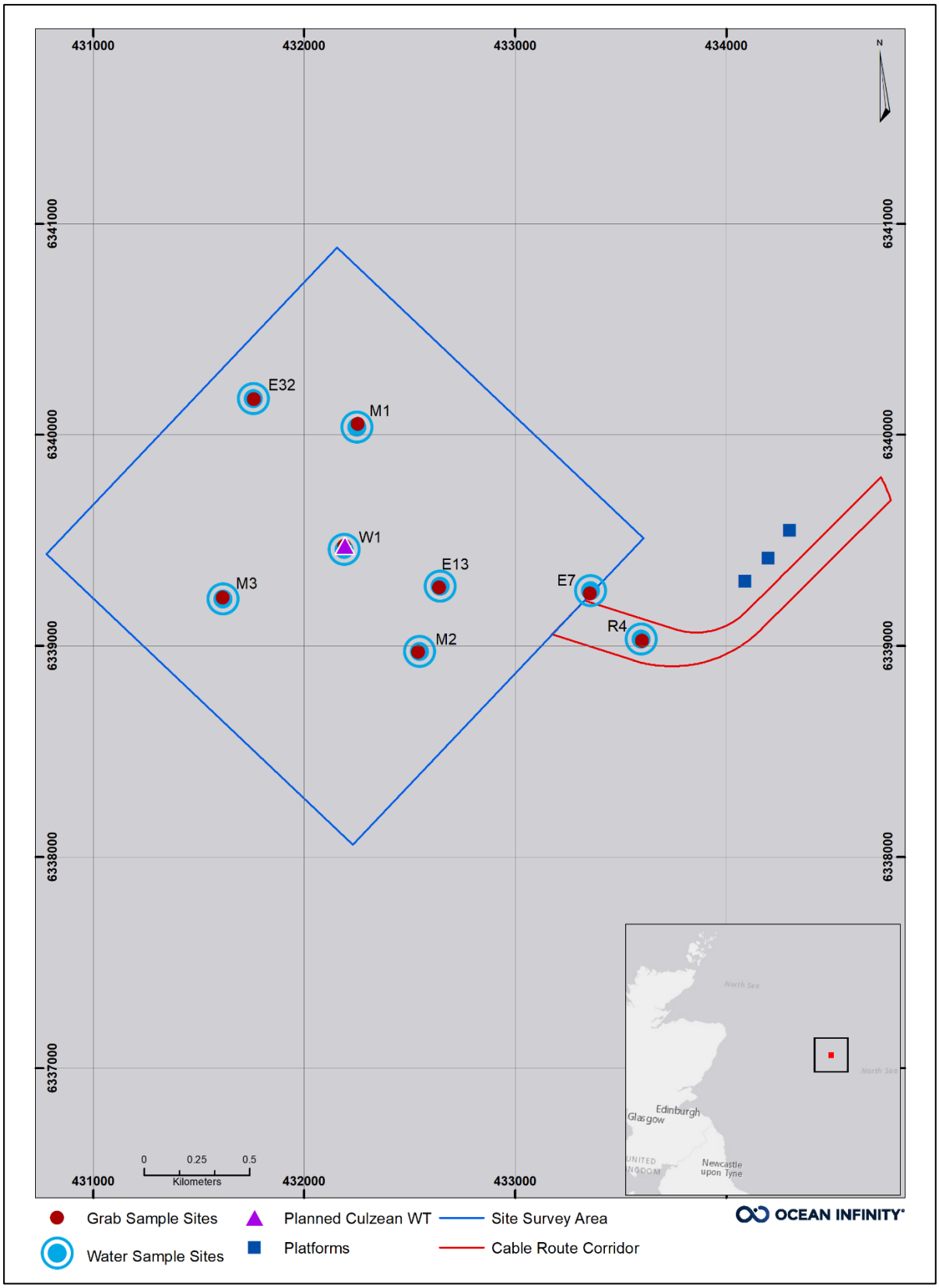


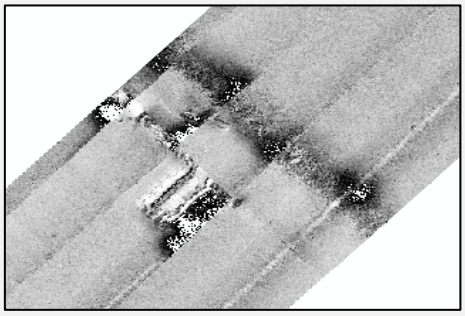
Figure 12 Overview of the conducted environmental sampling.

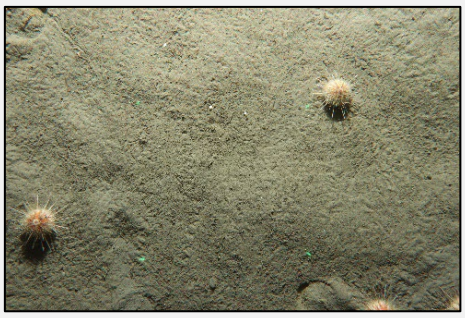
5.2 Summary of Identified Habitats

A total of one (1) EUNIS habitats, three (3) habitat complexes and one (1) artificial habitat were identified and delineated within the survey areas.

An overview of the identified habitats and sample sites is presented in Table 19 and further illustrated in Figure 15. The ID column in Table 19 defines the colour in the charts for the specific habitat type.

Table 19 Identified habitats within the surveyed area.

Habitat Image	ID	Habitat Classification	EUNIS Habitat Code	Site ID
		Constructed, industrial and other artificial habitats	J (V. 2012) *	
		Faunal communities in Atlantic offshore circalittoral sand	MD521	E7
		Faunal communities in Atlantic offshore circalittoral sand/ Seapens and burrowing megafauna in Atlantic circalittoral fine mud	MD521/ MC6216	M1, M2, E13 and R4
		Faunal communities in Atlantic offshore circalittoral sand/ *Brissopsis lyrifera* and *Amphiura chiajei* in Atlantic circalittoral mud	MD521/ MC6218	E32

Habitat Image	ID	Habitat Classification	EUNIS Habitat Code	Site ID
		Faunal communities in Atlantic offshore circalittoral sand/ *Gracilechinus acutus norvegicus* assemblage on Atlantic upper bathyal sand	MD521/ ME5213	M3, W1

**EUNIS 2012 habitat applied due to no equivalent habitat in the 2022 EUNIS classifications at the time of writing this report*

5.2.1 Sample Specific Habitats

For the sample specific habitats classification, a bottom-up approach was implemented to identify community patterns. The taxonomic assemblages from the acquired grab sample data indicate the presence of a primary and a secondary sample specific habitat across the survey area which are presented in Table 20.

The Particle Size Distribution (PSD) coupled with the taxa identified at each grab sample sites were not a match for the substrate component for the primary habitat identified, **MD6218** - *Paramphinome jeffreysii*, *Thyasira* spp. and *Amphiura filiformis* in Atlantic offshore circalittoral sandy mud. Although the PSD was not a match, the taxa present were a sufficient match with the qualifying descriptor.

The faunal composition further showed the potential presence of a secondary habitat, **MD5211** - *Maldanid polychaetes* and *Eudorellopsis deformis* in deep circalittoral sand or muddy sand.

There is a degree of overlap between **MD6218** and **MD5211** with regards to polychaetes and amphipods although **MD5211** comprises a higher presence of bivalves such as *Papillicardium minimum* and different species of Nuculidae.

Table 20 Sample-specific habitats within the surveyed area.

Notes	Habitat Classification	EUNIS Habitat Code	Sample ID
Variant on Muddy Sand.	*Paramphinome jeffreysii*, *Thyasira* spp. and *Amphiura filiformis* in Atlantic offshore circalittoral sandy mud	MD6218	W1, M1, M2, M3, E7, E13, E32 and R4
Variant with low *Eudorellopsis deformis*	*Maldanid polychaetes* and *Eudorellopsis deformis* in deep circalittoral sand or muddy sand.	MD5211	W1, E7

5.3 Area Descriptions

The habitat classifications within the Culzean site and route corridor were derived based on the geophysical data in combination with environmental sample sites (Figure 15). The interpreted habitats at the environmental sample sites were extrapolated to similar areas, where similarity was based on geophysical interpretations of substrate, texture, and topography.

For further details regarding results from the photo analyses see Appendix C and Section 5.4.

The depth within the Culzean site area ranges between 88.8 m to 92.4 m, and from 83.0 to 90.6 m along the cable route corridor (Figure 13). Small seabed depressions were noted scattered across both survey areas, representing the only notable features other than the jack-up spudcan depressions and existing infrastructure.

The seabed is quite homogenous within both survey areas, with some localised variations in the surface sediment composition. The backscatter intensity values exhibited limited variation with low reflectivity across a large spatial scale. Small-scale variability, where noticeable, was associated with features such as infrastructure, seabed depressions, furrows, occasional cobbles, and shell gravel (Figure 14).

The majority of the site area and export cable route comprises **MD521** - Faunal communities on Atlantic offshore circalittoral sand and/or muddy sand with some localised areas of coarse sediments in the north east of the Culzean site.

For the purpose of this report a number of habitat complexes have been introduced to better illustrate the small-scale substrate variation interpreted to be present.

Several of the noted taxon and the combination of these are currently only described within either the circalittoral or the upper bathyal levels of the EUNIS habitats classification. Thus, each assigned classification comprises **MD5 -** Offshore circalittoral sand as part of the complex to illustrate the depth band and substrate of the area and a more species-specific biotope to illustrate the faunal composition.

Notable taxa, as identified from the stills imagery and video acquired, were abundantly occurring sea pens *Pennatula phosphorea*, *Virgularia mirabilis*, sea urchins *Gracilechinus acutus*, *Brissopsis lyrifera* and likely a species of heart urchin, *Echinocardium chordatum* (Table 21). Further noted were rare occurrences of Ophiurida, Caridea, *Crangon* sp., Naticidae, *Hyalinoecia tubicola*, Polynoidae, Scaphopoda and *Pecten maximus*.

The majority of the site area and the western section of the route cable corridor are classified as habitat complex **MD521/MC6216** - Faunal communities on Atlantic offshore circalittoral sand/ Seapens and burrowing megafauna in Atlantic circalittoral fine mud. Occasional burrows, interpreted to be from Norway lobster *Nephrops norvegicus* were noted together with the Atlantic Hagfish *Myxine glutinosa*. The habitat complex **MD521**/**MC6216** matches the qualifying descriptors of the OSPAR habitat Sea-Pen & Burrowing Megafauna Communities.

The western and central sections of the site area are characterised by a frequent occurrence of *G. acutus* and classified as habitat complex **MD521/ME5213** - Faunal communities on Atlantic offshore circalittoral sand/ *Gracilechinus acutus norvegicus* assemblage on Atlantic upper bathyal sand.

The furrows interpreted in the northernmost sections extend into the central sections of the site area and show a species composition similar to **MD521/MC6218** -Faunal communities on Atlantic offshore circalittoral sand/ *Brissopsis lyrifera* and *Amphiura chiajei* in Atlantic circalittoral mud.

The western sections of the site area were classified as **MD521** - Faunal communities on Atlantic offshore circalittoral sand, a habitat which also dominates the eastern half of the cable route corridor (Figure 15).

The underwater installation and SSIV (Sub-Sea Underwater Intervention Valve) in the cable route corridor was delineated as **J** - Constructed, industrial and other artificial habitats (EUNIS, 2012).

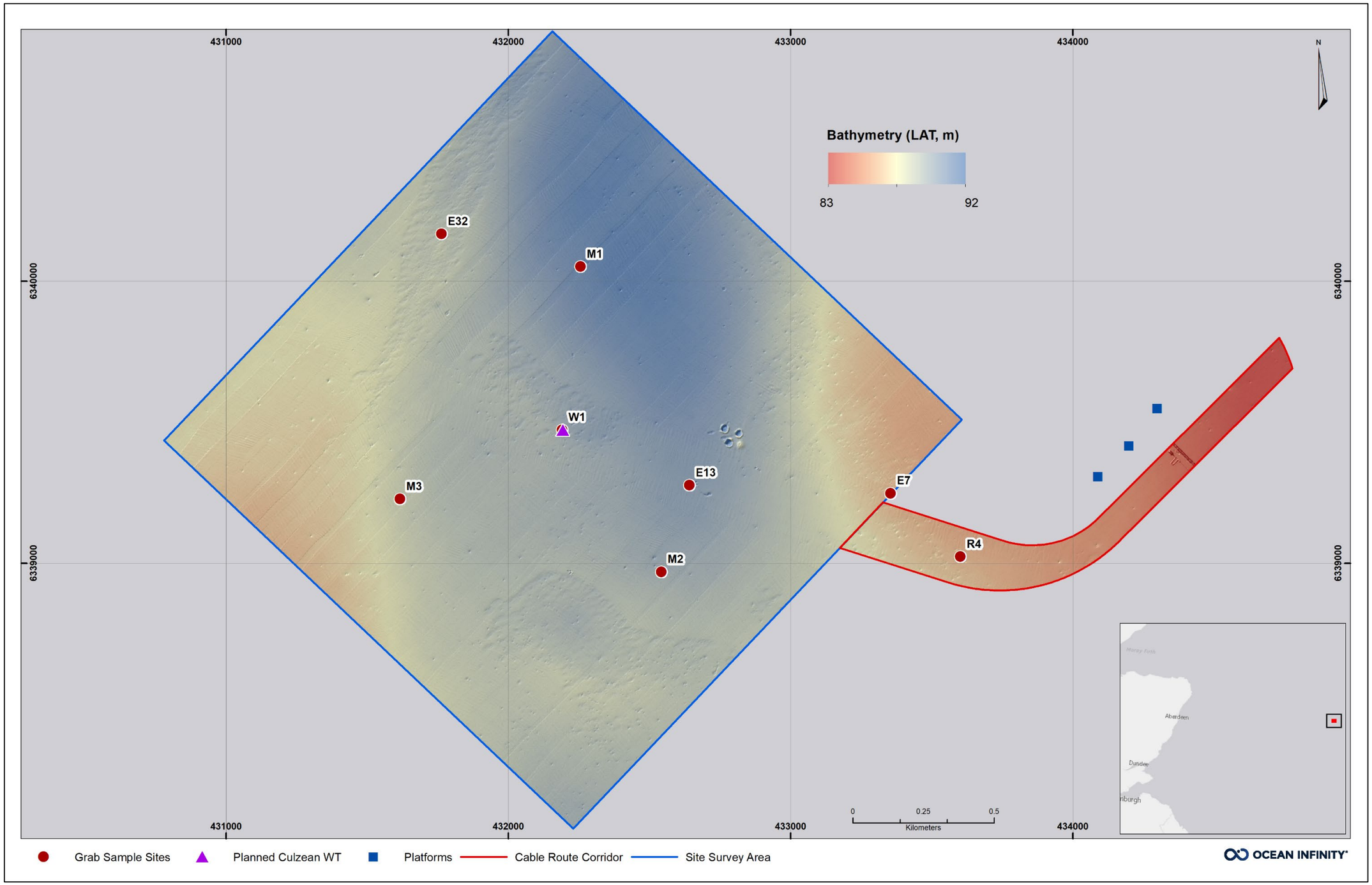


Figure 13 Bathymetric overview of the Culzean site and route corridor survey areas.

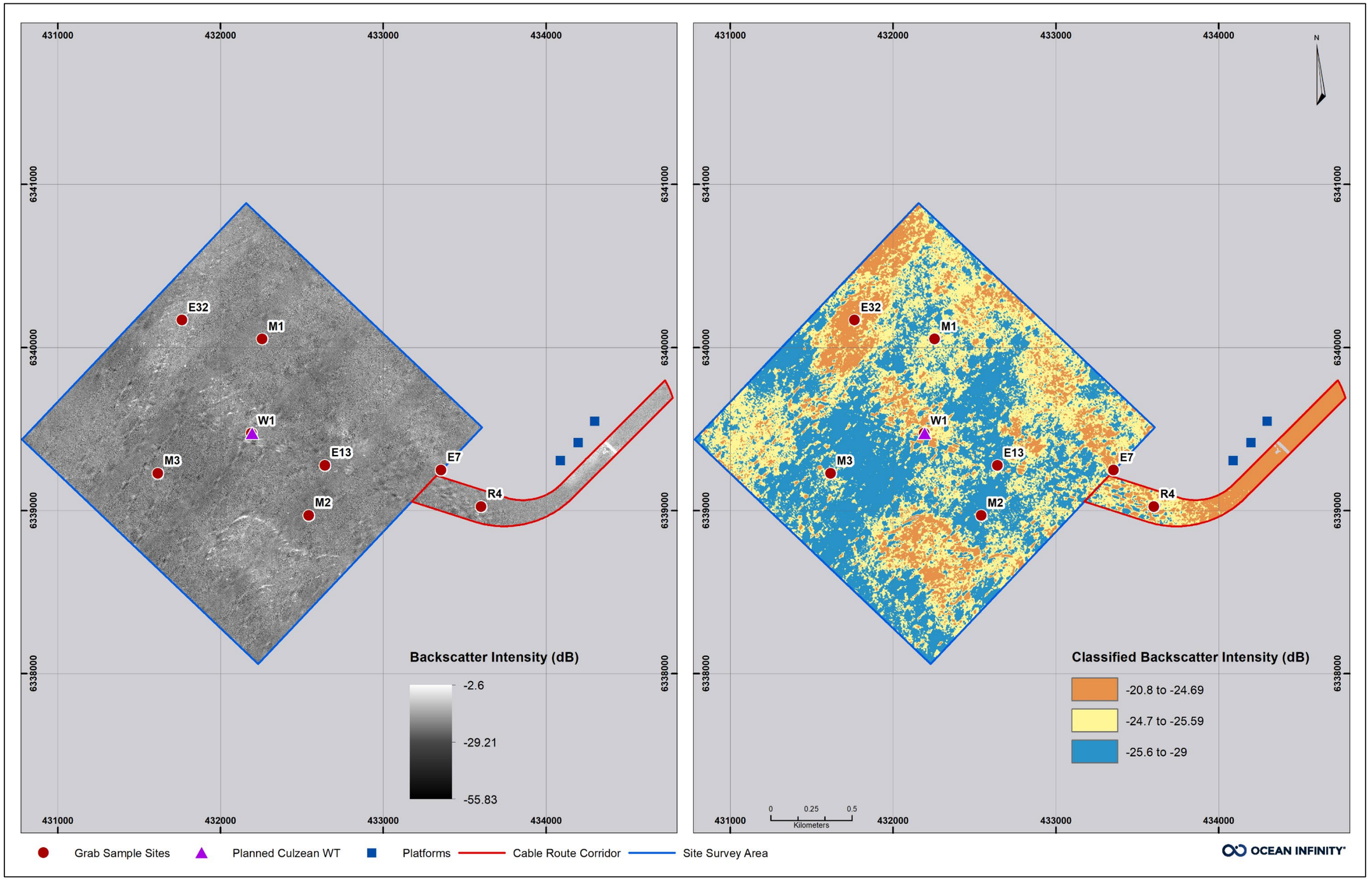


Figure 14 Overview of the raw and classified backscatter intensity within the Culzean site and route corridor survey areas.

Table 21 Example stills acquired throughout survey area sample sites.

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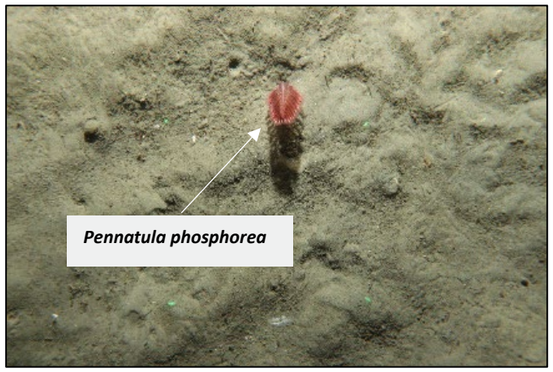
<td> 

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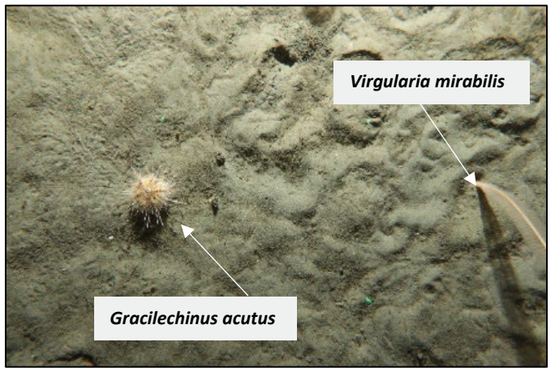
<td> 

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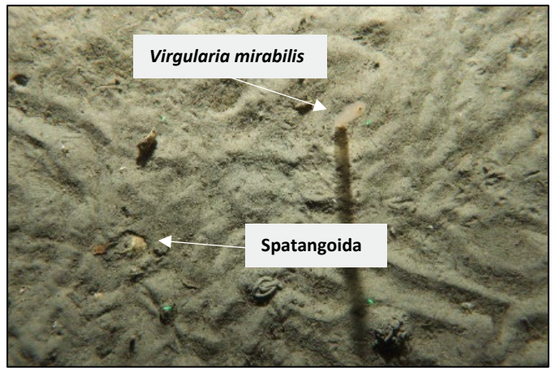
<td> 

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M1_SPT005</td>
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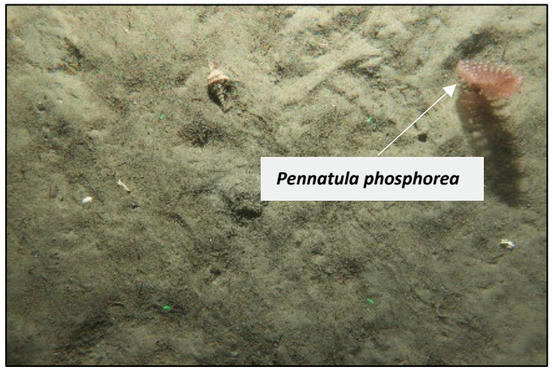
<td> 

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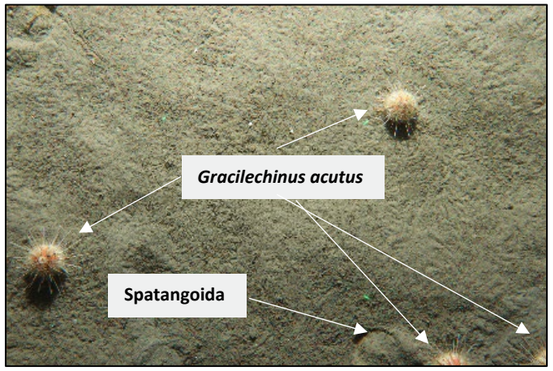
<td> 

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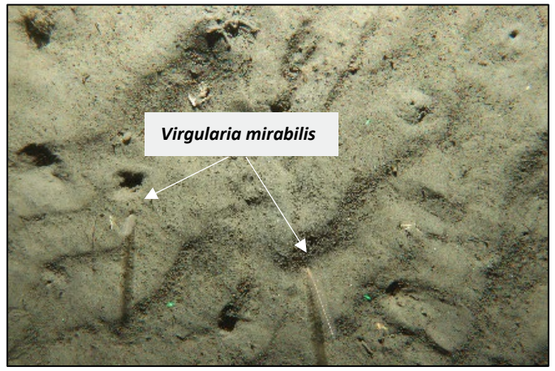
<td> 

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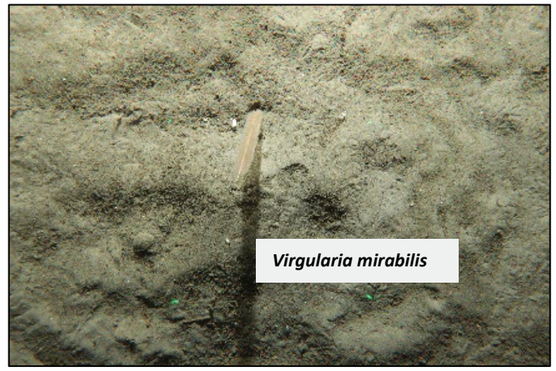
<td> 

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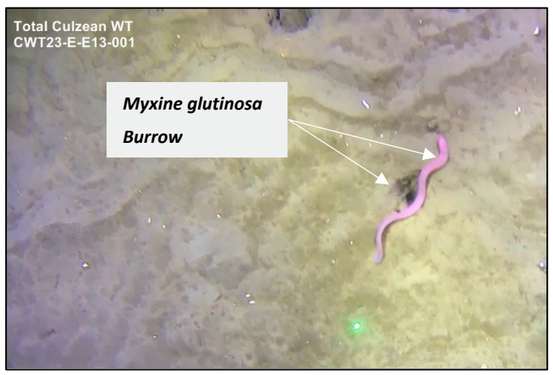
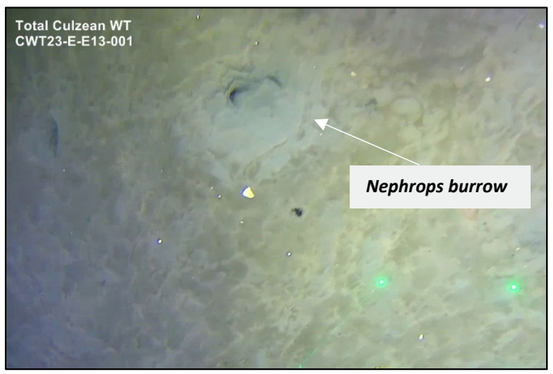
<td> 

Still extracted from E13 video (E432619.01; N6339279.92)</td>
<td> 

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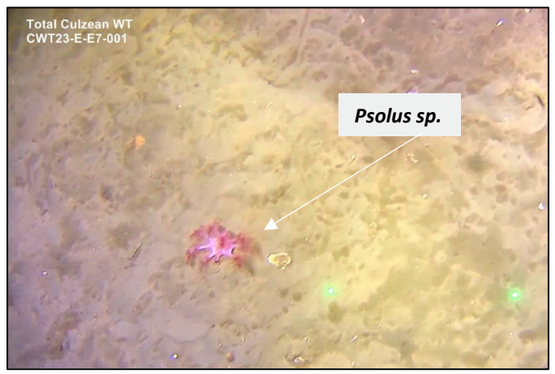
<td> 

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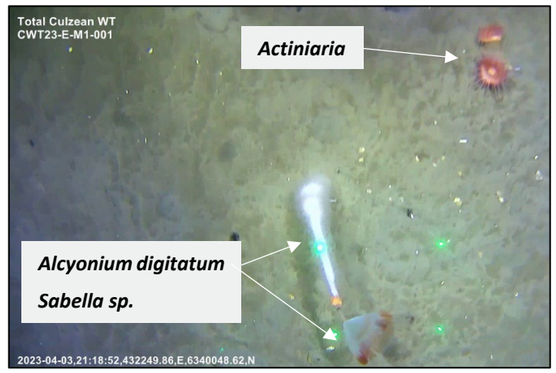
<td> 

Still extracted from M1 video (E432249.86; N6340048.62)</td>
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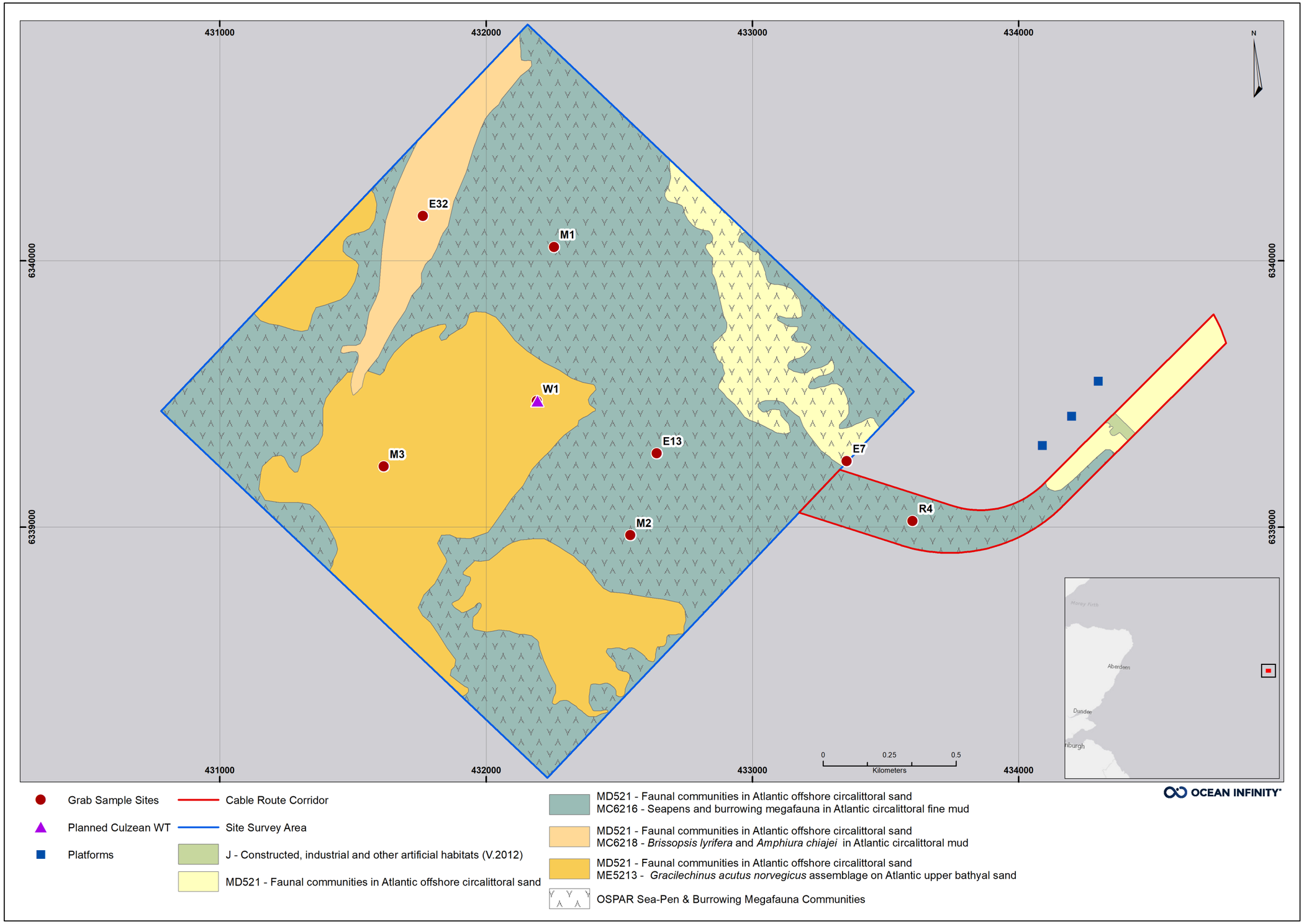


Figure 15 Overview of classified habitats within the Culzean site and route corridor.

5.4 Epibenthic Fauna from Visual Survey

The results from the analyses of the stills from DDV transect sites presented habitats generally dominated by sand and/or muddy sand with some localised areas of gravelly sediments with a presence of *Gracilechinus acutus* and *Virgularia mirabilis*. Conspicuous fauna was Ophiurida and Spatangoida mostly associated with fine sand or muddy sand.

All eight (8) sites had fauna recorded in the stills acquired. However, there was no colonial epifauna recorded in the stills. The number of taxa presented per phylum for all eight (8) sites along with the assigned habitats, are presented in Table 22.

The average number of taxa was three (3) per site. Figure 16 presents a still photo from site E13 (photo OI_728_DDV_CWT23_E13_SPT005), which had the highest number of taxa of all sites.

Table 22 Number of taxa per phyla per site and assigned habitats.

Site ID/Phylum	E13	E32	E7	M1	M2	M3	R4	W1
Habitat Code	MD521/ MC6216	MD521/ MC6218	M521	MD521/ MC6216	MD521/ MC6216	MD521/ ME5213	MD521/ MC6216	MD521/ ME5213
Echinodermata	3	2	2	3	3	2	3	2
Arthropoda	1	1	2	1	1	1	1	1
Cnidaria	1	1	1		1		1	1
Annelida	1			2	1		1	
Mollusca	1			1				1
Chordata								1
Grand Total	7	4	5	7	6	3	6	6

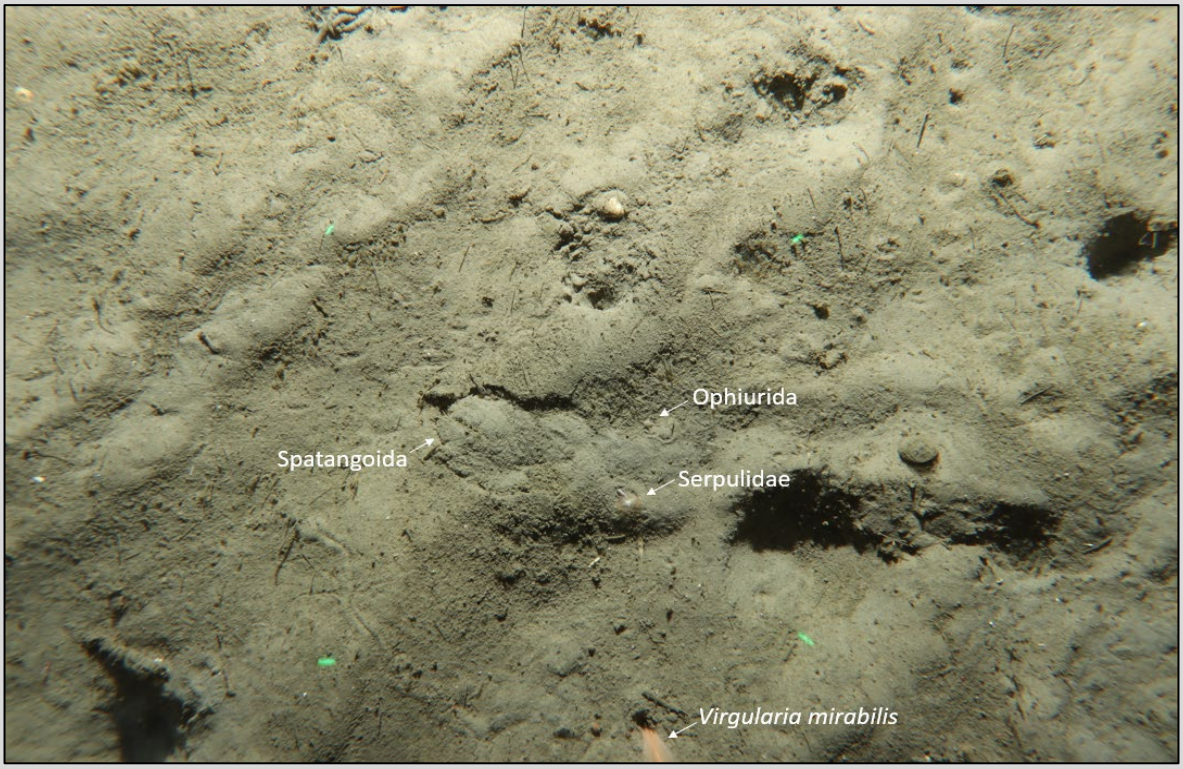


Figure 16 Site E13 photo OI_728_DDV_CWT23_E13_SPT005. It presents the highest number of taxa from the visual survey.

5.4.1 Non-Colonial Epibenthic Fauna in Site Stills

The distribution of abundance of the number of individuals recorded from the different phyla from the stills acquired is presented in Figure 17. The top 10 most abundant taxa are presented in Figure 18.

The most abundant phylum in the epibenthic fauna was Echinodermata, which contributed 54 % of all individuals recorded in the stills. Most of the abundance within the echinoderms was represented by Spatangoida, which constituted 36 %, followed by Ophiurida with 33 % of the abundance within the phylum. *Gracilechinus acutus* followed with 26 % of the abundance.

The second most abundant phylum was Cnidaria, with 13 % of all individuals recorded in the stills. The Sea pen *Virgularia mirabilis* constituted 65 % of the total abundance within the cnidarians.

The Arthropoda phylum was also the second most abundant phylum with the contribution of 13 % of all individuals recorded in the stills. The most abundant taxa within the arthropods were *Crangon* sp. which constituted 37 % of the total abundance.

The Annelida phylum contributed with 8 % of all individuals recorded in the stills. The most abundant taxa within the phylum were Serpulidae which constituted 42 % of the total abundance.

The Cordata phylum contributed with 7 % of all individuals recorded in the stills. Ascidiacea was the only taxa recorded in the phylum.

The phyla Mollusca contributed with 5 % of all individuals recorded in the stills. Scaphopoda was the most abundant taxa within the phylum with a contribution of 49 % of the total abundance.

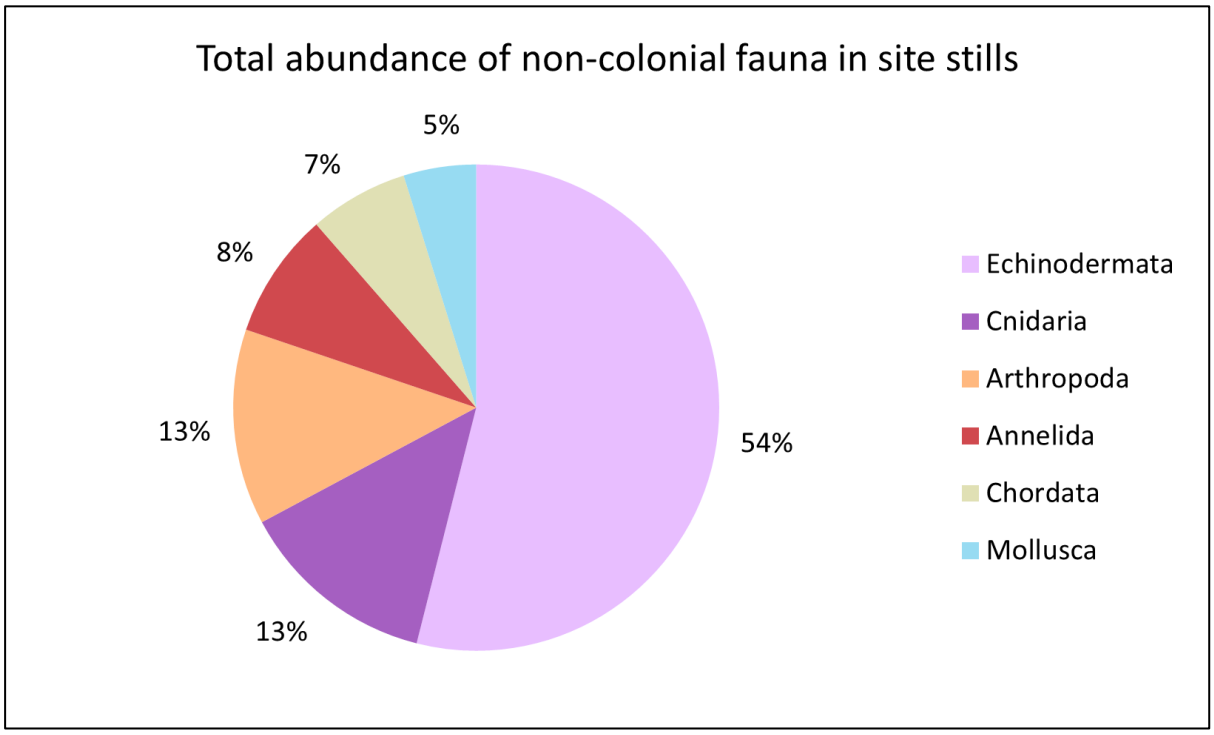


Figure 17 Total abundance of non-colonial fauna in site stills.

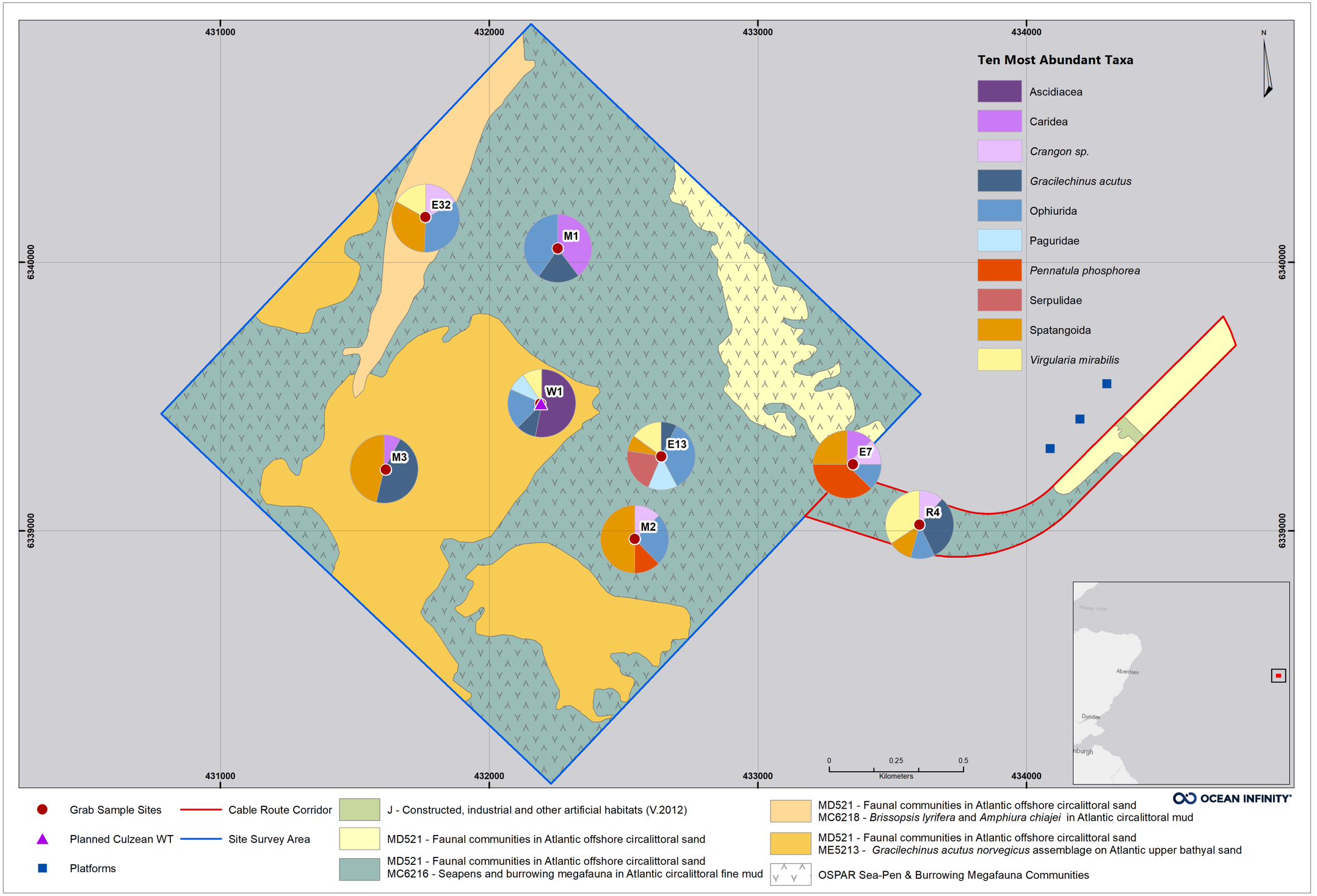


Figure 18 Overview of the ten most abundant taxa in stills per grab sample site.

The top 10 most frequently occurring non-colonial taxa across all sites are presented in Table 23. The order of Ophiurida was the overall most frequently occurring taxa, with a frequency of 88 % per site and 33 % per still. In total Ophiurida occurred at seven (7) sites and in 13 stills.

Table 23 Top 10 most frequently occurring non-colonial taxa across all sites.

Phylum	Taxa	Number of Sites of Occurrence	Frequency of Occurrence (%)	Number of Stills of Occurrence	Frequency of Occurrence (%)
Echinodermata	Ophiurida	7	88	13	33
Echinodermata	Spatangoida	6	75	11	28
Echinodermata	*Gracilechinus acutus*	5	63	8	20
Arthropoda	Crangon sp.	4	50	4	10
Cnidaria	*Virgularia mirabilis*	4	50	6	15
Arthropoda	Caridea	3	38	4	10
Arthropoda	Paguridae	2	25	3	8
Cnidaria	*Pennatula phosphorea*	2	25	4	10
Echinodermata	*Brissopsis lyrifera*	2	25	2	5
Annelida	*Hyalinoecia tubicola*	2	25	2	5

The average non-colonial fauna density (ind./m^2) for each site is presented per phylum in Figure 19. The average density, expressed as individuals per square meter (ind./m^2), varied from six (6) (ind./m^2) at site E32 to 16 (ind./m^2) at site E13. The average non-colonial fauna density for each site was 10.84 (SD=2.99) (ind./m^2).

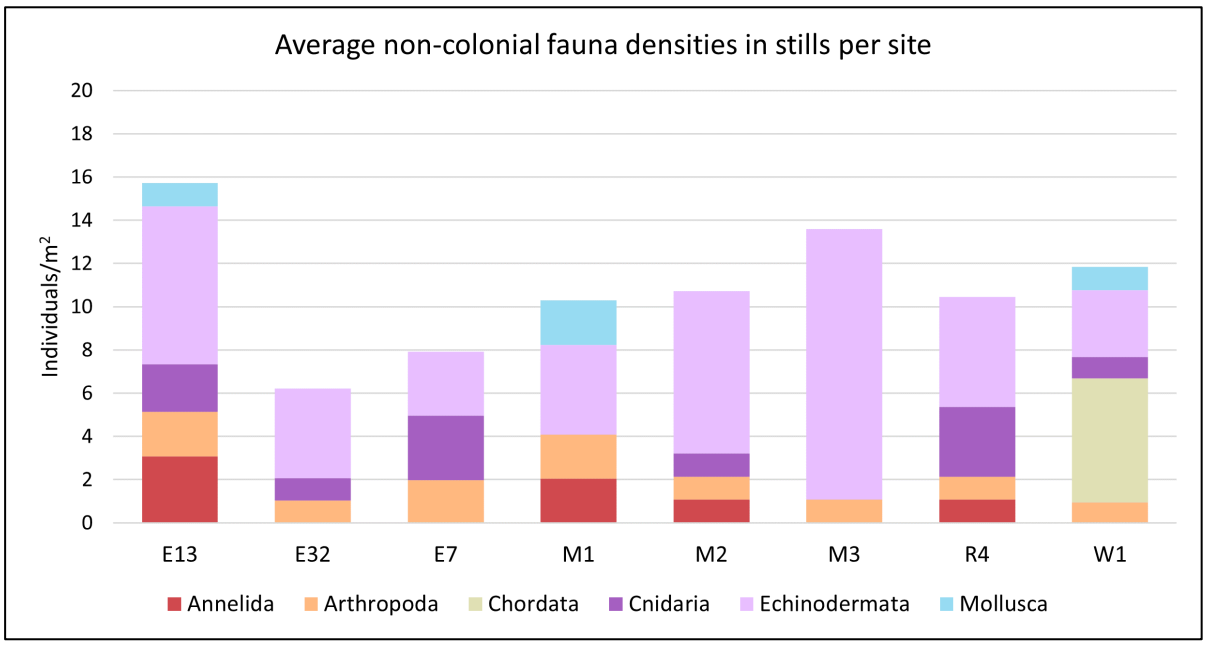


Figure 19 Average faunal densities (ind./m^2) in stills per site.

5.5 Particle Size Distribution

A total of eight (8) sites were selected for PSA sampling, all of which were successfully acquired. Detailed results from the PSA are presented in Table 24.

Fine sand was the dominating sediment fraction, with a mean content of 76.12 % (SD=3.54), followed by Silt which had a mean content of 20.49 % (SD=4.08). The Clay content was low with a mean content of 2.76 % (SD=0.53). Gravel had the lowest values with a mean content of 0.64 % (SD=1.36) (Table 24).

The results from the PSA analyses showed very little variation in the sediment composition between the sampled sites (Figure 20, Figure 21).

Table 24 Summary of PSA results.

Sample ID	BGS (1982) Classification (modified from Folk, 1954)	Depth (m)	Cumulative Sediment Fraction Group Classification (%)			
			Gravel	Sand	Silt	Clay
E13	Muddy Sand	90.35	0.06	72.39	24.31	3.25
E32	Slightly Gravelly Muddy Sand	89.82	3.96	81.55	12.72	1.76
E7	Muddy Sand	89.01	0.70	80.17	16.89	2.24
M1	Muddy Sand	90.70	0.10	75.29	21.78	2.83
M2	Muddy Sand	90.08	0.05	71.65	24.97	3.33
M3	Muddy Sand	89.66	0.11	78.06	19.19	2.64
R4	Muddy Sand	88.97	0.09	74.50	22.42	2.99
W1	Muddy Sand	90.08	0.02	75.33	21.60	3.05
Mean			**0.64**	**76.12**	**20.49**	**2.76**
SD			**1.36**	**3.54**	**4.08**	**0.53**
Min			**0.02**	**71.65**	**12.72**	**1.76**
Max			**3.96**	**81.55**	**24.97**	**3.33**
Median			**0.10**	**75.31**	**21.69**	**2.91**

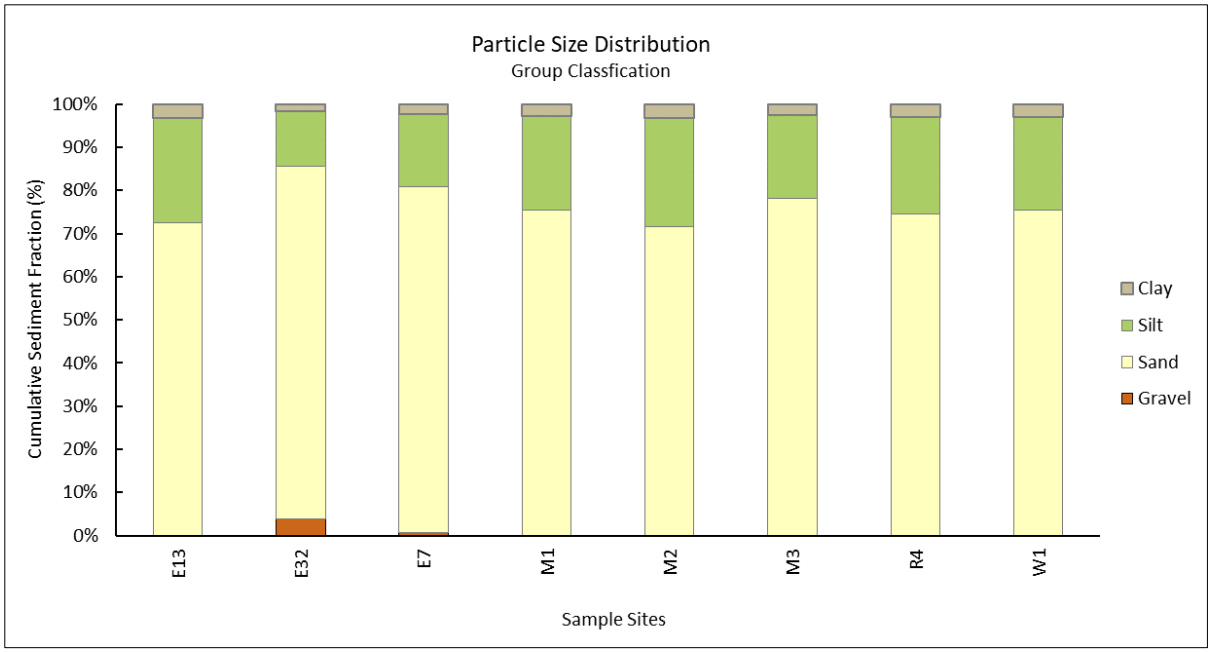


Figure 20 Cumulative particle size distribution.

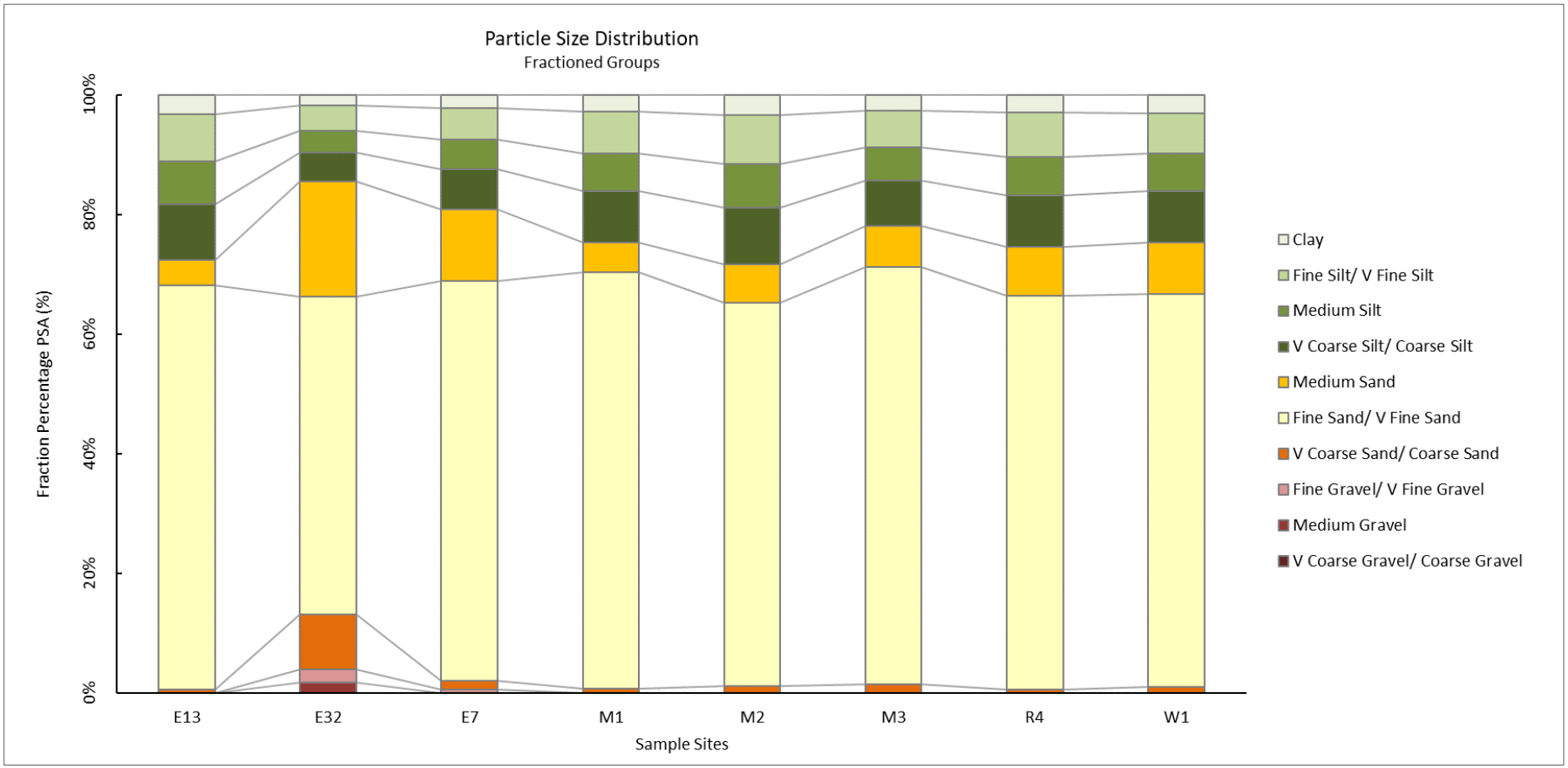


Figure 21 Sediment distribution as fraction percentage (Raw data used, prior to any statistical analyse).

5.6 Multivariate Analyses for Sediment

Multivariate analyses were undertaken on the Particle Size Distribution (PSD), to identify patterns in the sediment distribution. Analyses included hierarchical clustering employing the Euclidean distance resemblance matrix, SIMPROF analysis and Principal Component Analysis (PCA). The datasets were normalised prior to the analyses being performed.

The SIMPROF analysis of the sediment composition produced four distinct groups separating the eight (8) grab sample sites (Figure 22).

SIMPROF Group **a** comprised sand with the highest gravel content, corresponding to the Folk class Slightly Gravelly Muddy Sand. Groups **b**, **c** and **d** comprised sand with silt and clay with minor gravel content, corresponding to the Folk class Muddy Sand.

A PCA was carried out on the PSA results in order to identify spatial patterns and relationships between the sample sites (Figure 23). Each site was plotted against two principal component axes (PC1 and PC2) superimposed with their assigned Folk classification to determine the variability in sediment composition across the survey area.

Sites are distributed in the PCA plot according to their particle size composition in relation to each other and indicate the key, and often subtle, differences which contribute to their separation into SIMPROF groupings or different sediment classifications. Spatial differences along the PC1 axis refer mainly to dissimilarities in the silt and clay content, while spatial variability in the PC2 axis will be more influenced by dissimilarities in the sand and gravel content. PC1 was responsible for explaining 91.7 % of the variation and PC2 accounted for 2 % of the variation, indicating that silt and clay fraction was the main driver of the dissimilarities between groups.

Figure 23 indicates that sites classified as Muddy Sand according to the Folk classification, were influenced both by the silt and clay fraction and the sand fraction. Site E32, classified as Slightly Gravelly Muddy Sand, was separated from the other grab sites due to its low silt and clay component. The sites classified as Muddy Sand were further separated into three SIMPROF groupings, with dissimilarities in the clay and silt fraction being the main contributor for the separation of clusters c and d, and the sand fraction having more influence on the differentiation of group b.

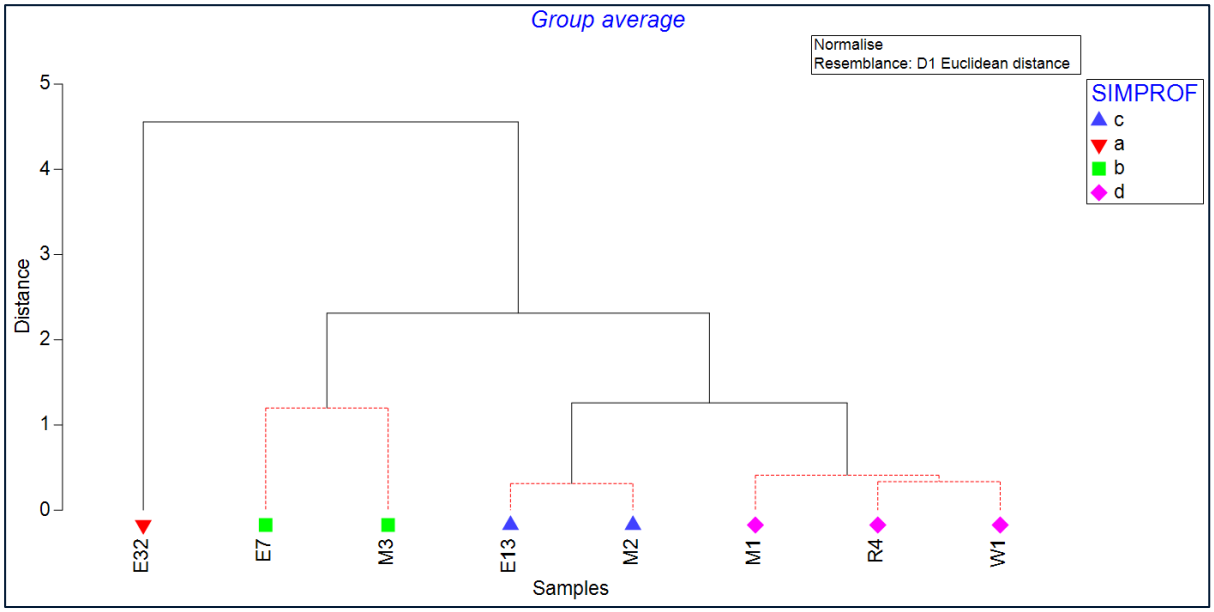


Figure 22 Dendrogram based on Euclidian distance for the sediment composition, showing SIMPROF groups with a 5 % significance level.

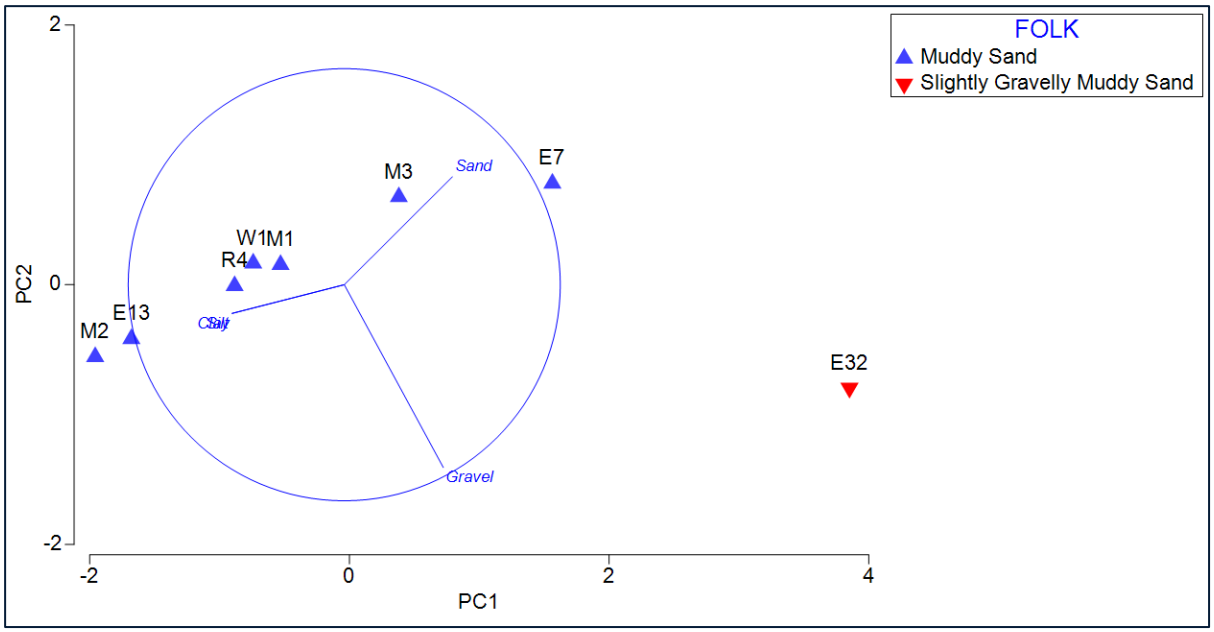


Figure 23 PCA plot of sediment composition, groups based on the Folk classification.

5.7 Sediment Chemical and Contaminant Analyses

Offshore sampling

Samples for chemical and contaminant analyses were successfully acquired at all eight (8) sites within the survey area.

5.7.1 Metals

Metal concentrations were overall low throughout the survey area, with no notable patterns of distribution (Table 25, Figure 24 and Figure 36).

Levels of mercury (Hg) were under the 0.01 mg/kg Limit of Detection (LoD) at all sample sites and has therefore been excluded from Table 25 and Figure 24, along with cadmium (Cd) and beryllium (Be), which also presented concentrations lower than the LoDs at the majority of the sites (Appendix F).

None of the metal concentrations exceeded the OSPAR Effect Range Low (ERL) levels (Table 25), below which they are considered not to have adverse effects on organisms (OSPAR, 2011). Although most values were above the UKOOA mean background levels recorded for fine sands in the central section of the North Sea (CNS), they were all well below the 95th percentile value for the same region (UKOOA, 2001) (Table 25).

Samples taken at site E13 showed particularly low metal concentrations compared to the other sites; whereas site E32 recorded slightly higher levels of As, Cu, Ni, Be and Fe (Table 25, Figure 24 and Figure 36). Strontium (Sr) was notably higher at E32 than at other sites (Figure 24).

Table 25 Metal concentrations (mg/kg dry weight) in samples with reference values. Highlighted cells indicate where threshold values have been exceeded.

Analytes	As	Cr	Cu	Pb	Ni	V	Zn	Al	Ba	Ba by fusion	Fe	Li	Sr
Units		mg/kg dry weight											
Method	ICPMSS	ICPMSS	ICPMSS	ICPMSS	ICPMSS	ICPMSS	ICPMSS	ICPSOIL	ICPSOIL	SUB_01	ICPSOIL	ICPSOIL	ICPSOIL
LoD	0.5	0.5	0.5	0.5	0.5	0.5	2	10	0.5	0.01	36	2	0.5
E13	<0.5	1.0	2.1	0.9	0.6	1.4	3.3	215	21.1	0.06	486	<2.0	2.2
E32	3.3	9.3	4.2	5.5	4.7	11.6	9.5	1900	71.6	0.03	5570	4.3	29.7
E7	2.2	7.6	4.0	5.5	3.5	9.9	8.2	1670	151.0	0.05	3960	3.5	15.6
M1	2.1	9.4	3.9	6.1	4.1	11.4	10.9	2220	178.0	0.05	4490	5.0	19.7
M2	2.3	10.2	4.1	6.7	4.7	12.5	13.4	2410	227.0	0.06	4570	5.5	22.0
M3	2.2	8.9	3.7	5.8	3.7	10.4	9.9	1900	131.0	0.05	4170	4.2	15.9
R4	2.5	9.4	3.9	6.6	4.7	11.7	11.6	2240	235.0	0.06	4700	5.0	20.5
W1	2.6	9.8	3.9	6.2	4.5	12.0	12.5	2230	146.0	0.05	4720	4.7	18.6
Mean	2.5	8.2	3.7	5.4	3.8	10.1	9.9	1848.1	145.1	0.05	4083.3	4.6	18.0
SD	0.4	3.0	0.7	1.9	1.4	3.6	3.2	702.8	72.6	0.01	1528.8	0.7	7.8
Min	2.1	1.0	2.1	0.9	0.6	1.4	3.3	215	21.1	0.03	486	3.5	2.2
Max	3.3	10.2	4.2	6.7	4.7	12.5	13.4	2410	235.0	0.06	5570	5.5	29.7
Median	2.3	9.4	3.9	6.0	4.3	11.5	10.4	2060	148.5	0.05	4530	4.7	19.2
	Reference Levels												
UKOOA Fine Sand CNS	-	7.60	1.55	5.39	3.20	9.11	8.78	-	169.31	-	3214	-	-
UKOOA 50th percentile CNS	-	7.17	2.00	6.65	4.00	12.00	10.45	-	117.50	-	3487	-	-
UKOOA 95th percentile CNS	-	31.04	6.00	16.70	19.00	31.30	32.59	-	523.20	-	11160	-	-
OSPAR ERL	-	81	34	47	-	-	150	-	-	-	-	-	-

**Where metal concentrations exceed more than one reference level, the higher one has been highlighted in the table.*

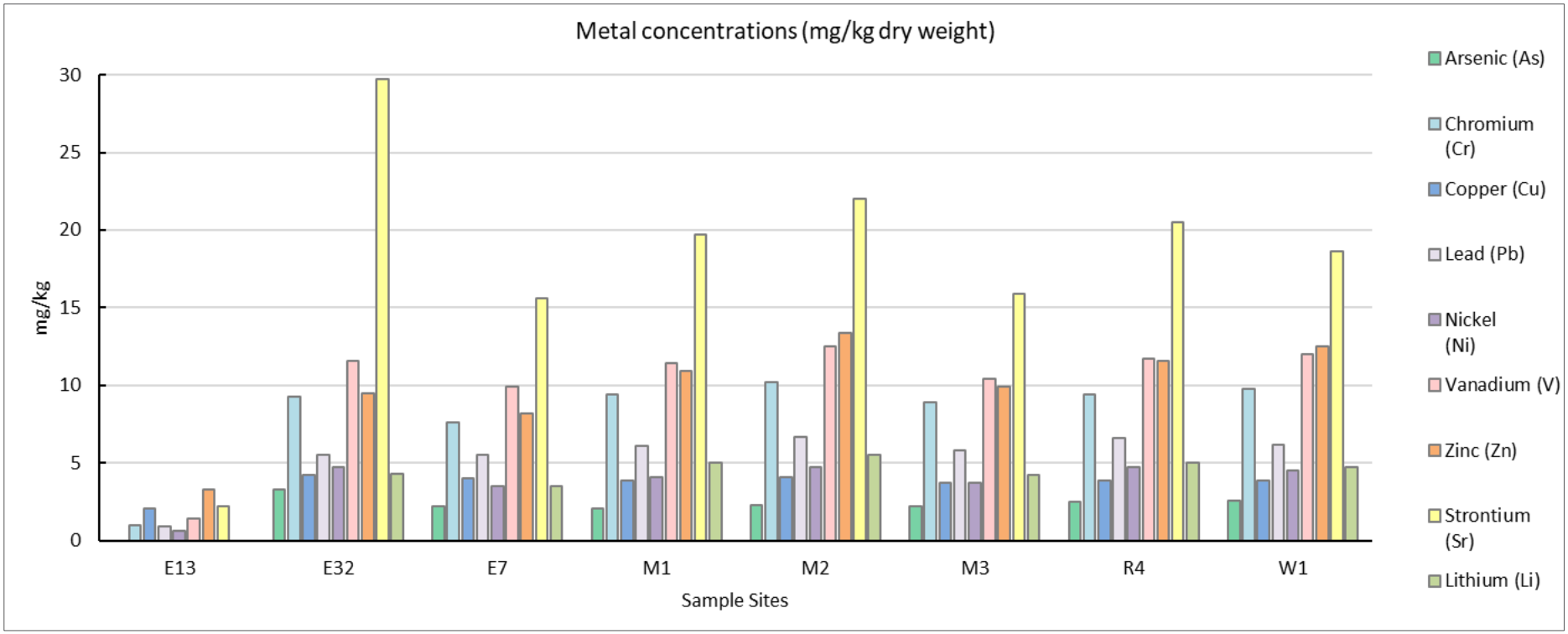


Figure 24 Concentrations of As, Cr, Cu, Pb, Ni, V, Zn, Sr and Li (mg/kg dry weight).

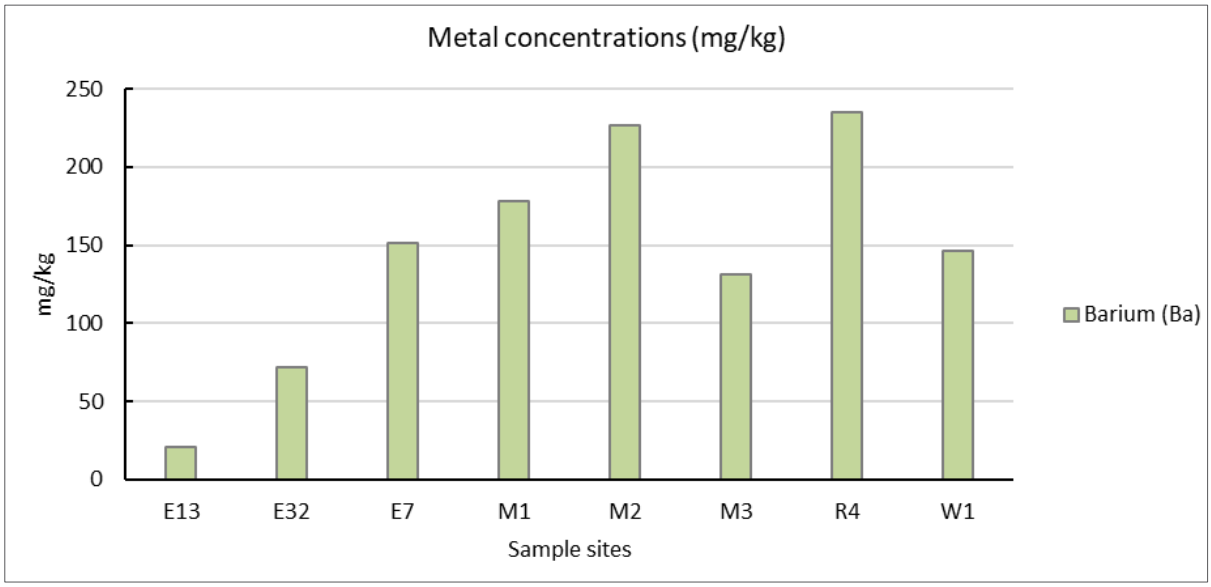


Figure 25 Concentration of Ba (mg/kg dry weight).

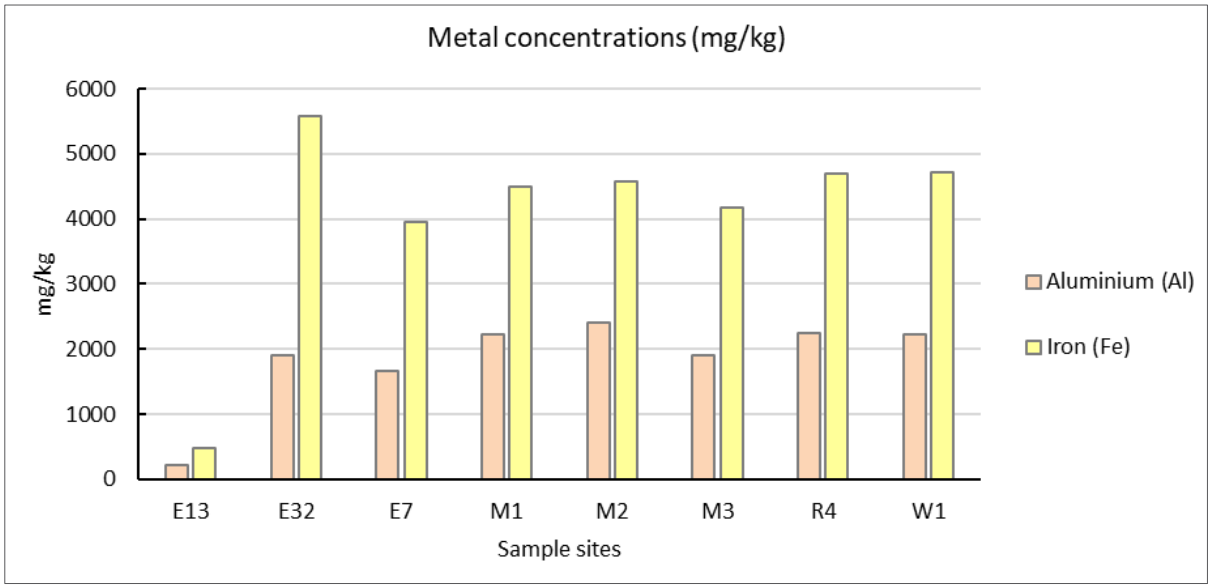


Figure 26 Concentrations of Al and Fe (mg/kg dry weight).

5.7.2 Organics

Total Organic Matter (TOM) and Total Organic Carbon (TOC) were relatively homogeneous across the survey area, with a mean content of 1.5 % (SD=0.3) and 0.24 % (SD=0.04), respectively (Table 26). All values were within what is expected for Central North Sea sediments, with six (6) of the sites showing concentrations above the UKOOA 50th percentile, while all remaining below the 95th percentile levels (UKOOA, 2001).

Moisture content showed equally limited variation throughout the survey area, ranging from 25.6 % at site E7 to 31.2 % at site M1 (Table 26). The slightly higher levels of moisture were generally attributable to slightly higher content of fine sediment in the samples (Table 24).

Table 26 Total organic matter (TOM), total organic carbon (TOC; % M/M) and total moisture (%) in samples with UKOOA reference values. Highlighted cells indicate where threshold values have been exceeded.

Analytes	Total Organic Matter	Total Organic Carbon	Moisture
Units	% M/M	% M/M	%
Method	Loss On Ignition (LOI)	WSLM59	ASC/SOP/303
LoD	0.2	0.02	0.2
E13	1.1	0.19	29.8
E32	1.5	0.25	26.5
E7	1.7	0.29	25.6
M1	1.0	0.21	31.2
M2	1.5	0.25	29.0
M3	1.8	0.29	27.9
R4	1.6	0.25	28.5
W1	1.8	0.21	28.2
Mean	1.50	0.24	28.34
SD	0.30	0.04	1.77
Min	1.00	0.19	25.60
Max	1.80	0.29	31.20
Median	1.55	0.25	28.35
UKOOA 50th percentile CNS	1.13	-	-
UKOOA 95th percentile CNS	4.48	-	-

5.7.3 Hydrocarbons

Results for hydrocarbon analyses are summarised and tabulated as Total Hydrocarbon Concentrations (THC), percentage of Unresolved Complex Mixture (UCM), nC_{10-20} and nC_{21-37} alkanes and total n-alkanes, Carbon Preference Index (CPI), Pristane (Pr) and Phytane (Ph) and their ratio (Pr/Ph), sum of NDP (naphthalenes, phenanthrenes and dibenzothiophenes), total Polycyclic Aromatic Hydrocarbons (PAH) and NDP/PAH ratio in Table 27, with individual alkanes (nC_{10-37}) listed in Appendix F.

A summary of the sixteen individual Polycyclic Aromatic Hydrocarbons designated by the Environmental Protection Agency (EPA PAH) as 'High Priority Pollutants' is presented in Table 28.

Total Hydrocarbon Content (THC)

The Total Hydrocarbon Content (THC) of the sediments showed little variability between sample sites, ranging from 7.37 µg/g at site E7 and 13.60 µg/g at site M1, with a mean of 10.25 µg/g (SD=2.38; Table 27).

All sites presented THC levels above the UKOOA 50th percentile for the Central North Sea (4.10 µg/g), and six (6) of these sample sites also exceeded the slightly higher UKOOA background levels for fine sands in the Central North Sea (CNS; 6.66 µg/g; Table 27) (UKOOA, 2001). However, the concentrations measured at all sample sites were still less than half of that of the UKOOA 95th percentile for the area (40.10 µg/g, Table 27) (UKOOA, 2001), remaining within recorded background levels. Furthermore, the THC concentrations were well below the 5000 µg/g established by the Dutch Ministry of Infrastructure and the Environment as the threshold below which the contamination of the sediment does not interfere with the chemical and ecological quality of the overlying water column.

Polycyclic Aromatic Hydrocarbon (PAH)

Polycyclic Aromatic Hydrocarbon (PAH) concentrations were relatively homogeneous throughout the survey area, ranging from 0.083 µg/g at site E32 to 0.194 µg/g at site M1, with a mean value of 0.144 µg/g (SD=0.038; Table 27).

Like the THC results, only two (2) sites (E32 and E7) showed PAH levels below the UKOOA mean background values for fine sands in the CNS (0.117 µg/g). These sites were also below the slightly lower UKOOA 50th percentile value for the area (0.109 µg/g), while all sites in the survey area presented PAH levels below that of the UKOOA 95th percentile (0.583 µg/g), therefore still within the recorded background values for the CNS (Table 27) (UKOOA, 2001).

The total n-alkanes concentrations were quite low (mean 0.39 µg/g, SD=0.09) and showed little variability between the sampled sites (Table 27). UKOOA background levels for fine sands (0.37 µg/g) were exceeded at all but three (3) sites (E32, E7 and M3), while concentrations recorded at every site varied between the slightly lower UKOOA 50th percentile (0.26 µg/g) and the 95th percentile (1.18 µg/g) for the CNS (Table 27) (UKOOA, 2001).

Pristane (Pr) and phytane (Ph) levels were low throughout, with phytane being below detection level at all but sites M1, and therefore only allowing the Pr/Ph ratio to be calculated for this sample site (Table 27).

The Carbon Preference Index (CPI) varied between 1.85 (E32) and 2.65 (M3), with the lowest values corresponding to the sample sites where THC and PAH were also lowest (Table 27). CPI was above the UKOOA mean level for fine sands in the CNS (2.03) at most sites, and all but site E32 presented values above the UKOOA 50th percentile for the area (1.86). Nevertheless, all sites were below the UKOOA 95th percentile reference value for the CNS (2.79) and considered to be within what is expected for the area (Table 27) (UKOOA, 2001).

In the absence of specific background levels for the area of interest, individual EPA PAH concentrations were compared to the Norwegian Environmental Agency (NEA) reference levels for the classification of environmental conditions in water (NEA, 2016, revised 2020). The thresholds established in the Canadian sediment quality guidelines by the Canadian Council of Ministers of the Environment (CCME, 2001), as well as the OSPAR ERL values (OSPAR, 2011) have also been included in Table 28, for comparison.

All EPA PAH concentrations were well below the OSPAR ERL and CCME reference levels, with most sites presenting levels within the NEA background range (class 1). Only sites M1 and M2 showed any values above background, with Indeno[123,cd]pyrene and Benzo[ghi]perylene within what is considered “Good” (class 2) by the NEA. These sites also presented the highest sum of the 16 EPA PAHs, although these were still well within the NEA background levels (class 1).

Table 27 Summary of hydrocarbon concentrations (µg/g) in samples with UKOOA. Highlighted cells indicate where threshold values have been exceeded.

Analytes	THC	UCM	nC_{10-20}	nC_{21-37}	total n-alkanes	CPI	Pristane (Pr)	Phytane (Ph)	Pr/Ph Ratio	NPD	Total PAH	NPD/4-6 ring PAH ratio
Units	µg/g	µg/g	µg/g	µg/g	µg/g	-	µg/g	µg/g	-	µg/g	µg/g	-
Method	ASC/SOP/303/306	-	ASC/SOP/303/306	ASC/SOP/303/306	ASC/SOP/303/306	-	ASC/SOP/303/306	ASC/SOP/303/306	-	ASC/SOP/303/304	ASC/SOP/303/304	-
LoD	0.10	-	0.001	0.001	0.028	-	0.001	0.001	-	0.014	0.034	-
E13	9.22	8.77	0.04	0.41	0.45	2.02	0.013	<0.001	-	0.024	0.156	0.18
E32	7.51	7.24	0.03	0.24	0.27	1.85	0.013	<0.001	-	<0.014	0.083	0.17
E7	7.31	7.04	0.03	0.24	0.27	1.90	0.010	<0.001	-	<0.014	0.098	0.15
M1	13.60	13.11	0.04	0.45	0.49	2.13	0.014	0.003	4.89	0.026	0.194	0.15
M2	12.60	12.13	0.03	0.44	0.47	2.48	0.012	<0.001	-	0.024	0.183	0.15
M3	8.83	8.46	0.01	0.35	0.37	2.65	0.009	<0.001	-	0.020	0.140	0.16
R4	10.80	10.38	0.05	0.37	0.42	2.36	0.013	<0.001	-	0.022	0.143	0.18
W1	12.10	11.71	0.04	0.35	0.39	2.34	0.012	<0.001	-	0.021	0.153	0.16
Mean	10.25	9.85	0.04	0.36	0.39	2.22	0.012	0.0	-	0.023	0.144	0.16
SD	2.38	2.31	0.01	0.08	0.09	0.29	0.002	-	-	0.002	0.038	0.01
Min	7.31	7.04	0.01	0.24	0.27	1.85	0.009	0.003	4.9	0.020	0.083	0.15
Max	13.60	13.11	0.05	0.45	0.49	2.65	0.014	0.003	4.9	0.026	0.194	0.18
Median	10.01	9.57	0.04	0.36	0.41	2.24	0.012	-	-	0.023	0.148	0.16
Reference Levels												
UKOOA Fine Sand CNS	8.66	-	-	-	0.37	2.03	-	-	-	-	0.117	-
UKOOA 50th percentile CNS	4.10	-	-	-	0.26	1.86	-	-	-	-	0.109	-
UKOOA 95th percentile CNS	40.10	-	-	-	1.18	2.79	-	-	-	-	0.583	-
Dutch RIVM	5000	-	-	-	-	-	-	-	-	-	-	-

**Where values exceed more than one reference level, the higher one has been highlighted in the table.*

Table 28 Total Polycyclic Aromatic Hydrocarbons (PAH; µg/kg) in samples with thresholds. Highlighted cells indicate where threshold values have been exceeded.

Analytes	Naphthalene	Acenaphthylene	Acenaphthene	Fluorene	Phenanthrene	Dibenzothiophene*	Anthracene	Fluoranthene	Pyrene	Benzo[a]anthracene	Chrysene	Benzo[b]fluoranthene	Benzo[k]fluoranthene	Benzo[e]pyrene*	Benzo[a]pyrene	Perylene *	Indeno[123,cd]pyrene	Dibenzo[a,h]anthracene	Benzo[ghi]perylene	Sum EPA 16
Units	µg/kg	µg/kg	µg/kg	µg/kg	µg/kg	µg/kg	µg/kg	µg/kg	µg/kg	µg/kg	µg/kg	µg/kg	µg/kg	µg/kg	µg/kg	µg/kg	µg/kg	µg/kg	µg/kg	µg/kg
Method	ASC/SOP/303/304																			-
LoD	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	-
E13	1.21	<1	<1	<1	3.19	<1	<1	4.59	3.61	2.13	3.49	11.1	10.8	7.12	3.77	1.37	17.3	1.90	17.8	80.89
E32	<1	<1	<1	<1	1.57	<1	<1	2.46	2.07	1.09	1.77	5.78	6.60	3.87	1.42	<1	9.55	1.26	8.77	42.34
E7	<1	<1	<1	<1	1.81	<1	<1	3.09	2.58	1.52	2.27	7.19	5.97	4.42	1.84	<1	12.4	1.29	11.5	51.46
M1	1.38	<1	<1	<1	3.17	<1	<1	5.68	4.38	2.79	4.13	14.5	11.9	8.57	4.07	1.72	23.9	2.54	21.4	99.84
M2	1.32	<1	<1	<1	3.23	<1	<1	5.04	3.95	2.54	3.92	13.3	10.3	7.94	4.01	1.59	22.5	2.43	20.5	93.04
M3	1.18	<1	<1	<1	2.13	<1	<1	3.48	2.90	1.79	2.54	8.83	8.87	5.95	3.15	1.03	17.4	2.32	15.4	69.99
R4	1.26	<1	<1	<1	2.64	<1	<1	3.71	3.02	1.79	2.86	10.6	7.68	6.46	3.58	1.27	18.7	2.54	16.3	74.68
W1	1.08	<1	<1	<1	2.57	<1	<1	4.23	3.32	2.17	3.44	12.0	9.55	6.96	3.73	1.68	19.9	2.24	17.5	81.73
Mean	1.2	-	-	-	2.5	-	-	4.0	3.2	2.0	3.1	10.4	9.0	6.4	3.2	1.4	17.7	2.1	16.1	74.2
SD	0.1	-	-	-	0.6	-	-	1.1	0.7	0.5	0.8	3.0	2.1	1.6	1.0	0.3	4.8	0.5	4.3	19.5
Min	1.08	-	-	0.00	1.57	-	-	2.46	2.07	1.09	1.77	5.78	5.97	3.87	1.42	1.03	9.55	1.26	8.77	42.34
Max	1.38	-	-	0.00	3.23	-	-	5.68	4.38	2.79	4.13	14.50	11.90	8.57	4.07	1.72	23.90	2.54	21.40	99.84
Median	1.24	-	-	-	2.61	-	-	3.97	3.17	1.96	3.15	10.85	9.21	6.71	3.66	1.48	18.05	2.28	16.90	77.79
Reference Levels																				
NEA 1 Background	0	0	0	0	0	-	0	0	0	0	0	0	0	-	0	-	0	0	0	0
NEA 2 Good	2	1.6	2.4	6.8	6.8	-	1.2	8	5.2	3.6	4.4	90	90	-	6	-	20	12	18	300
NEA 3 Moderate	27	33	96	150	780	-	4.8	-	84	60	-	-	-	-	183	-	-	27	-	2 000
NEA 4 Poor	1754	85	195	694	2500	-	30	400	840	501	280	140	135	-	230	-	63	273	84	6 000
NEA 5 Very Poor	8769	8500	19500	34700	25000	-	295	2 000	8 400	50 100	2 800	10 600	7 400	-	13 100	-	2 300	2 730	1 400	20 000
OSPAR ERL	160	-	-	-	240	190	85	600	665	261	384	-	-	-	430	-	240	-	85	-
CCME ISQG	34.6	5.87	6.71	21.2	86.7	-	46.9	113	153	74.8	108	-	-	-	88.8	-	-	6.22	-	-
CCME PEL	391	128	88.9	144	544	-	245	1494	1398	693	846	-	-	-	763	-	-	135	-	-

**Not EPA 16*

5.7.4 Polychlorinated Biphenyls (PCB)

Concentrations of Polychlorinated Biphenyls (PCB) were low throughout, with all sites presenting values below the 0.08 µg/kg limit of detection (LoD; Appendix F).

5.7.5 Organotins

None of the eight acquired samples in the survey area exceeded the 5 µg/kg LoD for any of the analytes (Dibutyltin (DBT), Tributyltin (TBT), Monobutyltin (MBT), Tetrabutyltin (TTBT) and Triphenyltin (TPT); Appendix F)

5.7.6 Pesticides (OCP)

Organochlorine pesticides (OCP) were below the 0.1 µg/kg LoD at all sites (Appendix F)

5.7.7 Brominated Flame Retardants (PBDE)

Concentrations of Polybrominated Diphenyl Ethers (PBDEs) or brominated flame retardants were below detection levels for all analytes at all eight sites (Appendix F).

5.8 Water Chemical and Contaminant Analyses

Water samples for Total Suspended Solids (TSS) and contaminant analyses were successfully acquired at all eight (8) sites within the survey area. An overview map of the water sample sites is presented in Figure 27.

For a detailed account of all the results, view Appendix H.

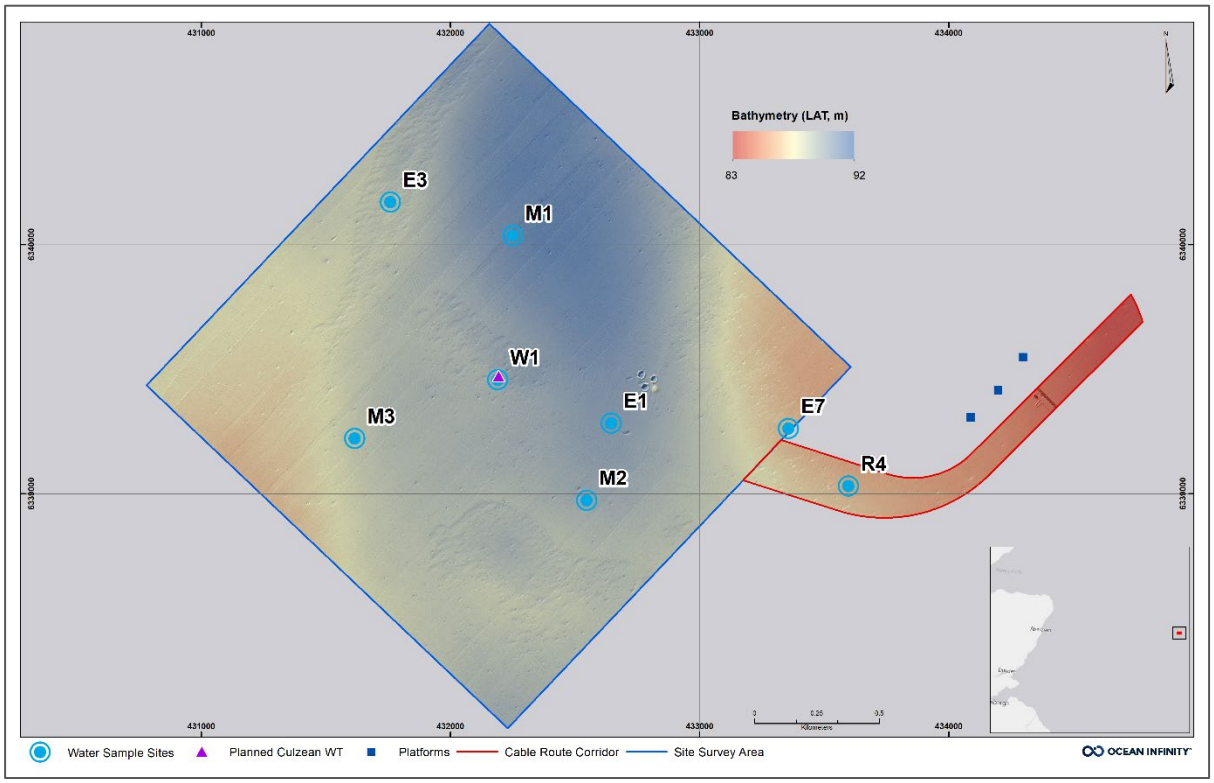


Figure 27 Overview of the distribution of water sample sites.

5.8.1 Total Suspended Solids

Water samples were collected to analyse levels of TSS at the surface and bottom of the water column (Figure 28). Water sample sites M1, M3, R4, and W1 showed values below 5 mg/l, which is below the instrument threshold and therefore do not appear in Figure 28.

Sites E13 and E7 showed higher levels of TSS in the bottom water than at the surface, whereas E32 and M2 were higher in the surface samples than the bottom. Measurable levels ranged from 7 mg/l to 22 mg/l in surface water and 26 mg/l and 28 mg/l in the bottom water at the two (2) sites where TSS was detected at depth (E13 and E7 respectively). For a detailed account of all the results, view Appendix G.

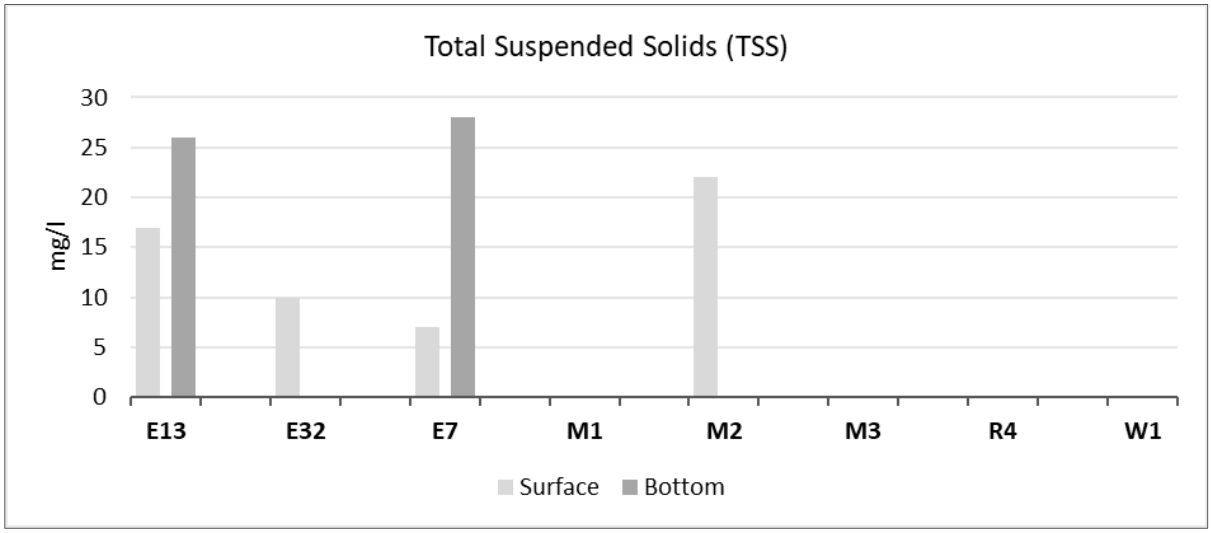


Figure 28 TSS levels (mg/l) for water samples sites.

5.8.2 Metals

Water samples were collected to analyse levels of heavy and trace metals at the surface and bottom of the water column. The majority of the analytes (Antimony (Sb), Cadmium (Cd), Cobalt (Co), Chromium (Cr), Manganese (Mn), Mercury (Hg), Tin (Sn), Aluminium (Al), Barium (Ba), Beryllium (Be), Iron (Fe) and Titanium (Ti)) were below the Limit of Detection (LoD) and are therefore not presented in Table 29. Copper (Cu), Lead (Pb) and Nickel (Ni) were below the LoD for all but a few samples, where the measured concentration was equal to the LoD (0.001 mg/l; Table 29) and below the reference thresholds. Where detectable, the concentrations of the different metals were generally low across the site and cable route (Figure 29 and Figure 30).

The only metal which showed concentrations in excess of the reference levels was Zinc (Zn; Table 29 and Figure 29, Figure 30), with six (6) samples being above the Annual Average (AA) of the UK Water Framework Directive (WFD) Environmental Quality Standards (EQS; 0.0079mg/l) (SEPA, 2018).

When comparing metal concentrations between depths in samples taken at the same site, Arsenic (As), Molybdenum (Mo) and Vanadium (V) remained consistent between the surface and bottom measurements (Figure 29 and Figure 30) across all sites. Selenium (Se) and Zinc (Zn) presented more variability between depths, although these were minimal, and levels still remained low (Figure 29 and Figure 30).

The concentrations of Total Sulphur, as SO_4, followed a similar trend, with all but one sample site presenting only minor variations across the survey area (Table 29, Figure 31 and Figure 32). The sample acquired near the seabed at site R4 presented a notably higher concentration of SO_4 compared all other water samples (5120 mg/l; Table 29, Figure 31 and Figure 32).

Table 29 Metal concentrations (mg/l) in samples with reference values. Highlighted cells indicate where threshold values were exceeded.

Analytes		As	Cu	Pb	Mo	Ni	Se	V	Zn	Total Sulphur as SO_4
Unit		mg/l								
Method		ICPMSSEA (Dissolved)	ICPMSSEA (Dissolved)	ICPMSSEA (Dissolved)	ICPMSSEA (Dissolved)	ICPMSSEA (Dissolved)	ICPMSSEA (Dissolved)	ICPMSSEA (Dissolved)	ICPMSSEA (Dissolved)	ICPWATVAR (Dissolved)
LoD		0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
E13	**Surface**	0.002	<0.001	<0.001	0.012	<0.001	0.002	0.002	0.005	2820
	Bottom	0.003	<0.001	<0.001	0.011	<0.001	0.008	0.002	0.005	2780
E32	**Surface**	0.002	<0.001	<0.001	0.011	<0.001	0.007	0.002	0.006	2880
	Bottom	0.003	<0.001	<0.001	0.012	<0.001	0.007	0.002	0.006	2790
E7	**Surface**	0.002	<0.001	<0.001	0.012	<0.001	0.007	0.002	0.009	2810
	Bottom	0.003	<0.001	<0.001	0.011	0.001	0.009	0.002	0.008	2830
M1	**Surface**	0.003	0.001	<0.001	0.011	<0.001	0.008	0.002	0.008	2710
	Bottom	0.002	<0.001	<0.001	0.011	<0.001	0.006	0.002	0.003	2800
M2	**Surface**	0.003	<0.001	<0.001	0.011	<0.001	0.005	0.002	0.007	2830
	Bottom	0.002	<0.001	0.001	0.011	<0.001	0.008	0.002	0.009	2850
M3	**Surface**	0.002	<0.001	<0.001	0.011	<0.001	0.005	0.002	0.012	2820
	Bottom	0.003	<0.001	<0.001	0.011	<0.001	0.006	0.002	0.005	2880
R4	**Surface**	0.003	<0.001	<0.001	0.011	<0.001	0.008	0.002	0.004	2890
	Bottom	0.003	<0.001	<0.001	0.011	<0.001	0.007	0.002	0.013	5120
W1	**Surface**	0.002	0.001	<0.001	0.011	<0.001	0.007	0.002	0.007	2840
	Bottom	0.002	<0.001	<0.001	0.011	<0.001	0.006	0.002	0.005	2830
Mean		0.003	0.001	-	0.011	-	0.007	0.002	0.007	2968
SD		0.001	0	-	0	-	0.002	0	0.003	576
Min		0.002	0.001	0.001	0.011	0.001	0.002	0.002	0.003	2710
Max		0.003	0.001	0.001	0.012	0.001	0.009	0.002	0.013	5120

Analytes	As	Cu	Pb	Mo	Ni	Se	V	Zn	Total Sulphur as SO_4
Unit	mg/l								
Method	ICPMSSEA (Dissolved)	ICPMSSEA (Dissolved)	ICPMSSEA (Dissolved)	ICPMSSEA (Dissolved)	ICPMSSEA (Dissolved)	ICPMSSEA (Dissolved)	ICPMSSEA (Dissolved)	ICPMSSEA (Dissolved)	ICPWATVAR (Dissolved)
LoD	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
Median	0.003	0.001	0.001	0.011	0.001	0.007	0.002	0.007	2830
Reference Levels									
WFD EQS UK (AA)*	0.025	0.00376	-	-	-	-	0.1	0.0079	-
WFD EQS EU (AA)**	-	-	0.0013	-	0.0086	-	-	-	-
WFD EQS EU (MAC)***	-	-	0.014	-	0.034	-	-	-	-

**WDF EQS UK (AA): Water Frame Directive Environmental Quality Standard for the UK (Annual Average)* (SEPA, 2018)

***WDF EQS EU (AA): Water Frame Directive Environmental Quality Standard for the EU (Annual Average)* (SEPA, 2018)

****WDF EQS EU (MAC): Water Frame Directive Environmental Quality Standard for the EU (Maximum Allowed Concentration)* (SEPA, 2018)

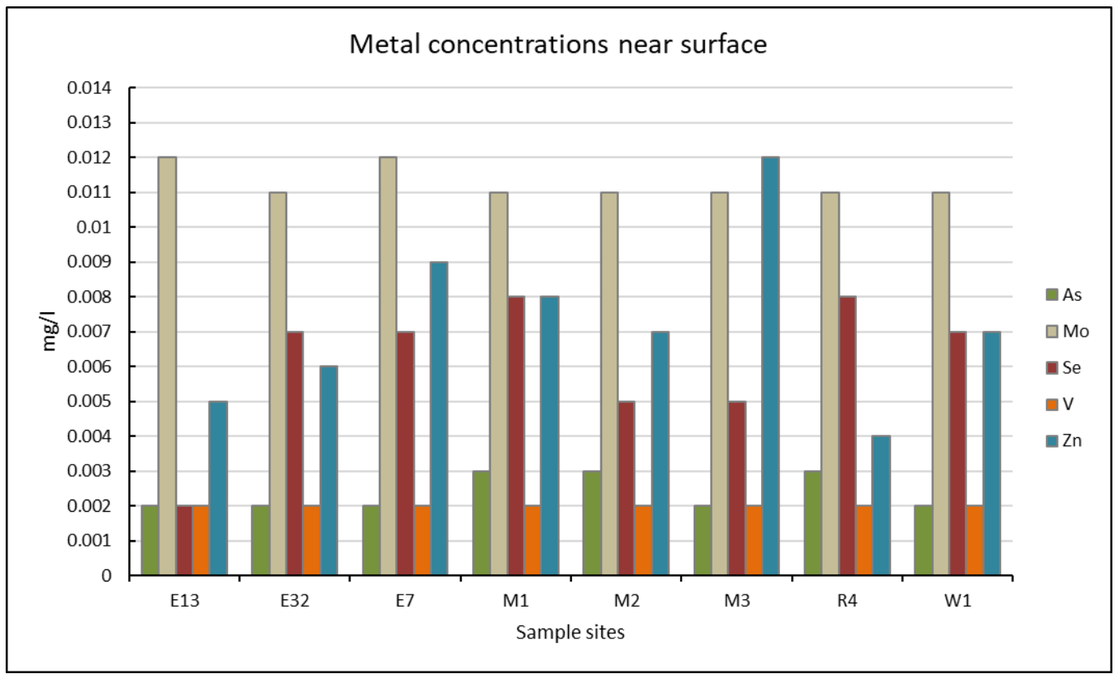


Figure 29 Metal concentrations in surface water samples.

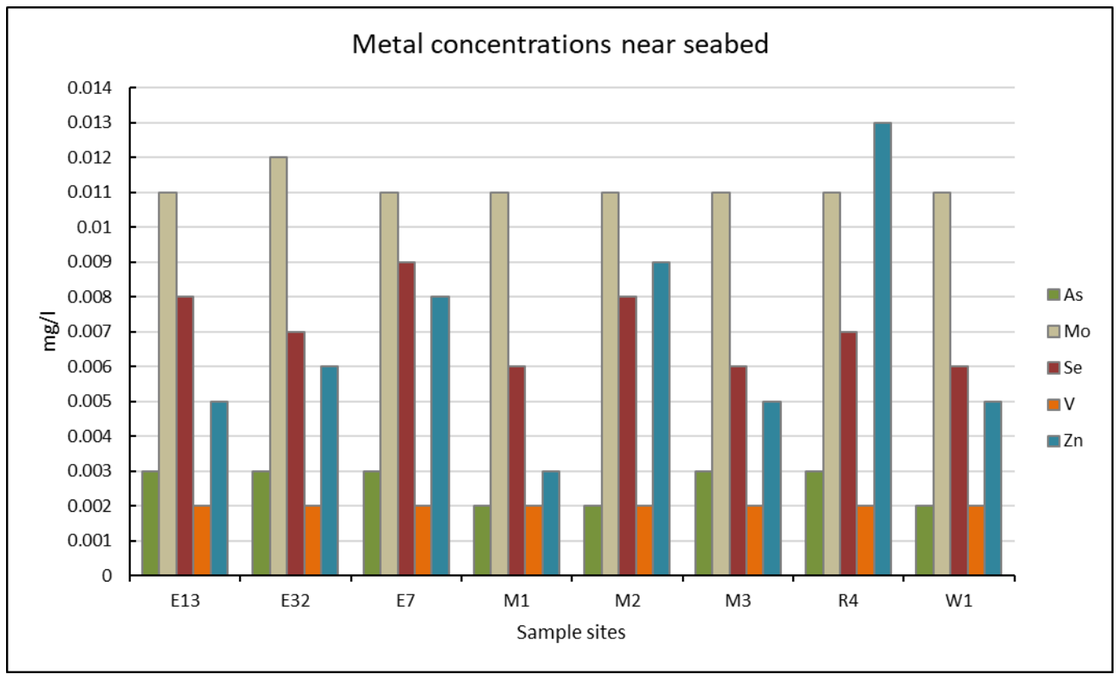


Figure 30 Metal concentrations in bottom water samples.

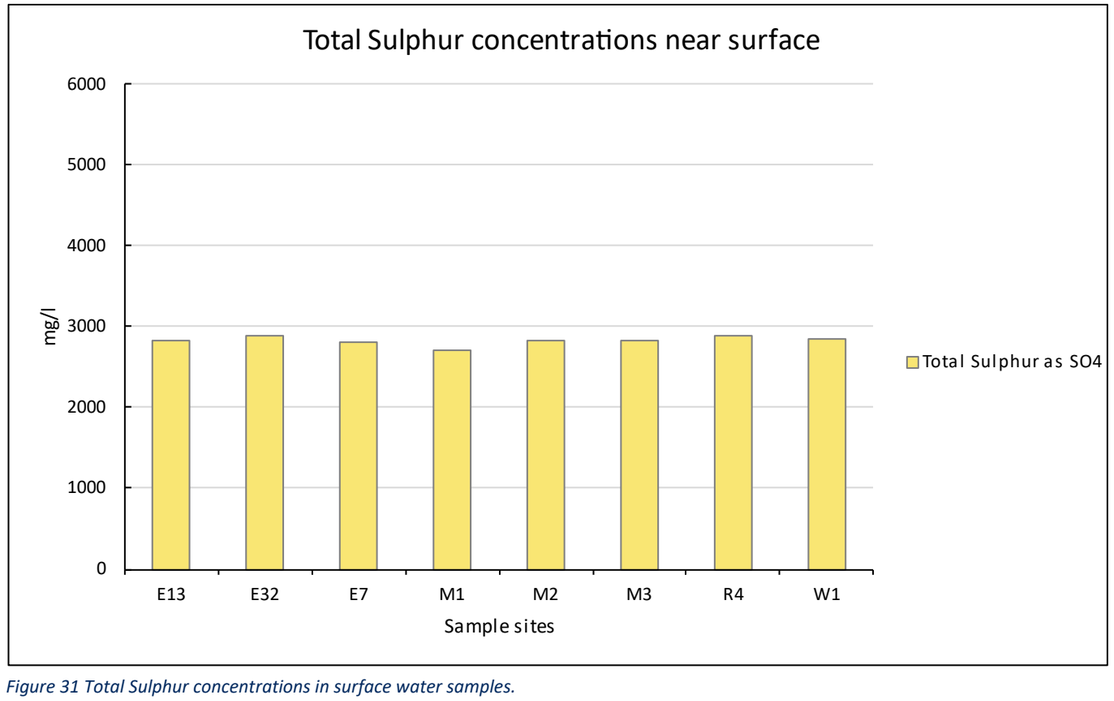


Figure 31 Total Sulphur concentrations in surface water samples.

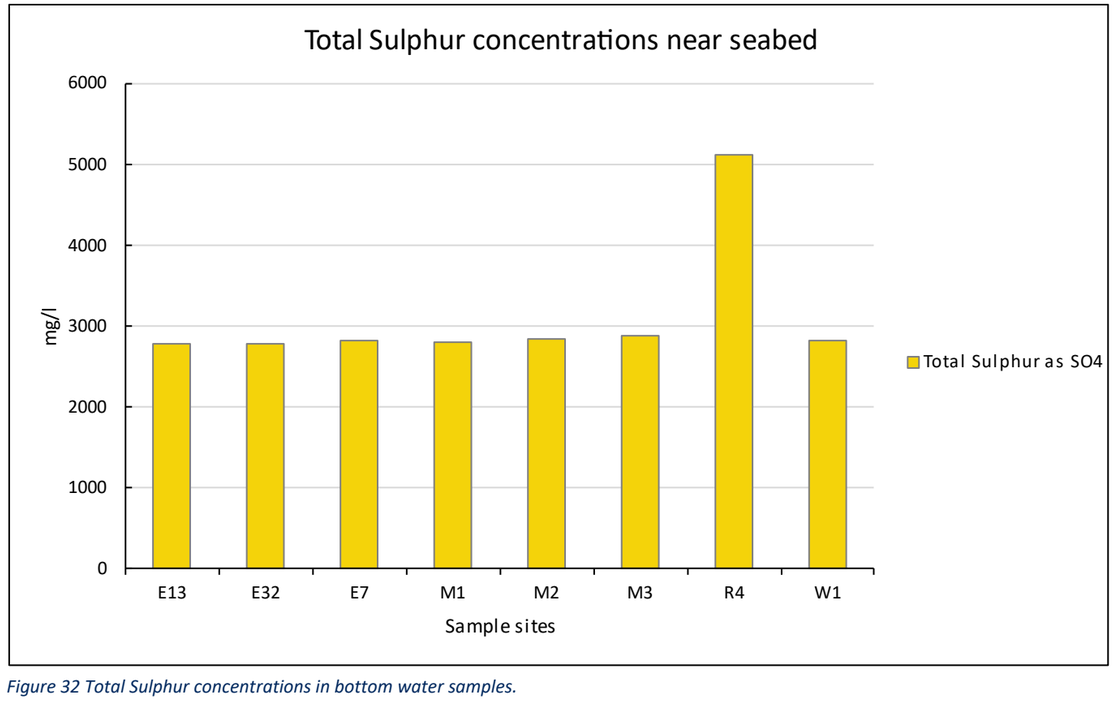


Figure 32 Total Sulphur concentrations in bottom water samples.

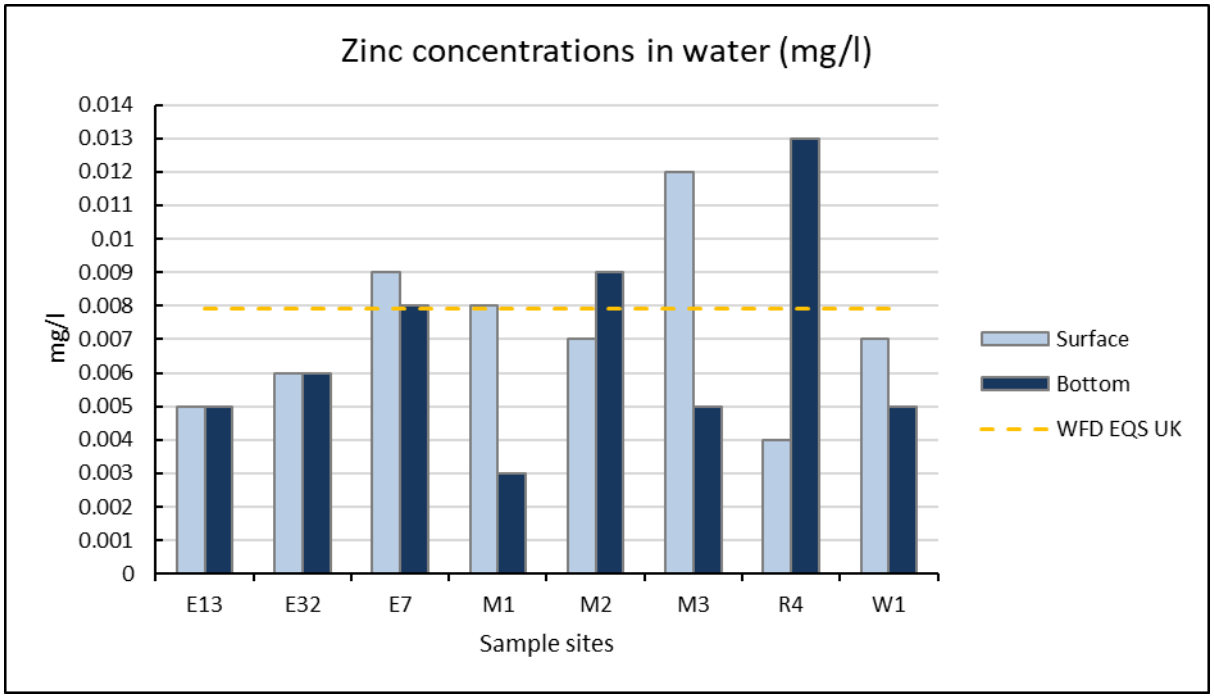


Figure 33 Zinc (Zn) concentrations in surface and bottom samples, with UK WFD EQS.

5.8.3 Hydrocarbons

Results for hydrocarbon analyses are tabulated in Appendix H with all analysed components, including Total Hydrocarbons (THC), Polycyclic Aromatic Hydrocarbons (PAH), n-alkanes, Pristane (Pr) and Phytane (Ph) being below the limit of detection (LoD).

Total Hydrocarbon Content (THC)

Total Hydrocarbons were analysed in all eight (8) water samples, resulting in low concentrations throughout, with all levels below the 100 µg/l detection limit.

Polycyclic Aromatic Hydrocarbons (PAH)

Total Polycyclic Aromatic Hydrocarbons (PAH) were equally low, presenting results below the LoD (34 µg/l) in all samples collected.

All other analysed components, such as total n-alkanes, Pr, Ph and individual Environment Protection Agency (EPA) PAHs were also below LoD (28 µg/l, 1 µg/l, 1 µg/l, 1 µg/l respectively).

5.9 Statistical Analyses from Grab Samples

5.9.1 Non-Colonial Fauna

The non-colonial epifauna was identified to the lowest taxonomic level possible and the individuals were enumerated. The infauna and non-colonial epifauna were combined and analysed together. When analysing phyletic composition, the following phyla: Phoronida, Nemertea, Cnidaria, Hemichordata, Platyhelminthes and Chordata was combined into the group “Others”.

All grab sample sites, and replicate samples comprised sufficient sample volume and were included in the statistical analyses.

The colonial epifauna was identified to the lowest taxonomic level possible. The colonial epifauna was recorded as absent/present and analysed separately. The results are presented in 5.9.10. A full list of species from the grab samples is presented in Appendix D.

5.9.2 Phyletic Composition

The phyletic composition of the non-colonial fauna identified from the grab samples is illustrated in Figure 34, and Figure 35, and summarised in Table 30. Annelida had the highest abundance, followed by Mollusca and Arthropoda. These three phyla contributed to 82 % of the recorded individuals. Annelida had the highest diversity, followed by Mollusca and Arthropoda. These three phyla contributed to 88 % of the recorded taxa.

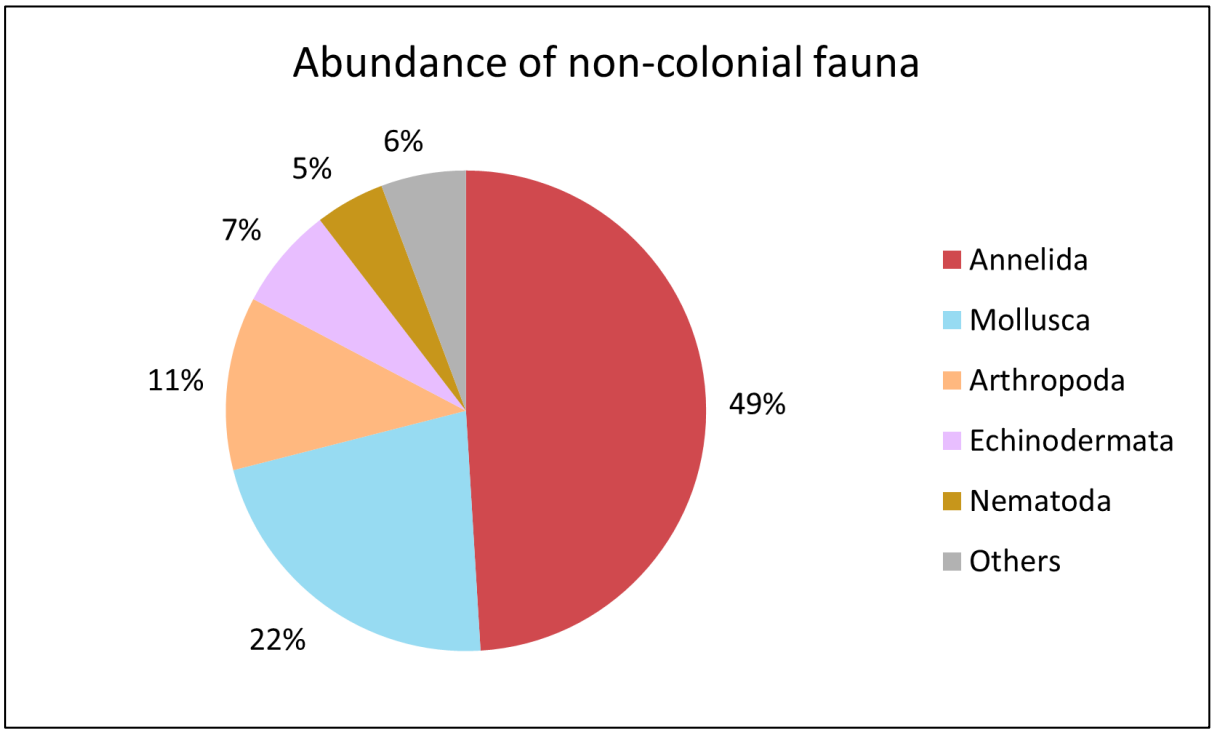


Figure 34 Abundance of non-colonial fauna from grab samples.

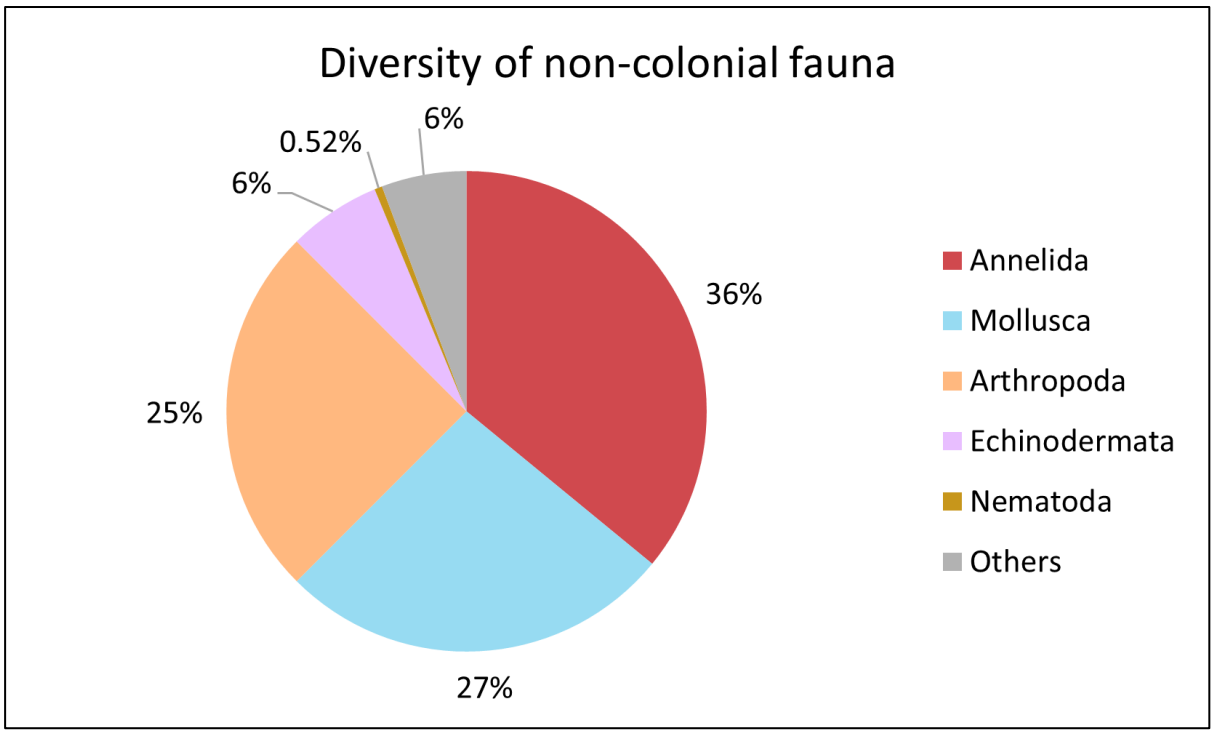


Figure 35 Diversity of non-colonial fauna from grab samples.

Table 30 Phyletic composition of non-colonial fauna from grab samples.

Phylum	Number of Taxa	Abundance (Total Number of Individuals)
Annelida	69	1697
Mollusca	51	761
Arthropoda	48	404
Echinodermata	12	239
Nematoda	1	162
Others	11	198

A list of the ten most abundant taxa, with total abundance and frequency of occurrence, is presented in Table 31 and the distribution within the survey area is illustrated in Table 30. The most abundant taxon is the annelid *Paramphinome jeffreysii*, with a total of 809 individuals recorded, and the species occurred in 100 % of the grab samples.

Table 31 The ten most abundant taxa from grab samples and frequency of occurrence.

Phylum	Taxa	Total Abundance	Mean Abundance	SD	Frequency of Occurrence (%)
Annelida	*Paramphinome jeffreysii*	809	50.56	21.07	100
Annelida	*Galathowenia oculata*	189	11.81	6.51	100
Arthropoda	*Harpinia antennaria*	163	10.19	6.18	100
Nematoda	Nematoda	162	10.13	6.24	100
Echinodermata	Amphiuridae (juvenile)	144	9.00	4.34	100
Mollusca	Nuculidae (juvenile)	139	8.69	7.11	69
Mollusca	*Papillicardium minimum*	111	6.94	5.99	100
Annelida	*Pholoe assimilis*	99	6.19	4.75	88
Phoronida	Phoronis	97	6.06	5.99	88
Annelida	*Spiophanes kroyeri*	90	5.63	4.69	100

A list of the ten most frequently occurring taxa, with total abundance, is presented in Table 32. The most frequently occurring taxon was the annelid *Paramphinome jeffreysii*, which occurred in 100 % of the grab samples, with a total abundance of 809 individuals.

Table 32 The ten most frequently occurring taxa from grab samples and total abundance.

Phylum	Taxa	Frequency of Occurrence (%)	Total Abundance
Annelida	*Paramphinome jeffreysii*	100	809
Annelida	*Galathowenia oculata*	100	189
Arthropoda	*Harpinia antennaria*	100	163
Nematoda	Nematoda	100	162
Echinodermata	Amphiuridae (juvenile)	100	144
Mollusca	*Papillicardium minimum*	100	111
Annelida	*Spiophanes kroyeri*	100	90
Annelida	*Spiophanes bombyx*	100	81
Echinodermata	*Amphiura filiformis*	100	62
Annelida	*Levinsenia gracilis*	94	48

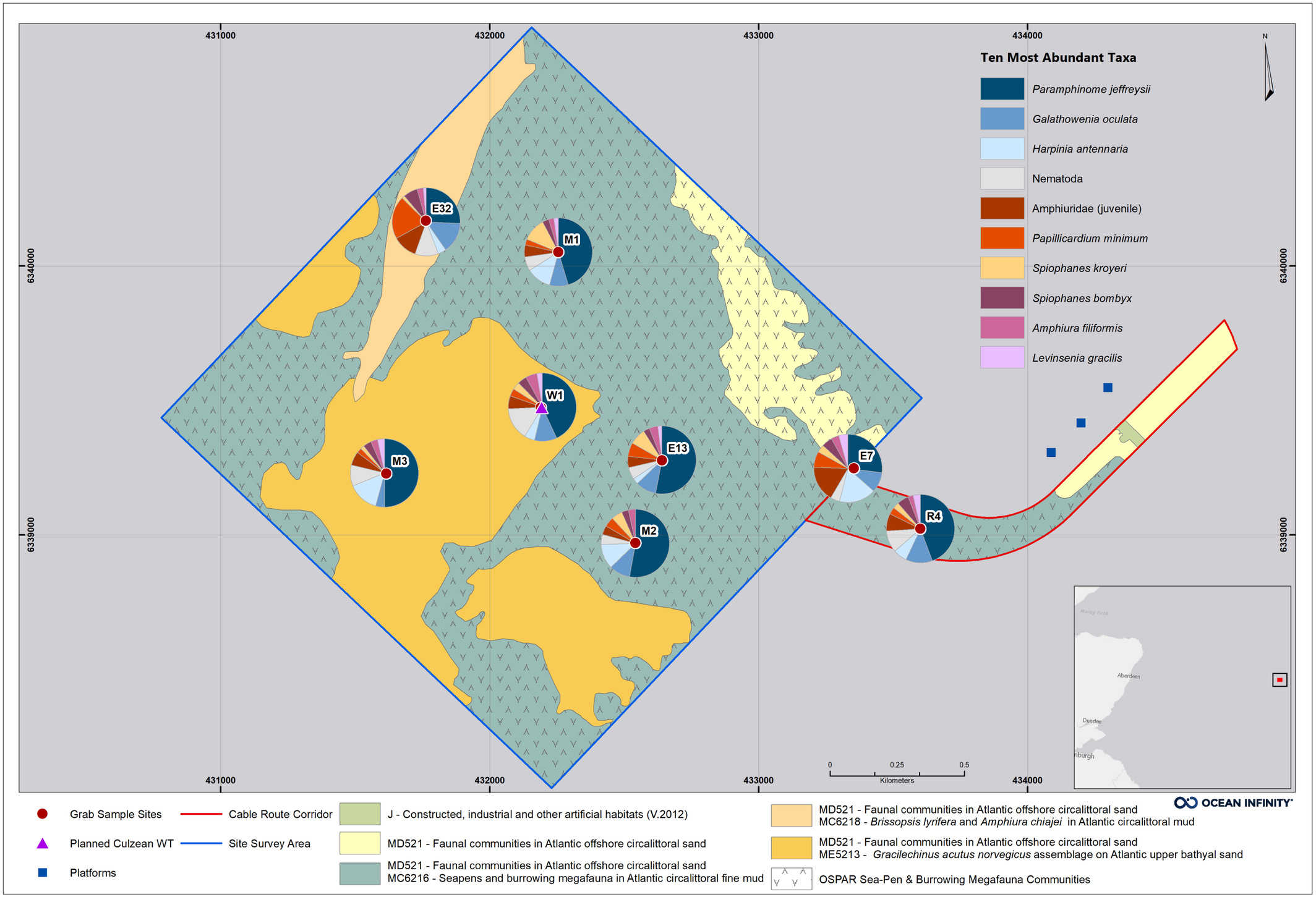


Figure 36 Overview of the ten most abundant taxa per grab sample site.

5.9.3 Univariate Statistical Analyses

Univariate analyses were performed to assess the non-colonial faunal richness, diversity, evenness and dominance. The results of the univariate analyses are presented in Table 33.

The number of Taxa (S) per site varied with a mean of 53 (SD= 8) where R4_F1 contained the highest number of Taxa (66 different taxa) and M3_F1 the lowest (38 different taxa). An overview of the number of Taxa (S) identified per grab sampling site and replicate sample is presented in Figure 37.

The number of individuals (N) per site (expressed per 0.1 m^2) varied with a mean of 216 (SD= 50) where R4_F2 contained the highest number of individuals (313 individuals) and M3_F1 the lowest with 123 individuals. An overview of the number of individuals (N) identified per grab sampling site and replicate sample is presented in Figure 38.

The species richness measured with Margalef's diversity index (D) varied between 7.69 and 11.47 with E13_F1 having the highest value of 11.47. Pielou's evenness index (J') ranged from 0.74 to 0.88, with E7_F1 having the highest value of 0.88.

The Shannon-Wiener index (H') ranged from 2.81 to 3.45, with W1_F2 having the highest value of 3.45. An overview of the Shannon-Wiener index (H') identified per grab sampling site and replicate sample is presented in Figure 39. Simpson's index of dominance (1-λ) varied from 0.87 to 0.95, with E7_F1 having the highest value of 0.95.

Table 33 Univariate indices of species richness, diversity and evenness for fauna in a single grab sample per site.

Sample ID	Number of Taxa (S)	Number of Individuals (N)	Margalef's Richness Index (D)	Pielou's Evenness Index (J')	Shannon-Wiener Index (H')	Simpson's Index of Dominance (1-λ)
E13_F1	64	243	11.47	0.83	3.44	0.93
E13_F2	53	251	9.41	0.79	3.12	0.90
E32_F1	47	179	8.87	0.82	3.16	0.93
E32_F2	50	197	9.28	0.82	3.21	0.93
E7_F1	45	160	8.67	0.88	3.33	0.95
E7_F2	51	153	9.94	0.81	3.18	0.92
M1_F1	58	249	10.33	0.76	3.09	0.89
M1_F2	55	221	10.00	0.85	3.40	0.94
M2_F1	47	229	8.47	0.78	3.01	0.90
M2_F2	48	186	8.99	0.81	3.15	0.91
M3_F1	38	123	7.69	0.82	2.97	0.90
M3_F2	44	218	7.99	0.74	2.81	0.87
R4_F1	66	295	11.43	0.80	3.35	0.93
R4_F2	62	313	10.62	0.79	3.26	0.91
W1_F1	59	243	10.56	0.79	3.24	0.91
W1_F2	59	201	10.94	0.85	3.45	0.94
Mean	53	216	9.67	0.81	3.20	0.92
SD	8	50	1.18	0.03	0.18	0.02
Min	38	123	7.69	0.74	2.81	0.87
Max	66	313	11.47	0.88	3.45	0.95
Median	52	220	9.68	0.81	3.19	0.92

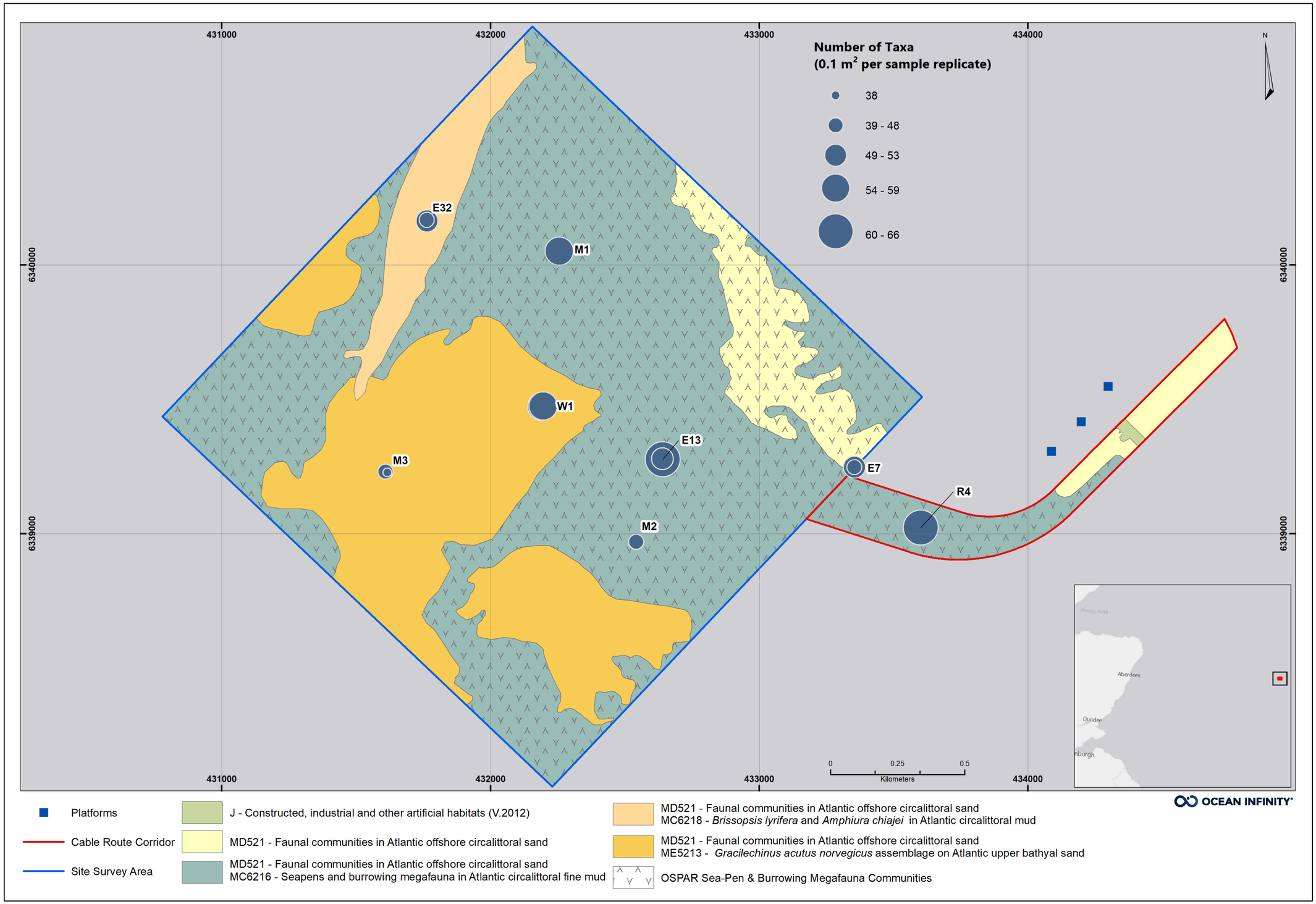


Figure 37 Overview of the Number of Taxa (S) per grab sample replicate.

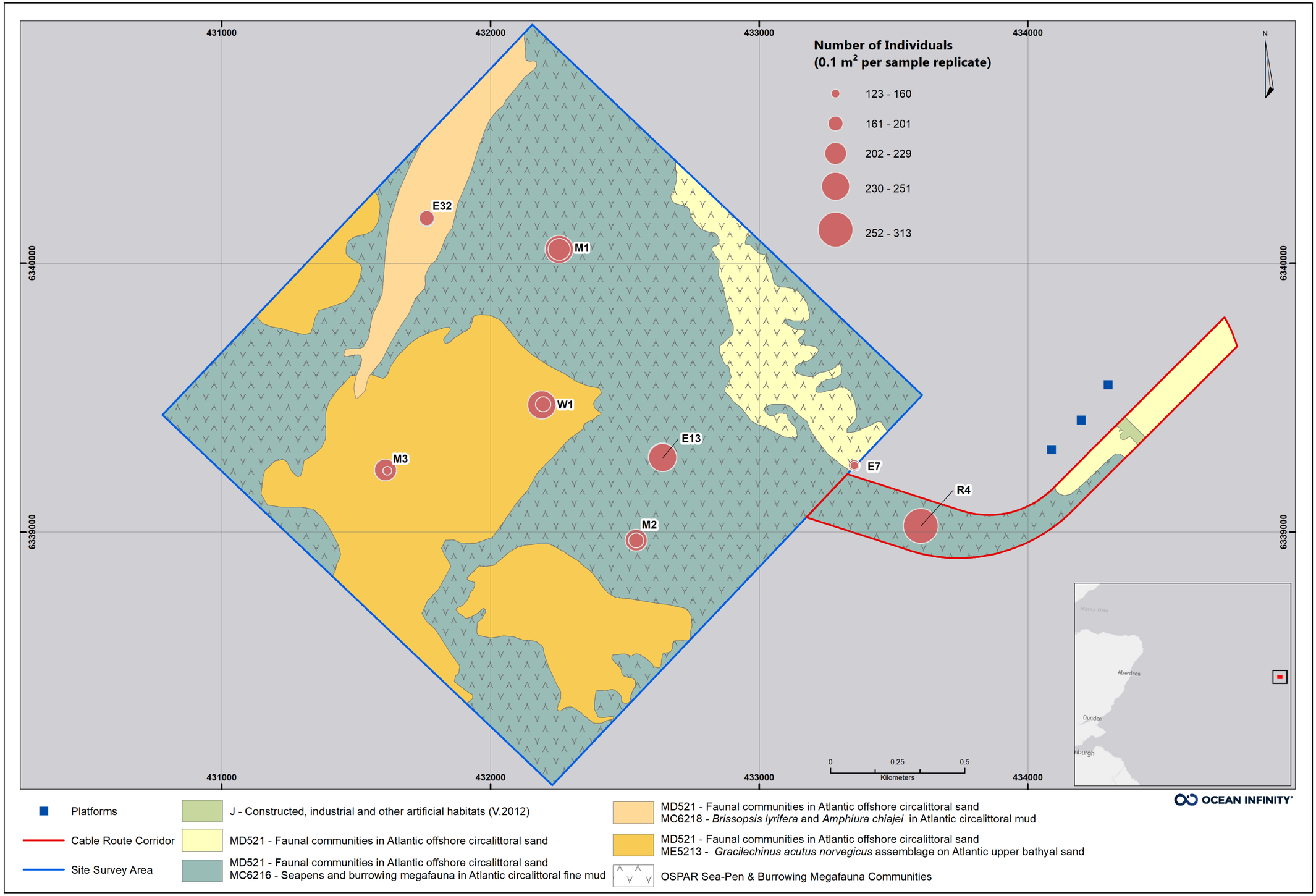


Figure 38 Overview of the Number of Individuals (N) per grab sample replicate.

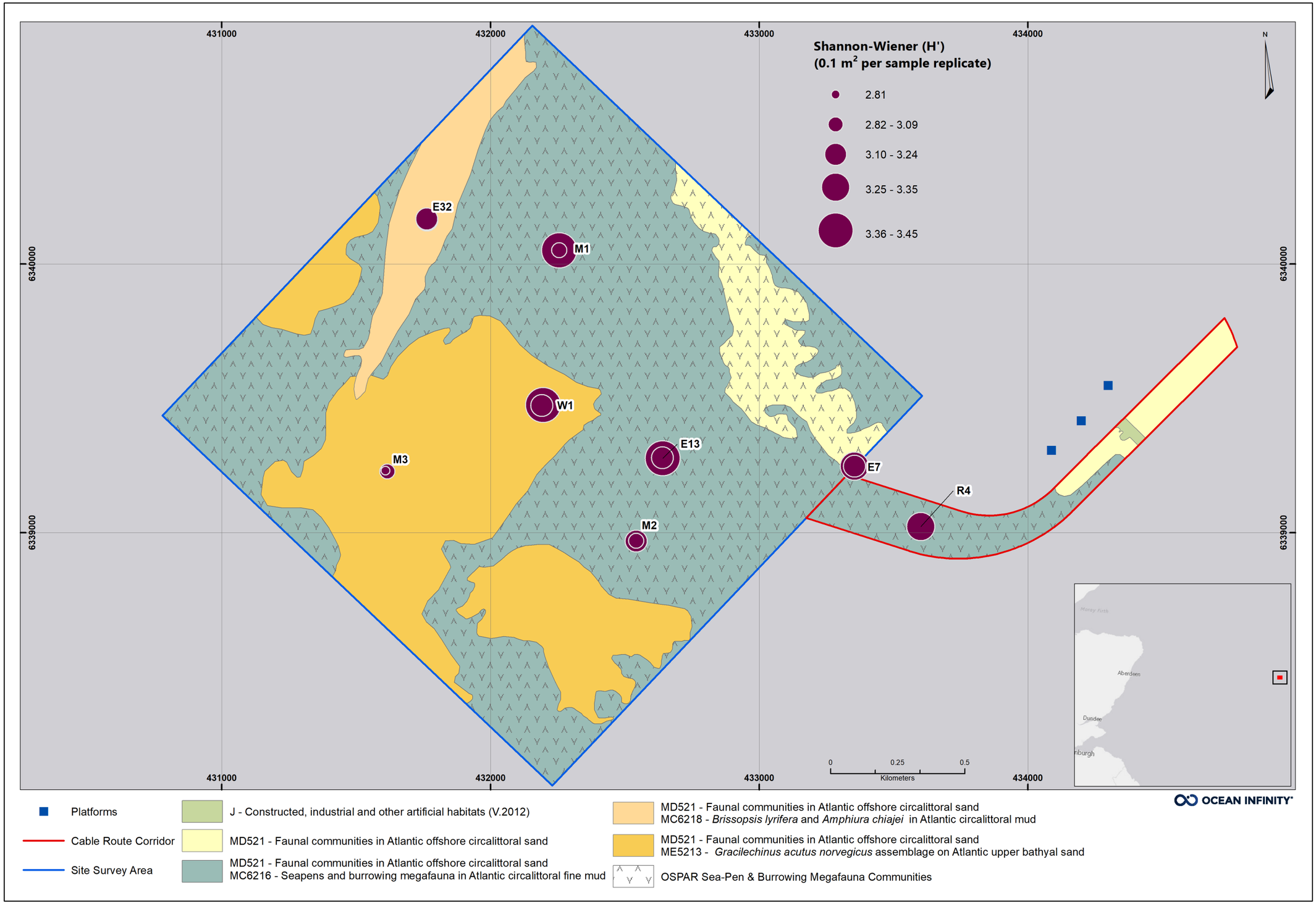


Figure 39 Overview of the Shannon-Wiener Index (H') per grab sample replicate.

5.9.4 Multivariate Statistical Analyses

Square root transformation was applied to the dataset before calculating the Bray-Curtis similarity measures in the SIMPROF and SIMPER analyses. The transformation was applied to prevent abundant species from influencing the Bray-Curtis similarity index measures excessively and to take the rarer species into account (Clarke & Gorley, PRIMER v7: User Manual/Tutorial. Plymouth: PRIMER-E., 2015). The statistical analyses were based on macrofaunal data derived from the taxonomic analyses of the grab samples.

5.9.5 SIMPROF Cluster Analyses

The SIMPROF analyses of the non-colonial faunal composition produced three (3) statistically distinct groups (black lines) and is presented in a hierarchical dendrogram in Figure 40.

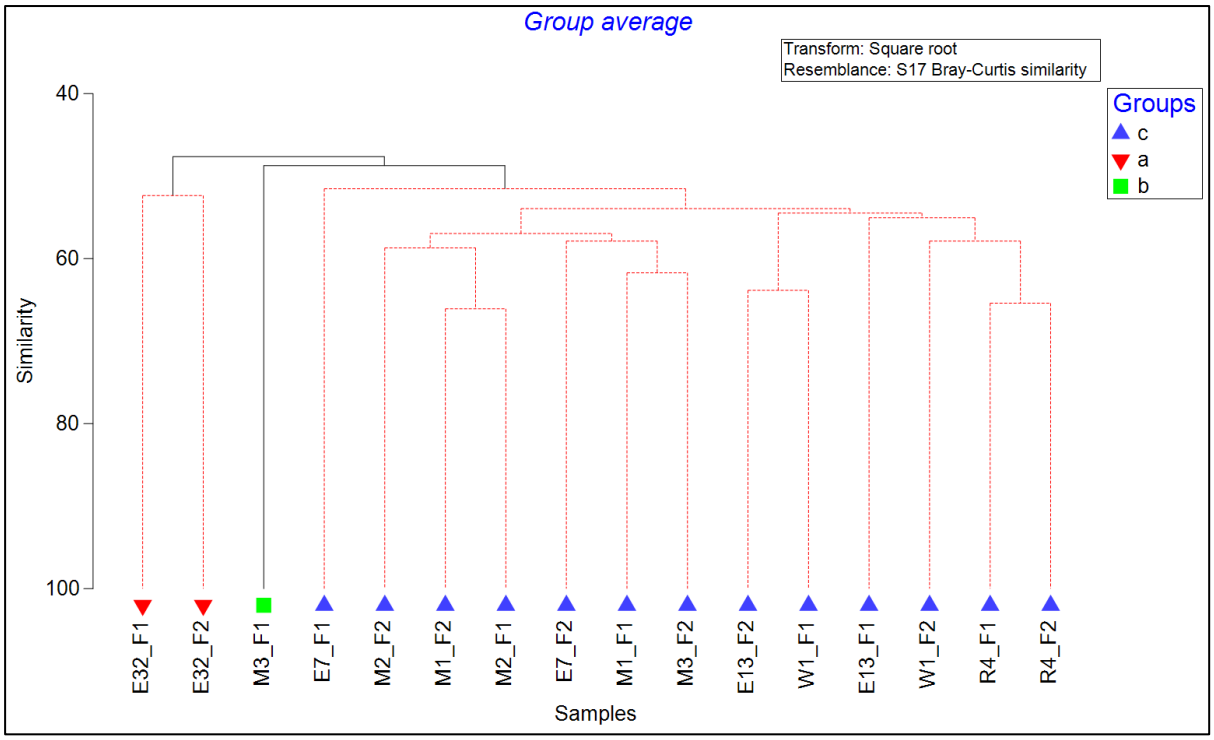


Figure 40 SIMPROF dendrogram of non-colonial fauna from grab sample sites.

5.9.6 Non-Metric Multi-Dimensional Scaling (MDS)

The nMDS-plot reflects the dendrogram (Figure 40) and displays the similarity between grab sample sites at 20 % to highlight homogeneous species composition. Sample similarity is further explored in the nMDS-plot in Figure 41.

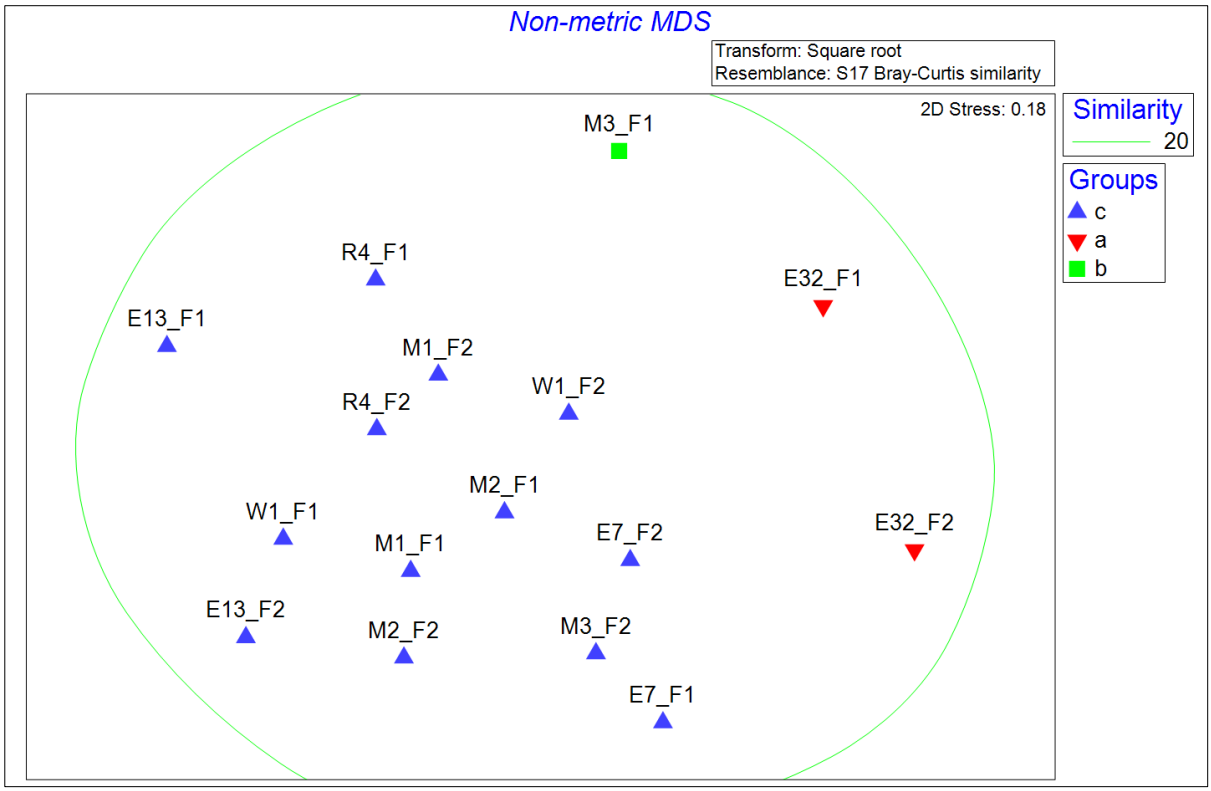


Figure 41 nMDS-plot of non-colonial faunal composition from grab sample sites with group based on the SIMPROF analysis.

5.9.7 SIMPER Results

A SIMPER test, displaying the percentage contribution of the most important species seen in the Bray-Curtis similarity test is presented in Table 34 with species abundance for each SIMPROF group. Average abundance refers to the square root transformed data and is expressed per 0.1 m^2 within the multivariate groups.

Table 34 Summary of characteristics of the non-colonial faunal groups from grab samples derived from the SIMPER test.

Group	Sample ID	Depth (m)	Species	Average Abundance	Contribution (%)
a **Average similarity: 52.35**	E32_F1 and E32_F2	90, 90	*Paramphinome jeffreysii*	5.14	11.38
			Papillicardium minimum	4.57	10.07
			Galathowenia oculata	3.79	7.12
			Nematoda	3.29	5.81
			Amphiuridae (juvenile)	3.35	5.81
			Spiophanes bombyx	2.64	5.81
			Phoronis	3.29	5.81
			Harpinia antennaria	2.00	4.75
			Retusa umbilicata	1.73	4.11
			Scoloplos armiger	1.73	4.11

b **Less than 2 samples in group**	M3_F1	90	-	-	-
c **Average similarity: 54.86**	E13_F1, E13_F2 E7_F1, E7_F2 M1_F1, M1_F2 M2_F1, M2_F2 M3_F2, R4_F1 R4_F2, W1_F1 W1_F2	90, 90 89, 89 91, 91 90, 90, 90, 89 89, 90 90	*Paramphinome jeffreysii* *Galathowenia oculata* *Harpinia antennaria* Amphiuridae (juvenile) Nematoda *Pholoe assimilis* *Papillicardium minimum* Nuculidae (juvenile) *Spiophanes bombyx* *Spiophanes kroyeri*	7.30 3.32 3.22 2.91 3.05 2.64 2.24 2.72 2.19 2.37	12.57 5.37 5.29 5.00 4.73 4.26 3.91 3.73 3.67 3.56

SIMPROF Group **a** comprised two (2) samples E32_F1 and E32_F2, located in the northernmost section of the site survey area at 90 meters depth. These samples were located in the identified habitat **MD521/MC6218** - Faunal communities on Atlantic offshore circalittoral sand/ *Brissopsis lyrifera* and *Amphiura chiajei* in Atlantic circalittoral mud. The annelid *Paramphinome jeffreysii* were the most abundant species and had the highest contribution (11.38 %) within group **a**.

Group **b** comprised a single sample M3_F1, located in the western site survey area at 90 meters depth, in **MD521/ME5213** - Faunal communities on Atlantic offshore circalittoral sand/ *Gracilechinus acutus norvegicus* assemblage on Atlantic upper bathyal sand. The annelid *P. jeffreysii* were the most abundant species and had the highest contribution (28.46 %) within group **b**.

Group **c** consisted of thirteen samples (E13_F1, E13_F2, E7_F1, E7_F2, M1_F1, M1_F2, M2_F1, M2_F2, M3_F2, R4_F1, R4_F2, W1_F1, W1_F2) distributed in the site survey area and along the cable route corridor, with a depth range between 89 - 90 m. These samples are included in the assigned habitats **MD521/MC6216** - Faunal communities on Atlantic offshore circalittoral sand/ Seapens and burrowing megafauna in Atlantic circalittoral fine mud, **MD521** - Faunal communities on Atlantic offshore circalittoral sand and **MD521/ME5213** - Faunal communities on Atlantic offshore circalittoral sand/ *Gracilechinus acutus* norvegicus assemblage on Atlantic upper bathyal sand. The annelid *P. jeffreysii* were the most abundant species and had the highest contribution (12.57 %) within group **c**.

5.9.8 Relationship Between Physical and Biological Data

The relationship between physical and biological (faunal abundance data from grab samples) data was assessed by applying the BEST analysis from the PRIMER suite. The BEST test identifies which of the variables best explains the observed macrofaunal distribution and groupings. As these variables are measured on different scales the physical data was normalised prior to analysis. This process takes each entry of a single variable, subtracts the mean and then divides by the standard deviation for that variable. This is carried out to bring the data on to a common scale and allow for the use of Euclidean distance measures.

A total of eight (8) sample sites were selected for the BEST analysis, where both physical and biological data was sampled. Selected variables in the BEST test included depth and PSA.

One test was carried out with the number of variables limited to one (1) to determine which single physical variable had the strongest correlation to the biological data. A second test was carried out with the maximum number of trial variables was set to three (3). The null hypothesis (H_0) is that there is no relationship between

the variables included in the test. Results of the BEST analysis for Single and Multiple variables are presented in Table 35.

Results presented for single variables gave a global correlation coefficient (σ) of 0.855 for V Coarse Sand/ Coarse Sand. The significance level was 0.2 % which means that the null hypothesis of 'no agreement in multivariate pattern between physical and biological data' can be rejected at p<1%. The variables % V Coarse Silt/ Coarse Silt and % Medium Silt followed with a correlation (σ) of 0.798 and 0.738, respectively. This indicates that V Coarse Sand/ Coarse Sand best explains the patterns observed based on the substrate as the single variable.

Results presented for multiple variables gave a global correlation (σ) of 0.867 for the combined variables V Coarse Sand/ Coarse Sand, Medium Gravel. In addition, the three (3) combined variables V Coarse Sand/ Coarse Sand, Medium Gravel, V Coarse Gravel/ Coarse Gravel also presented a global correlation (σ) of 0.867. The significance level was 0.5 % which means that the null hypothesis of 'no agreement in multivariate pattern between physical and biological data' can be rejected at p<1%. The variables V Coarse Sand/ Coarse Sand and the variables V Coarse Sand/ Coarse Sand, V Coarse Gravel/ Coarse Gravel followed both with a correlation (σ) of 0.855, respectively.

A higher the global correlation coefficient (σ) indicates a stronger relationship. Thus, where values exceed 0.7, there is considered to be a moderate to strong linear correlation between the physical variable and the fauna abundance data. The significance level of this correlation, for these tests p<0.2 % and p<0.5 %, indicates that the correlation is statistically significant which means that there is less than 5 % likelihood that the null hypothesis is true and that the relationship between the variables is random.

Chemicals and contaminants variables did not exceed any threshold values and are therefore not listed in Table 35.

Table 35 BEST test of physical data and biological data for single and multiple variables.

Max nr of trail variables	Number of Variables	Spearman Correlation (σ)	Physical Variables
Single variables Global Test (σ): 0.855 Significance: 0.2 %	1	0.855	V Coarse Sand/ Coarse Sand
	1	0.798	V Coarse Silt/ Coarse Silt
	1	0.738	Medium Silt
	1	0.737	Fine Gravel/ V Fine Gravel
	1	0.717	Clay
Multiple variables Global Test (σ): 0.867 Significance: 0.5 %	2	0.867	V Coarse Sand/ Coarse Sand, Medium Gravel
	3	0.867	V Coarse Sand/ Coarse Sand, Medium Gravel, V Coarse Gravel/ Coarse Gravel
	1	0.855	V Coarse Sand/ Coarse Sand
	2	0.855	V Coarse Sand/ Coarse Sand, V Coarse Gravel/ Coarse Gravel
	2	0.838	V Coarse Sand/ Coarse Sand, Fine Gravel/ V Fine Gravel

5.9.9 Multivariate Statistical Analyses EUNIS

Similarities between the macrofaunal data and EUNIS habitats are further explored in a hierarchical dendrogram presented in Figure 42, and in the nMDS-plot, presented in Figure 43.

Analyses in sub-section 5.9.9 are conducted on the faunal composition from grab sampling sites but with groups superimposed with EUNIS habitats (Table 19). Additionally, multivariate analyses were conducted using the sample specific EUNIS habitats (Table 20) presented in Figure 44 and Figure 45.

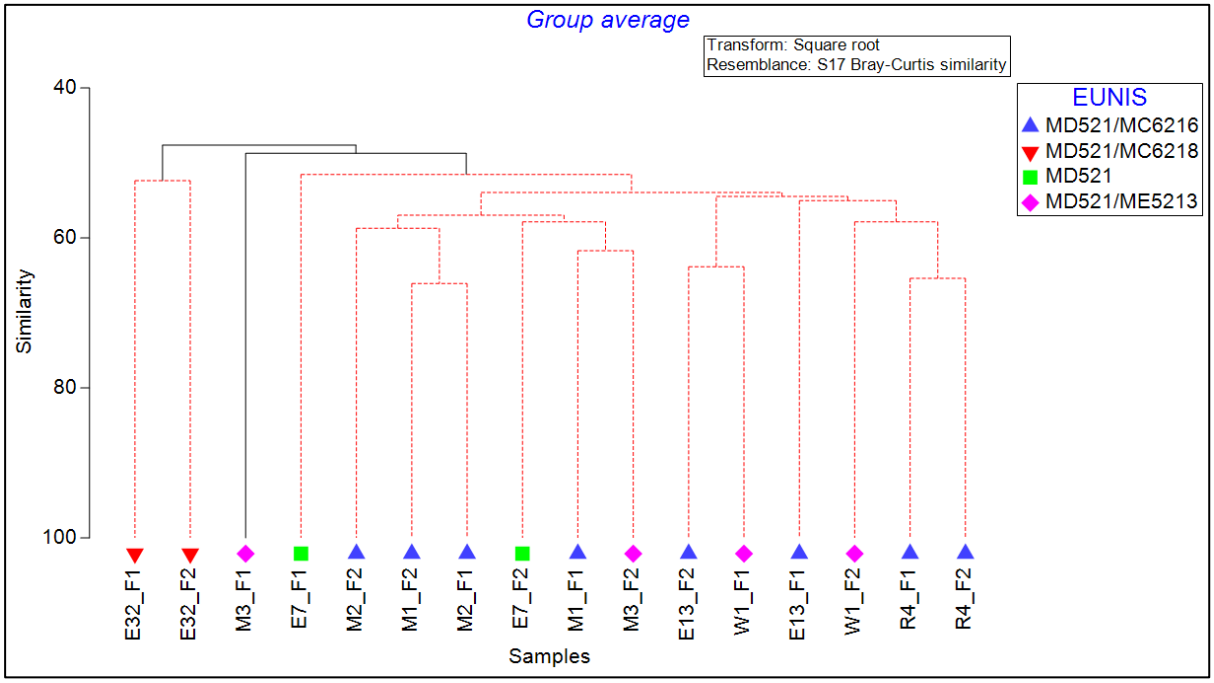


Figure 42 SIMPROF dendrogram of non-colonial faunal composition from grab sampling sites superimposed with EUNIS habitats.

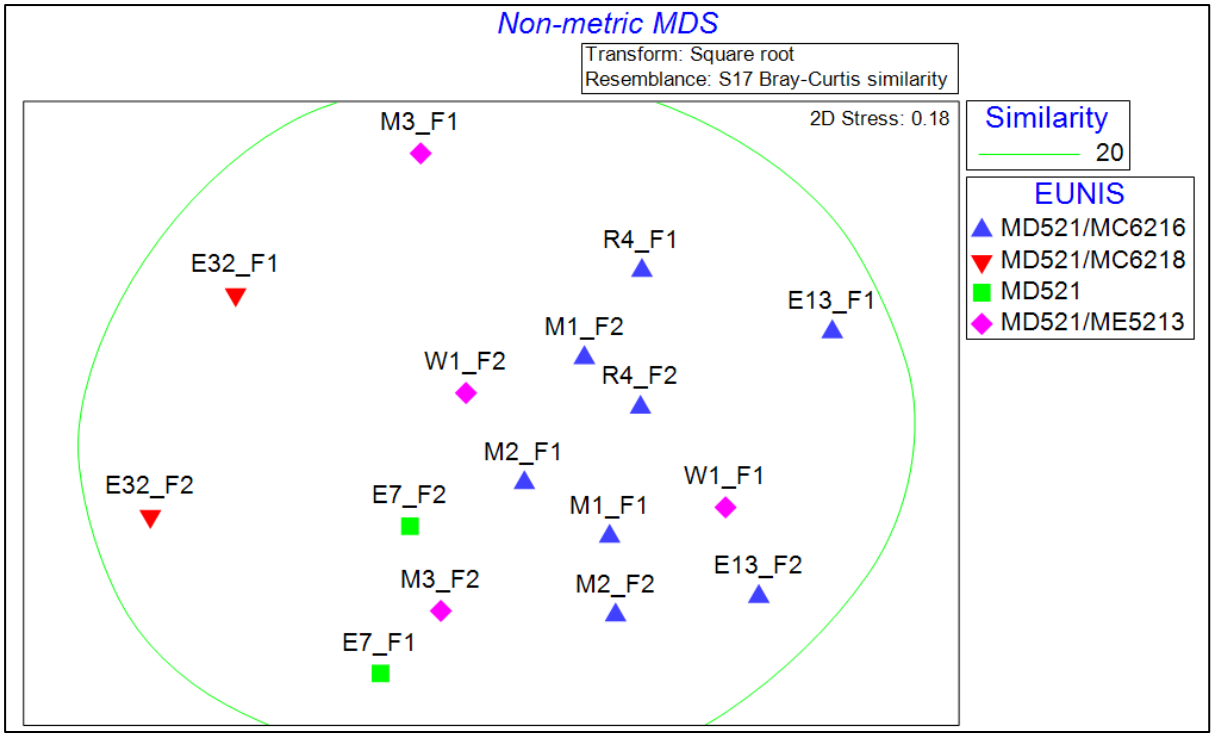


Figure 43 nMDS-plot of non-colonial faunal composition from grab samplings sites superimposed with EUNIS habitats.

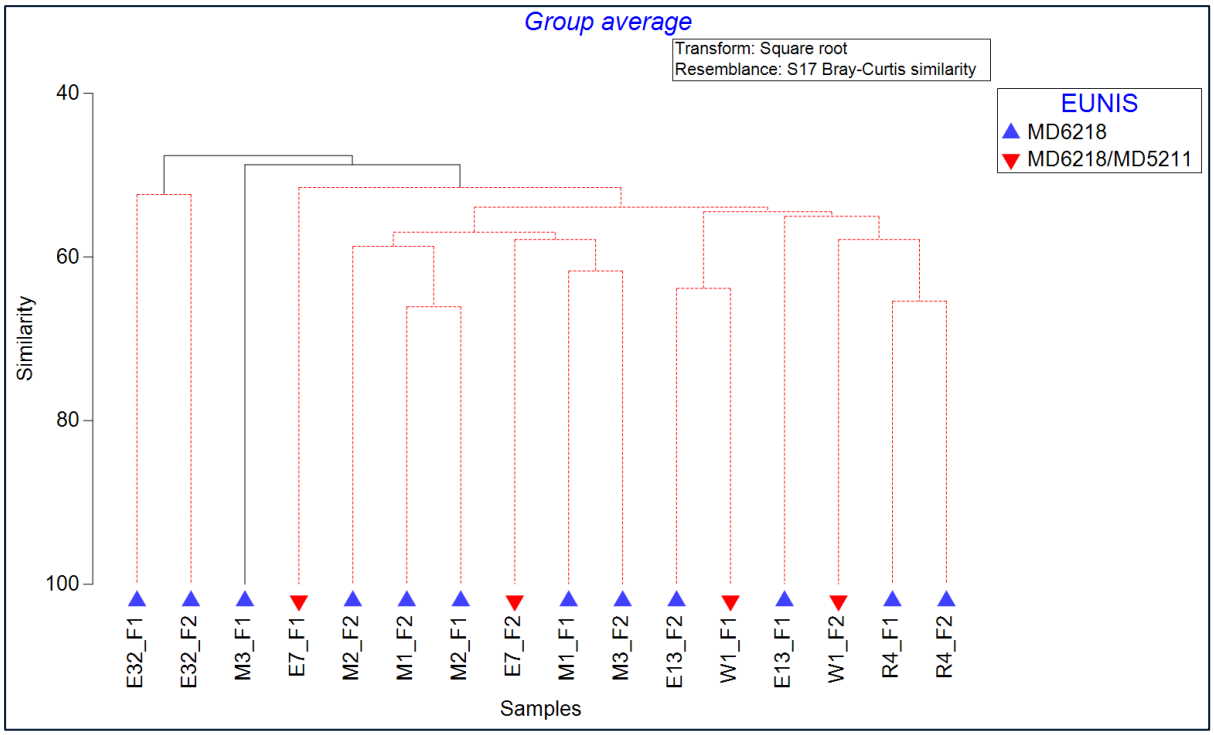


Figure 44 SIMPROF dendrogram of non-colonial faunal composition from grab sampling sites superimposed with sample specific EUNIS habitats.

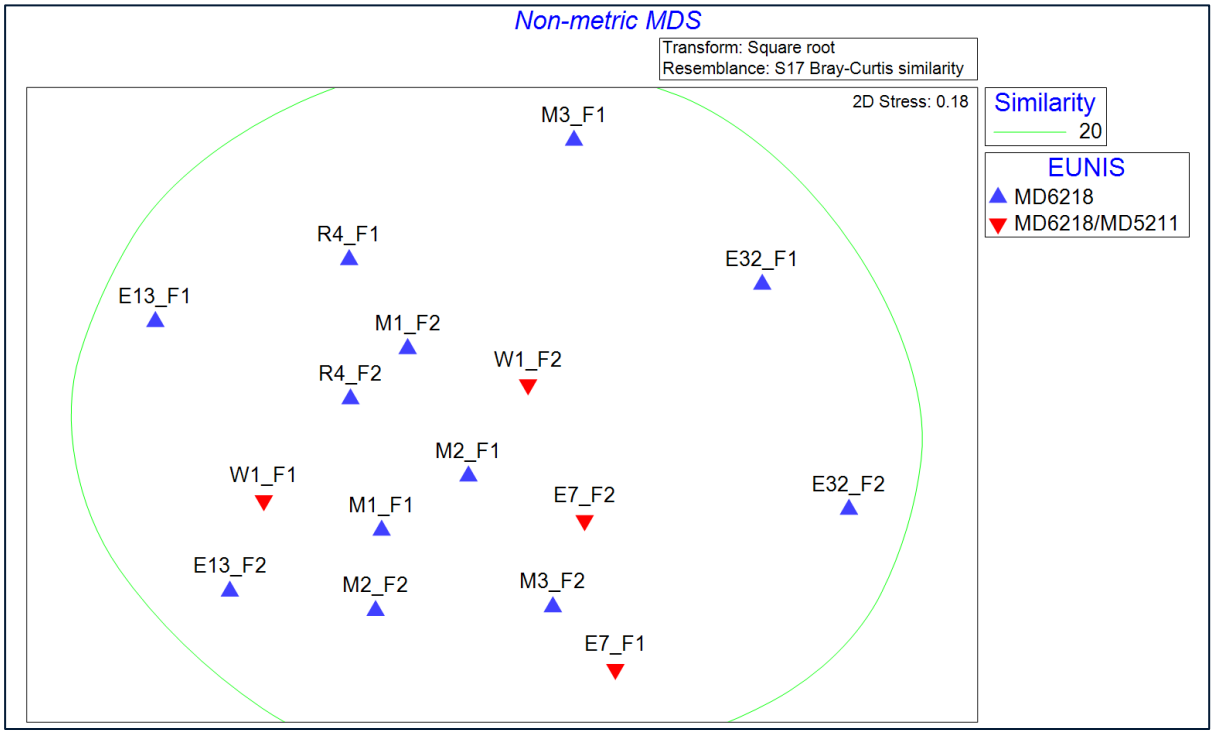


Figure 45 nMDS-plot of non-colonial faunal composition from grab samplings sites superimposed with sample specific EUNIS habitats.

5.9.10 Sessile Colonial Epifauna from Grab Samples

The phyletic composition of sessile colonial epifauna identified from grab samples is summarised in Table 36 and illustrated in Figure 46 and Figure 47.

A total of four (4) major phyla were identified. The dominant phylum was Cnidaria which constituted 63 % of the total taxa. Bryozoa, Porifera and Entoprocta followed with 13 % respectively of the total taxa. In total eight (8) different taxa were identified.

Abundance was also dominated by Cnidaria with a total of 14 colonies, followed by Bryozoa with six (6) colonies and Porifera with two (2) colonies. Entoprocta contributed with one (1) colony.

Table 36 Phyletic composition of colonial epifauna from grab samples.

Phylum	Number of Taxa	Abundance of Colonies
Cnidaria	5	14
Bryozoa	1	6
Porifera	1	2
Entoprocta	1	1
Total	8	23

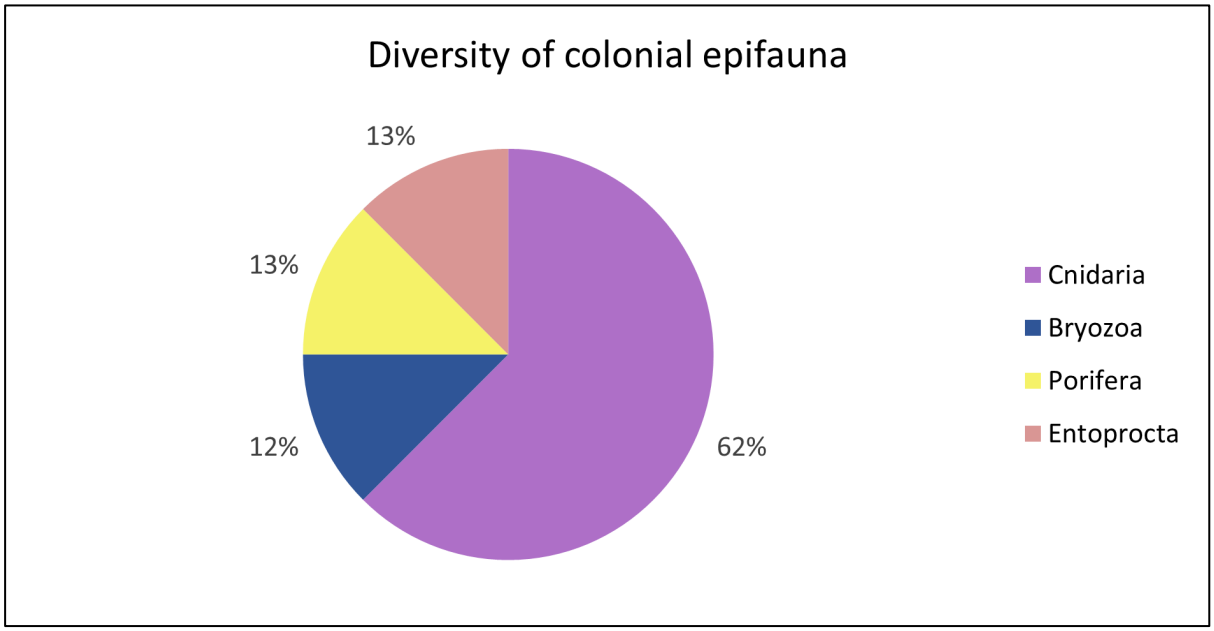


Figure 46 Diversity of colonial epifauna from grab samples.

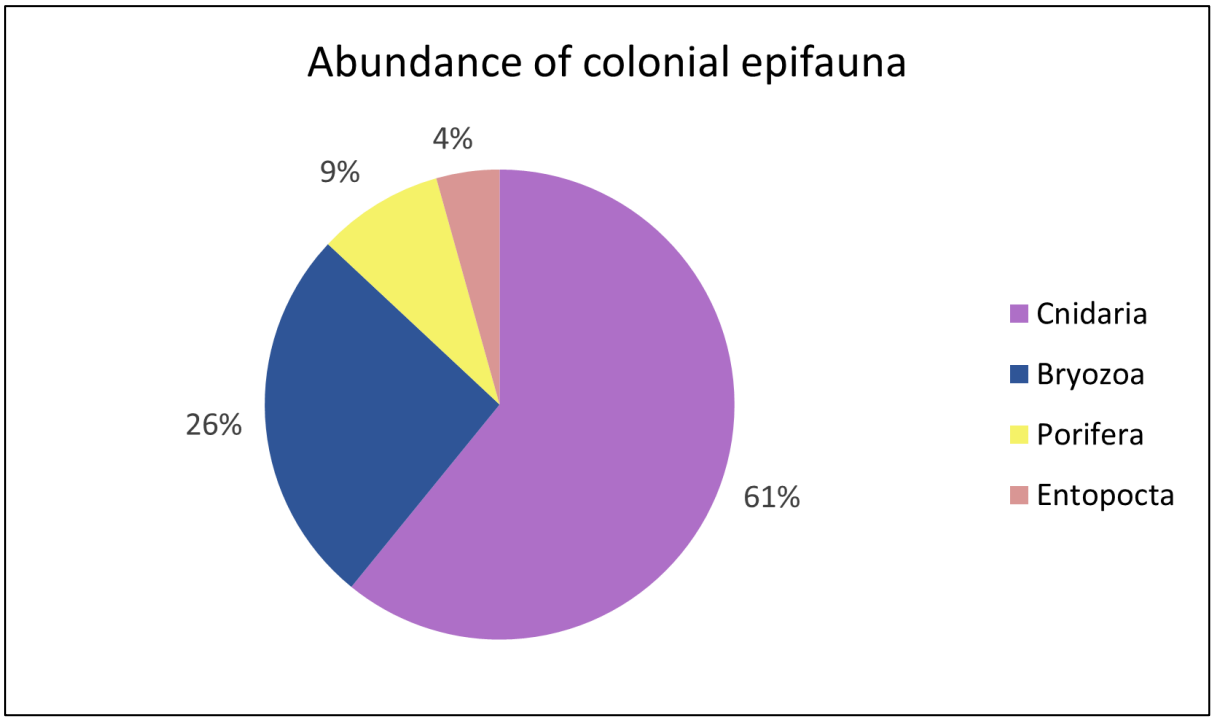


Figure 47 Abundance of colonial epifauna from grab samples.

5.9.11 Biomass

The non-colonial fauna species biomass expressed as blotted wet weight (g per 0.1 m²) is illustrated in Figure 48, and summarised in Table 37. Biomass was grouped into the major groups Echinodermata, Mollusca, Annelida, Phoronida, Hemichordata and "Others". The group "Others" included the phyla Arthropoda, Cnidaria Nemertea, Platyhelminthes and Nematoda.

Following the NMBAQC Taxonomic Discrimination Protocol, Ascidiacea were not weighted and included in the biomass analysis (Worsfold, Hall, & O'Reilly, 2010).

The biomass was dominated by Echinodermata, which accounted for 72 % of the total biomass. This was primarily due to the presence of two (2) large specimens of the Sea potato *Echinocardium cordatum* in samples M1_F2 and M2_F1 weighing 10.0901 g and 15.9228 g respectively. Both specimens constituted 41 % of the total echinoderm weight.

The second largest group was Mollusca, accounting for 15 % of the total biomass. The relatively large *Astarte sulcata*, weighing 3.9132 g in sample W1_F1 contributed to 5 % of the total mollusc biomass. Annelida accounted for 10 % of the total biomass, followed by Phoronida and Hemichordata with 1 % and 0.5 % respectively. The group "Others" accounted 1.4 %.

Within the group "Others", Arthropoda constituted 0.49 % of the total biomass. Cnidaria contributed 0.45 %, Nemertea 0.44 %, Platyhelminthes 0.01 % and Nematoda 0.01 % respectively of the total biomass. Non-colonial fauna biomass varied between 0.6395 g/0.1 m² in sample E7_F1 to 17.9573 g/0.1 m² in sample M2_F1. The mean biomass across all sites was 4.0846 g/0.1 m² (SD= 5.2905). The spatial distribution of biomass across the survey area is illustrated in Figure 49.

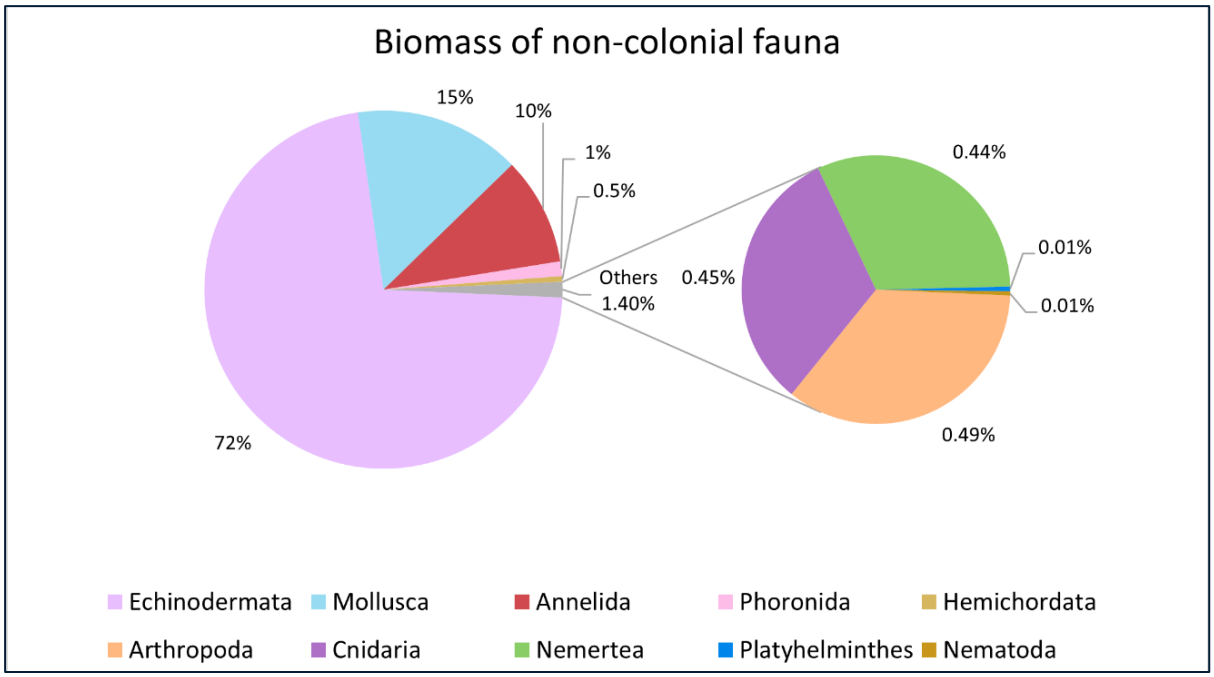


Figure 48 Total biomass (blotted wet weight in g/0.1 m²) composition of major phyla in the left pie chart and group "Others" in the right pie chart.

Table 37 Biomass (blotted wet weight in g/0.1 m²).

Sample ID	Echinodermata	Mollusca	Annelida	Phoronida	Hemichordata	Others	Total
E13_F1	9.6930	0.0605	0.9347	0.0000	0.0000	0.0460	10.7342
E13_F2	11.3954	0.3121	0.8522	0.0229	0.0000	0.2892	12.8718
E32_F1	0.0998	1.0054	0.1528	0.0100	0.0000	0.0360	1.3040
E32_F2	0.0893	0.1749	0.4029	0.0799	0.0000	0.0208	0.7678
E7_F1	0.2035	0.0660	0.2867	0.0141	0.0000	0.0692	0.6395
E7_F2	0.1533	0.0929	0.6949	0.0019	0.0000	0.0158	0.9588
M1_F1	0.2197	0.1302	0.8492	0.0113	0.0000	0.0409	1.2513
M1_F2	10.5455	1.2877	0.2663	0.0551	0.0038	0.1192	12.2776
M2_F1	16.4962	0.3941	0.4728	0.5839	0.0000	0.0103	17.9573
M2_F2	4.5391	0.3620	0.2718	0.0308	0.0000	0.0289	5.2326
M3_F1	0.0588	1.5012	0.3023	0.0000	0.0000	0.0806	1.9429
M3_F2	0.1097	2.9327	0.1622	0.0323	0.0000	0.0254	3.2623
R4_F1	0.1304	0.0242	0.9771	0.0287	0.3292	0.0788	1.5684
R4_F2	3.8343	0.1246	0.6521	0.0266	0.0650	0.2043	4.9069
W1_F1	0.1844	3.9597	0.6679	0.0979	0.0000	0.0951	5.0050
W1_F2	5.0060	0.6504	0.5453	0.1568	0.0491	0.0588	6.4664
Total	62.7584	13.0786	8.4912	1.1522	0.4471	1.2193	87.1468
Mean	3.9224	0.8174	0.5307	0.0720	0.0279	0.0762	5.4467
SD	5.3012	1.1372	0.2799	0.1428	0.0827	0.0748	5.2905
Min	0.0588	0.0242	0.1528	0.0000	0.0000	0.0103	0.6395
Max	16.4962	3.9597	0.9771	0.5839	0.3292	0.2892	17.9573
Median	0.2116	0.3371	0.5091	0.0277	0.0000	0.0524	4.0846

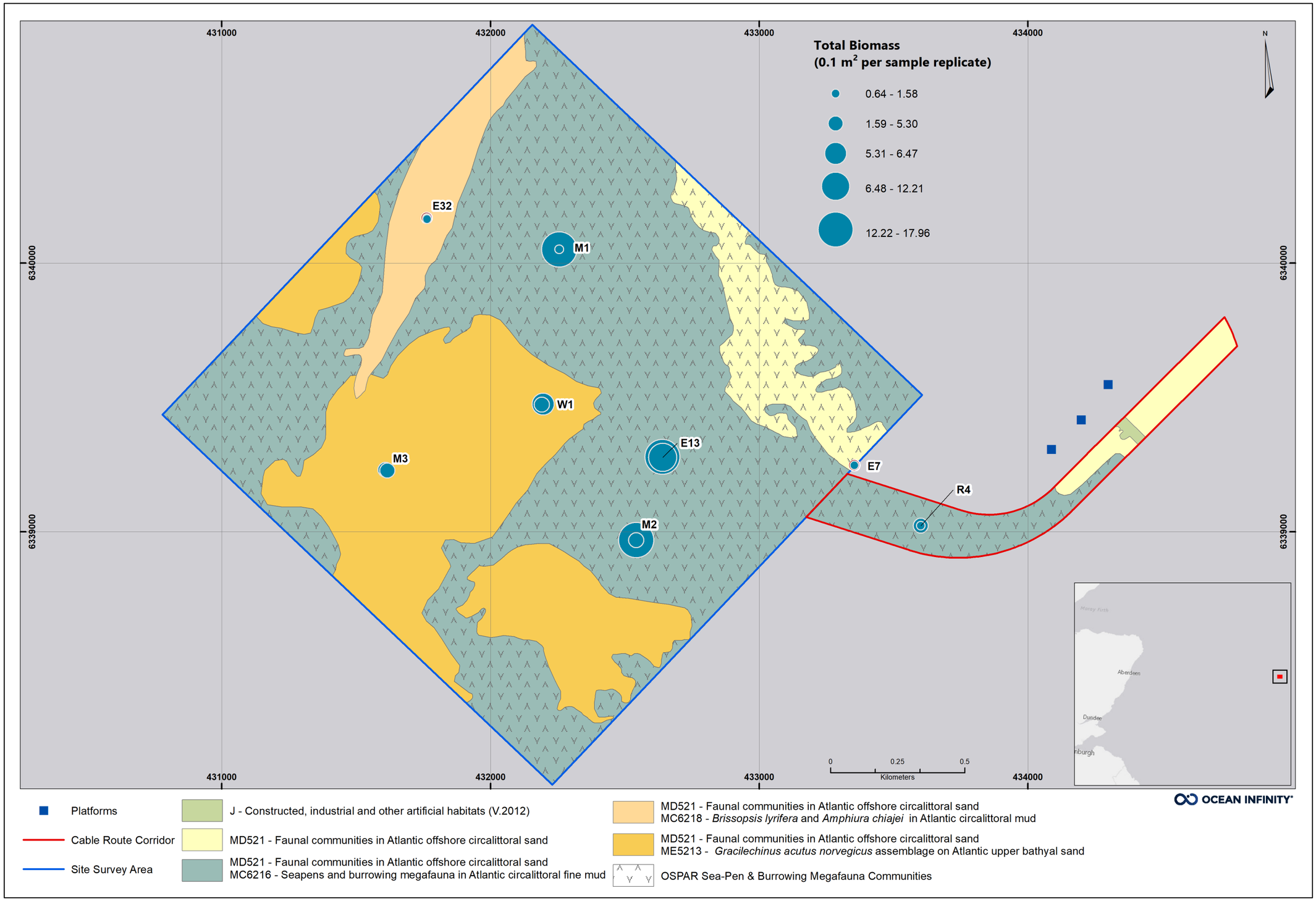


Figure 49 Overview of the total biomass (g/0.1 m²) per grab sample replicate.

5.10 Environmental DNA Results

Water sampling for environmental DNA (eDNA) analyses was acquired at all eight (8) sites and samples were collected from the Top and Bottom of the water column at each of these sites. Additionally, sediment samples for eDNA analyses were collected at each of the eight (8) grab sample sites.

A total of assays (5) assays were targeted for the water samples; Marine Water Vertebrates (12S gene), Marine Water Eukaryotes (18S gene), Marine Water Invertebrates (CO1 gene), Marine Water Fish (12S gene) and the Mammals (12S gene). A total of two (2) assays were targeted for the sediment samples; Marine Invertebrates (18S gene) and Bacteria (16S gene).

5.10.1 eDNA Water Samples

Invertebrates

Water samples from all sampled sites, except the top sample at E32_003, were successfully sequenced and analysed for invertebrates. The composition of Operational Taxonomic Units (OTU) read counts recorded from the different phyla from the acquired invertebrate eDNA water samples is presented in Figure 50. Read counts for the invertebrates were dominated by Arthropoda which constituted 50 % and a total of 39 603 read counts. The majority of the read counts within Arthropoda were represented by the copepods *Paracalanus parvus,* contributing with 50 %, followed by *Calanus finmarchicus* and *Microcalanus pusillus* contributing with 26 % and 12 % respectively. Second largest phyla for invertebrates read counts was Echinodermata contributing with 43 % and a total of 34 117 read counts. The majority of the read counts within Echinodermata was represented by the sea urchins *Echinocardium cordatum* contributing with 63 %, followed by *Echinocardium flavescens* contributing with 36 %.

The group “Others” consisted of Mollusca, Phoronida and Ctenophora and is presented in Figure 51.

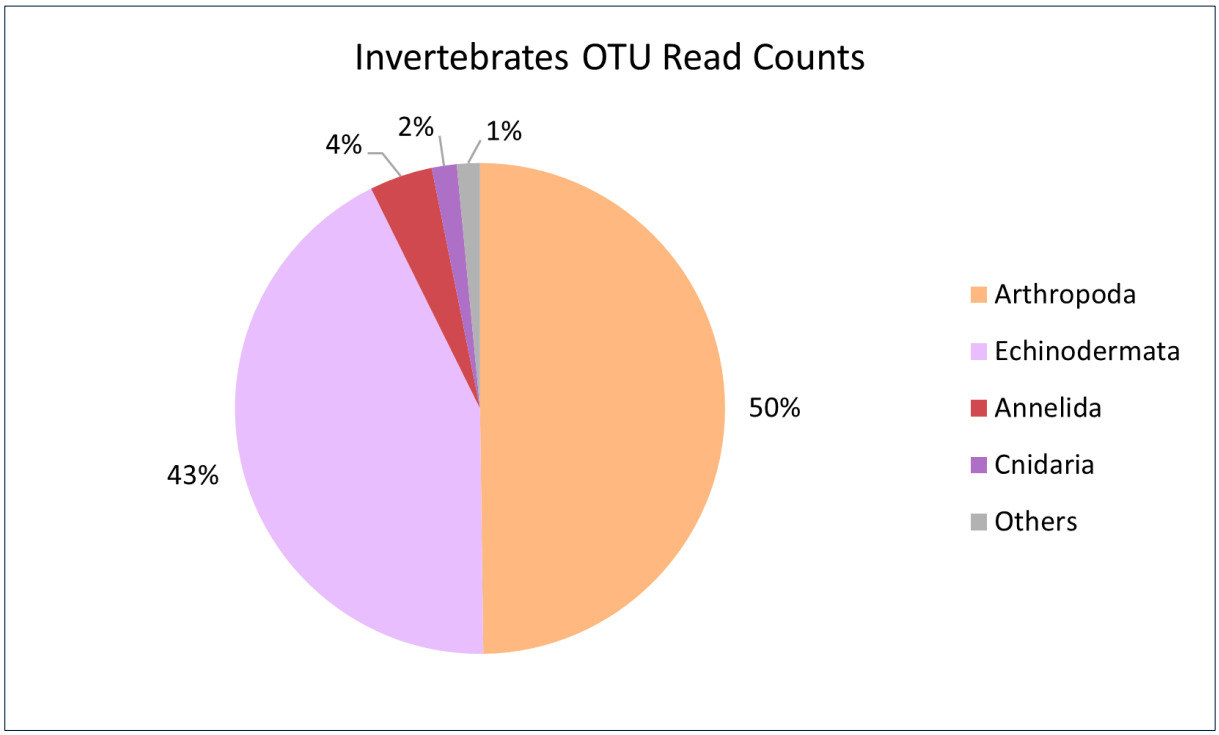


Figure 50 Read counts for invertebrates in eDNA water samples.

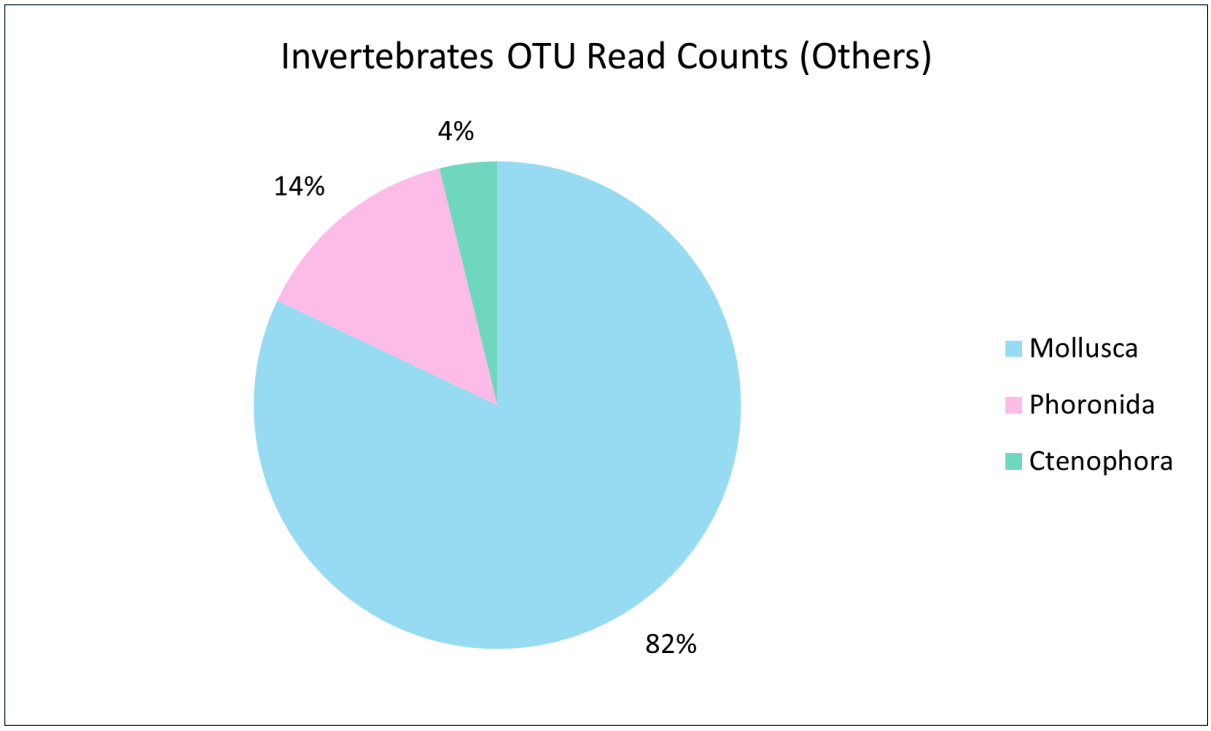


Figure 51 Read counts for invertebrates (Others) in eDNA water samples.

Vertebrates

Five (5) out of the eight (8) water sample sites were successfully sequenced and analysed for Vertebrates, these were:

- Top E_E13_001
- Top and Bottom E_E32_003
- Bottom E_M3_002
- Bottom R4_001
- Bottom W1_001

The composition of OTU read counts recorded for vertebrates is presented in Figure 52. Because the vertebrates category only comprised of one phylum, Chordata, this dataset only contains identification to Family level. The majority of the read counts were represented by the Cod Family Gadidae, constituting 89 % with a total of 36 411 read counts out of which 1669 read counts were identified as the Norway Pout *Trisopterus esmarkii*. Second most recorded family were the Ling fishes Lotidae, contributing with 5 %, and a total of 2117 read counts. Species identified within Lotidae were *Molva molva* and *Ciliata Mustela*. One marine mammal family, the seal Phocidae, was also identified within this assay contributing with 0.3 % and a total of 125 read counts.

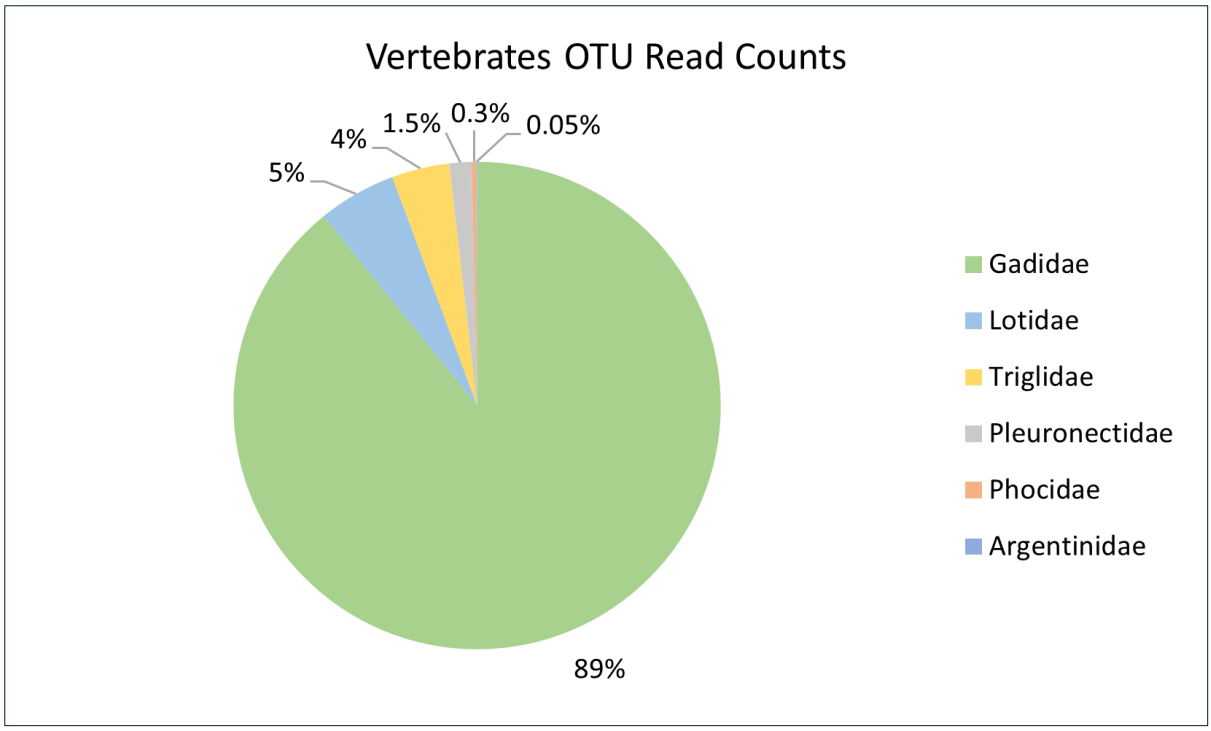


Figure 52 Read counts for vertebrates in eDNA water samples.

Eukaryotes

All water sample sites were successfully sequenced and analysed for eukaryotes. A total of 24 different phyla were identified from the eukaryotes eDNA samples, of which 12 phyla including one (1) unidentified group were grouped into "Others". The phyletic composition of OTU read counts recorded for eukaryotes is presented in Figure 53 and Figure 54. Read counts for the eukaryotes were dominated by algae Ochrophyta, contributing with 39 % and a total of 529 014 read counts, followed by Arthropoda with 37 % and a total of 496 724 read counts, and Echinodermata with 6 % and 76 323 read counts.

Ochrophyta was dominated by the diatoms *Thalassiosira* and *Skeletonema* contributing with 40 % and 36 % respectively. Arthropoda was dominated by the copepods Calanidae, *Oithona similis*, and Metridinidae, contributing with 52 %, 19 %, and 14 % respectively.

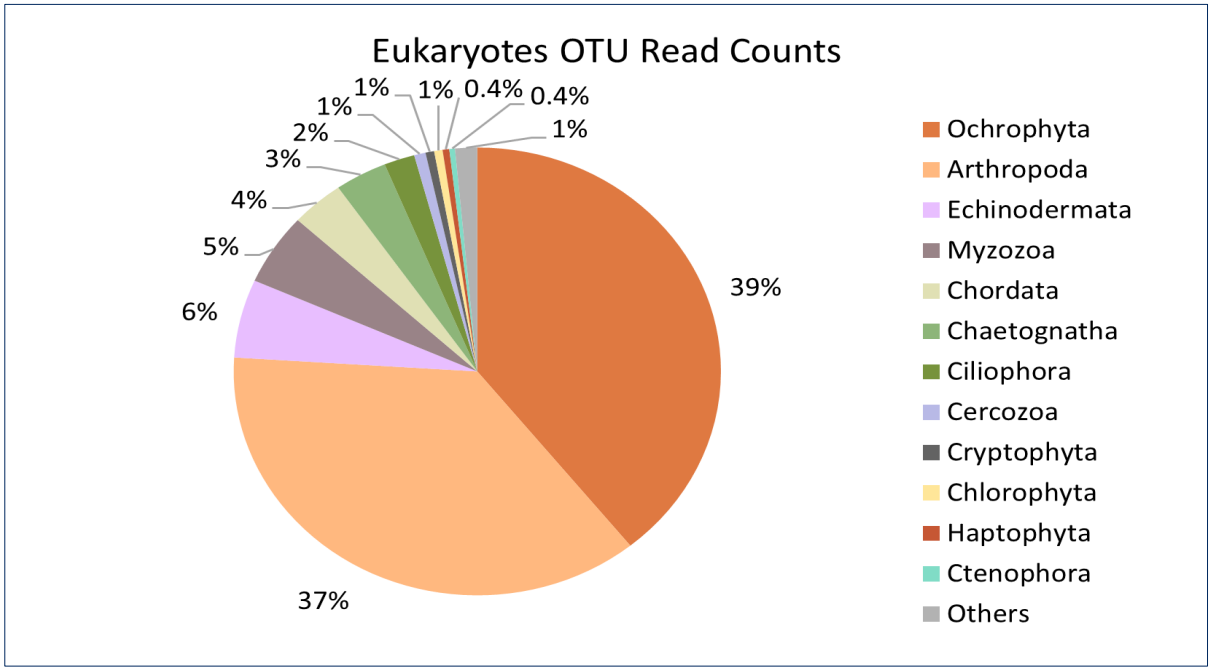


Figure 53 Read counts for eukaryotes in eDNA water samples.

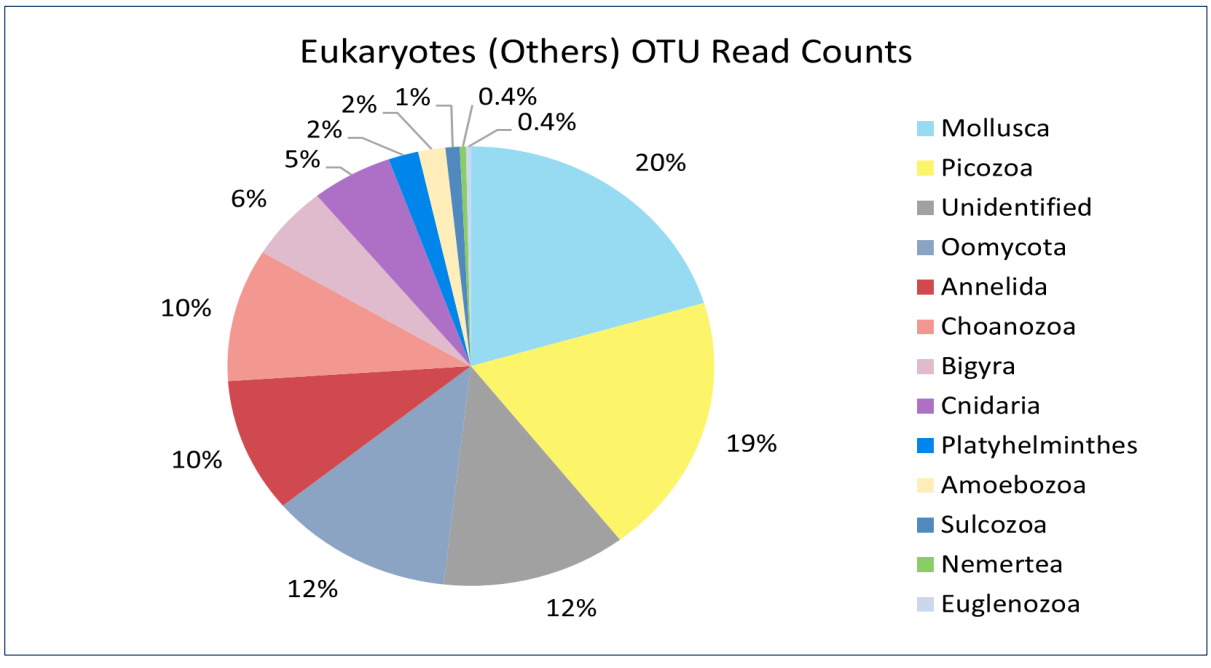


Figure 54 Read counts for eukaryotes (Others) in eDNA water samples.

Fish

Target Sequence reads for fish were successfully obtained from three (3) out of the eight (8) sampled sites, these were: Bottom E7_001, Bottom E_M1_002, and Bottom M2_001. The presence of Fish by OUT read counts recorded from acquired eDNA water samples is presented in Figure 55, and the distribution per sample site is presented in Figure 56.

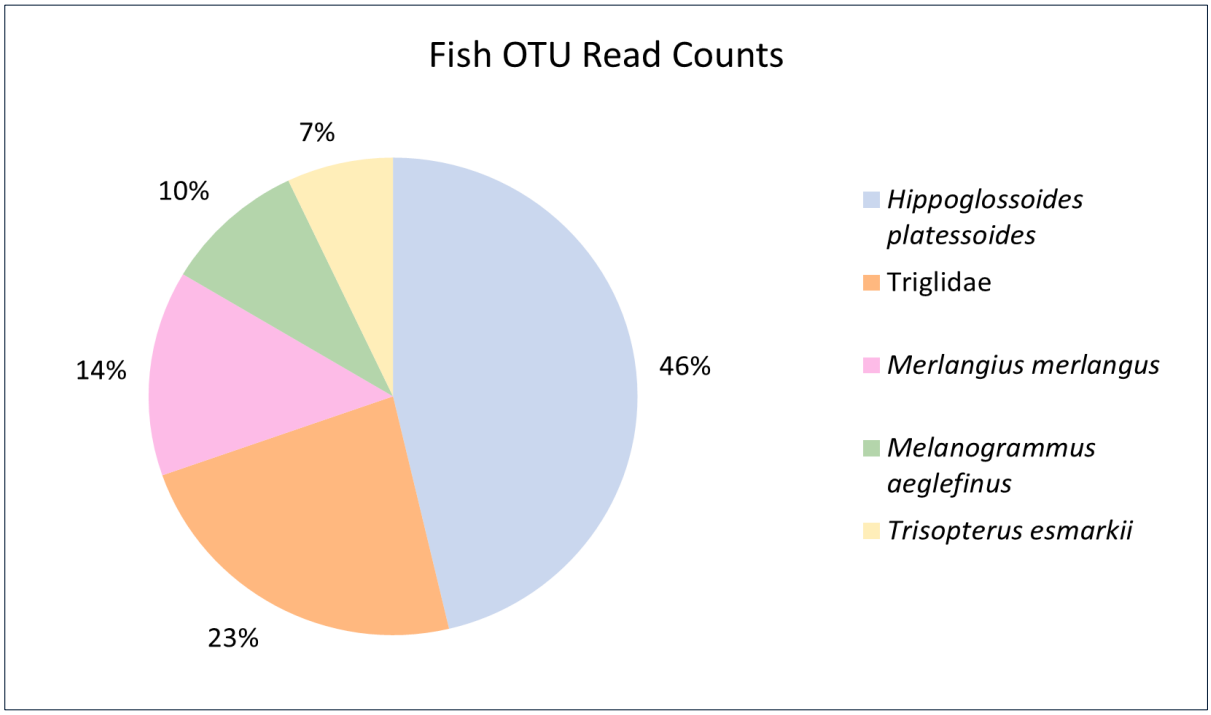


Figure 55 Read counts for fish in eDNA water samples.

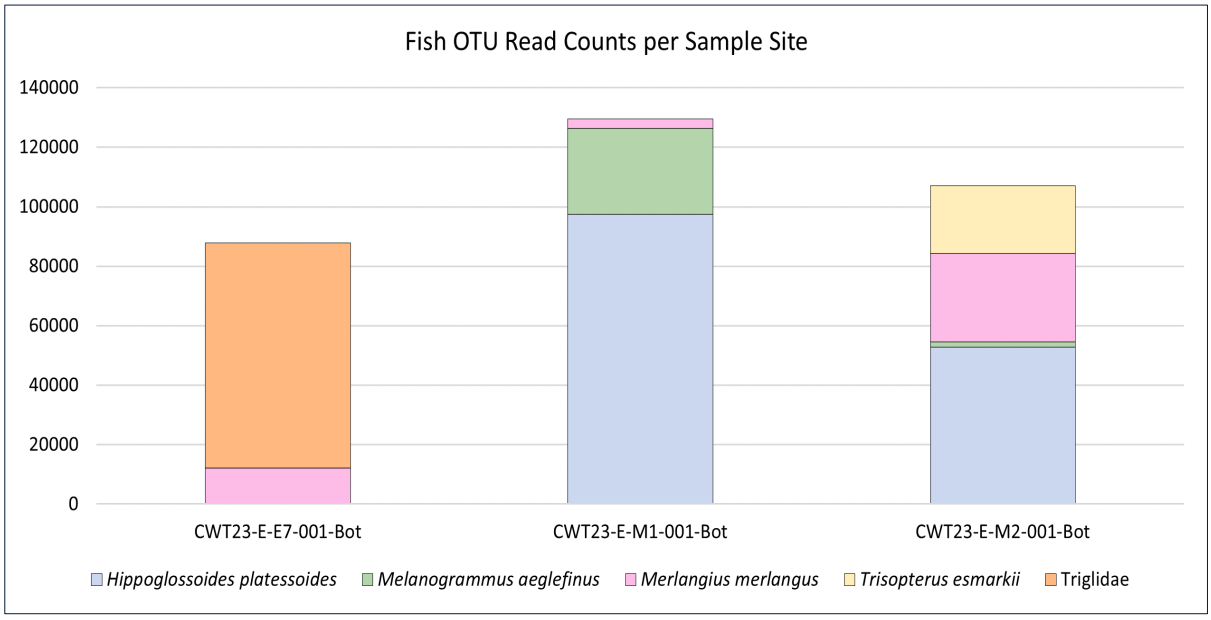


Figure 56 Read counts for fish, per species and sample site.

Mammals

The eDNA results from the marine mammal assay were inconclusive, for further details view Appendix I.

5.10.2 eDNA Sediment Samples

Bacteria

All grab sample sites were successfully sequenced and analysed for bacteria. The depth of taxonomic identification of bacteria varied greatly. A total of 413 different OTUs were identified of which 85 % could be identified to phylum. The distribution of OUT read counts recorded from the different bacterial phyla from the grab samples acquired is presented in Figure 57. Ten (10) different phyla were grouped into "Others" and are presented in Figure 58. OTUs only identified as "Bacteria" is mentioned as "Bacteria Phy".

The most abundant phylum was Proteobacteria, which contributed with 42 % and a total of 156 996 read counts. The majority of the read counts within the proteobacteria was represented by the class Gammaproteobacteria, which constituted 43 %. Only one (1) species of proteobacteria could be identified, which was *Methyloceanibacter stevinii*.

The second most abundant phylum that could be identified was Actinobacteriota, with 10 % of all read counts. The majority of the read counts within Actinobacteriota were represented by the class Actinomycetia which constituted 50 %. Only one (1) species of Actinobacteriota could be identified, which was *Ilumatobacter nonamiensis*.

The group "Others" contributed with 3 % of all recorded read counts and comprised Verrucomicrobiota, Desulfobacterota, Nitrospinota, Gemmatimonadota, Cyanobacteria, Firmicutes, Latescibacterota, Nitrospirota, Moduliflexota, and Fusobacteriota.

The geographical distribution of the bacteria is presented in Figure 59.

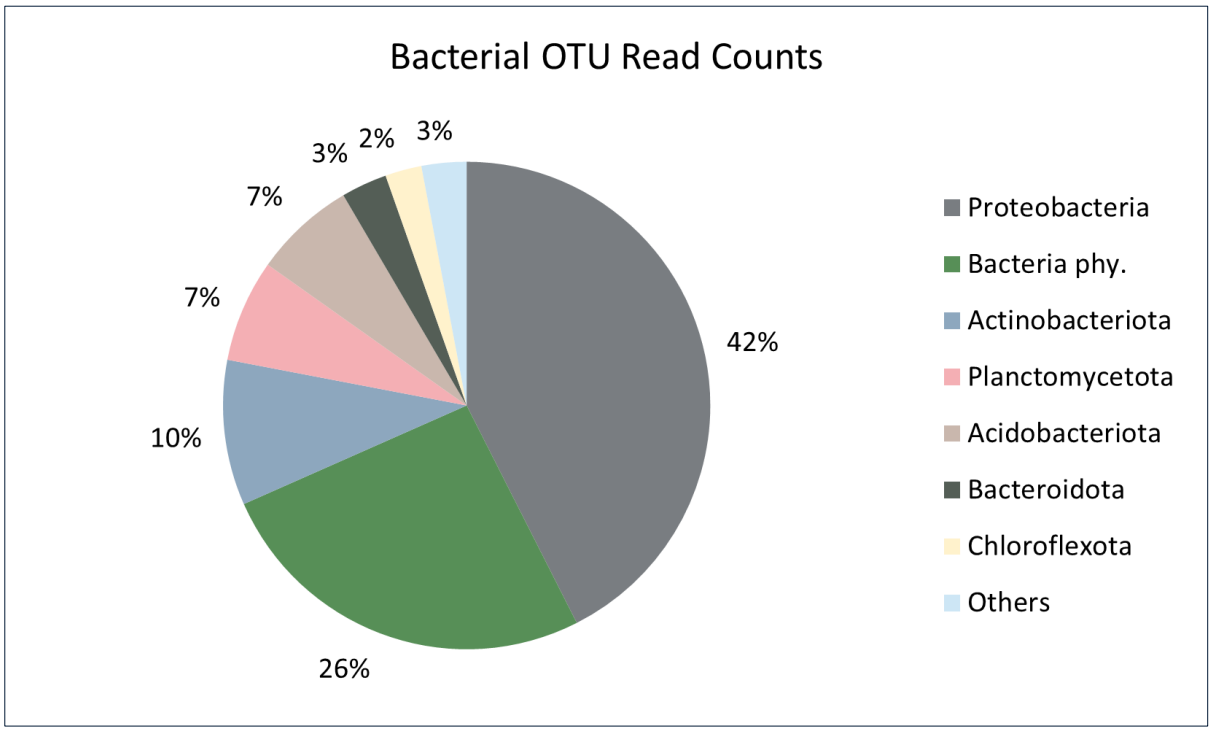


Figure 57 Read counts for bacteria in eDNA sediment samples.

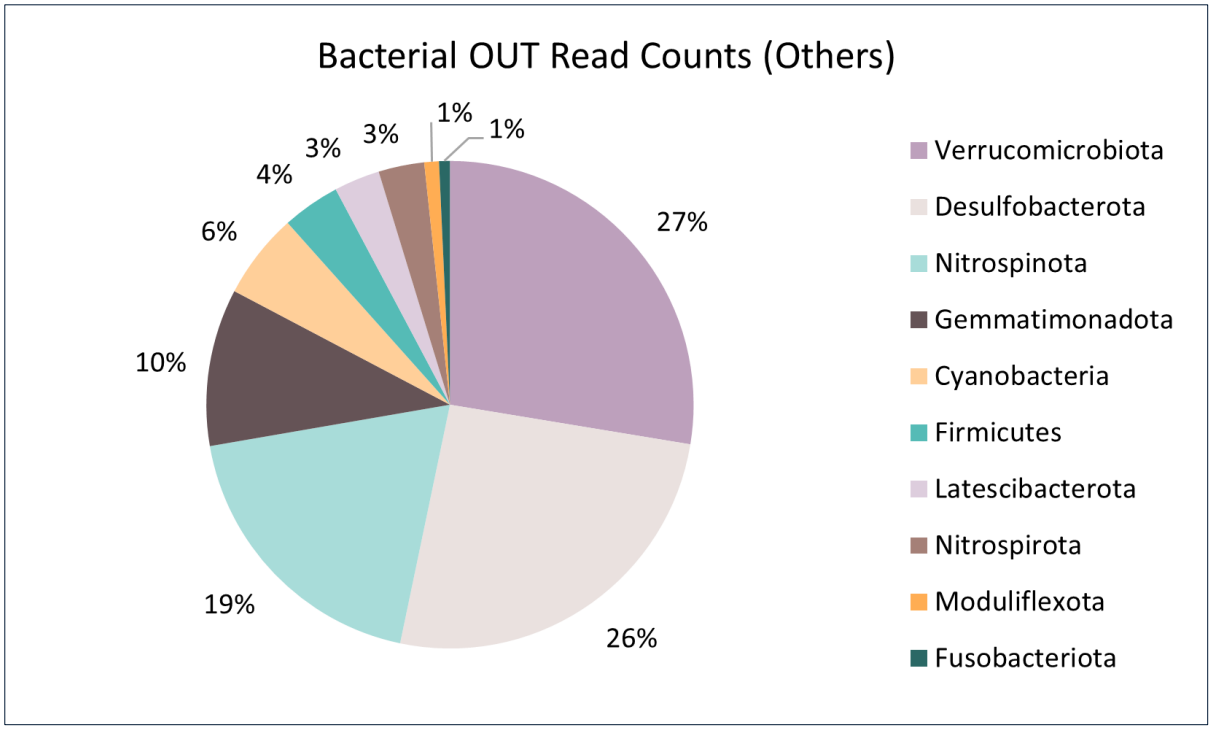


Figure 58 Read counts for bacteria (Others) in eDNA sediment samples.

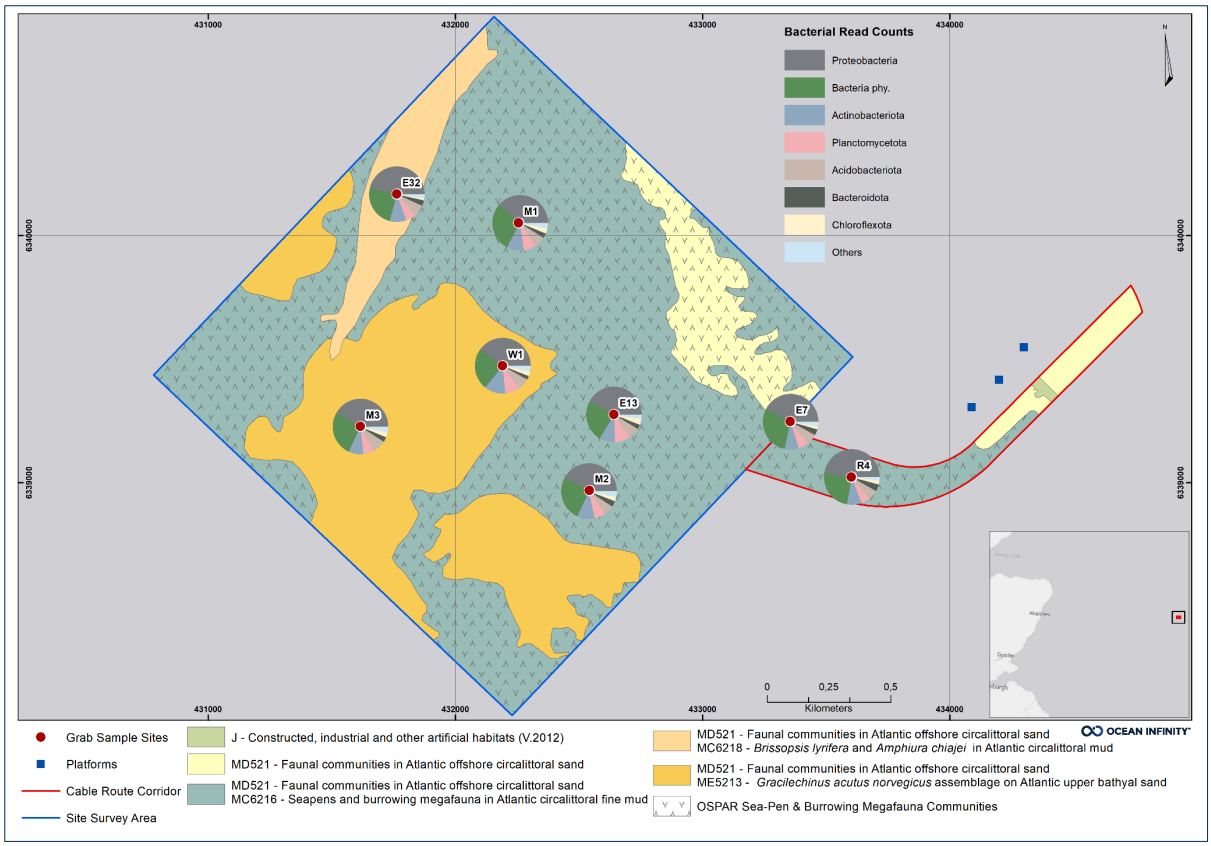


Figure 59 Geographical distribution of bacterial read counts from sediment sample sites.

Invertebrates

All the grab sample sites were successfully sequenced and analysed for invertebrates. The distribution of OTU read counts recorded from the different phyla from the acquired invertebrate samples is presented in Figure 60.

The highest read counts Phylum in the invertebrate samples was Annelida, which contributed 87 % of all read counts recorded in the samples. The great majority of the read counts within the annelids was represented by the order Amphinomida, which constituted 63 % of the read counts within the phylum. The species *Laonice sarsi* followed with 33 % of the read counts within the phylum.

The second most abundant phylum was Mollusca, with 6 % of all read counts recorded in the samples. The mussel *Abra nitida* constituted 53 % of the read counts within the molluscs.

The Echinodermata phylum contributed with 5 % of all read counts recorded in the samples. The most abundant taxa within the phylum were the brittle star *Amphiura filiformis* which constituted 84 % of the read counts.

The phyla Nemertea contributed with 2 % of all read counts recorded in the samples. *Hubrechtella dubia* was the most abundant taxa within the phylum, contributing 79 % of the read counts.

The group Others, which constituted of the Cnidaria, Arthropoda and Hemichordata, contributed with 0.3 % of all read counts recorded in the samples. The distribution between the three phyla in the group Others is presented in Figure 61. The genus *Bougainvillia* had the highest read counts within the phylum Cnidaria and the copepod *Microcalanus pusillus* within the phylum Arthropoda with a contribution of 81 % and 43 % respectively. *Glossobalanus marginatus* was the only taxa recorded in the phylum Hemichordata.

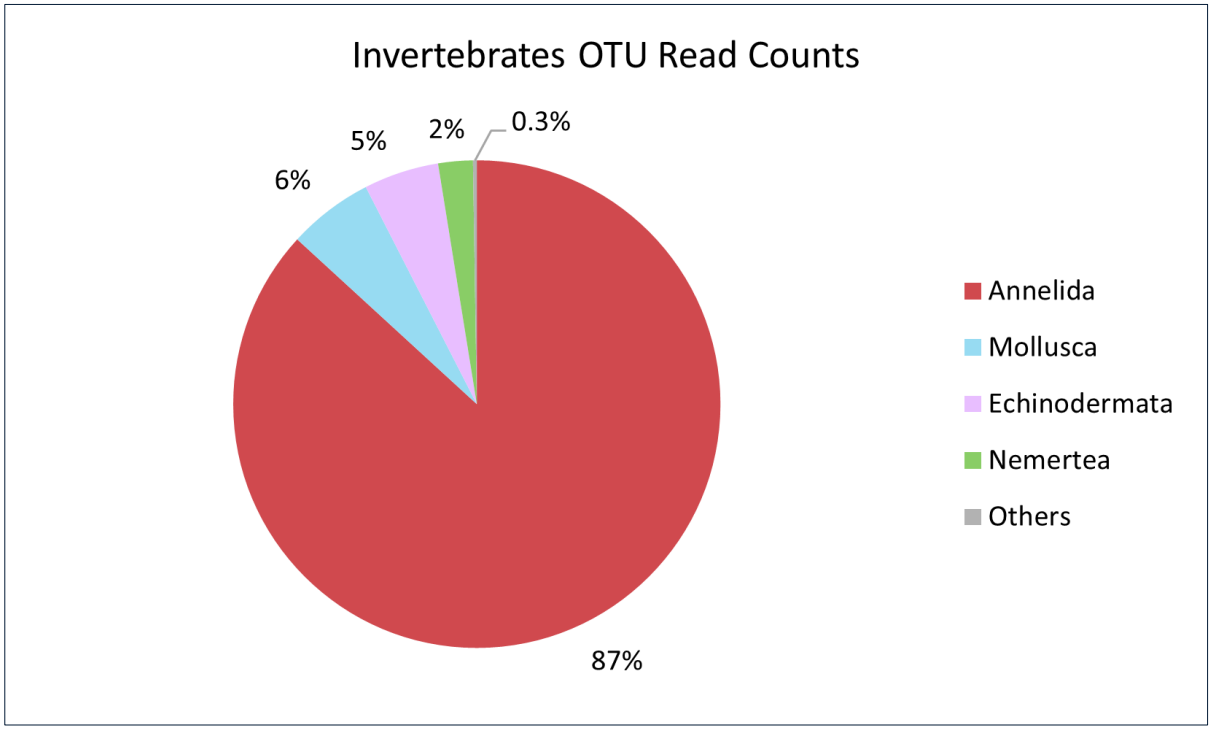


Figure 60 Read counts for invertebrates in eDNA sediment samples.

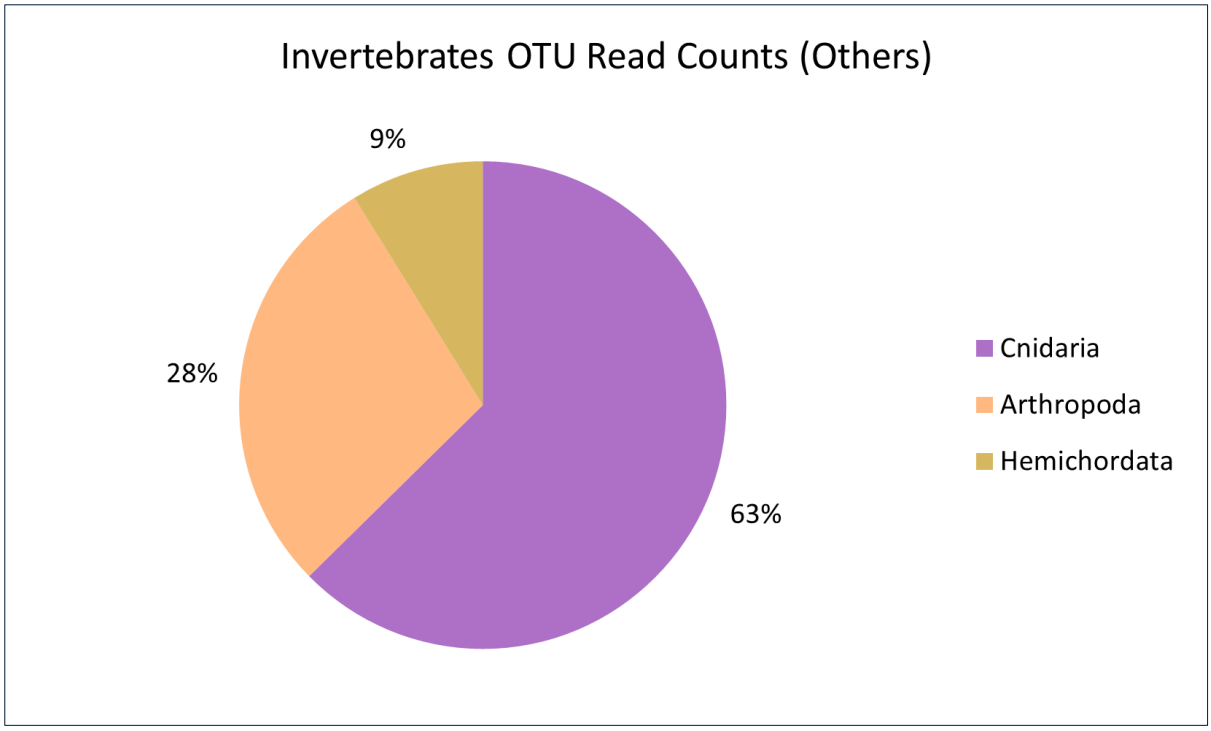


Figure 61 Read counts for invertebrates in the group "Others" in eDNA sediment samples.

5.11 Comparative Data

An Environmental Baseline Survey was carried out in the Culzean area in 2013 by Gardline Environmental Ltd (Gardline, 2013a; Gardline, 2013b). The following chapter on comparative data comprises analytical results from the current Environmental Baseline Survey (2023) and includes a comparison to the results presented by Gardline. Three (3) of the 2023 sampling sites (E13, E32 and E7) were selected in order to provide a comparison with the corresponding 2013 sampling sites (ENV13, ENV32 and ENV7).

5.11.1 Particle Size Distribution

The Particle Size Analysis (PSA) was conducted via two different methods for the respective surveys. The 2013 samples were analysed using the Malvern Mastersizer 2000 laser diffraction particle sizer while the 2023 samples were analysed with a combination of wet sieving and Coulter LS13320 laser diffraction methods.

The PSA results show a minimal variation between the datasets (Figure 62). Fine sand was the dominating sediment fraction at all three sites in both surveys, followed by Silt and Clay. Gravel content was the lowest recorded fraction (Table 38). The sample acquired at E32, during the 2023 survey, comprised a Gravel content of 3.96 % compared to the 2013 sample ENV32 which comprised a Gravel content of 0.54 %. A review of the backscatter data shows that the 2013 sample was acquired approximately 2 m east of E32, in an area of slightly lower reflectivity.

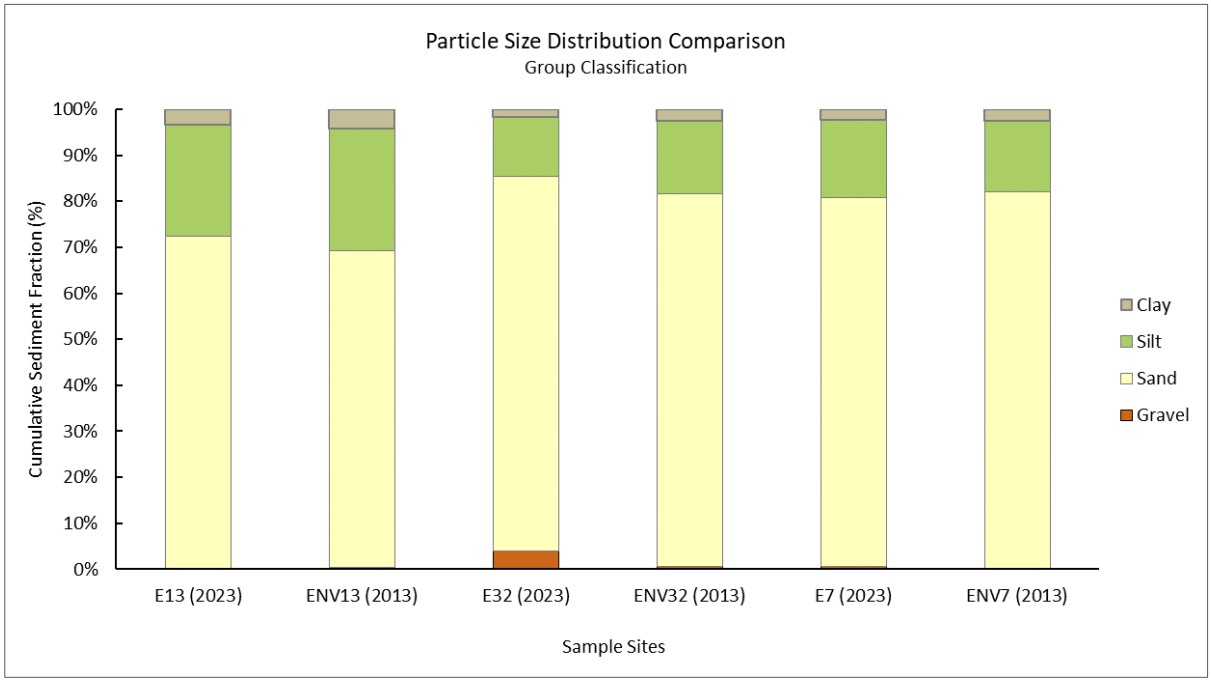


Figure 62 Cumulative particle size distribution for comparative data.

Table 38 Summary of comparative PSA results.

Sample ID	BGS (1982) Classification (modified from Folk, 1954)	Depth (m)	Cumulative Sediment Fraction Group Classification (%)			
			Gravel	Sand	Silt	Clay
E13 (2023)	Muddy Sand	90.35	0.06	72.39	24.31	3.25
ENV13 (2013)	Muddy Sand	90.34	0.34	68.83	26.73	4.11
E32 (2023)	Slightly Gravelly Muddy Sand	89.82	3.96	81.55	12.72	1.76
ENV32 (2013)	Muddy Sand	89.80	0.54	81.16	15.89	2.41
E7 (2023)	Muddy Sand	89.01	0.70	80.17	16.89	2.24
ENV7 (2013)	Muddy Sand	89.01	0.26	81.80	15.39	2.55

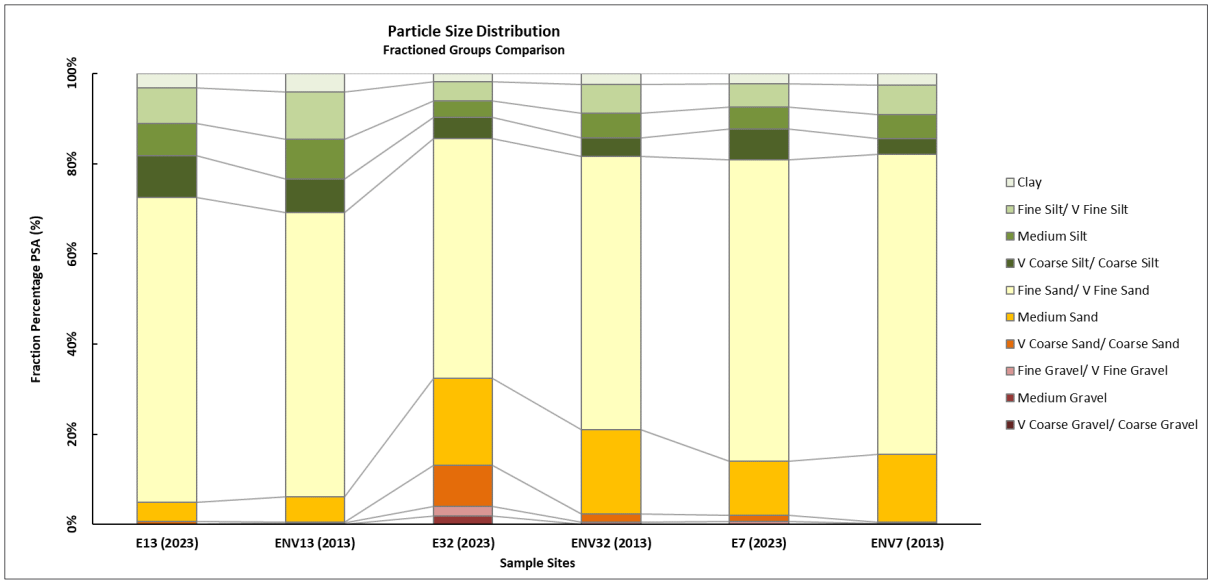


Figure 63 Sediment distribution as fraction percentage for comparative data.

5.11.2 Metals

Metal concentrations between datasets were quite variable, with samples taken in 2013 generally having higher metal concentrations than those taken in 2023 (Table 39, Figure 64, Figure 65 and Figure 66), suggesting a decrease in heavy and trace metal concentrations in the last decade at the three compared sites.

Cadmium (Cd) and mercury (Hg) were similarly low in both datasets, being below or close to their Limit of Detection (LoD) and therefore were excluded from graphs (Table 39). Samples collected in 2013 at ENV13 and ENV7 presented higher concentrations of all metals than the samples collected in the same location during the current survey (E13 and E7 respectively; Table 39, Figure 64, Figure 65 and Figure 66). Sites ENV13 and E13 showed the largest difference in metal concentrations between surveys, with decreases between 2013 and 2023 ranging from 76 % in Cu to 99 % in Al. While in the current survey, E13 showed the lowest metal content out of all the sites, ENV13 presented the highest metal concentrations out of the three comparable sites from the 2013 survey.

When comparing the results obtained for ENV32 and E32, copper and nickel showed a slight increase, although in both cases concentrations were below the UKOOA 95th percentile for the CNS (UKOOA, 2001) and the OSPAR ERL values (OSPAR, 2011), and considered within background levels (Table 39 and Figure 64). Concentrations of all other metals decreased at this site between 2013 and 2023.

The most notable difference in metal concentrations between surveys was that of aluminium (Al), which showed a decrease of between 90 % (E32) and 99 % (E13) since 2013 (Table 39 and Figure 65). Differences in barium (Ba) and vanadium (V) were also noteworthy, with Ba decreasing 63 - 96% and V decreasing 65 - 96 % since the 2013 Gardline survey (Gardline, 2013b).

Table 39 Metal concentrations for comparative data (mg/kg dry weight).

Analytes	As	Cd	Cr	Cu	Pb	Hg	Ni	V	Zn	Al	Ba
Units	mg/kg dry weight										
Method	ICPMSS	ICPMSS	ICPMSS	ICPMSS	ICPMSS	ICPMSS	ICPMSS	ICPMSS	ICPMSS	ICPSOIL	ICPSOIL
LoD	0.5	0.04	0.5	0.5	0.5	0.01	0.5	0.5	2	10	0.5
E13	<0.5	<0.04	1.0	2.1	0.9	<0.01	0.6	1.4	3.3	215	21.1
E32	3.3	<0.04	9.3	4.2	5.5	<0.01	4.7	11.6	9.5	1900	71.6
E7	2.2	<0.04	7.6	4.0	5.5	<0.01	3.5	9.9	8.2	1670	151.0
Gardline 2013 data											
ENV13	4.5	0.1	17.5	8.8	11.5	0.01	6.3	39.8	20.8	18900	510
ENV32	3.5	0.1	14.7	5.3	9.3	<0.01	4.3	35.2	17.4	19040	413
ENV7	3.9	<0.1	14.6	3.2	9.5	0.02	4.5	33.1	18.0	19900	390
Reference levels											
UKOOA Fine Sand CNS	-	0.02	7.60	1.55	5.39	0.04	3.20	9.11	8.78	-	169.31
UKOOA 50th percentile CNS	-	0.02	7.17	2.00	6.65	0.01	4.00	12.00	10.45	-	117.50
UKOOA 95th percentile CNS	-	0.12	31.04	6.00	16.70	0.12	19.00	31.30	32.59	-	523.20
OSPAR ERL	-	1.2	81	34	47	0.15	-	-	150	-	-

**Where metal concentrations exceed more than one reference level, the higher one has been highlighted in the table.*

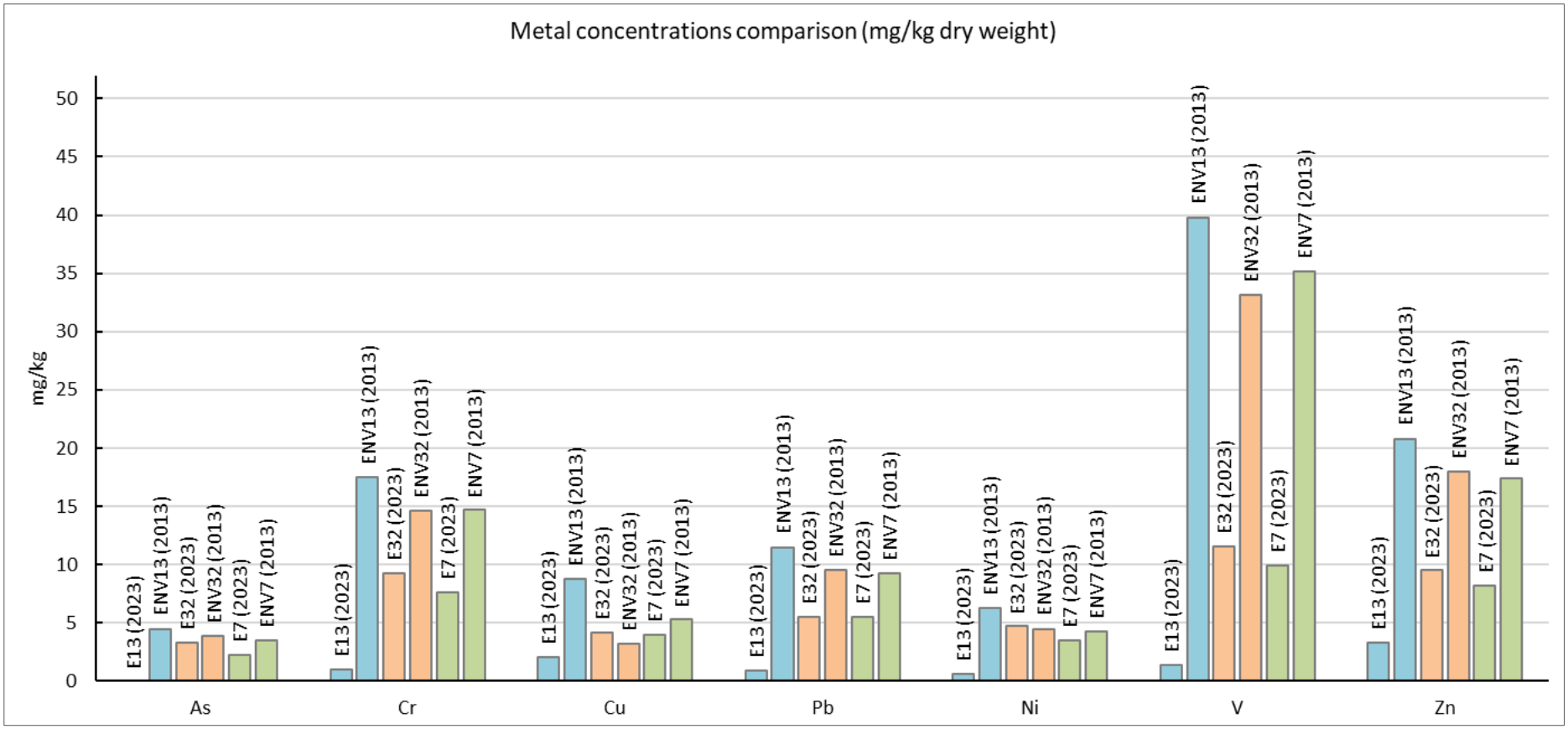


Figure 64 Concentrations of As, Cr, Cu, Pb, Ni, V and Zn for comparative data.

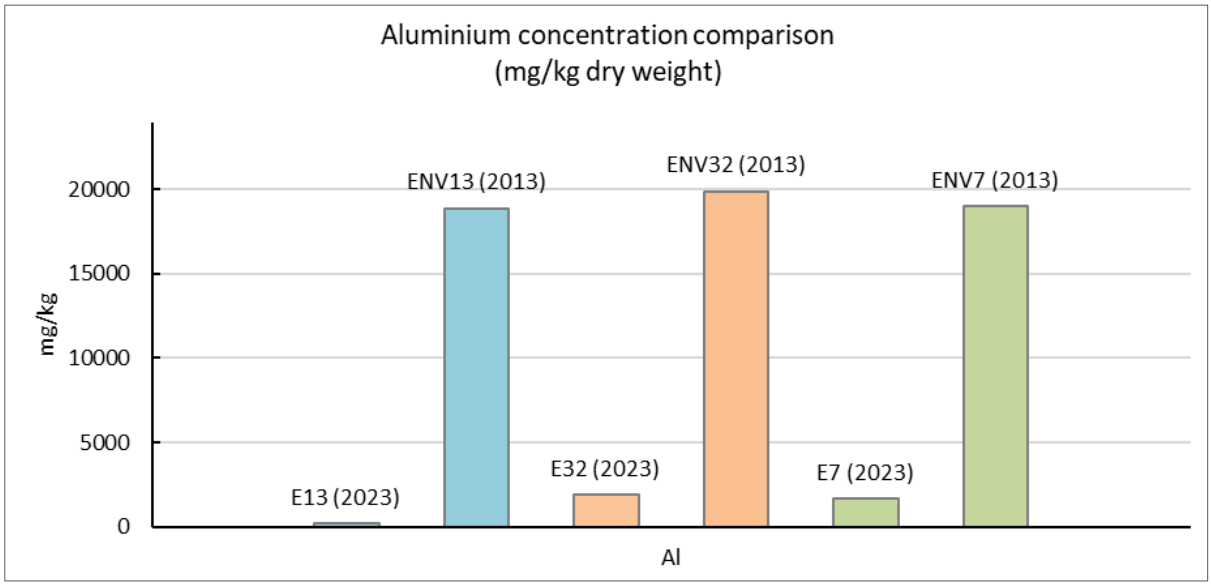


Figure 65 Concentration of Al for comparative data.

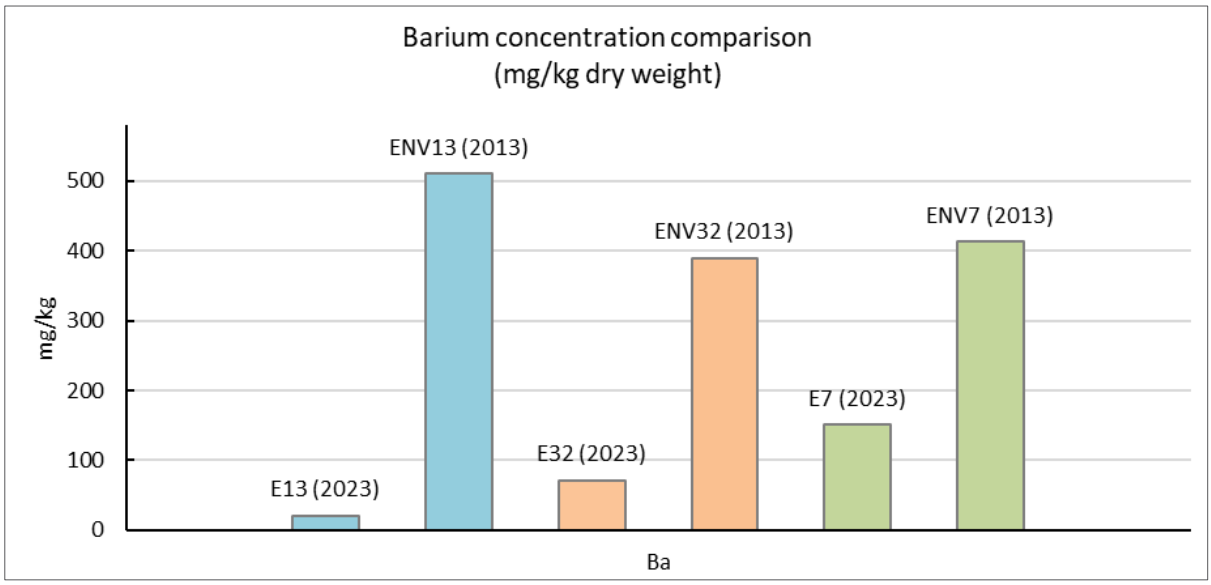


Figure 66 Concentration of Ba for comparative data.

5.11.3 Organics

Total Organic Matter (TOM) and Total Organic Carbon (TOC) content showed minimal variation between datasets (Table 40; Figure 67), with TOM levels remaining within background levels for this sector of the North Sea (UKOOA, 2001) in both surveys.

Table 40 Total organic matter and total organic carbon (% M/M) for comparative data.

Analytes	Total Organic Matter	Total Organic Carbon
Units	% M/M	% M/M
Method	Loss On Ignition (LOI)	WSLM59
LoD	0.2	0.02
E13	1.1	0.19
E32	1.5	0.25
E7	1.7	0.29
Gardline 2013 data		
ENV13	1.9	0.35
ENV32	1.4	0.26
ENV7	1.4	0.23
Reference levels		
UKOOA 50th percentile CNS	1.13	-
UKOOA 95th percentile CNS	4.48	-

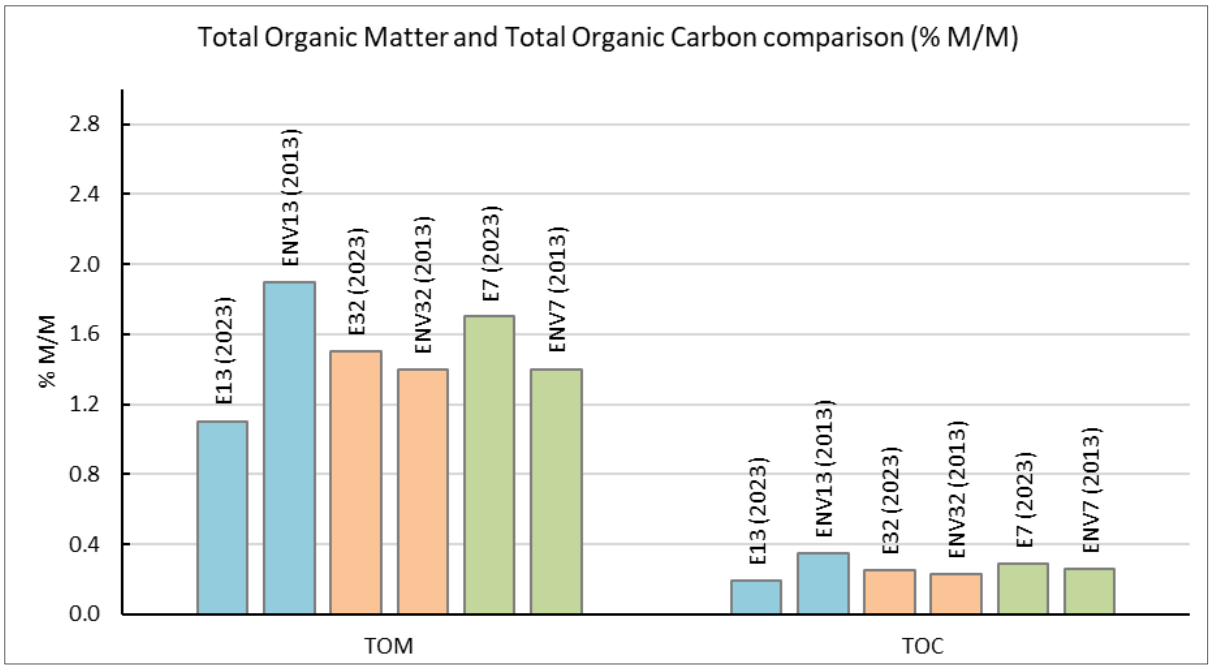


Figure 67 Total Organic Matter and Total Organic Carbon for comparative data.

5.11.4 Hydrocarbons

The results for hydrocarbon analyses were similar between the two datasets (Table 41), with Total Hydrocarbon concentration (THC), Unresolved Complex Mixture (UCM) and different alkane groups showing minimal differences. The Carbon Preference Index (CPI) showed a decrease since the 2013 survey, with values previously having been above the UKOOA 95th percentile for this region (Table 41) (UKOOA, 2001). Although Pristane has remained invariable, Phytane was previously above detection limits, allowing the Pr/Ph ratio to be calculated for sites sampled in the Gardline survey (Gardline, 2013b). Although phytane was detected in the previous survey, these values were still below those of pristane, indicating a general biogenic influence.

Results for total Polycyclic Aromatic Hydrocarbons (PAH) presented a notable decrease in values at the three compared points in the last decade, with concentrations being reduced between 24 % at E13 and 47 % at E32.

Table 41 Summary of hydrocarbon concentrations (µg/g) for comparative data.

Analytes	THC	UCM	nC_{10-20}	nC_{21-37}	total n-alkanes	CPI	Pristane (Pr)	Phytane (Ph)	Pr/Ph Ratio	NPD	Total PAH	NPD/4-6 ring PAH ratio
Units	µg/g	µg/g	µg/g	µg/g	µg/g	-	µg/g	µg/g	-	µg/g	µg/g	-
Method	ASC/SOP/303/306	-	ASC/SOP/303/306	ASC/SOP/303/306	ASC/SOP/303/306	ASC/SOP/303/306	ASC/SOP/303/306	ASC/SOP/303/306	ASC/SOP/303/306	ASC/SOP/303/304	ASC/SOP/303/304	ASC/SOP/303/304
LoD	0.10	-	0.001	0.001	0.028	1	0.001	0.001	1	0.014	0.034	-
E13	9.22	8.77	0.04	0.41	0.45	2.02	0.013	<0.001	-	0.024	0.156	0.18
E32	7.51	7.24	0.03	0.24	0.27	1.85	0.013	<0.001	-	<0.014	0.083	0.17
E7	7.31	7.04	0.03	0.24	0.27	1.90	0.010	<0.001	-	<0.014	0.098	0.15
Gardline 2013 data												
ENV13	9.3	8.8	0.036	0.453	0.488	4.1	0.020	0.006	3.6	0.022	0.204	0.12
ENV32	6.7	6.3	0.031	0.312	0.344	3.5	0.011	0.003	4.4	0.017	0.156	0.12
ENV7	6.5	6.2	0.034	0.288	0.322	3.9	0.013	0.003	4.5	0.012	0.130	0.10
Reference Levels												
UKOOA Fine Sand CNS	8.66	-	-	-	0.37	2.03	-	-	-	-	0.117	-
UKOOA 50th percentile CNS	4.10	-	-	-	0.26	1.86	-	-	-	-	0.109	-
UKOOA 95th percentile CNS	40.10	-	-	-	1.18	2.79	-	-	-	-	0.583	-
Dutch RIVM	5000	-	-	-	-	-	-	-	-	-	-	-

**Where values exceed more than one reference level, the higher one has been highlighted in the table.*

5.11.5 Non-Colonial Fauna

To further compare potential similarities and dissimilarities between the current Environmental Baseline Survey (2023) and the Gardline Environmental Ltd survey (Gardline, 2013a; Gardline, 2013b) a series of comparisons were conducted on the macrofaunal datasets. Both datasets were treated equally using square root transformation and including juveniles. The comparison includes two (2) replicates from each comparative site from both datasets.

5.11.6 Species Composition

The compared species composition of the non-colonial fauna identified from the grab samples is illustrated in Figure 68 and Figure 69, and summarised in Table 42. Species abundance was higher in all 2013 samples compared to 2023. The replicate sample ENV32 MFA presented the highest abundance of all samples, with a total of 668 individuals. E7_F2 had the lowest abundance with a total of 153 individuals recorded. The total number of taxa was higher in all 2013 samples, with ENV32 MFA having the highest diversity of all samples, with a total of 87 different taxa. E7_F1 had the lowest number of taxa with a total of 45 different taxa.

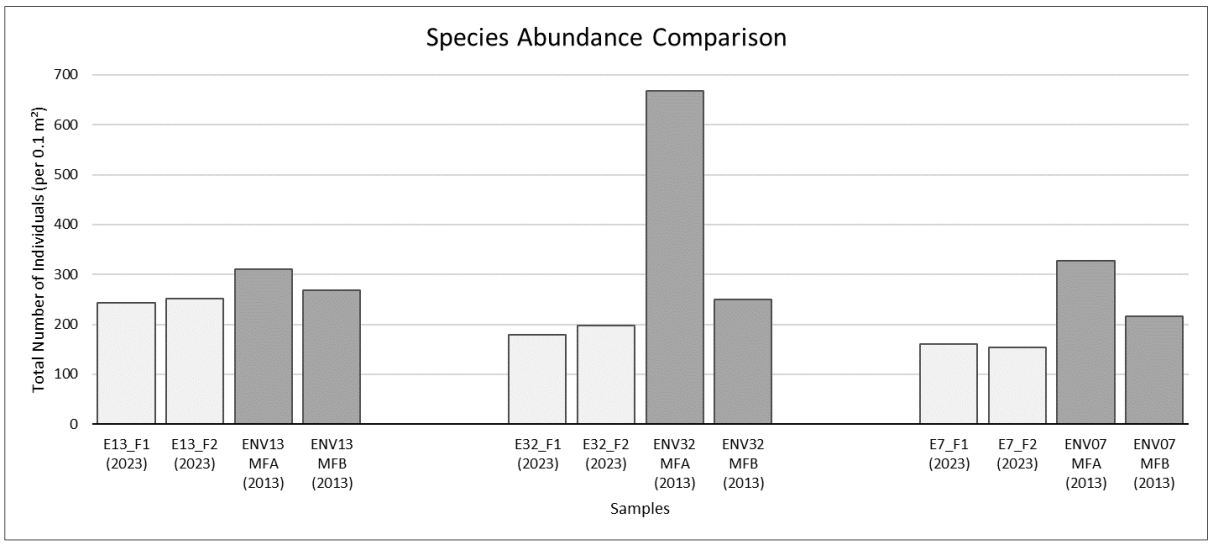


Figure 68 Abundance of non-colonial fauna from compared grab samples expressed per replicate sample per 0.1 m².

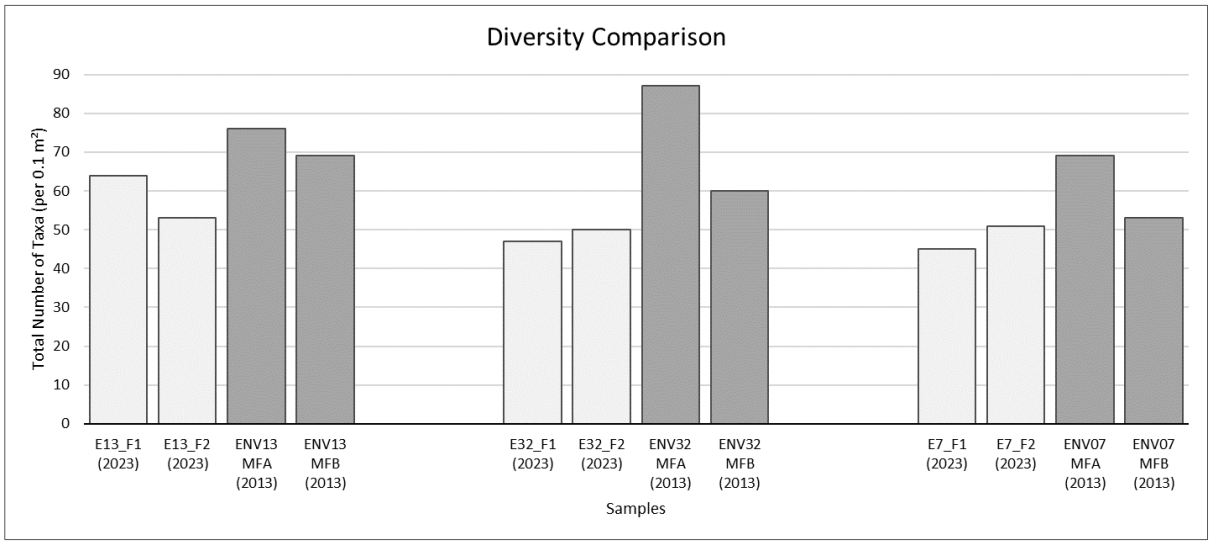


Figure 69 Diversity of non-colonial fauna from compared grab samples expressed per replicate sample per 0.1 m².

Table 42 Species composition of non-colonial fauna from compared grab samples.

Site ID	Number of Taxa	Abundance (Total Number of Individuals)
E13_F1 (2023)	64	243
E13_F2 (2023)	53	251
ENV13 MFA (2013)	76	311
ENV13 MFB (2013)	69	269
E32_F1 (2023)	47	179
E32_F2 (2023)	50	197
ENV32 MFA (2013)	87	668
ENV32 MFB (2013)	60	250
E7_F1 (2023)	45	160
E7_F2 (2023)	51	153
ENV07 MFA (2013)	69	327
ENV07 MFB (2013)	53	216

A list of the ten most abundant taxa, with total abundance and frequency of occurrence for the three compared sites is presented for the 2013 samples in Table 43 and for the 2023 samples in Table 44. The most abundant taxon in both the 2013 and 2013 samples was the annelid *Paramphinome jeffreysii*, with a total of 475 individuals recorded in 2013 samples and 226 in 2023. The species occurred in 100 % of the 2013 and 2023 samples that were compared.

Table 43 The ten most abundant taxa from compared grab samples from 2013, together with the frequency of occurrence.

Phylum	Taxa	Total Abundance	Mean Abundance	SD	Frequency of Occurrence (%)
Annelida	*Paramphinome jeffreysii*	475	79.17	59.70	100
Annelida	*Galathowenia oculata*	323	53.83	40.95	100
Annelida	*Spiophanes bombyx*	117	19.50	15.10	100
Mollusca	*Adontorhina similis*	91	15.17	14.33	100
Annelida	*Pholoe assimilis*	68	11.33	13.94	100
Annelida	*Pseudopolydora paucibranchiata*	61	10.17	7.94	83
Mollusca	*Axinulus croulinensis*	49	8.17	4.17	100
Echinodermata	Ophiuroidea (juvenile)	40	6.67	5.39	83
Annelida	*Amphictene auricoma*	32	5.33	4.89	100
Annelida	*Spiophanes kroyeri*	29	4.83	4.22	100

Table 44 The ten most abundant taxa from compared grab samples from 2023, together with the frequency of occurrence.

Phylum	Taxa	Total Abundance	Mean Abundance	SD	Frequency of Occurrence (%)
Annelida	*Paramphinome jeffreysii*	226	37.67	22.24	100
Mollusca	*Papillicardium minimum*	70	11.67	7.63	100
Annelida	*Galathowenia oculata*	69	11.50	5.99	100
Echinodermata	Amphiuridae (juvenile)	64	10.67	5.13	100
Mollusca	Nuculidae (juvenile)	63	10.50	8.80	67
Nematoda	Nematoda	44	7.33	5.43	100
Arthropoda	*Harpinia antennaria*	43	7.17	5.34	100
Phoronida	Phoronis	38	6.33	6.09	83
Mollusca	*Ennucula tenuis*	33	5.50	8.50	67
Annelida	*Pholoe assimilis*	33	5.50	5.75	67

5.11.7 Univariate Statistical Analyses

Univariate analyses were performed to assess the non-colonial faunal richness, diversity, evenness and dominance for the compared sites. Simpson's Index of Dominance was included as (λ) and (1-λ) for the comparison due to the fact that the current Environmental Baseline Survey (2023) applied (1-λ) and Gardline Environmental Ltd (Gardline, 2013b) applied (λ). The results of the univariate analyses for the compared sites are presented in Table 45.

Table 45 Univariate indices for comparative sites.

Sample ID	Number of Taxa (S)	Number of Individuals (N)	Margalef's Richness Index (D)	Pielou's Evenness Index (J')	Shannon-Wiener Index (H')	Simpson's Index of Dominance (λ)	Simpson's Index of Dominance (1-λ)
E13_F1 (2023)	64	243	11.47	0.83	3.44	0.07	0.93
E13_F2 (2023)	53	251	9.41	0.79	3.12	0.10	0.90
ENV13 MFB (2013)	69	269	12.15	0.79	3.33	0.08	0.93
ENV13 MFA (2013)	76	311	13.07	0.81	3.50	0.06	0.94
E32_F1 (2023)	47	179	8.87	0.82	3.16	0.07	0.94
E32_F2 (2023)	50	197	9.28	0.82	3.21	0.07	0.94
ENV32 MFA (2013)	87	668	13.22	0.67	2.98	0.14	0.87
ENV32 MFB (2013)	60	250	10.69	0.77	3.17	0.11	0.90
E7_F1 (2023)	45	160	8.67	0.88	3.33	0.05	0.95
E7_F2 (2023)	51	153	9.94	0.81	3.18	0.08	0.93
ENV07 MFA (2013)	69	327	11.74	0.74	3.15	0.09	0.91
ENV07 MFB (2013)	53	216	9.67	0.74	2.93	0.11	0.89

5.11.8 Multivariate Statistical Analyses

Square root transformation was applied to the dataset before calculating the Bray-Curtis similarity measures in the SIMPROF and SIMPER analyses. The transformation was applied to prevent abundant species from influencing the Bray-Curtis similarity index measures excessively and to take the rarer species into account (Clarke & Gorley, PRIMER v7: User Manual/Tutorial. Plymouth: PRIMER-E., 2015). The statistical analyses were based on macrofaunal data derived from the taxonomic analyses of the grab samples for the compared sites from 2013 (ENV13, ENV32 and ENV7) and 2023 (E13, E32 and E7).

5.11.9 SIMPROF Cluster Analyses

The SIMPROF analyses of the non-colonial faunal composition produced three (3) statistically distinct groups (black lines) and is presented in a hierarchical dendrogram in Figure 70.

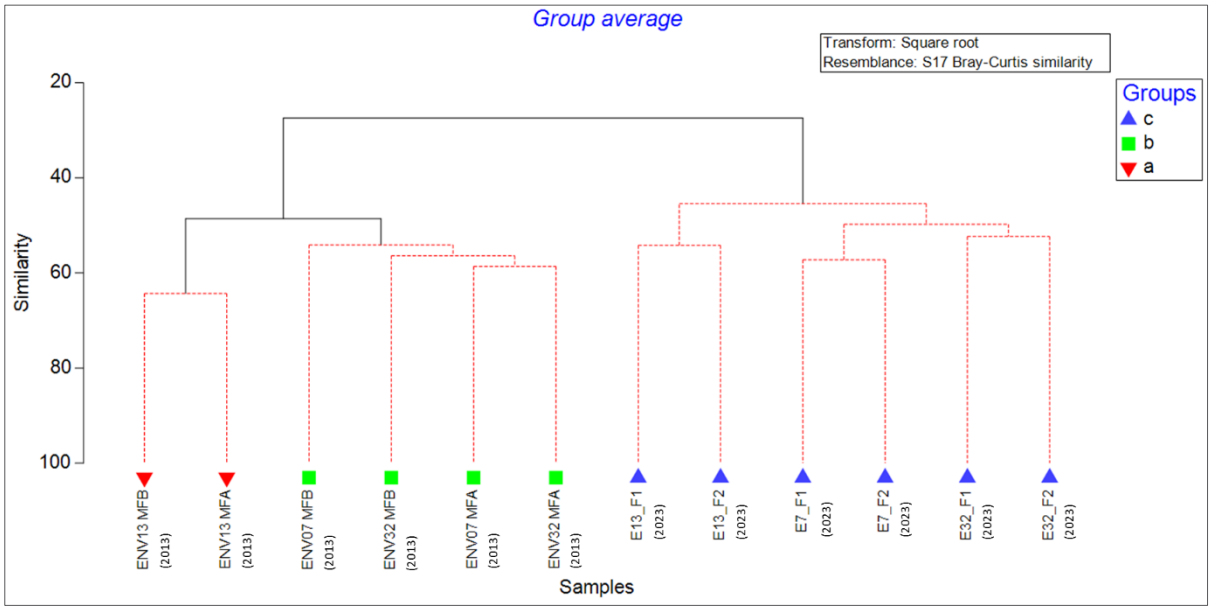


Figure 70 SIMPROF dendrogram of non-colonial faunal composition at comparative sites.

5.11.10 Non-Metric Multi-Dimensional Scaling (MDS)

The nMDS-plot reflects the dendrogram (Figure 70) and displays the similarity between the compared grab sample sites at 20 % to highlight homogeneous species composition. Sample similarity is further explored in the nMDS-plot in Figure 71.

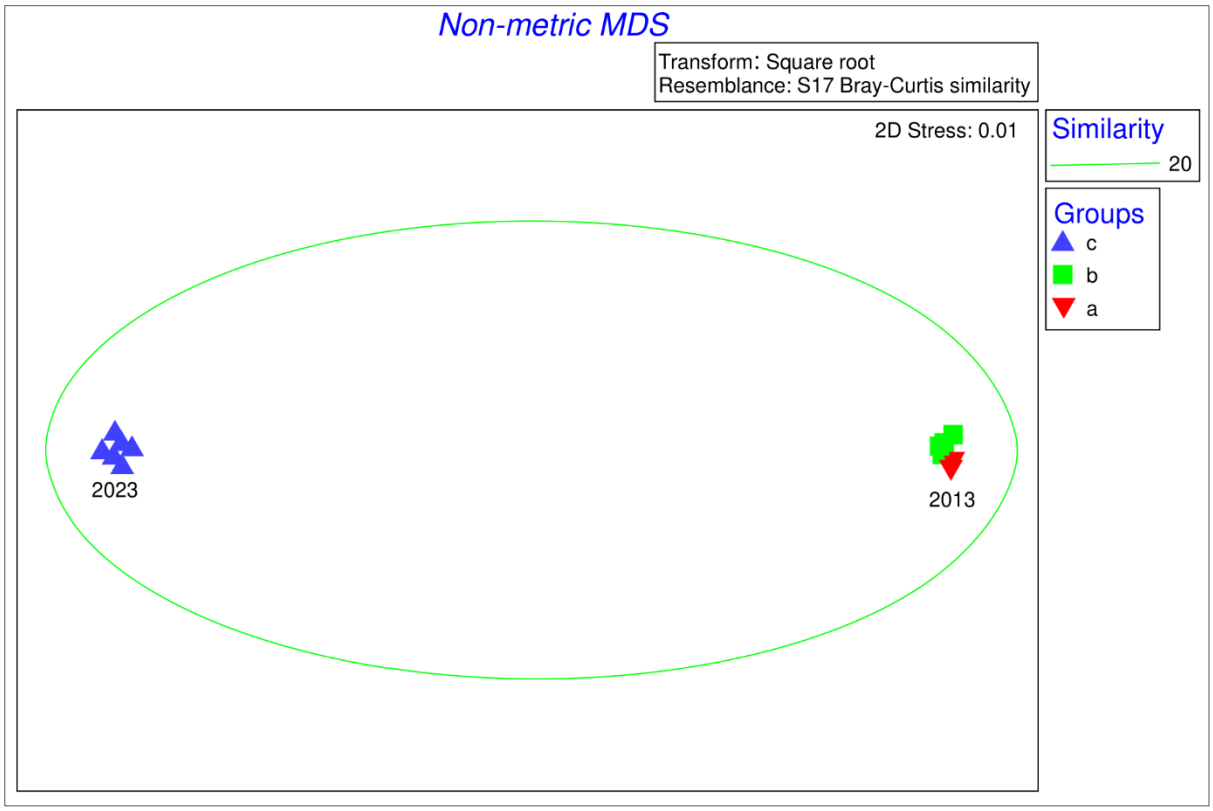


Figure 71 Comparative nMDS composition of non-colonial fauna with groups based on the SIMPROF analysis.

5.11.11 SIMPER Results

A SIMPER test for the compared sites, displaying the percentage contribution of the most important species seen in the Bray-Curtis similarity test is presented in Figure 63 with species abundance for each SIMPROF group. Average abundance refers to the square root transformed data and is expressed per 0.1 m^2 within the multivariate groups.

Table 46 Summary of characteristics of the non-colonial faunal groups from compared samples derived from the SIMPER test.

Group	Sample ID	Depth (m)	Species	Average Abundance	Contribution (%)
a **Average similarity: 64.33**	ENV13 MFB and ENV13 MFA	91	*Paramphinome jeffreysii*	6.95	8.45
			Adontorhina similis	5.74	7.14
			Galathowenia oculata	5.51	5.05
			Axinulus croulinensis	3.53	4.32
			Thyasira equalis	3.44	3.91
			Notomastus latericeus	3.13	3.45
			Abyssoninoe hibernica	2.65	3.45
			Pseudopolydora paucibranchiata	2.85	2.92
			Kurtiella tumidula	2.12	2.61
			Glycera alba	1.87	2.26
b **Average similarity: 55.63**	ENV07 MFA, ENV07 MFB, ENV32 MFA, ENV32 MFB,	94, 94, 94, 94	*Paramphinome jeffreysii*	9.28	11.20
			Galathowenia oculata	7.62	20.17
			Spiophanes bombyx	5.13	26.90
			Ophiuroidea (juvenile)	2.87	30.53
			Pholoe assimilis	3.41	33.79
			Axinulus croulinensis	2.39	36.85
			Pterolysippe vanelli	2.3	39.87
			Amphictene auricoma	2.55	42.75
			Adontorhina similis	2.35	45.34
			Spiophanes kroyeri	2.28	47.83
c **Average similarity: 64.33**	E13_F1, E13_F2, E32_F1, E32_F2, E7_F1, E7_F2	90, 90, 90, 90, 89, 89	*Paramphinome jeffreysii*	6.95	8.45
			Adontorhina similis	5.74	7.14
			Galathowenia oculata	5.51	5.05
			Axinulus croulinensis	3.53	4.32
			Thyasira equalis	3.44	3.91
			Notomastus latericeus	3.13	3.45
			Abyssoninoe hibernica	2.65	3.45
			Pseudopolydora paucibranchiata	2.85	2.92
			Kurtiella tumidula	2.12	2.61
			Glycera alba	1.87	2.26

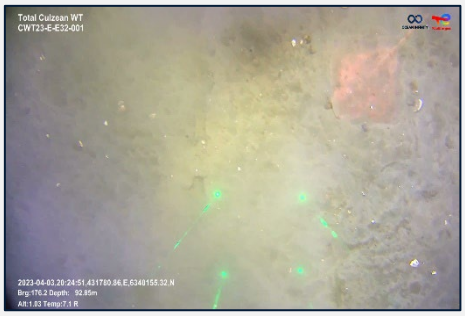
5.12 Potential Areas and Species of Interest

The habitats and species identified which correspond to those defined in the EC's Habitats Directive, the OSPAR List of Threatened and/or Declining Species and Habitats, Scottish PMF, and SBL are listed in Table 47 and Table 48.

Table 47 Potential habitats of conservation interest identified.

Image	Habitat	ANNEX I/OSPAR/PMF/SBL	Site ID
	Sea-Pen & Burrowing Megafauna Communities.	OSPAR PMF Burrowed Mud	R4, E7, E13, M2 and M1

Table 48 Potential species of conservation interest identified.

Image	Species	ANNEX I/OSPAR/PMF/SBL	Site ID
	Rajidae Possibly *Leucoraja circularis*	PMF/ SBL	E32
N/A	*Arctica islandica* juvenile	OSPAR/ PMF	E13-F2, R4-F1 and R4-F2

5.12.1 Habitats Directive

No habitats listed within the Annex I of the Habitats Directive (EEA, 2019; EUR 28, 2013) were identified within the site survey area or along the cable route corridor.

5.12.2 OSPAR and PMF

Burrowed Mud

The habitat Sea pen and burrowing megafauna communities is included in the List of Threatened and/or Declining Species and Habitats (OSPAR, 2008). It is considered under threat and/or decline in region II, the Greater North Sea (OSPAR, 2010). Sea pen and burrowing megafauna communities are a component biotope within the PMF habitat Burrowed Mud (Tyler-Walters, et al., 2016).

Sea pens and burrowing megafauna communities are characterised by a substrate comprising of fine circalittoral sand or mud, occurring in relatively sheltered areas.

The bioturbation from burrowing megafauna occurring in these habitats facilitates oxygenation deep down in the sediment and allows for a great diversity of smaller organisms to survive.

Prevalent features in this environment include burrowing mounds from crustaceans such as *Nephrops norvegicus, Calocaris macandreae* or *Callianassa subterranea*, as well as epifauna such as sea pens, *Virgularia mirabilis, Pennatula phosphorea*, and various types of echinoderms (OSPAR, 2010). In undisturbed areas the larger sea pen, *Funiculina quadrangularis* is more common (Tyler-Walters, et al., 2016).

The site survey area and cable route corridor comprised of sandy mud and muddy sand. Dominating species were burrowing and top gracing urchins along with sea-pens and occasional sea cucumbers. Burrows were present and observed in video on sites: E7, E13, M1, M2 and R4.

Burrows ranged from large entrance and exit holes as well as single vertical holes which are indicative of a *Nephrops norvegicus* presences. Presence of infauna excrement casts was also observed, however no burrowing animals were observed. Table 21 contains example species and burrows seen in video and photos.

During the 2013 survey no areas were interpreted to meet the qualifying descriptors of the OSPAR Sea pen and burrowing megafauna habitat. Species as well as areas of bioturbation and faunal burrows were however noted which is in line with the findings of the current survey.

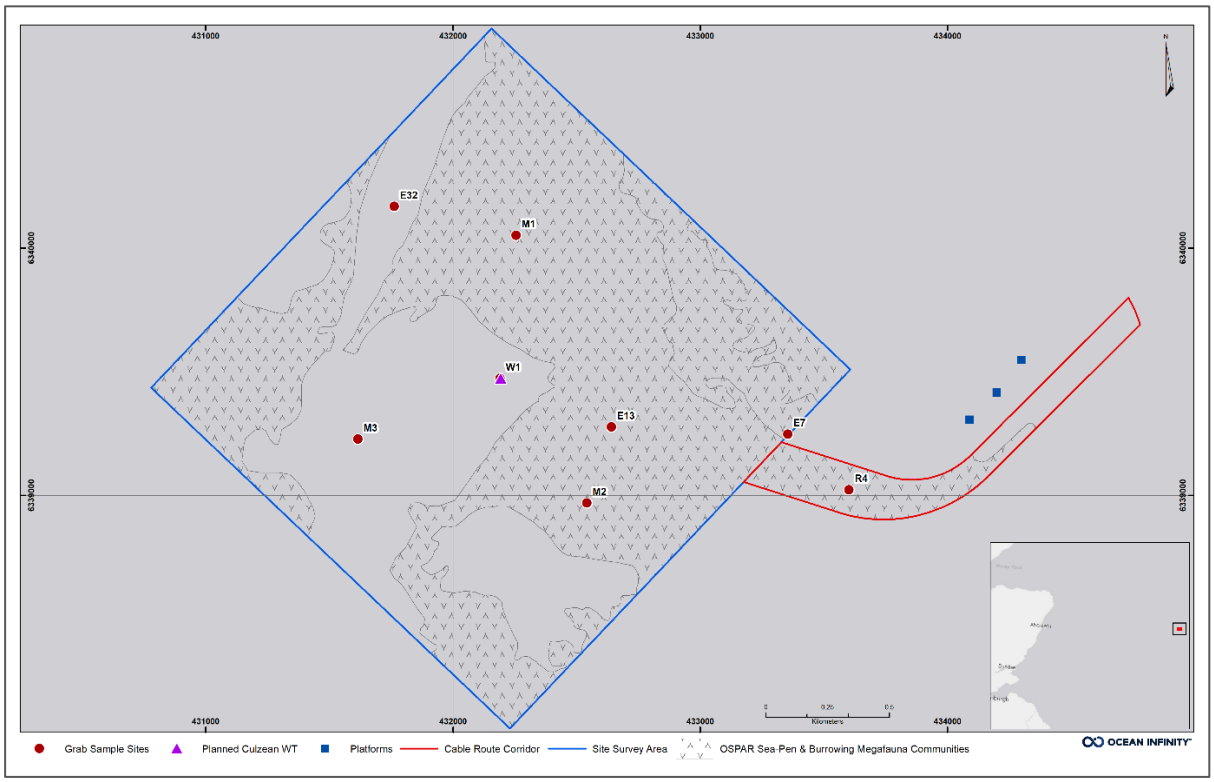


Figure 72 Delineation of OSPAR habitat Sea-pens and burrowing megafauna.

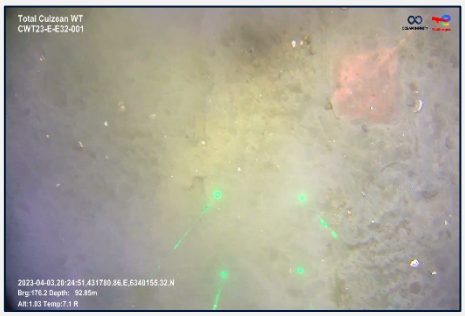
Arctica islandica

Juvenile *A. islandica* were identified in grab samples from sites E13 and R4. A total of four (4) juvenile individuals were identified in sample replicates E13-F2 (1 ind.), R4-F1 (1 Ind.) and R4-F2 (2 Ind). *A. islandica* is typically found in sand/ sandy mud or coarse sand habitats from the low intertidal zone to 400 m throughout the North Sea. The broad-scale habitats where *A. islandica* was identified was **MD521/ MC6216** - Faunal communities in Atlantic offshore circalittoral sand/ Seapens and burrowing megafauna in Atlantic circalittoral fine mud. Sparse presence of *Arctica islandica* was noted during the 2013 survey and as within the current survey all identified individuals were juveniles.

5.12.3 IUCN Red List

One taxon corresponding to those defined by the IUCN Red List as "Threatened" was identified in the survey area and is listed in Table 49.

Table 49 IUCN Red List taxa of concern identified within the survey areas.

Image	Description	IUCN Status	OSPAR/PMF/SBL	Site ID
	Rajidae Possibly *Leucoraja circularis*	Endangered	PMF/ SBL	E32

Leucoraja circularis

A sandy ray, possibly *Leucoraja circularis,* was identified in the video at sample site E32. *L. circularis* belongs to the family Rajidae which is commercially designated in the United Kingdom (UK). The species *L. circularis* is considered "Endangered" according to the IUCN Red List (McCully, Ellis, Walls, & Fordham, 2015). It is also threatened by overfishing and as unwanted by-catch.

6. Conclusions and Discussion

Sampling was conducted as part of the Benthic Environmental survey for TotalEnergies E&P North Sea UK Ltd (TotalEnergies) within the Culzean field, located approximately 230 kilometres off the coast of Aberdeen, Scotland in the Central North Sea.

Benthic sampling was performed at eight (8) pre-selected sites using a combination of Drop Down Video transects and grab sampling. In addition to benthic sampling, water sampling for eDNA and contaminants was conducted at the same locations.

The depth within the Culzean site area ranges between 88.8 m to 92.4 m, and from 83.0 to 90.6 m along the cable route corridor. Small seabed depressions were noted scattered across both survey areas and represent the only notable features other than the jack-up spudcan depressions within the site survey area and existing infrastructure within the route cable corridor.

The seabed within both the site area and route cable corridor is quite homogenous with some localised variations in the surface sediment composition. The backscatter intensity values exhibited limited variation with low reflectivity, across a large spatial scale. Small-scale variability, where noticeable, was associated with features such as infrastructure, seabed depressions, furrows, occasional cobbles and shell-gravel. A total of one (1) EUNIS habitat, three (3) habitat complexes and one (1) artificial habitat were identified and delineated within the survey area.

Statistical analyses conducted on the epibenthic fauna from the visual survey, showed the highest number of taxa, with a total of seven (7) different taxa, at site E13. The most abundant phyla of non-colonial fauna in stills images were Echinodermata with 54 %, followed by Cnidaria and Arthropoda with 13 % and 13 %, respectively. The Ophiurida was the overall most frequently occurring taxa, with a frequency of 88 % per site and 33 % per stills image. The density of non-colonial fauna in the stills imagery varied from six (6) (ind./m^2) at sites E32 to 16 (ind./m^2) at site E13. The average non-colonial fauna density per site still was 10.84 (SD=2.99) (ind./m^2). No listing of species and their abundances from the stills imagery was available from the 2013 survey, thus no quantitative comparison could be conducted.

The sediment composition had limited variation at the survey area. Fine sand/V Fine sand was the dominant sediment fraction. Grab sample site E32 presented slightly higher gravel content, with a total of 3.96 % gravel. The PCA plot mainly grouped the sites based on the silt and clay content and to a lesser extent on sand to gravel ratio. The Particle Size Analysis (PSA) results showed minimal variation between the 2013 Gardline dataset and the current survey for the three (3) sample sites included in the comparison, fine sand was the dominating sediment fraction, followed by Silt and Clay. Sample E32 from the 2023 survey, comprised the highest Gravel content of all compared samples with a total of 3.96 %. This could potentially be explained by the fact that the 2013 sample (ENV32) was acquired approximately 2 m east of the 2023 sample (E32).

Metal concentrations in sediment were generally low, with all grab samples showing values within background ranges for the Central North Sea (UKOOA, 2001) and below the OSPAR Effect Range Low (ERL) (OSPAR, 2011). Site E13 presented lower concentrations than other sample sites in all analysed metals, while location E32 showed slightly higher concentrations of most metals compared to other sites. Strontium (Sr) was the most notably elevated at E32, although levels were still considered within natural concentrations. This metal is most often associated with carbonate rocks and is naturally present in marine sediment, with fluctuating biogenic inputs such as the dissolution of carbonate sediment and continental weathering (Wierzbowski, 2015). Metal concentrations were generally lower in the current survey than in the samples acquired in 2013 for the three sites chosen for comparison. Levels of Aluminium (Al) presented the most notable differences between datasets, with higher concentrations in the previous survey.

Total Organic Matter (TOM) and Total Organic Carbon (TOC) both remained fairly consistent throughout the survey area, with values for TOM remaining within the Central North Sea background levels (UKOOA, 2001). Predictably, moisture content in samples showed a slight increase with the percentage of fines, since finer sediments tend to have a higher absorptive capacity.

Total Hydrocarbons (THC), total Polycyclic Aromatic Hydrocarbons (PAH) and n-alkane concentrations again showed little variation across the survey site, with all values being within background levels for the area (UKOOA, 2001) and below other comparable thresholds. The Carbon Preference Index (CPI) at all sample sites was above 1, indicating a general dominance of biogenic compounds over petrogenic compounds throughout the survey area. Phytane (Pr), which is often associated with oil contamination and not commonly found in natural marine environments, was below the limit of detection (LoD) for all but one grab sample.

TOM and TOC content, as well as hydrocarbon concentrations, showed minimal changes since the 2013 survey, with values remaining low at the three (3) compared sample sites.

Levels of polychlorinated biphenyls (PCB), organotins (DBT, TBT, MBT, TTBT & TPT), pesticides (OCP) and brominated flame retardants (PBDE) were below limits of detection for all analytes at every sample site.

Water samples were collected for Total Suspended Solids (TSS) and chemical analyses at the top and bottom of the water column for every site. TSS were relatively low across the survey area, with most samples below the detection limit. Where detected, TSS were higher in the bottom samples, potentially due to resuspension of seabed sediment. The TSS in the surface sediment often relates to planktonic organisms in the water column.

Heavy and trace metal concentrations in water samples were low throughout, with most being below or equal to their LoD. Zinc (Zn) was the only metal to exceed any of the water quality thresholds, presenting levels above the UK Annual Average (AA) Water Framework Directive (WFD) Environment Quality Standards (EQS) in six (6) of the samples collected. However, this threshold could be considered conservative, as concentrations of Zn in seawater can be highly variable. The main source of Zn in the marine environment is through aerial deposition, often resulting in high levels of Zn in seawater, particularly in the North Sea, which receives a yearly flux of Zn of almost 80.000 $\mu g/m^2$ (Neff, 2002).

Total Sulphur as SO_4 concentrations in water were within the normal range for seawater throughout the survey (Ministry of Environment Province of British Columbia, 2013), with the exception of the bottom water acquired at site R4, which presented a higher level of this analyte. High sulphate concentrations in water close to the seabed can be related to the presence of anoxic sediment below the surface, which is released into the water column via bioturbation of sediment-dwelling organisms (Brimblecombe, 2014).

The phyletic composition from grab samples, regarding both the total number of taxa and abundance, was dominated by Annelida. The most abundant taxa were the annelid *Paramphinome jeffreysii* which had a total abundance of 809 individuals and occurred in 100 % of the grab samples.

Comparing results from the eDNA samples to the non-colonial infauna grab samples revealed the phyla Nematoda, Phoronida and Platyhelminthes recorded in the grab samples were absent in the eDNA analyses. Annelida had the highest abundance in the non-colonial infauna grab samples (section 5.9.2), and the highest read counts for sediment invertebrate eDNA (section 5.10.2), with a contribution of 49 % for the grab samples and 87 % for the eDNA. This suggests a correlation between annelid abundance and number of read counts.

Comparing results from colonial infauna grab samples, the phylum Bryozoa, which had the highest abundance in the grab samples, was not recorded by the eDNA. Additionally, the phyla Entoprocta and Phoronoida were neither recorded in the eDNA. Explanation for the absence of these species in eDNA samples can be lack of shed DNA in the sediment and/or missing reference sequences, preventing detection.

Pielou's Evenness index and Simpson's Index of Dominance had a limited variation, whereas Margalef's Richness Index and Shannon-Wiener index presented slightly higher variation across the grab samples. The number of taxa and the number of individuals varied between 38 - 66 taxa and 123 - 313 (ind./m^2), respectively per grab sampling site.

The SIMPROF analysis of the non-colonial faunal composition produced three (3) statistically distinct groups. The sample similarity explored in the nMDS-plot presented a stress value of 0.18 which is considered a still useful ordination with a relatively low prospect of a misleading interpretation, the nMDS might have contradicted the resemblance matrix (Clarke & Gorley, 2015).

In the results of the BEST analysis limited to a single variable, V Coarse Sand/ Coarse Sand was the most distinguished variable with a global correlation (σ) of 0.855 and was the statistically significant variable for the distribution of the biological data. The strength of this correlation is considered highly correlated (Taylor, 1990).

In the results of the BEST analysis using multiple variables, the combined variables V Coarse Sand/ Coarse Sand, Medium Gravel and the combined variables V Coarse Sand/ Coarse Sand, Medium Gravel, V Coarse Gravel/ Coarse Gravel, both presented a global correlation (σ) of 0.867 and were statistically significant variables for the distribution of the biological data. The strength of this correlation is considered highly correlated (Taylor, 1990).

Cnidaria dominated the phyletic composition of the sessile colonial epifauna in grab samples, both regarding the number of taxa and abundance of colonies.

The non-colonial fauna species biomass was dominated by Echinodermata with 72 % of the total biomass, followed by Mollusca with 15 %. Non-colonial fauna biomass varied between 0.6395 g/0.1 m^2 in sample E7_F1, to 17.9573 g/0.1 m^2 in sample M2_F1. The non-colonial fauna biomass expressed as mean value across all grab samples sites was 5.4467 g/0.1 m^2 (SD=5.2905).

The compared species composition, regarding both the total number of taxa and abundance, presented higher values in all 2013 samples compared to 2023. The replicate sample ENV32 MFA presented the highest abundance and number of taxa in all compared samples with a total of 668 individuals and 87 different taxa.

The most abundant taxon was the annelid *Paramphinome jeffreysii*, with a total of 475 individuals recorded in 2013 samples and 226 individuals in 2023. The species occurred in 100 % of the compared samples from both the 2013 and 2023 samples.

The univariate indices for the three compared sites presented lower values in 2023 compared to 2013 for the number of individuals and the number of taxa.

Pielou's Evenness index presented slightly higher values in 2023 compared to 2013 indicating a more even species community. Margalef's Richness Index was slightly lower in the 2023 samples and the Shannon-Wiener index and Simpson's Index of Dominance ((λ) and (1-λ)) showed no significant difference between the 2013 and 2023 samples.

The SIMPROF analysis of the non-colonial faunal composition for the compared samples produced three (3) statistically distinct groups. The sample similarity explored in the compared nMDS-plot presented a stress value of 0.01 which is considered an excellent representation of the matrix data (Clarke & Gorley, 2015) with no prospect of a misleading interpretation, the nMDS don't contradict the resemblance matrix. In the compared nMDS plot, the 2013 and 2023 samples are clearly clustered together, although they are still located within 20 % similarity.

The OSPAR habitat Sea-pens and burrowing megafauna was identified in the site survey area and cable route corridor. The habitat is widespread in the surrounding seas around the Culzean site and covers more than 50 % of the Culzean area. The remaining seabed mainly comprised sea pens, heart urchins and sea urchins with minor to no burrows present. The absence of burrows could suggest a difference in sediment composition. During the 2013 survey, no areas were assessed to be in line with the qualifying descriptors of the OSPAR habitat Sea-pens and burrowing megafauna. This could in part be due to that the OSPAR background documents and guidance (OSPAR, 2010) described the habitat as primary "plains of fine mud" whilst the seabed within the Culzean site comprised muddy sand. In 2014 the JNCC sought to provide further clarification to the Sea-pens and burrowing megafauna and the definition of these concluding that the habitat had been observed in sandier sediments. Guidance was provided that where the relevant faunal composition was identified these areas could be classified as Sea-pens and burrowing megafauna with less weight on the substrate component. It is also possible that the presence of sea-pens was not as dense as noted within the current survey. The sites subject to comparison from 2013 did not indicate a presence of sea-pens.

From the image and video data, one species listed by IUCN Red List as "Threatened" was identified: Rajidae (possibly *Leucoraja circularis*). Sparse *A. islandica* (juvenile) were identified in grab samples from sites E13 and R4, which is similar to the 2103 survey where only juvenile *A. islandica* were identified.

Overall, the survey area presented a homogeneous seabed comprised mainly of fine to very fine sand. Contaminants were low throughout, with all concentrations within background levels for this region of the North Sea. The variations in faunal abundance and species richness of the sediment samples, as well as in the fauna observed in the photographic data, are likely driven by the natural variability in seabed composition found in the area, as demonstrated by the correlations between biological and physical indices resulting from the BEST tests. The data collected on the survey is considered to be consistent with a relatively uncontaminated seabed.

7. Reservations and Recommendations

The results detailed within this report are based on the field grab sample site descriptions and analyses of the photo and video recordings. The data has been reviewed in conjunction with the geophysical data (SSS and MBES) and interpretations. It should be noted that there is some natural limitation in the accuracy of interpretations and delineation of habitats. Where considered applicable, the sampling results have been extrapolated to surrounding areas exhibiting similarity as interpreted from the geophysical data.

The EUNIS 2022 Habitat classifications are currently under review and therefore a number of categories from the 2012 version have not yet been included. These categories include Inland waters, Wetlands, Constructed, industrial and other artificial habitats and Complexes. For the purpose of this report, infrastructure within the cable route corridor has been delineated as per the 2012 EUNIS Habitat **J** - Constructed, industrial and other artificial habitats.

For eDNA it is worth noting that each OTU is presented with a number of read counts, i.e., the number of reads assigned to a specific OTU. Whether the read counts correlates to species abundance and/or biomass is highly debated and depends on several factors such as filter type and PCR method, water temperature and type of organism (Di Muri, et al., 2020).

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Appendix A Sample Position List

Appendix B Grab Field Protocols

Appendix C Photo Identification Results

Appendix D Grab Identification Results

Appendix E Particle Size Analysis Results

Appendix F Chemical Analyses Results (Sediment Samples)

Appendix G Total Suspended Solids Results (Water Samples)

Appendix H Chemical Analyses Results (Water Samples)

Appendix I eDNA Results