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Firth of Tay Benthic Survey 2024: Technical Report

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Abbreviations

BIIGLE	Bio-Image Indexing and Graphical Labelling Environment
BSH	Broadscale Habitat
CLOC	Clear Liquid Optical Chamber
DDC	Drop-Down Camera
EUNIS	European Nature Information System
GPS	Global Positioning System
HD	High Definition
INNS	Invasive Non-Native Species
JNCC	Joint Nature Conservation Committee
LED	Light-Emitting Diode
NE	Natural England
NMBAQC	NE Atlantic Marine Biological Analytical Quality Control
OEL	Ocean Ecology Ltd
PSD	Particle Size Distribution
QAF	Quality Assurance Framework
RAMS	Risk Assessment Method Statement
RHDHV	Royal Haskoning DHV
SAC	Special Area of Conservation
SBAS	Satellite based Augmentation System
SPA	Special Protection Area
UPS	Uninterruptable Power Supply

Non-Technical Summary

Ocean Ecology Limited (OEL) was contracted by Forth Ports to undertake a two-phase benthic sampling survey at a series of stations within and surrounding the proposed Lady Shoal dredge area. The key focus of the environmental characterisation survey for the Lady Shoal survey was to provide a comprehensive baseline dataset characterising assessment of the habitats and associated biological communities for future monitoring using Drop-Down Camera (DDC) and grab sampling techniques, as well as identifying Annex I habitats if present. Due to the known presence of the priority marine feature (PMF) 'blue mussel beds; in proximity to the Lady Shoal dredge area, a key focus of the survey was in identifying and mapping these features.

The Lady Shoal dredge area lies within the boundary of the Firth of Tay and Eden Estuary Special Area of Conservation (SAC) and Firth of Tay and Eden Estuary Special Protection Area (SPA).

The DDC survey resulted in the collection of 457 still images and 19 videos totalling 196 minutes for subsequent seabed imagery analysis. Across the survey area, six European Nature Information System (EUNIS) habitat complexes were identified in the seabed imagery, including three Level 4 habitats: EUNIS MC124 'Faunal communities on variable salinity Atlantic circalittoral rock', EUNIS A5.14 'Circalittoral coarse sediment', EUNIS MC42 'Atlantic circalittoral mixed sediment' and three Level 5 biotopes: EUNIS MC1241 'Cushion sponges and hydroids on turbid tide-swept sheltered Atlantic circalittoral rock', EUNIS MC3211 '*Pomatoceros triqueter* with barnacles and bryozoan crusts on Atlantic circalittoral unstable cobbles and pebbles' and EUNIS MC2235 '*Mytilus edulis* beds on Atlantic circalittoral sediment'. The most commonly occurring epifauna observed in the seabed imagery across the survey area was the sea star *Asterias rubens*, identified as present in 194 images, followed by the bivalve *Mytilus edulis*, identified as present in 161 images.

Evidence of Annex I reef was identified with low confidence across the proposed Lady Shoal dredging area based on seabed imagery. Low confidence *M. edulis* reef and *M. edulis* Annex I biogenic reef was also observed throughout the survey area. The PMF 'Blue mussel beds' were identified within 30 images situated primarily to the west of the survey area at 5 transects in total.

Of the 15 stations sampled by grab, 14 were representative of BSH A5.4 'Mixed Sediment' and one station were representative of the BSH A5.1 'Coarse Sediment'. The most dominant sediment textural group observed was Muddy Sandy Gravel (msG) followed by Gravelly Muddy Sand (gmS) and Sandy Gravel (sG). The macrobenthic community was characterised by 148 different taxa and a total of 27,715 individuals recorded. Nematodes were the most abundant taxon and were found in all samples together with the polychaete *Pholoe inornata*, Nemertea, and the barnacle *Balanus crenatus*.

Biomass was dominated by molluscs most likely due to the high abundance of juveniles of Mytilidae (mussels). This was most likely due to the presence of a known mussel bed in the area that could have encourage recruitment of juvenile mussels, particularly to the east of the Lady Shoal dredging area.

Conversely adults of *Mytilus edulis* (blue mussels) were not as abundant in the grab samples collected across the survey area. To note that blue mussel on their own are not afforded protection and it is only blue mussel beds that qualify as a PMF habitat. Two invasive non-native species (INNS) were recorded across the survey area: the Darwin's barnacle *Austrominius modestus* and the sand-gaper *Mya arenaria*. The former is known to compete for space with UK native barnacles and spread rapidly. Due to the presence of mixed sediments, the macrobenthic community present across the survey area was quite diverse as mixed sediments offer several niches for a variety of organisms to colonise. It was therefore not possible to assign the community to any known biotope, however stations within Survey Area B to the west of the Lady Shoal dredging area were characterised by the presence of oligochaetes and polychaetes, amphipods and barnacles while stations within the Lady Shoal dredging area were characterised by the presence of several barnacle and polychaete species, sea snails, amphipods and brittle stars.

1. Introduction

1.1. Project Overview

Forth Ports have proposed a new dredge area within the Lady Shoal area of the River Tay, associated within the Forth Ports operated Port of Dundee. To support dredge activity licencing, a benthic survey of the proposed Lady Shoal dredge area was required to accurately characterise the benthic habitats and describe the macrobenthic communities present within this area.

Ocean Ecology Limited (OEL) was contracted by Forth Ports to undertake a two-phase benthic sampling survey consisting of drop-down camera (DDC) sampling and grab sampling at a series of stations within and surrounding the proposed Lady Shoal dredge area.

1.2. Aims and Objectives

The key focus of the environmental characterisation survey for the Lady Shoal survey was to provide a comprehensive baseline dataset characterising the habitats and associated biological communities for future monitoring using Drop-Down Camera (DDC) and grab sampling techniques.

The key objectives of the survey were to:

- Provide an initial description of the seabed habitats within the survey area, including described biotopes, biodiversity, function, abundance, extent, species richness, representativeness, rarity, and sensitivity.
- Identify and assess the status of species and habitats of conservation importance, including Annex I protected species and habitats (such as *Sabellaria spinulosa* biogenic reef or stony reef), and Annex V species of the Habitats Regulations, species listed under Schedule 5 of the Wildlife & Countryside Act, OSPAR species and habitats and designated features of the Marine Protected Area (MPA) network (e.g., Special Area of Conservation (SAC);
- Identify the PMF 'blue mussel beds', and
- Confirm the presence / absence of any epibenthic invasive non-native species (INNS), species non-native to UK waters and species non-native to the local habitat types (e.g., hard-substrate specialists in a wider sedimentary habitat).

1.3. Site Information

The Lady Shoal dredge area encompasses a dredge box approximately 0.86 km² in area, stretching 4.8 km in length within the River Tay downstream of the Port of Dundee.

2. Current Understanding

2.1. Designated Sites and Sensitive Habitats

The Lady Shoal dredge area lies within, or within proximity to three Marine Protected Areas (MPAs) (Figure 2):

- Firth of Tay and Eden Estuary Special Area of Conservation (SAC)
- Firth of Tay and Eden Estuary Special Protection Area (SPA)
- Outer Firth of Forth and St Andrews Bay Complex SPA

The Firth of Tay and Eden Estuary (SAC)

The SAC is approximately 15,441.63 hectares and includes a variety of habitats such as marine areas, sea inlets, tidal rivers, estuaries, mudflats, sandflats, salt marshes, coastal sand dunes, and inland water bodies. The Tay is the least-modified of the large east coast estuaries in Scotland, while the Eden estuary represents a smaller 'pocket' estuary. The area is also crucial for its harbour seal (*Phoca vitulina*) population, with around 600 adults, representing approximately 2% of the UK population. These seals utilise the sandbanks within the estuary to rest, pup, and moult.

Firth of Tay and Eden Estuary Special Protection Area (SPA)

As an SPA, the Firth of Tay and Eden Estuary support internationally and nationally important numbers of wintering waterfowl and waders. Species such as the bar-tailed godwit (*Limosa lapponica*) and common redshank (*Tringa totanus*) are present in significant numbers during migration and wintering periods. The estuary also serves as a resting area for greylag geese (*Anser anser*).

Outer Firth of Forth and St Andrews Bay Complex SPA

The Outer Firth of Forth and St Andrews Bay Complex is an extensive SPA located in the southeast coast of Scotland. It extends from Arbroath in the North to St Abb's Head in the South and encompasses the Firth of Forth, the outer Firth of Tay and St Andrews Bay. This SPA provides many habitats and ecological niches such as firths, inlets and sandy bays that are used by seabirds and waterbirds during different stages of their life cycle. The site is designated for the protection of 21 seabird and waterbird species including non-breeding waterfowl like the red-throated diver (*Gavia stellata*) and breeding seabirds like the common tern (*Sterna hirundo*).

Annex I *Mytilus* reef and Priority Marine Feature (PMF) Habitat 'Blue Mussel Bed'

The EC Habitats Directive (CEC 2013) definition of Annex I 'Reef', (Holt et al. 1998) described biogenic reef as:

"Solid, massive structures which are created by accumulations of organisms, usually rising from the seabed, or at least clearly forming a substantial, discrete community or habitat which is very different

from the surrounding seabed. The structure of the reef may be composed almost entirely of the reef building organism and its tubes or shells, or it may to some degree be composed of sediments, stones and shells bound together by the organisms”....

Annex I biogenic reef habitats protected in Europe under the EC Habitats Directive are generally formed by gregarious species of polychaetes (e.g., *Sabellaria* spp., *Serpula vermicularis*), cold-water coral (*Desmophyllum pertusum*), or bivalves (Mytilidae). This refers specifically to *Mytilus edulis* forming beds, which may also constitute the Priority Marine Feature (PMF) habitat ‘Blue Mussel Bed’, provided they occur as cohesive beds, not as individuals.

The distribution of *Mytilus edulis* within the Firth of Tay and Eden Estuary has been mapped using data from previous surveys, with records attributed to their qualification as protected features of protected areas within the Scottish MPA network.

This existing survey has already identified areas of potential mussel bed presence. However, as noted in the previous survey findings, there is potential for further modelling to refine the understanding of mussel bed distribution, particularly in areas with cobbles and boulders that could support *M. edulis* (Figure 3). These habitats may have the potential to qualify as Annex I biogenic reefs under the EC Habitats Directive.

2.2. Existing Habitat Mapping

The 2023 EUSeaMap broad-scale predictive model classifies and maps intertidal and subtidal habitats according to the European Nature Information Systems (EUNIS) classification criteria. The system is able to identify keystone species that have been evidenced to inhabit areas with certain environmental conditions and can therefore act as an indicator, allowing inferences of overall community composition. EUSeaMap mapping from within the Lady Shoal dredge area predicts the survey area as MC32 ‘Atlantic circalittoral coarse sediment’, MC223 ‘Bivalve reefs in the Atlantic circalittoral zone’, MC2235 ‘*Mytilus edulis* beds on Atlantic circalittoral sediment’ and MC42 ‘Atlantic circalittoral mixed sediment’ (EMODnet 2023) (Figure 1).

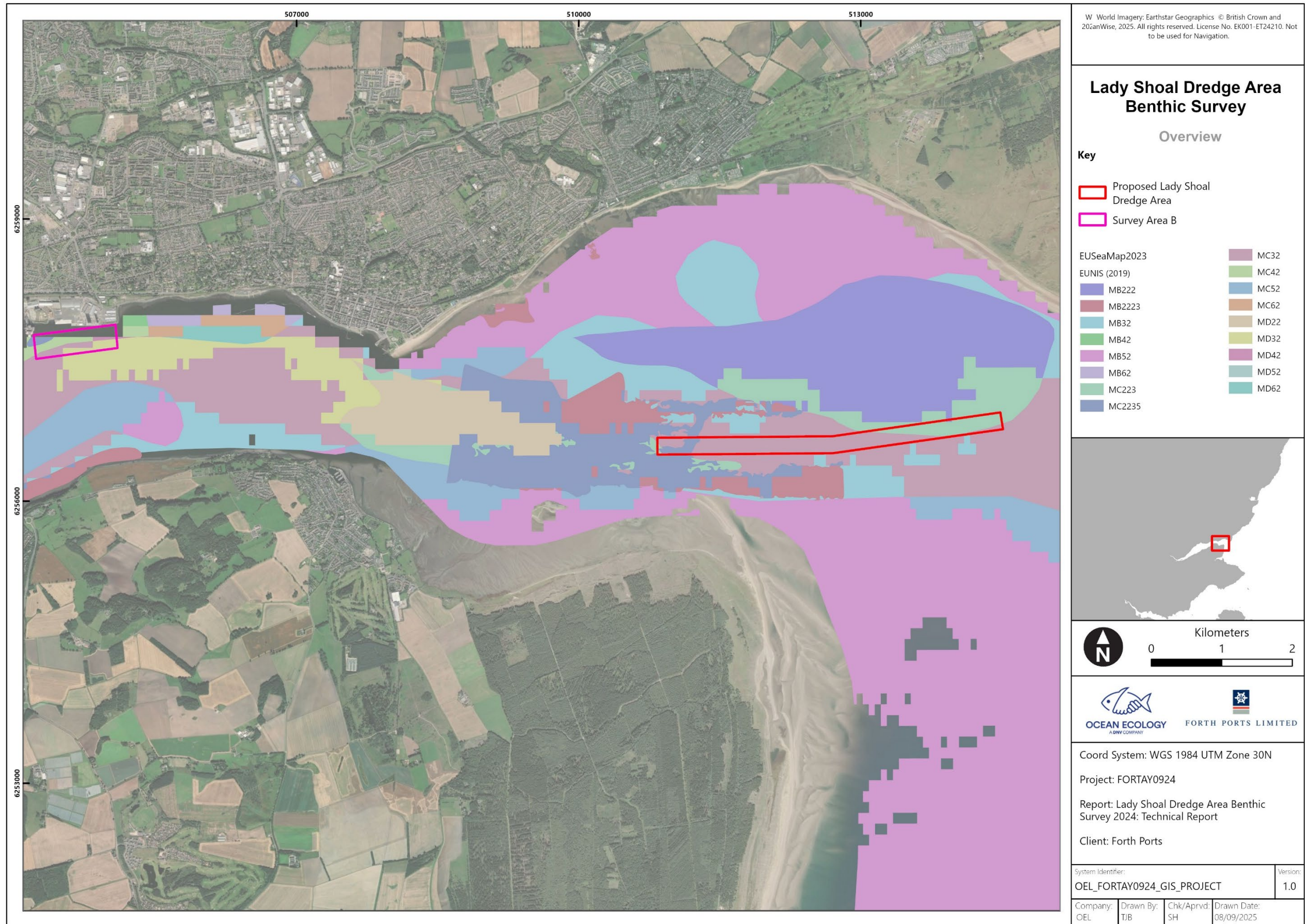


Figure 1 Lady Shoal survey area overview, including predicted habitats (EUSaMap2023).

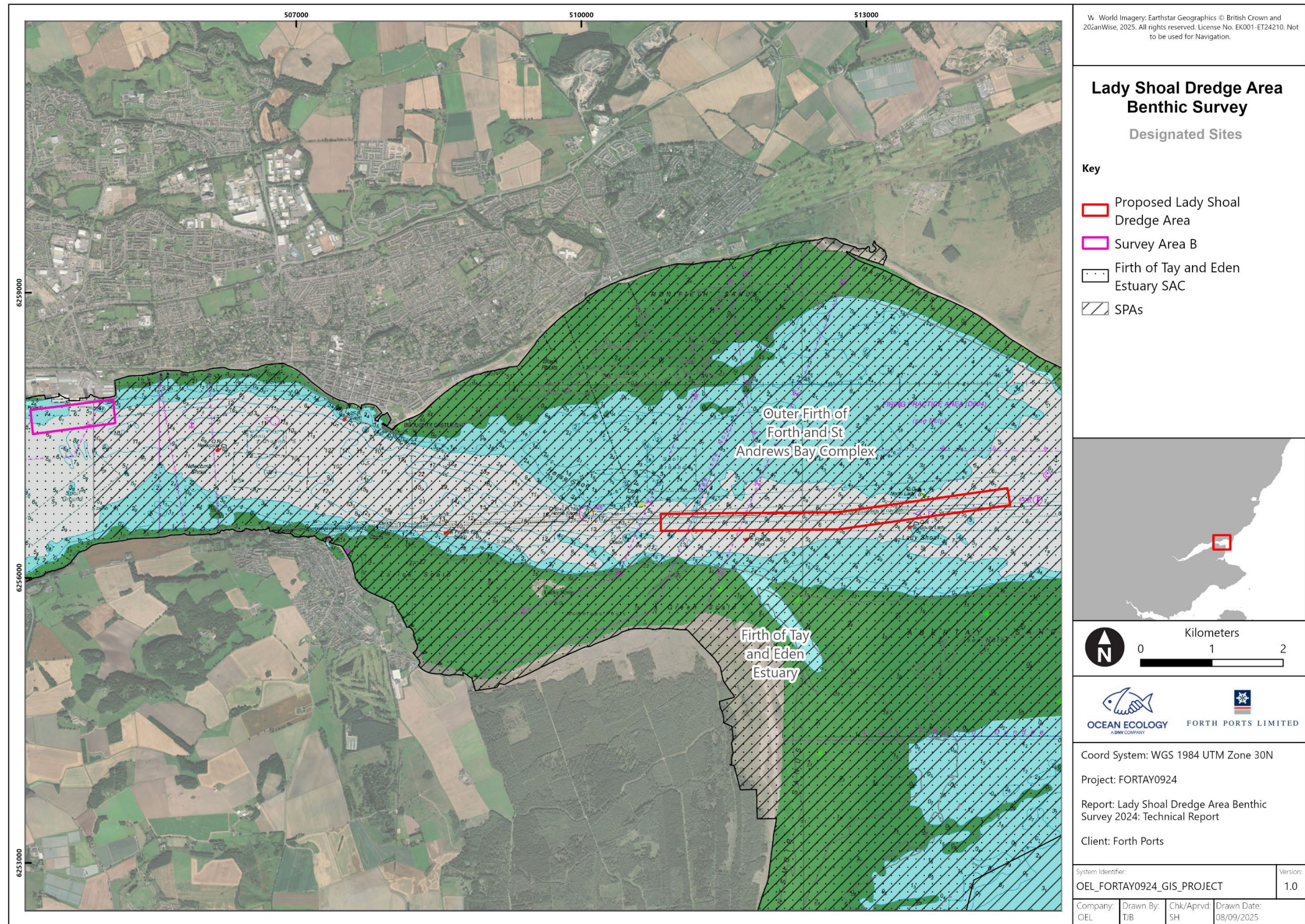


Figure 2 Lady Shoal survey area overview, including sampling design and designated sites.

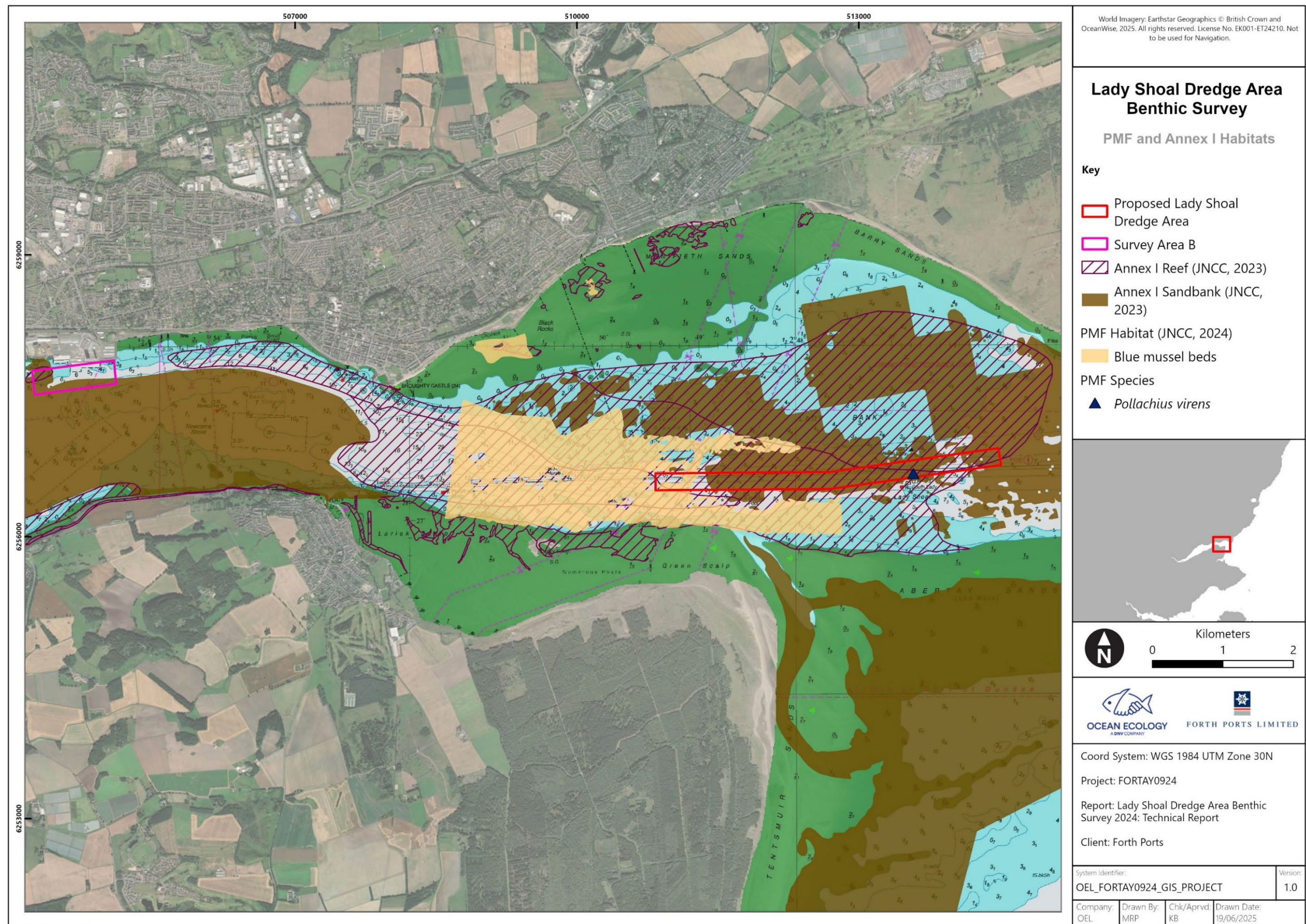


Figure 3 PMF and Annex I Habitats withing the survey area.

3. Survey Design

3.1. Sampling Design and Rationale

A two-phase sampling approach was developed by Haskoning (formerly known as Royal HaskoningDHV) on behalf of Forth Ports to include DDC and grab sampling at 15 stations within and surrounding the proposed Lady Shoal dredge area. Stations were positioned to provide representative sampling of predicted EUNIS habitats (EUSeaMap 2023, Figure 1) as well as broad coverage of the dredge area. Following consultation between RHDHV and NatureScot, additional sampling was recommended by NatureScot at two stations (ST015 and ST016), specifically targeting potential blue mussel (*Mytilus edulis*) beds in the area. Additionally, Survey Area B was established at the request of the port authorities Forth Ports to assess potential dredging locations while the main primary survey was being conducted. It is considered separate from the primary dredge area.

A 100 m DDC transect was run at each benthic sampling station. Transects intersected the sampling station target location at the transect mid-point (50 m), and data was reviewed in real time to determine a suitable location to perform grab sampling at each station.

Following completion of the DDC transects, grab sampling was undertaken using a 0.1 m² mini-Hamon grab. Samples were retained for subsequent macrobenthic and particle size distribution (PSD) analyses, as detailed further in Section 3.4.2. Target benthic sampling stations are listed in Table 1 and presented spatially within Figure 1.

Table 1 Proposed benthic sampling stations for the Lady Shoal dredge area survey.

Station ID	Latitude	Longitude
ST001	56.453842	-2.839331
ST002	56.453081	-2.831217
ST003	56.453121	-2.827614
ST004	56.454089	-2.823175
ST005	56.453569	-2.815428
ST006	56.454064	-2.802839
ST007	56.453489	-2.792675
ST008	56.454772	-2.780622
ST009	56.455022	-2.770139
ST010	56.456461	-2.831861
ST011	56.456864	-2.786925
ST012	56.455633	-2.759742
ST013	56.451106	-2.785664
ST014	56.451469	-2.823158
ST015*	56.45321	-2.82036
ST016*	56.45167	-2.82036

* Denotes a NatureScot requested station targeting *M. edulis* beds.

3.2. Sampling Approach

At all benthic sampling stations, DDC sampling was conducted in advance of grab sampling to identify a suitable location to target for grab sampling and ensure the seabed was clear of any subsea infrastructure or sensitive features.

If features of interest (e.g., *M. edulis* beds) were observed in the DDC footage, transects were extended in an attempt to capture the extent of the feature. Transects were extended within the assumed zone of influence of the proposed activity, which may have extended beyond the area initially proposed for the benthic sampling stations. Additional *ad hoc* transects were also surveyed, perpendicular to planned transects, to better understand the extent of the feature of interest. The requirement for additional transects was determined in the field.

Grab sampling was conducted, following pre-screening via DDC, to collect samples for subsequent macrobenthic and PSD analyses.

3.3. Timing

The survey of the Lady Shoal Dredge area was undertaken between the 4th and 7th of November 2024.

4. Survey Methods

4.1. Survey Vessel

The survey was conducted aboard OEL's dedicated 10.0 m MCA Category 2 coded survey vessel *Seren Las* (Plate 1 and Table 2). The vessel was mobilised from Tayport Harbour and operated out at sea on a 12-hour daylight operations basis.

Table 2 Vessel details of *Seren Las*.

Vessel Name	<i>Seren Las</i>
Call Sign	MDAH2
MMSI	235087047
Mobilisation Port	Tayport Harbour
Length	10.0 m
Beam	3.5 m



Plate 1 OEL's dedicated survey vessel *Seren Las*.

4.2. Survey Navigation

Project positional data was recorded and collected in the WGS84 UTM 30N coordinate reference system.

Surface positioning aboard the *Seren Las* was determined using a Hemisphere V104s Global Positioning System (GPS) compass system. The Hemisphere V104s internal GPS receiver utilised a minimum of four GPS satellites, managing the navigation information required to obtain a position within 3 m at 95% accuracy. The V104s automatically tracked Satellite-Based Augmentation System (SBAS) differential correction to improve position accuracy to >1 m at 95% accuracy. The V104s included an integrated gyro and two tilt sensors to provide an accurate heading for navigation software.

4.3. Navigation Software

A vessel-based positioning system was employed utilising EIVA NaviPac V4.6.5 software to ensure the accurate positioning of the vessel and camera system. A navigation screen, displaying EIVA Helmsman Display, was provided at the helm position of the vessel for the skipper as well as for the ecologist/surveyor in the wheelhouse.

Fixed offsets were measured for the deployment of the camera from the aft A-frame. An Ultra-Short Baseline (USBL) system was not required due to the shallow water depths, meaning the camera system was at a negligible offset from the vessel's stern (i.e., the deployment point).

4.4. Drop-Down Camera Sampling

4.4.1. Drop-Down Camera System

Seabed imagery was acquired at each station using OEL's SubC Rayfin PLE camera system, set up to obtain 1080p High Definition (HD) video and 20 Megapixel (MP) still images. The camera system (Plate 2) consisted of a SubC Imaging Rayfin PLE camera mounted in a clear liquid optical chamber (CLOC) filled with freshwater to ensure high-quality imagery was obtained. The CLOC was height- and angle-adjustable, providing a variety of options for view, lighting, and focal length to maximise data quality with respect to prevailing conditions. LED strip lamps and a 10 cm point laser scaling array were projected into the field of view. The camera was connected via a 150m umbilical to a topside computer and powered by an Uninterruptible Power Supply (UPS) to ensure data loss was prevented should the vessel lose power or in case of a power surge. A full redundancy SubC Rayfin PLE camera system was stored onboard for use if required.

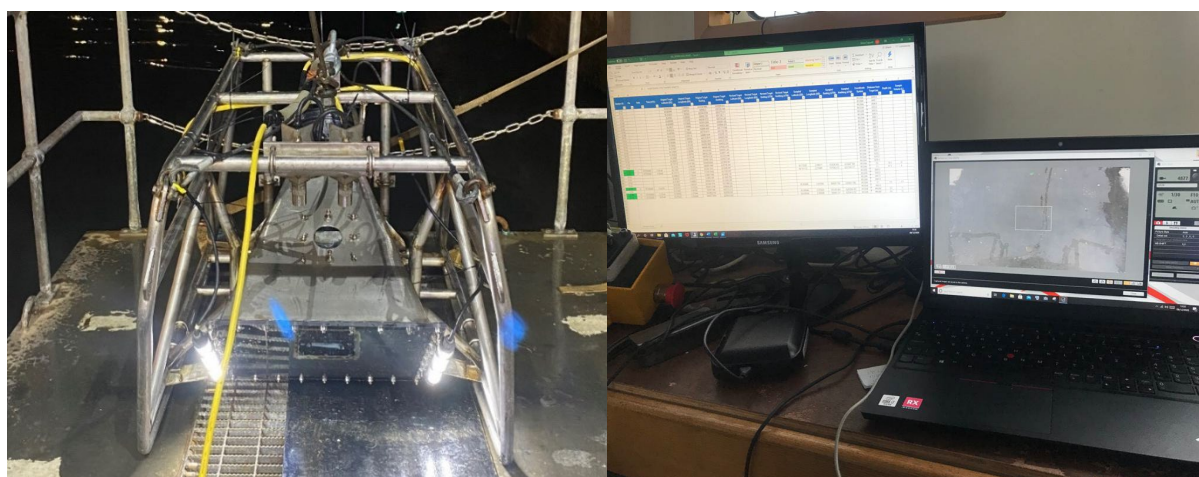


Plate 2 Left: OEL CLOC camera system. Right: The camera system topside setup.

4.4.2. Seabed Imagery Collection

All DDC transects were sampled in consideration of the Joint Nature Conservation Committee (JNCC) epibiota remote monitoring operational guidelines (Hitchin et al., 2015).

At each DDC transect, the camera was deployed to the seabed over the target start/end transect location and slowly 'flown' just above the seabed along the transect to obtain both continuous video footage and still images. The camera was flown (or used a bed-hopping approach where visibility/tide did not allow) above the seabed at a consistent speed of 0.5 knots (0.25 m/s). Video and still images were acquired in either plan-view or with a slight oblique angle, depending on visibility on site, with the setting chosen to best facilitate accurate imagery analysis. Photographs were taken at approximately 5–10 m intervals, or more frequently with changing seabed type/habitat, with accompanying video recorded throughout the length of the transect.

All footage underwent a preliminary review *in situ* by the onboard ecologist(s). Videos were recorded in a digital format, and detailed notes were taken of visible sediment conditions and seabed features, obvious fauna, and habitat related features whilst in the field, prior to detailed analysis in the laboratory. Whenever a photograph was taken, a positional fix was recorded.

4.5. Grab Sampling

4.5.1. Grab Sampler

A 0.1 m² mini-Hamon grab was used to obtain macrobenthic and sediment samples at each of the proposed grab sampling locations. All grab sampling was undertaken in line with the latest version (2.7) of the POSEIDON benthic sampling protocols¹.

4.5.2. Grab Sample Collection

At all stations, a single 0.1 m² sample of approximately 10 L was collected and sub-sampled for subsequent macrobenthic and PSD analyses. The macrobenthic sample was elutriated through a 0.5 mm sieve mesh, fixed with 4–10% buffered formalin solution, and retained for macrobenthic analysis in the laboratory. The grab sampler was deployed from the hydraulic 'A' frame on the aft deck of *Seren Las*.

To ensure consistency in sampling, grab samples were screened by the lead Ecologist and considered unacceptable if:

- Sample replicates were less than 5 L. i.e., the sample represents less than approx. a half of the 10 L capacity of the grab used.
- The jaws failed to close completely or were jammed open by an obstruction, allowing fines to pass through (washout or partial washout).

¹ Available [here](#).

- The sample was taken at an unacceptable distance from the target location (beyond 20 m).
- There was obvious contamination of the sample from survey equipment, paint chips etc.

Should a grab fail, a second and third attempt were conducted at the same site of the first failed attempt before abandoning the station. No pooling of samples took place.

Initial grab sample processing was undertaken in line with the following methodology:

- Initial visual assessment of sample size and acceptability was made.
- Photograph of the sample with station details and scale bar was taken.
- A minimum of 200 ml and maximum of 500 ml of the sample was removed as a PSD sub-sample and transferred to a labelled and sealed plastic container and frozen at -20°C in an onboard freezer.
- Sample emptied onto 0.5 mm sieve net laid over 4.0 mm sieve table and washed through using gentle rinsing with seawater hose.
- Backwashed into a suitable sized sample container with 10% formalin solution added to fix the sample prior to laboratory analysis.
- Sample containers were clearly labelled internally and externally with date, sample ID and project name.

Detailed field notes and digital photographs were taken of each station and will include station number, fix number, number of attempts, water depth. Visual descriptions of sediment type were made (using the Folk classification categories) at the time of sampling, together with estimates of sample volume. Any notable or obvious fauna present was also be recorded in the field notes.

Formalin was used in full adherence to the OEL RAMS which sets out the appropriate minimum Personal Protective Equipment (PPE) requirements when using formalin and the procedure to follow in the event of a spill.

5. Laboratory Analysis & Interpretation

5.1. Seabed Imagery Analysis

All seabed imagery analysis was undertaken using the Bio-Image Indexing and Graphical Labelling Environment ([BIIGLE](#)) annotation platform (Langenkämper et al. 2017) and in consideration of the JNCC epibiota remote monitoring interpretation guidelines (Turner et al. 2016) and the latest [NMBAQC/JNCC Epibiota Quality Assurance Framework \(QAF\) guidance and identification protocols](#). Analysis of still images and videos was undertaken in two stages. The “Tier 1” level consisted of labels that referred to the whole image being assigned, providing appropriate metadata for the image, these included labels such as image quality, broad scale habitat (BSH), EUNIS habitat assigned in line with Parry (2019) and INNS.

In addition, if biogenic or geogenic reef was observed in the video footage and seabed imagery, an Annex I reef assessment was also undertaken to determine whether habitats meet the definitions of Annex I reef habitats as detailed in Table 3 and Table 4. The latest JNCC guidance on the characterisation of ‘low resemblance’ Annex I stony reef was also considered (Golding et al. 2020). The annotation label tree used during analysis had major headings for each of the reef types.

The second stage, “Tier 2”, was used to assess abundance of conspicuous epibiota, to assign percentage cover of ‘reef’ types by drawing polygons to inform the habitat assessment process and undertake a burrowing assessment (where required).

Table 3 Characteristics of stony reef (Irving 2009).

Characteristic	‘Reefiness’			
	Not a Reef	Low	Medium	High
Composition (proportion of boulders/cobbles (>64 mm))	<10 %	10-40 % matrix supported	40-95 %	>95 % clast-supported
Elevation	Flat seabed	<64 mm	64 mm - 5 m	>5 m
Extent	<25 m ²	>25 m ²		
Biota	Dominated by infaunal species	>80 % of species present composed of epibiotal species		

Table 4 Characteristics of *Sabellaria spinulosa* reef (Gubbay 2007).

Characteristic	'Reefiness'			
	Not a Reef	Low	Medium	High
Elevation (cm)	< 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

5.1.1. Tier 1 Analysis

The first stage, "Tier 1", consisted of assigning labels that referred to the whole image, providing appropriate metadata for the image. Metadata "Image Labels" include:

- Broadscale Habitat (BSH) type.
- Substrate type (and percentage cover in 10% intervals).
- Bedforms present.
- The presence of any Annex I habitats, HOCl and INNS.
- The presence of any visible impacts or other modifiers (such as discarded fishing gear or marine litter (as per the Marine Strategy Framework Directive (MSFD) categories), visible physical damage to the seabed, evidence of strong currents, non-native species, etc.).
- Image quality categories (including "Not Analysable" category).

Depending on the presence of reef, this also included:

- Extent: As it is not possible to fully determine the extent of reef habitats from a single image alone this label was used to identify areas that are highly unlikely to constitute reef habitats. An example is an image that shows a large boulder being preceded and succeeded by images of unconsolidated sandy sediments.
- Biota: Labels assigned to determine whether epifauna dominate the biological community observed.
- Elevation: Labels assigned depending on reef type. Laser points will be used to assist in the assignment of categories.

5.1.2. Tier 2 Analysis

The second stage, "Tier 2", was used to assess epibiotical abundance data as "annotations" within each image for visible flora and fauna. This was undertaken as follows:

- Using the BIIGLE Annotation Platform enumeration of all visible taxa was undertaken using points for enumerable "count" taxa and polygons for ground-covering taxa; to enable calculation of percentage cover for these taxa.
- Where an individual of a "count" taxon overlaid a ground-cover taxon, this individual was still counted (i.e., a point annotation was added for the count taxa over the polygon of the ground-cover taxon).
- To assist the Tier 1 analysis of reef presence, polygons were drawn at the Tier 2 stage to delineate percentage cover of biogenic and geogenic reef features.

- Identification of any INNS and species non-native to UK waters. Information was also included on species non-native to the local habitat types (e.g., hard-substrate specialists in a wider sedimentary habitat).

The substratum observed in each still image was recorded as a percentage cover of CATAMI (Althaus et al. 2015) substratum types where possible.

Determination of sediment type (such as coarse, mixed, sand etc.) was facilitated using the adapted Folk sediment trigon (Long 2006) incorporated into a sediment category correlation table. Percentage cover of the different substrate types was used to determine and assign EUNIS codes and BSH.

5.2. Particle Size Distribution (PSD) Analysis

PSD analysis of the sediment samples was undertaken by in-house laboratory technicians at OEL's NMBAQC participating laboratory in line with NMBAQC best practice guidance (Mason 2016).

Frozen sediment samples were first transferred to a drying oven and thawed at 80°C for at least 6 hours before visual assessment of sediment type. Before any further processing (e.g., sieving or sub-sample removal), samples were mixed thoroughly with a spatula and all conspicuous fauna (>0.5 mm) which appeared to have been alive at the time of sampling were removed from the sample. A representative sub-sample of the whole sample was then removed for laser diffraction analysis before the remaining sample screened over a 1 mm sieve to sort coarse and fine fractions. The >0.5 mm fraction was then returned to a drying oven and dried at 80°C for at least 24 hours before dry sieving.

Once dry, the sediment sample were run through a series of Endecott BS 410 test sieves (nested at 0.5 ϕ intervals) using a Retsch AS200 sieve shaker to fractionate the samples into particle size classes. The dry sieve mesh apertures used are given in Table 5.

Table 5 Sieve series employed for PSD analysis by dry sieving.

Sieve aperture (mm)												
63	45	32	22.5	16	11.2	8	5.6	4	2.8	2	1.4	1

The sample was then transferred onto the coarsest sieve at the top of the sieve stack and shaken for a standardised period of 20 minutes. The sieve stack was checked to ensure the components of the sample had been fractionated as far down the sieve stack as their diameter would allow.

The sub-sample for laser diffraction was first screened over a 0.5 mm sieve and the fine fraction residue (<0.5 mm sediments) transferred to a suitable container and allowed to settle for 24 hours before excess water syphoned from above the sediment surface until a paste texture was achieved. The fine fraction was then analysed by laser diffraction using a Beckman Coulter LS13 320.

The dry sieve and laser data was then merged for each sample with the results expressed as a percentage of the whole sample. Once data was merged, PSD statistics and sediment classifications were generated from the percentages of the sediment determined for each sediment fraction using Gradistat v9 software.

Sediment descriptions are defined by their size class based on the Wentworth classification system (Wentworth 1922) (Table 6). Statistics such as mean and median grain size, sorting coefficient, skewness and bulk sediment classes (percentage silt, sand and gravel) were derived following the Folk classification (Folk 1954).

Table 6 The classification used for defining sediment type based on the Wentworth Classification System (Wentworth 1922).

Wentworth Scale	Phi Units (ϕ)	Sediment Types
>64 mm	< -6	Cobble and boulders
32 – 64 mm	-5 to -6	Very Coarse Pebble
16 – 32 mm	-4 to -5	Coarse Pebble
8 – 16 mm	-3 to -4	Medium Pebble
4 - 8 mm	-3 to -2	Small Pebble
2 - 4 mm	-2 to -1	Granule
1 - 2 mm	-1 to 0	Very coarse sand
0.5 - 1 mm	0 – 1	Coarse sand
250 - 500 μm	1 – 2	Medium sand
125 - 250 μm	2 – 3	Fine sand
63 - 125 μm	3 – 4	Very fine sand
31.25 – 63 μm	4 – 5	Coarse silt
15.63 – 31.25 μm	5 – 6	Medium silt
7.813 – 15.63 μm	6 – 7	Fine silt
3.91 – 7.81 μm	7 – 8	Very Fine silt
0.98 – 3.91 μm	8 – 10	Clay

5.3. Macroenthic Analysis

All elutriation, extraction, identification, and enumeration were undertaken at OEL's NMBAQC scheme participating laboratory in line with the NMBAQC Processing Requirement Protocol (Worsfold & Hall 2010). All processing information and macroenthic records were recorded using OEL's cloud-based data management application [ABACUS](#) that employs [MEDIN](#) validated, controlled vocabularies ensuring all sample information, nomenclature, qualifiers, and metadata are recorded in line with international data standards.

For each macroenthic sample, the excess formalin was drained off into a labelled container over a 0.5 mm mesh sieve in a well-ventilated area. The samples were then re-sieved over a 0.5 mm mesh sieve to remove all remaining fine sediment and fixative. The low-density fauna was then separated by elutriation with freshwater, poured over a 0.5 mm mesh sieve, transferred into a Nalgene and preserved in 70 % Industrial Denatured Alcohol (IDA). The remaining sediment from each sample was subsequently separated into 0.5 mm, 1 mm, 2 mm and 4 mm fractions and sorted under a stereomicroscope to extract any remaining fauna (e.g., high-density bivalves not 'floated' off during elutriation).

All fauna present was identified to species level, where possible, and enumerated by trained benthic taxonomists using the most up to date taxonomic literature and checks against existing reference collections. Nomenclature utilises the live link within ABACUS to the World Register of Marine Species ([WoRMS](#)) web services to ensure the most up to date taxonomic classifications are recorded. Colonial fauna (e.g., hydroids and bryozoans) were identified to species level where possible and recorded as present (P). For subsequent data analysis, taxa recorded as P were given the numerical value of 1.

Biomass was measured as blotted wet weight in grams to at least 4 decimal places for all countable taxa (i.e., at species level where possible). As a standard, the conventional conversion factors as defined by (Eleftheriou & Basford 1989) was applied to biomass data to provide equivalent dry weight biomass (Ash Free Dry Weight (AFDW)).

The conversion factors applied are as follows:

- Annelida = 15.5%
- Crustacea = 22.5%
- Mollusca = 8.5%
- Echinodermata = 8.0%
- Miscellaneous = 15.5%

5.3.1. Data Truncation and Standardisation

The macrobenthic taxon list was checked using the R package “*worms*” (Holstein 2018) to check against WoRMS taxon lists and standardise species nomenclature. Once the species nomenclature was standardised in accordance with WoRMS-accepted species names, the species list was examined carefully by a senior taxonomist to truncate the data, combining species records where differences in taxonomic resolution were identified.

5.3.2. Pre-Analysis Data Treatment

All data were collated in excel spreadsheets and made suitable for statistical analysis. All data processing and statistical analysis was undertaken using R v 1.2 1335 (R Core Team, 2022) and PRIMER v7 (Clarke & Gorley 2015) software packages.

In accordance with the OSPAR Commission guidelines (OSPAR 2004) records of colonial, meiofaunal, parasitic, egg and pelagic taxa (e.g., epitokes and larvae) were recorded, but were excluded when calculating diversity indices and conducting multivariate analysis of community structure.

Newly settled juveniles of macrobenthic species may at times dominate the macrobenthos, however the OSPAR (2004) guidelines suggest they should be considered an ephemeral component due to heavy post-settlement mortality and not therefore representative of prevailing bottom conditions (OSPAR 2004). OSPAR (2004) further states that “Should juveniles appear among the ten most dominant organisms in the data set, then statistical analyses should be conducted both with and without these in order to evaluate their importance”. As juveniles clams of the family Anomiidae, juveniles barnacles of the order Balanomorphs and juveniles mussels of the family Mytilidae were among the top ten of the most dominant taxa across survey area, a 2STAGE analysis was conducted to compare the two data sets (with and without juveniles). This analysis revealed a 90 % similarity between the two datasets which is below the OSPAR threshold of 95 % therefore juveniles were removed from the dataset for all further analyses and discussion as including them would skew the analyses in favour of an ephemeral component of the community. However, due to the high counts of juveniles of Mytilidae, these counts data have been analysed separately to aid in the identification and mapping of potential mussel beds within the survey area.

In accordance with NMBAQC PRP (Worsfold & Hall 2010), Nematoda were recorded during the macrobenthic analysis and included in all datasets for all further analyses and discussion.

5.3.3. Univariate Statistics

The DIVERSE routine in PRIMER v7 was used to calculate diversity indices for the macrobenthic data including:

- Number of species (S): the number of taxa present in a sample, with no indication of relative abundances.
- Number of individuals (N): total number of individuals counted.
- Shannon-Wiener index (H'): measures the uncertainty in predicting the identity of the next species withdrawn from a sample.
- Species Richness, Margalef's index (d): a measure of the number of taxa present for a given number of individuals. The higher the index, the greater the diversity.
- Simpson's Diversity Index (1- λ): a measure of the probability of choosing two individuals from a sample that are different taxa. Ranges from zero (minimum diversity) to one (maximum diversity).
- Pielou's evenness (J'): indicates how evenly the individuals in a sample are distributed. J' is a range of zero to one. The less variation in the samples, the higher J' value.

These univariate indices enable the reduction of large datasets into useful metrics, which can be used to describe and compare community structures.

5.3.4. Multivariate Statistics

Prior to multivariate analyses, data were displayed as a shade plot with linear grey-scale intensity proportional to macrobenthic abundance (Clarke et al. 2014) to determine the most efficient pre-treatment (transformation) method. Macrobenthic abundance data from grab samples were square-root transformed to prevent taxa with intermediate abundances from being discounted from the analysis, whilst allowing the underlying community structure to be assessed.

The PRIMER v7 software package (Clarke & Gorley 2015) was utilised to undertake the multivariate statistical analysis on the biotic macrobenthic dataset. To fully investigate the multivariate patterns in the biotic data, macrobenthic assemblages were characterised based on their community composition, with hierarchical clustering and non-metric multidimensional scaling (nMDS) used to identify groupings of sampling stations that could be grouped together as a habitat type or community. SIMPER (similarities-percentage) analysis was then applied to identify which taxa contributed most to the similarity within that habitat type or community. A detailed description of analytical routines is provided in Appendix III.

5.3.5. Determining EUNIS Classifications

Sampling stations were grouped based on their macrobenthic assemblage composition using hierarchical clustering; the SIMPER routine was then applied to identify key and characterising taxa that contributed the most to the similarity within each group. EUNIS classifications were

then assigned to each sampling station based on their macrobenthic group and key, characterising taxa as well as based on their sediment type and composition following the latest JNCC guidance (Parry 2019).

6. Results

All grab samples were successfully collected as planned except for stations 8 and 9, where sampling was not possible due to rocks in the grab jaws owing the particularly coarse substrate. The DDC survey resulted in the collection of 457 still images and 19 videos totalling 196 minutes for subsequent seabed imagery analysis.

6.1. Imagery Analysis

DDC video and still logs are presented in Appendices I and II, respectively. Example imagery is presented in Plate 3. The full DDC imagery proforma is provided in Appendix III.

Across the survey area, three Level 4 habitats complexes were identified in the seabed imagery: EUNIS MC124 'Faunal communities on variable salinity Atlantic circalittoral rock', EUNIS MC32 'Atlantic circalittoral coarse sediment', EUNIS MC42 'Atlantic circalittoral mixed sediment' and three Level 5 biotopes: EUNIS MC1241 'Cushion sponges and hydroids on turbid tide-swept sheltered Atlantic circalittoral rock', EUNIS MC3211 '*Pomatoceros triqueter* with barnacles and bryozoan crusts on Atlantic circalittoral unstable cobbles and pebbles' and EUNIS MC2235 '*Mytilus edulis* beds on Atlantic circalittoral sediment' (Figure 4 - Figure 7).

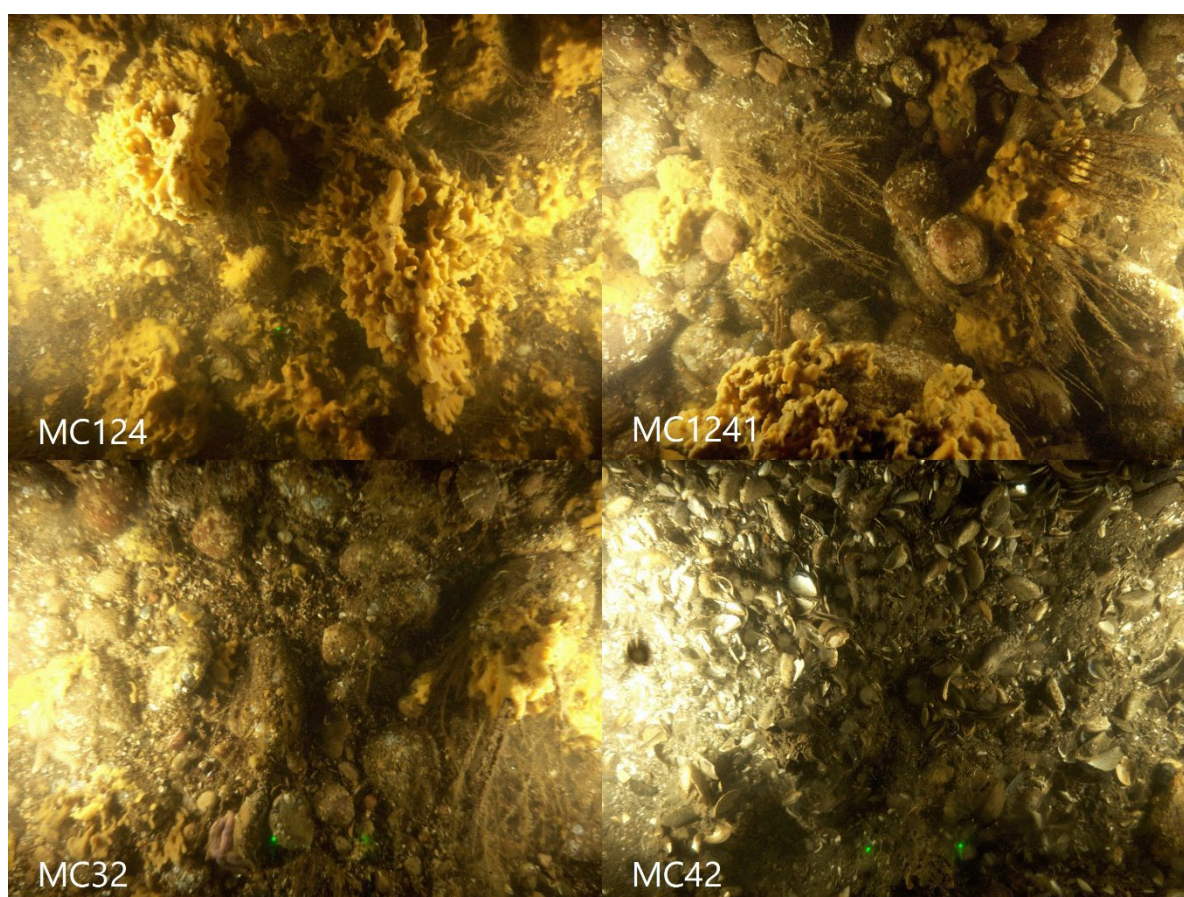


Plate 3 Example seabed imagery collected by DDC during the Lady Shoal benthic survey.

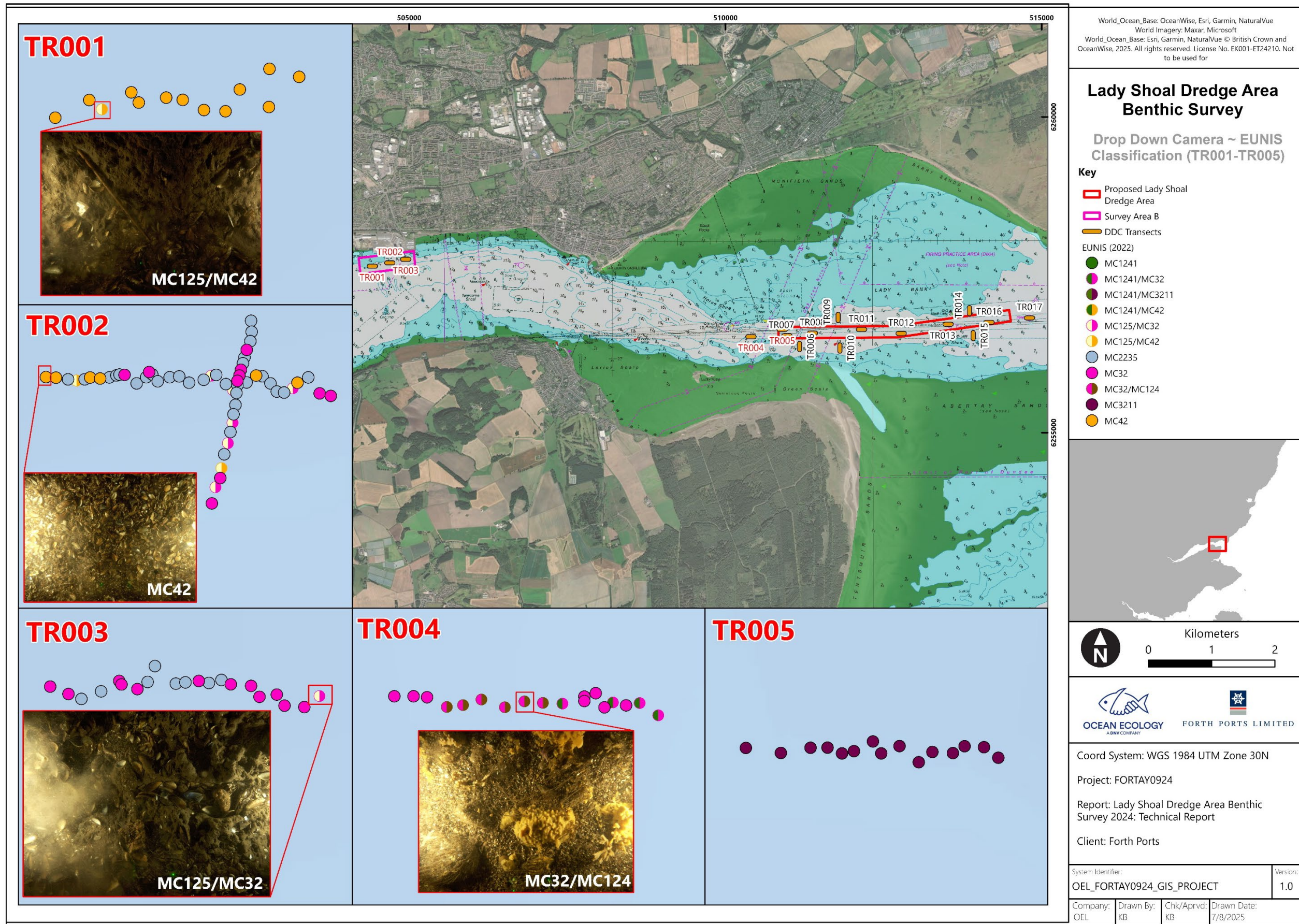


Figure 4 EUNIS classifications assigned to DDC footage along each of the transects sampled across the Lady Shoal survey area (1/4).

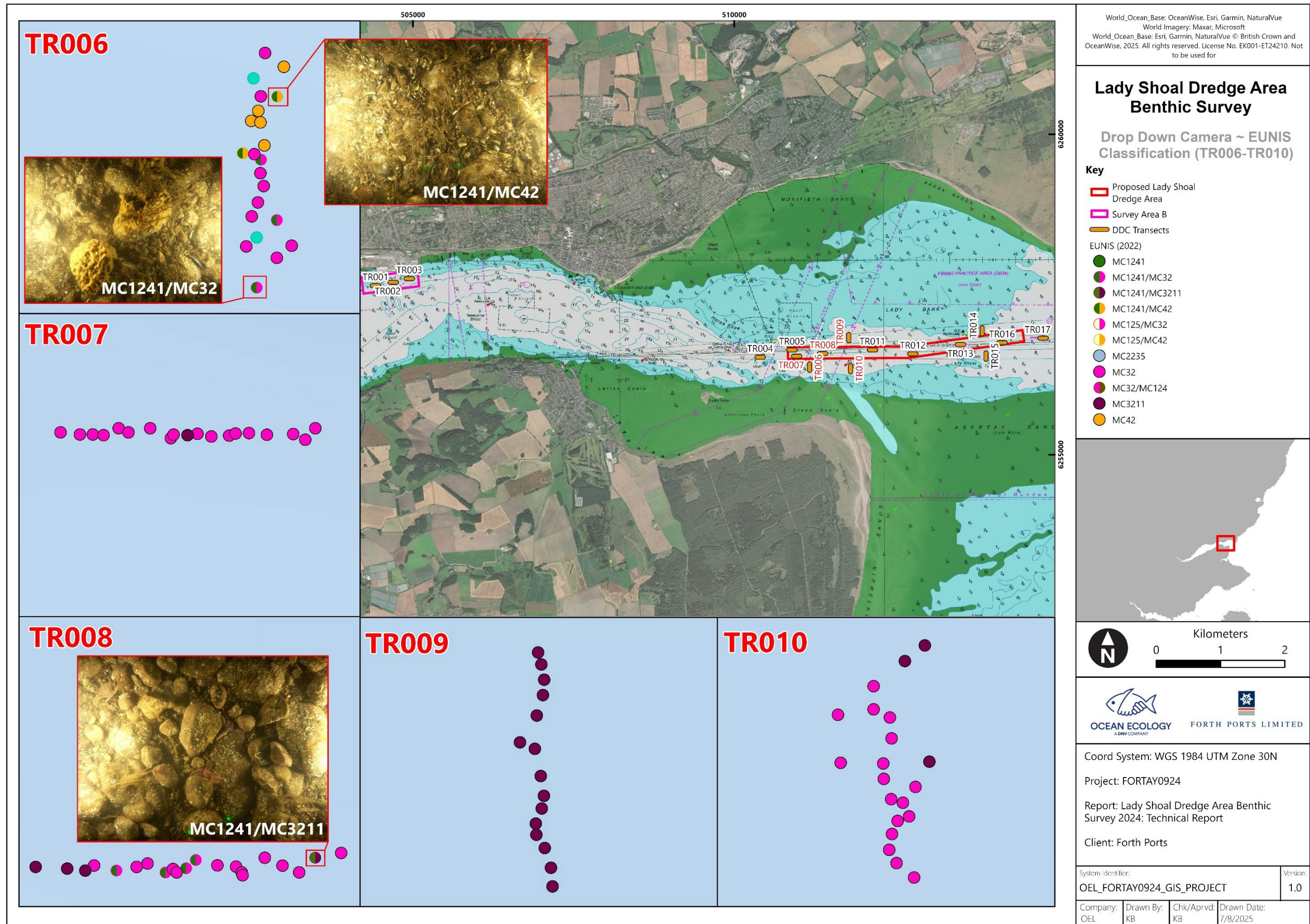


Figure 5 EUNIS classifications assigned to DDC footage along each of the transects sampled across the Lady Shoal survey area (2/4).

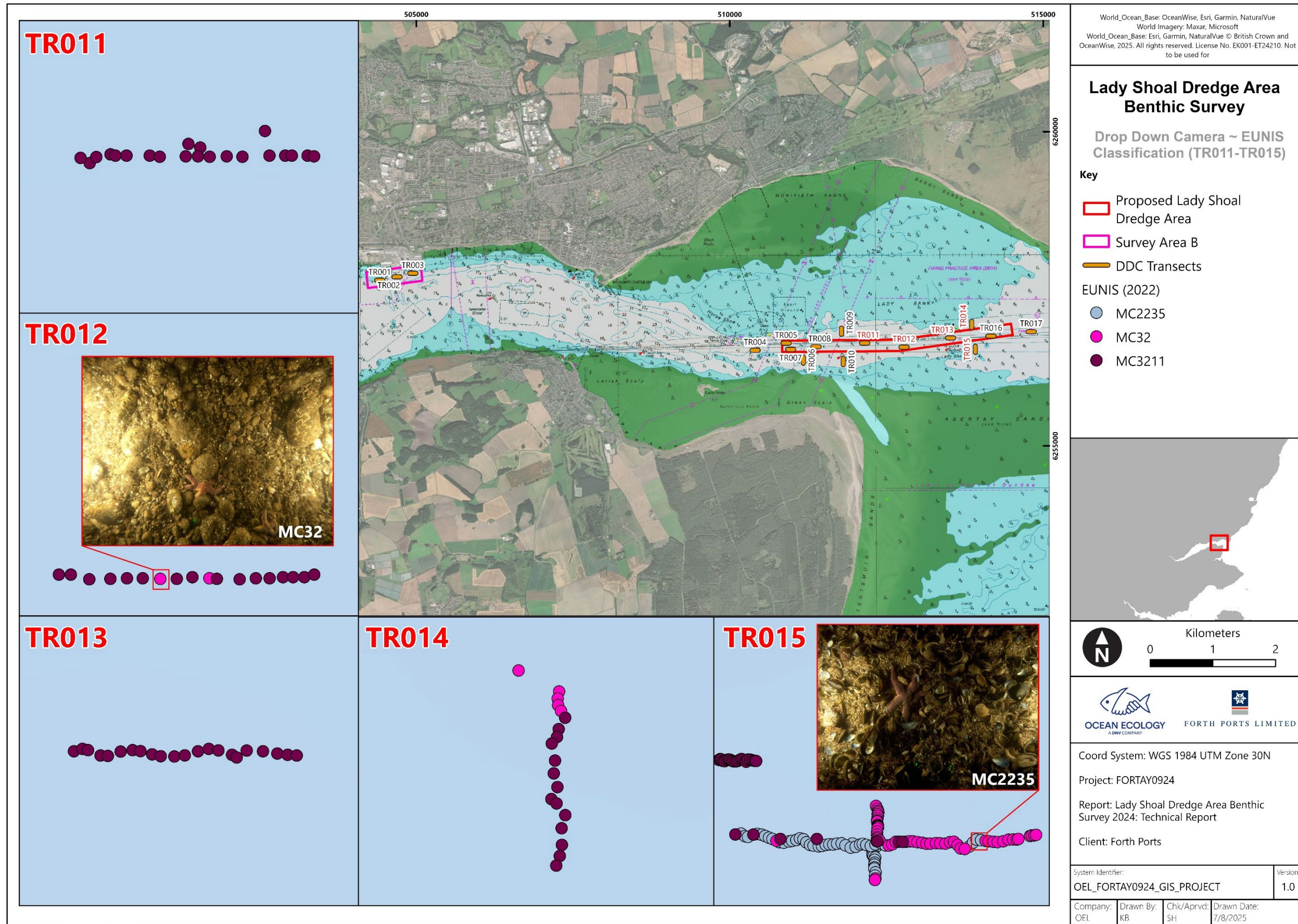


Figure 6 EUNIS classifications assigned to DDC footage along each of the transects sampled across the Lady Shoal survey area (3/4).

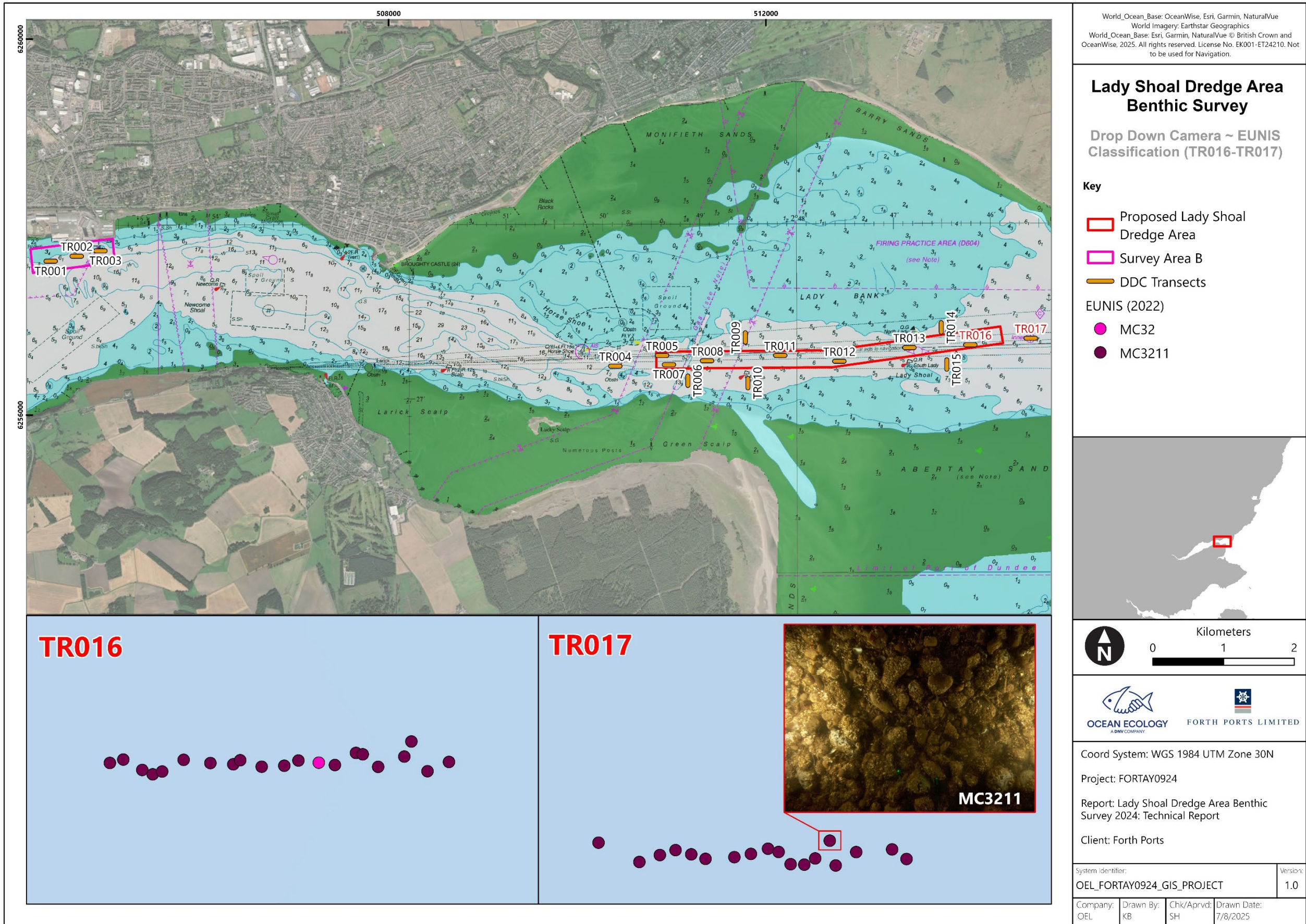


Figure 7 EUNIS classifications assigned to DDC footage along each of the transects sampled across the Lady Shoal survey area (4/4).

6.1.1. Epifauna

The most commonly occurring epifauna observed in the seabed imagery across the survey area was the sea star *Asterias rubens*, identified in 194 images, with a maximum count of 63 individuals observed along TR015. This was followed by the blue mussel *M. edulis* identified in 161 images, with a maximum count of 126 individuals observed along transect TR015. The true crab *Carcinus maenas* was identified in 87 images, with a maximum count of 3 individuals observed along transects TR015 and TR005. Nine images showed no visible fauna, and in 16 images, the biota assessment was not possible either because the image was not analysable or because the visual quality was very poor and only allowed for the substrate to be assessed not the epifauna.

When considering the abundance and diversity across the different transects, TR015 had the highest abundance, with 3,342 individuals observed, followed by TR002 with 1,083 individuals. In terms of diversity, TR004 had the highest taxa diversity, with 12 different taxa identified, while TR002 and TR015 both supported a diversity of 11 taxa each.

The full list of taxa present is provided in Appendix IV.

6.1.2. Annex I Reef Assessment

A full reef assessment was conducted on all images to determine whether habitats met the definitions of Annex I reef habitats as detailed in Table 3 and Table 4. The full Annex I reef assessment is provided in Appendix V. Evidence of Annex I reef was identified in 166 out of 454 images across 17 transects. Annex I low stony reef was predominantly observed on the west side of the survey area, with features recorded at 12 transects across 160 images, followed by Annex I medium stony reef, which was identified at only 4 transects and in 4 images. Low confidence *Mytilus* sp. reef and *Mytilus* sp. were also observed throughout the survey area. This is summarised in Table 7 and displayed in Figure 8 - Figure 11.

Table 7 Annex I Reef Assessment Summary.

Annex I	EUNIS	Images	Transects
Low Stony	MC124, MC1241	160	TR004, TR005, TR006, TR007, TR008, TR009, TR010, TR011, TR012, TR013, TR014, TR015
Medium Stony	MC1241	4	TR005, TR006, TR008, TR009
Low confidence <i>Mytilus</i> reef	MC2235	1	TR003
<i>Mytilus</i> sp.	MC2235	1	TR015

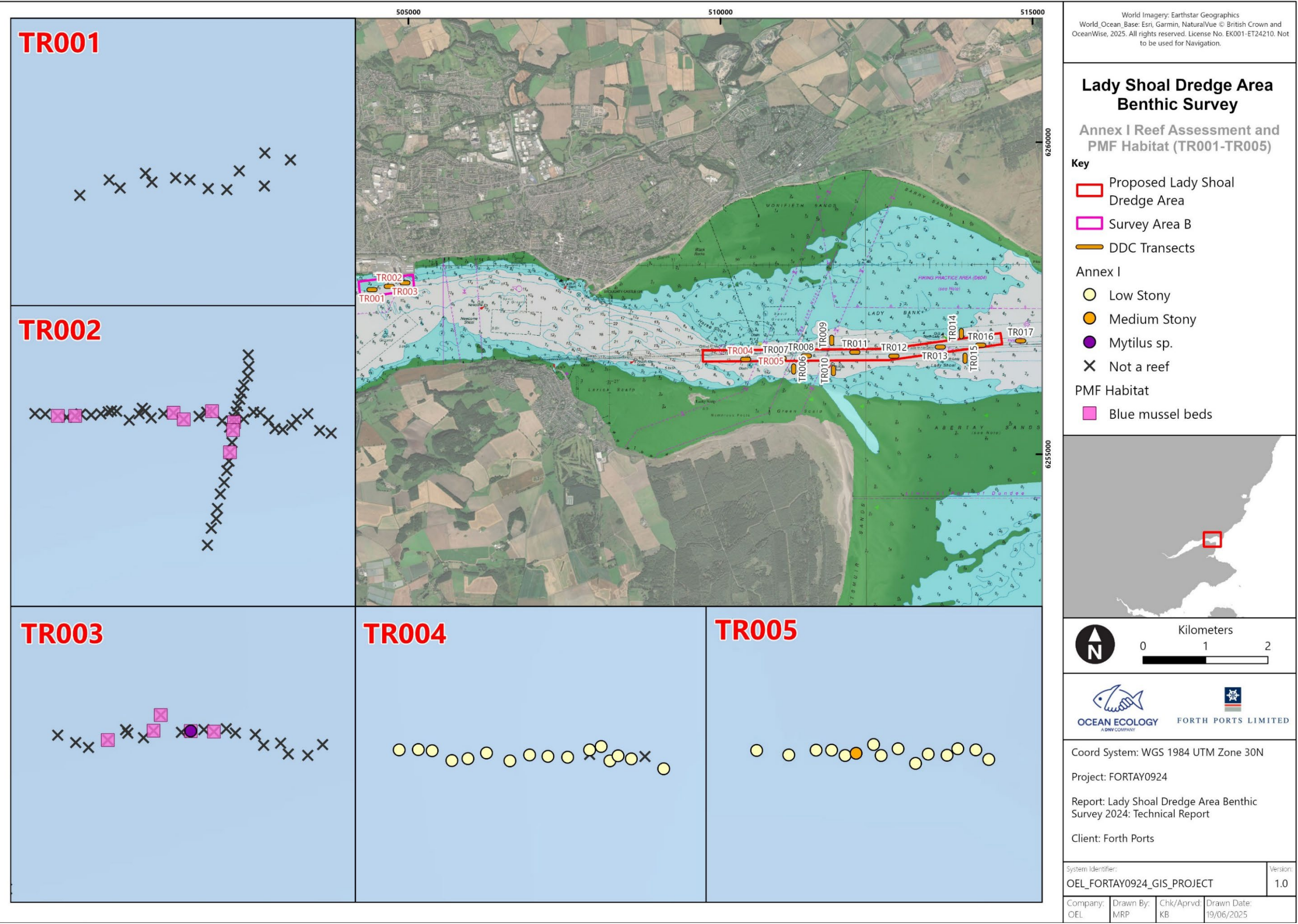


Figure 8 Annex I Reef assessment and PMF habitat throughout the survey area (1/4).

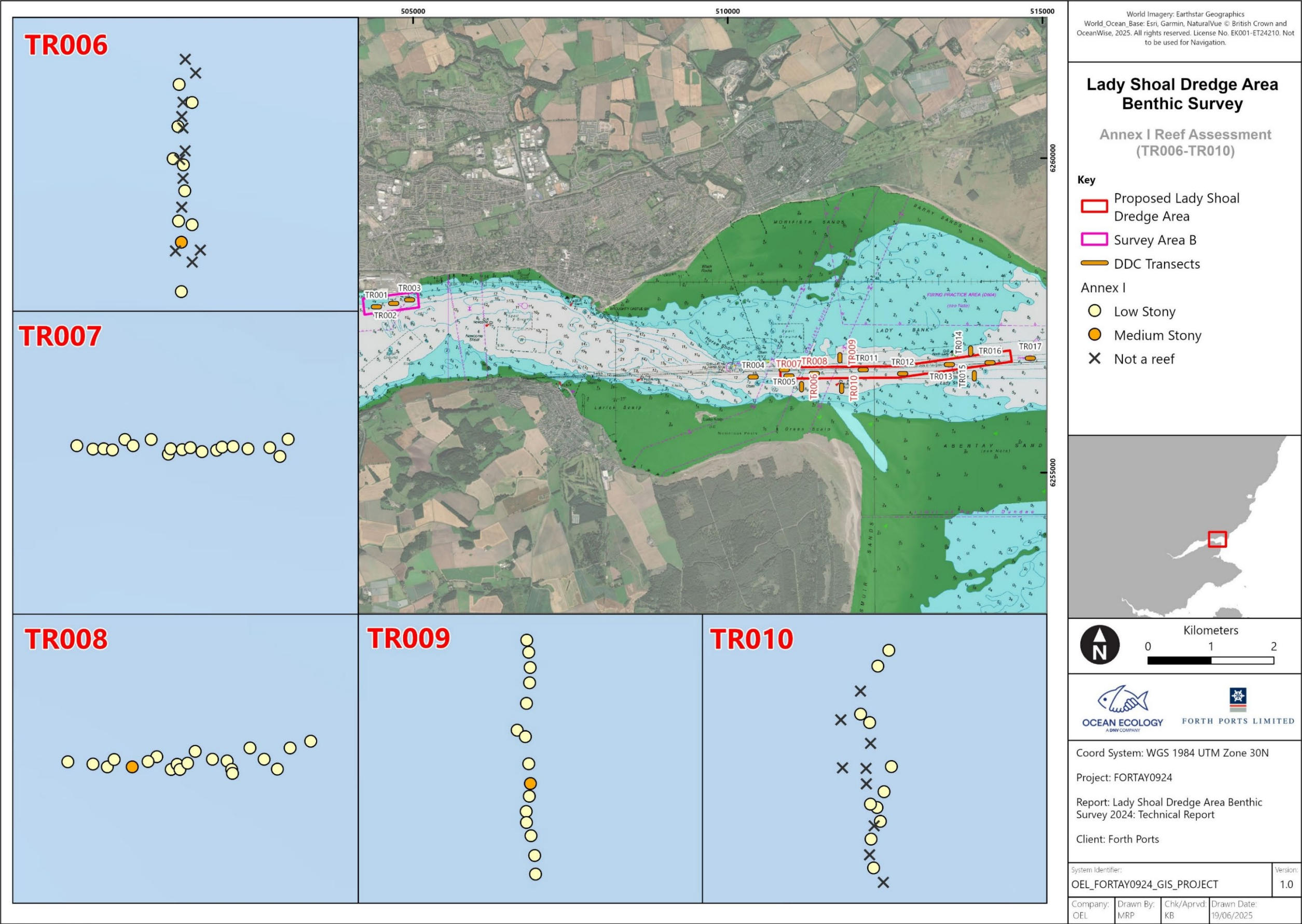


Figure 9 Annex I Reef assessment and PMF habitat throughout the survey area (2/4).

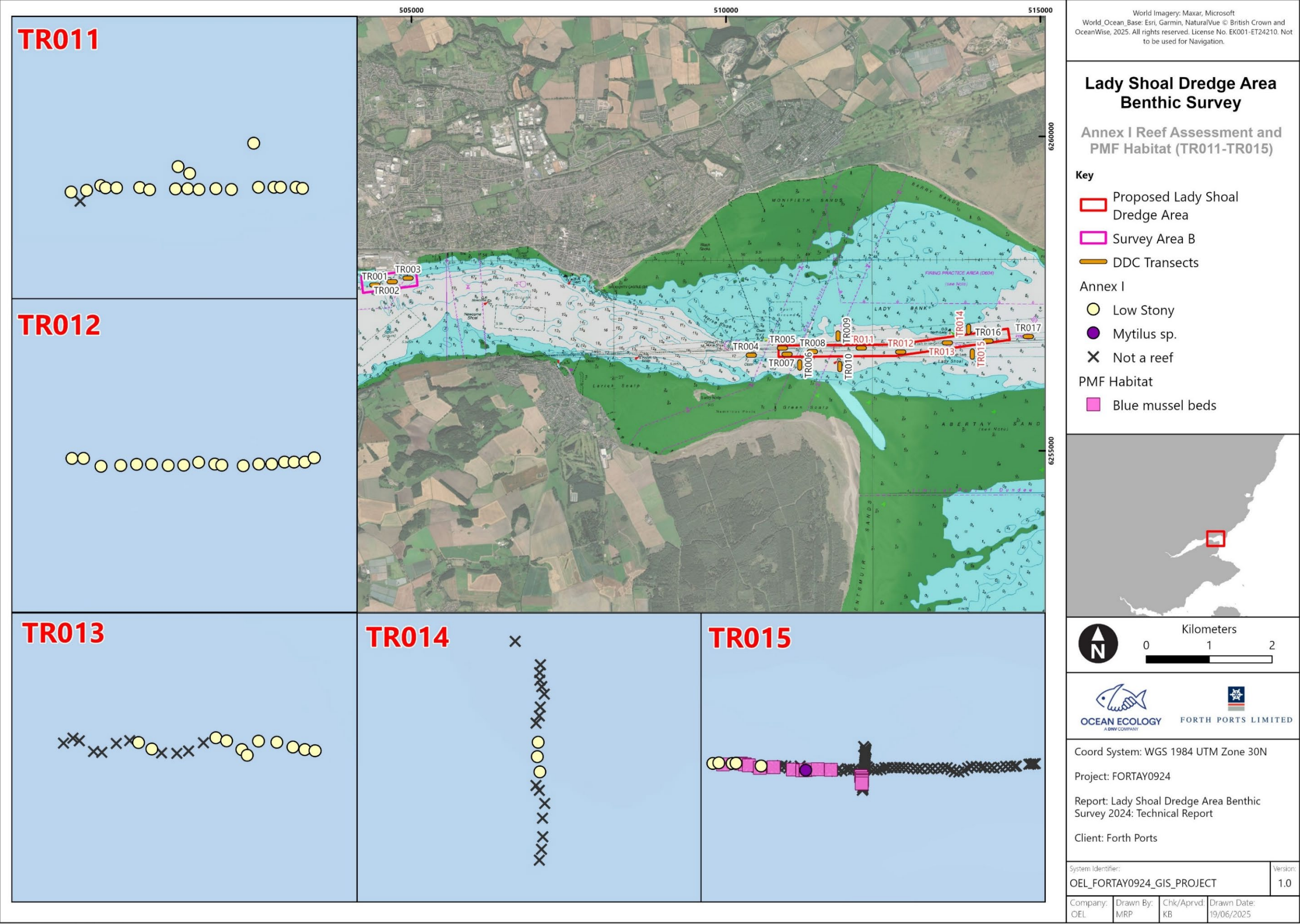


Figure 10 Annex I Reef assessment and PMF habitat throughout the survey area (3/4).

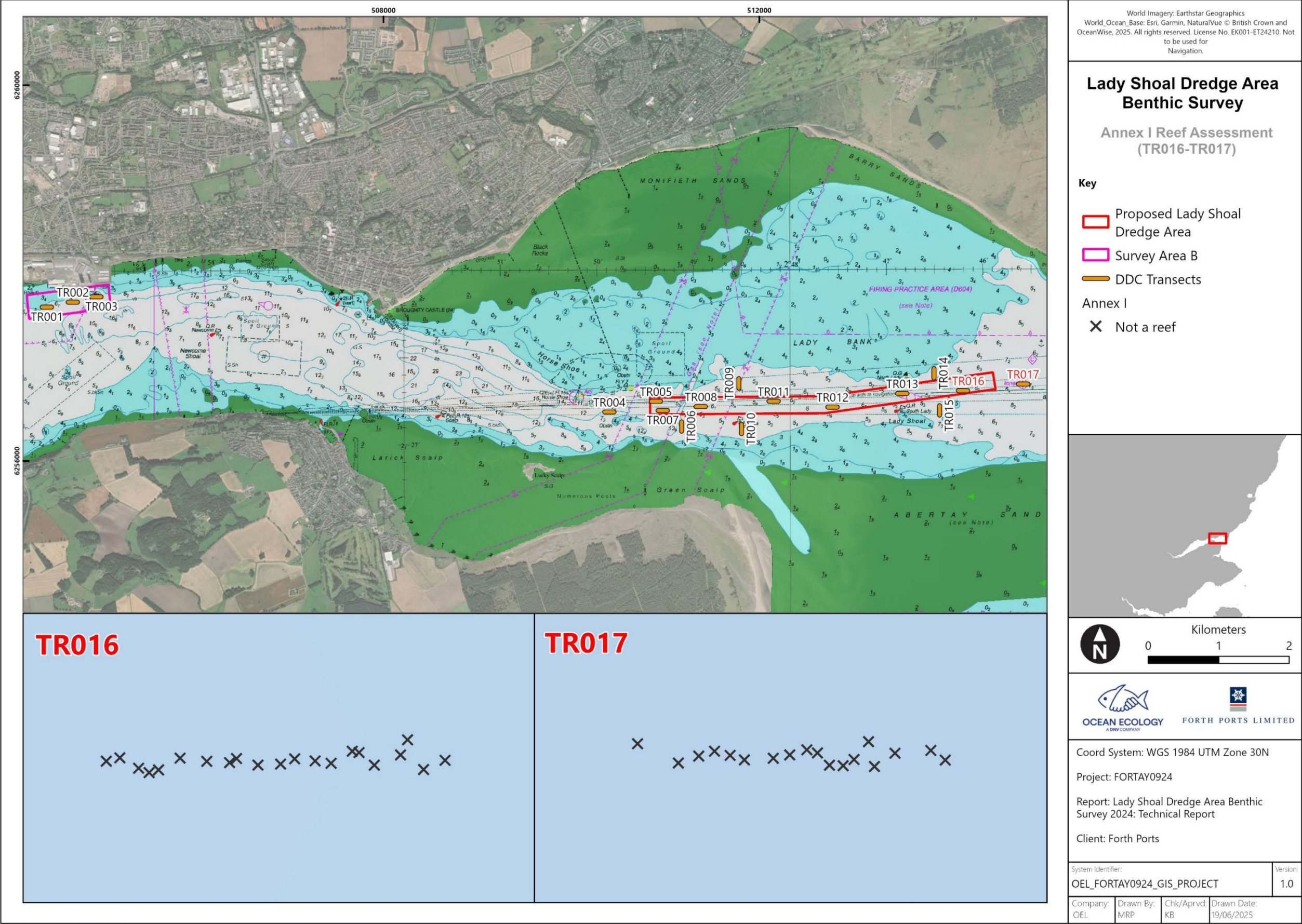


Figure 11 Annex I Reef assessment and PMF habitat throughout the survey area (4/4).

6.1.3. PMF

The PMF 'Blue mussel beds' was identified within 30 images situated primarily to the west of the survey area along three transects: TR002, TR003 and TR015 (Figure 8 and Figure 10). Transects TR002 and TR003 were located in 'Survey Area B' within the Port of Dundee and TR015 to the eastern extent of the Lady Shoal survey area but outside of the specified dredge area. Example images of this PMF habitats are provided in Plate 4. The PMF 'Blue mussel beds' was assigned to EUNIS biotope MC2235 '*Mytilus edulis* beds on sublittoral sediment'.

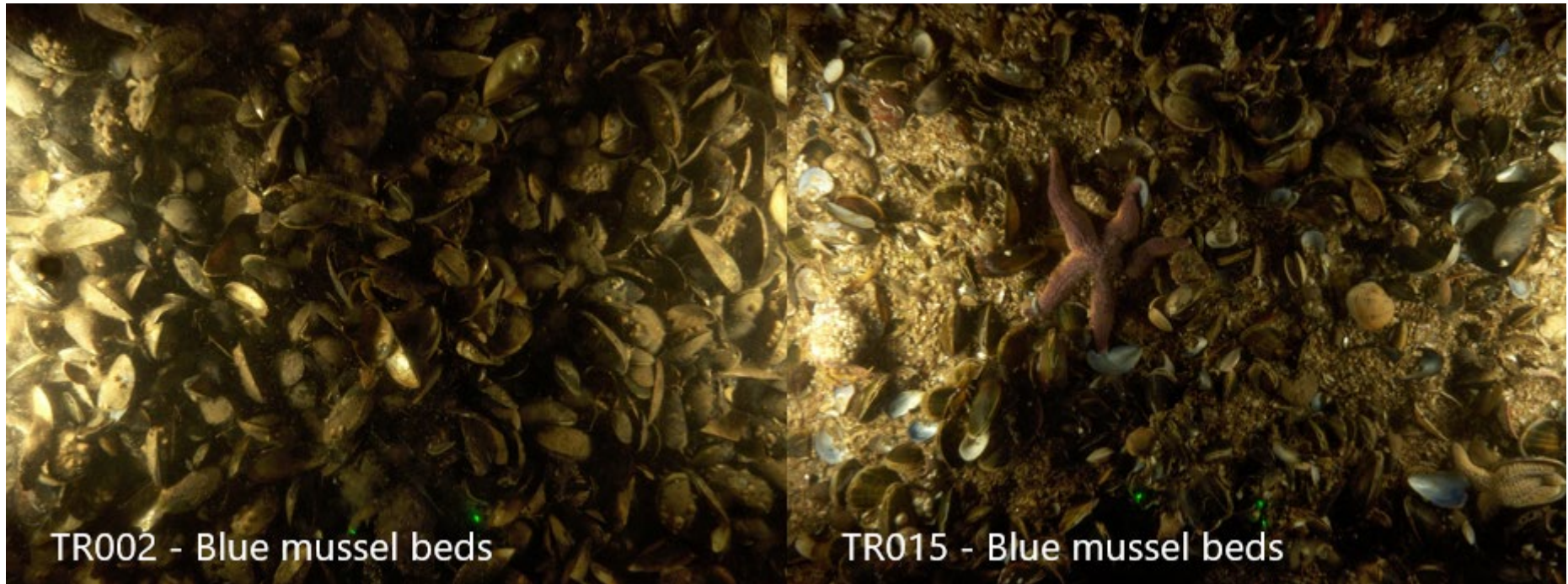


Plate 4 Example imagery of the PMF habitat 'Blue mussel bed' identified across Survey Area B (TR002) and the outside of the proposed Lady Shoal dredging area. (TR1015).

6.2. Sediment PSD

In total, 15 sediment samples were analysed for full particle size classification. Example images of all sampled sediment types are presented in Plate 5 with full grab logs provided in Appendix VI and sample photographs provided in Appendix VII. Particle size data provided in Appendix VIII and summary data provided in Appendix IX.

6.2.1. Sediment Type

Sediment types, as classified using the Folk triangle (Folk, 1954) for each station sampled across the survey area are presented in Figure 12. Each Folk classification was converted to BSH type (EUNIS Level 3) using the adapted Folk triangle (Long 2006). Sediments were generally mixed across the survey area and characterised by variable contributions of sand, mud and gravel.

Of the 15 stations sampled, 14 were representative of BSH A5.4 'Mixed Sediment' and one station were representative of the BSH A5.1 'Coarse Sediment'. The most dominant sediment textural group observed was Muddy Sandy Gravel (msG) followed by Gravelly Muddy Sand (gmS) and Sandy Gravel (sG) (Figure 13 and Figure 14).

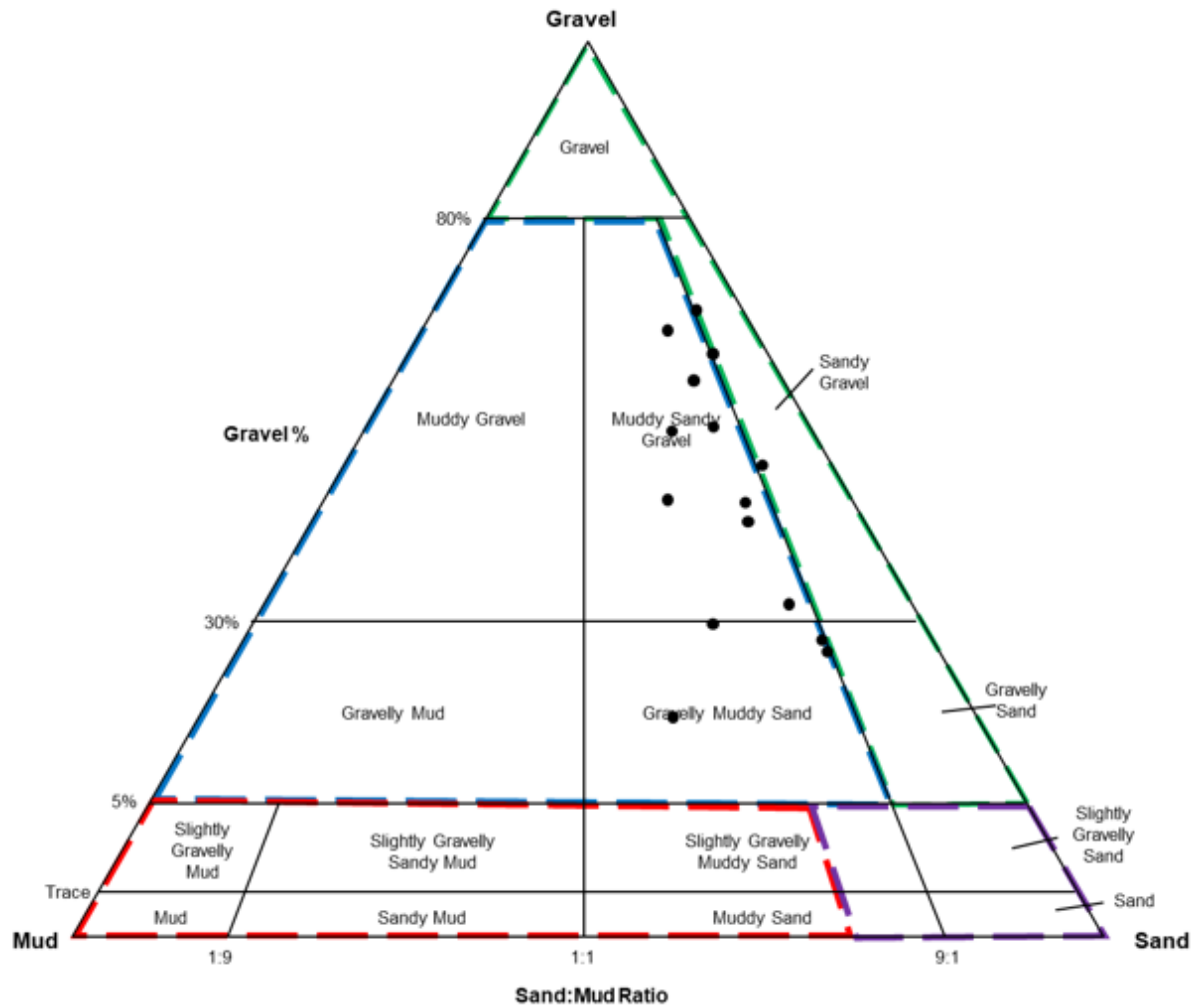
All stations were very poorly sorted due to the contribution of both sand and mud.

6.2.2. Sediment Composition

Sediments were dominated by gravel with variable contribution of mud (0 % – 31 %) and sand content (23 % - 64 %). The percentage contribution of gravels (> 2 mm), sands (63 µm to 2 mm), and fines (< 63 µm) at each station are presented in Figure 15 and Figure 16. The mean proportion ($\pm$ Standard Error, SE) of sands across all stations was 41.82 % ($\pm$ 3.30%). The mean ($\pm$ SE) mud content across the survey area was 10.97 % ($\pm$ 1.91 %) and gravel content was 47.21 % ($\pm$ 4.19 %).



Plate 5 Example images of sampled sediment types across the survey area.



EUNIS Broad Scale Habitats (BSH) (Level 3)

A5.4	Mixed Sediment	A5.3	Mud and Sandy Mud
A5.1	Coarse Sediment	A5.2	Sand and Muddy Sand

Figure 12 Folk (1954) triangle classifications of sediment gravel percentage and the sand-to-mud ratio of samples collected across the survey area, overlain by the modified Folk triangle for determination of mobile sediment BSHs under the EUNIS 2008 habitat classification system (adapted from (Long 2006)).

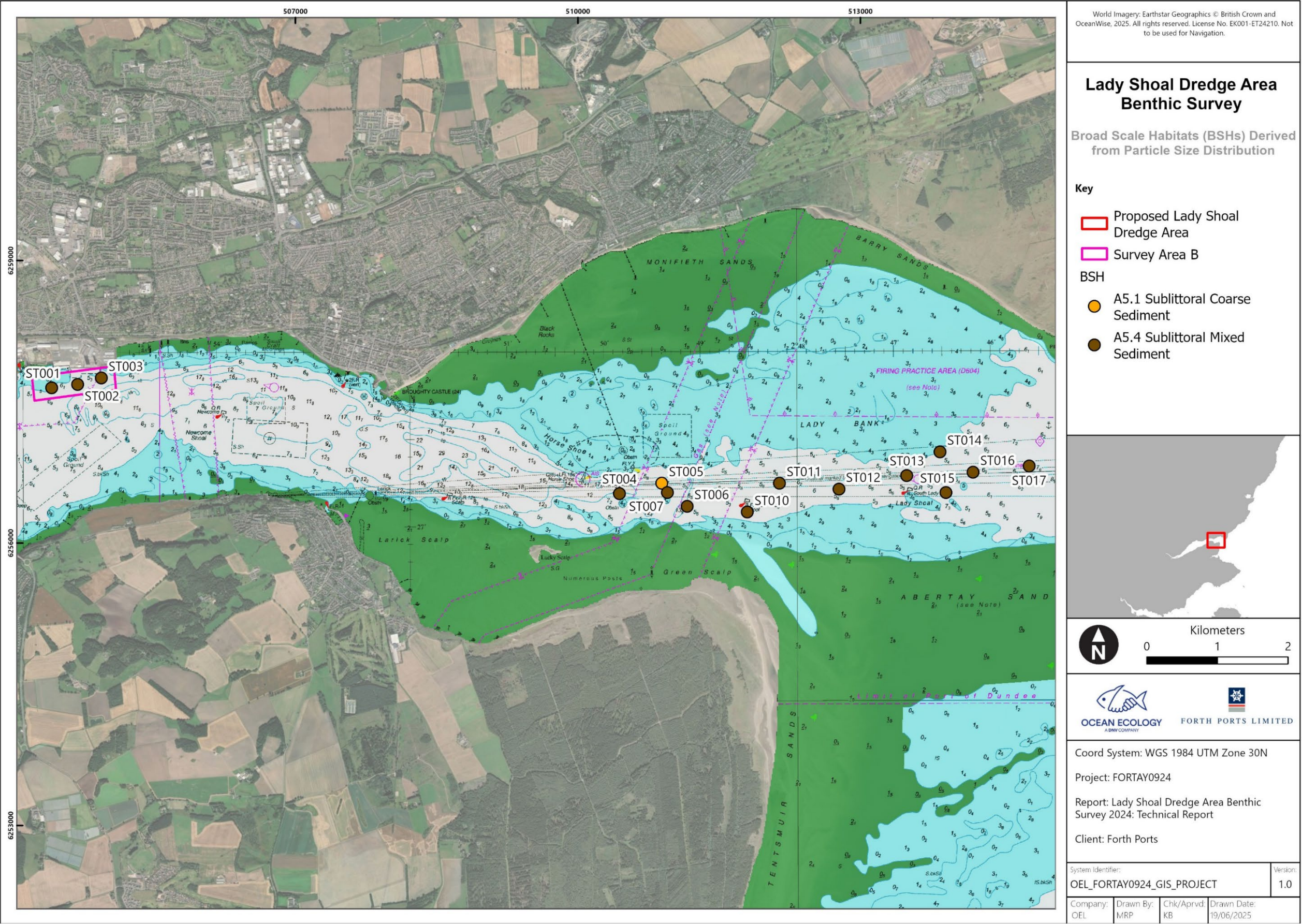


Figure 13 BSHs derived from PSD analysis samples collected across the survey area.

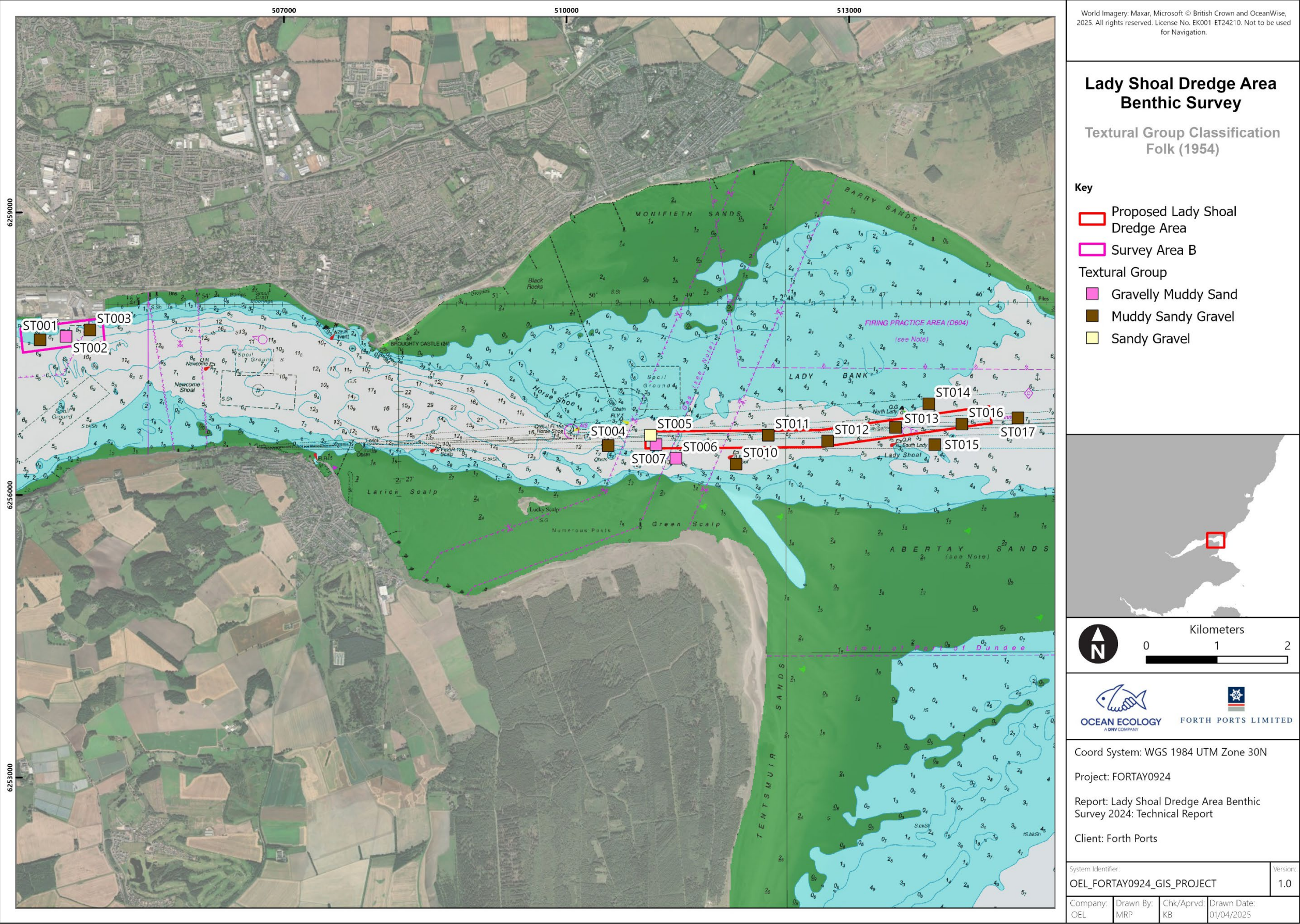


Figure 14 Textural Groups as determined from PSD analysis of samples collected across the survey area.

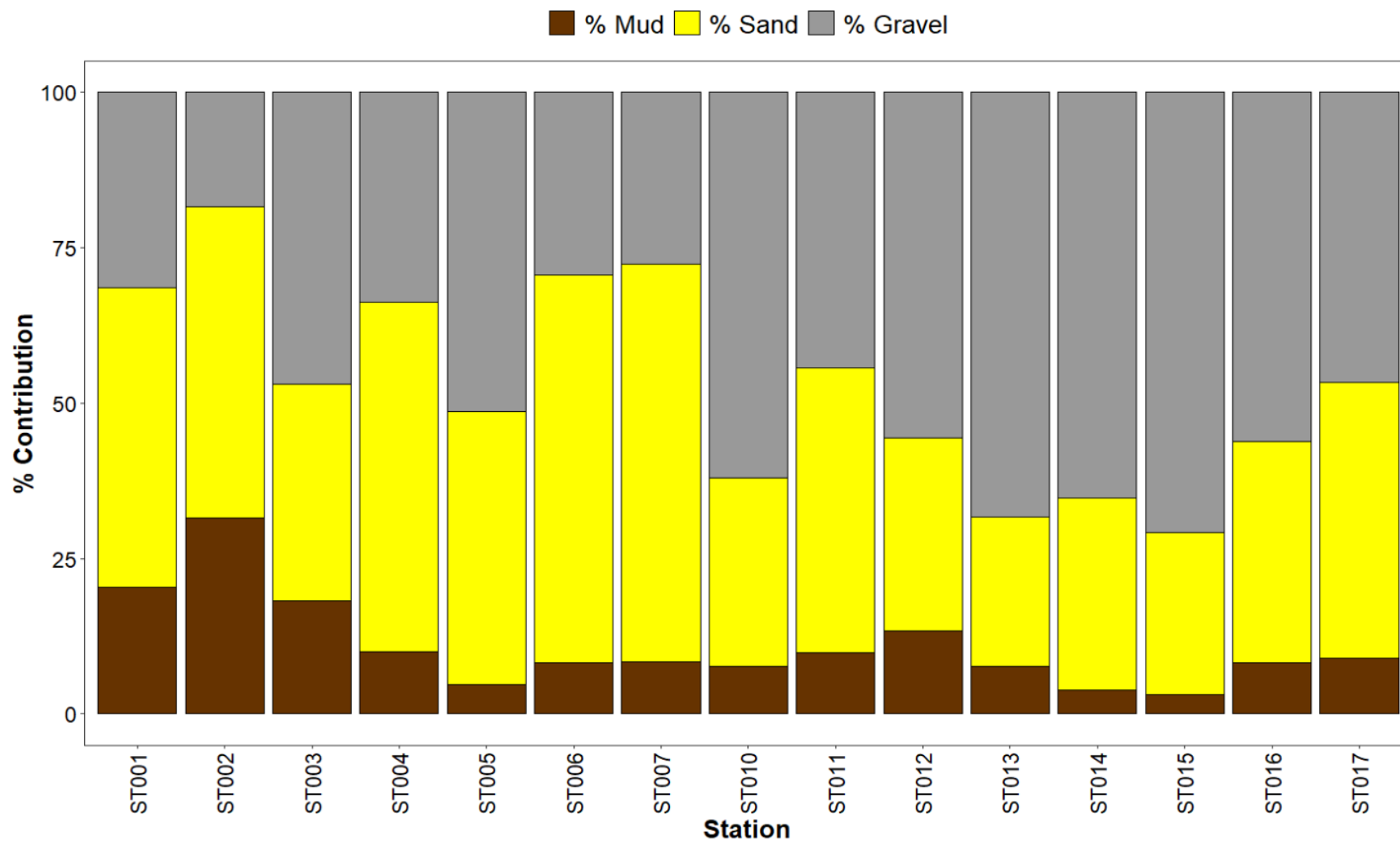


Figure 15 Relative contribution of major sediment fractions (Gravel, Sand, Mud) by volume at each sampling stations

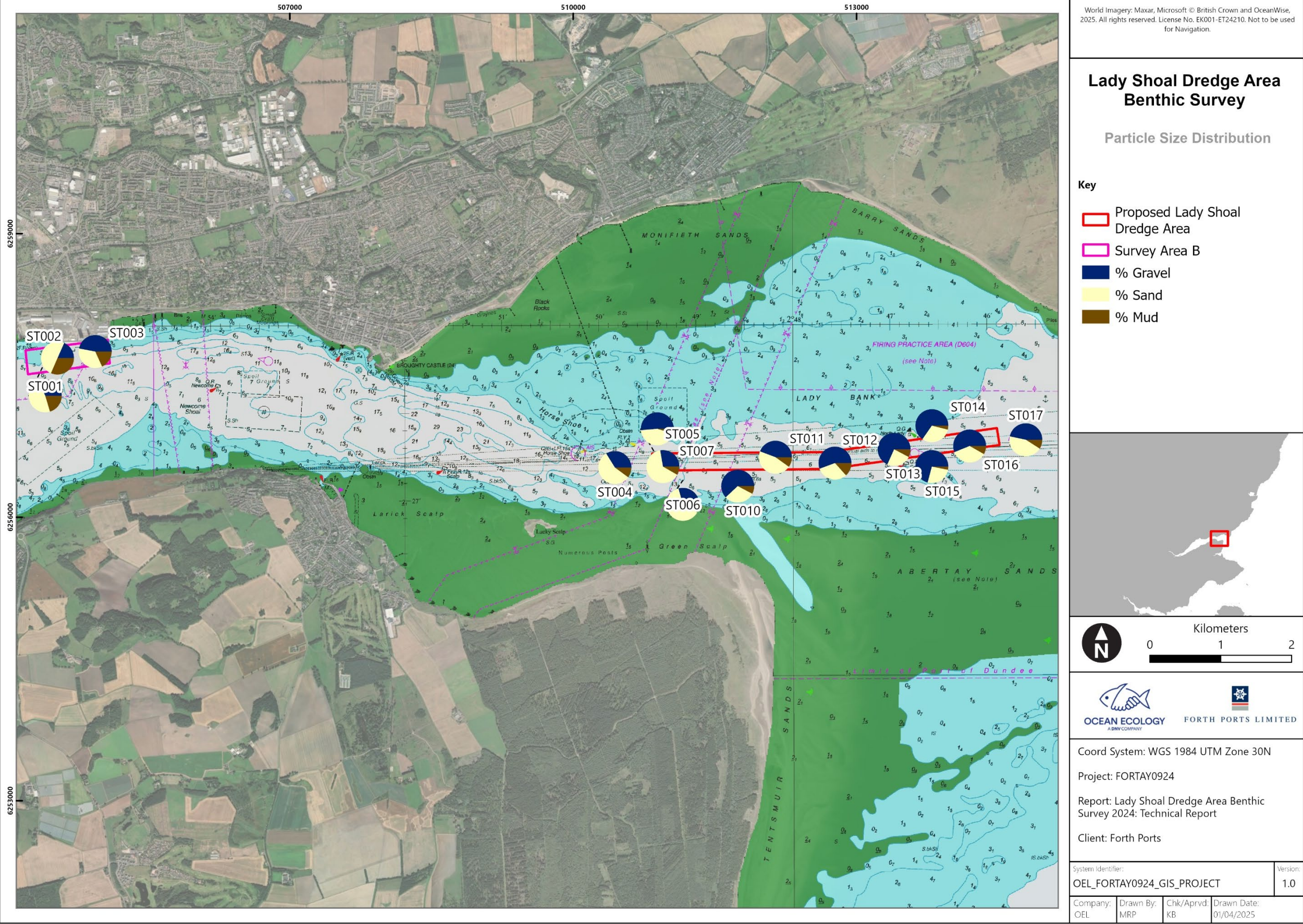


Figure 16 Particle size distribution (%Gravel, %Sand and %Mud) across the survey area.

6.3. Macrobenthos

6.3.1. Macrobenthic Composition

The macrobenthic assemblage identified across the survey area from the samples collected across the 15 grab stations included a total of 27,715 individuals and 148 taxa. The mean ($\pm$ SE) number of taxa per station was 52 ± 5 , mean ($\pm$ SE) abundance per station was $1,848 \pm 416$ and mean ($\pm$ SE) biomass per station was 3.2079 ± 2.3594 gAFDW. To note that while juvenile specimens were removed from the analysis of abundance and diversity as explained in section 5.3.2, they were retained when measuring biomass.

The full abundance matrix is provided in Appendix X. The biomass (gAFDW) of each major taxonomic group (Annelida, Crustacea, Mollusca, Echinodermata, and Miscellaneous) in each sample collected is presented in Appendix XI.

Figure 17 shows the main macrobenthic taxa characterising the stations. Nematodes were the most abundant taxa accounting for 46 % of all individuals recorded. This taxon accounted for the maximum abundance and average density per sample. Nematodes were also one of the most frequently occurring taxa across the survey area along with the polychaete *Pholoe inornata*, Nemertea, and the barnacle *Balanus crenatus*, occurring in 100% of the samples.

Figure 18 illustrates the relative contributions to total abundance, diversity, and biomass of the major taxonomic groups in the macrobenthic community sampled across the survey area. Miscellaneous taxa dominated abundance due to the large numbers of individuals from the phyla Nematoda and Nemertea, accounting for 47% of all individuals recorded. This was followed by Crustacea, which accounted for 27% of all individuals recorded. Annelida dominated diversity as they accounted for 46% of all taxa recorded, whilst biomass was dominated by Mollusca, contributing to 90% of the total biomass. This was likely due to the large numbers of juveniles recorded, particularly *Mytilus edulis* (see section 6.3.3) but also adult *M. edulis* and the sea snail *Onoba* spp., particularly *Onoba semicostata*, which were amongst the top 10 taxa recorded across samples.

Figure 19 represents total abundance, diversity, and biomass across the survey area. Stations ST0007 and ST0004 exhibited the highest abundance and diversity, recording 6,151 and 3,411 individuals, and 78 and 79 taxa, respectively. The full suite of univariate diversity indices calculated is provided in Table 8. Additionally, station ST002 had the highest recorded biomass at 36.1726 gAFDW, much higher than all other stations (< 2.0 gAFDW).

Table 8 Univariate diversity indices for stations sampled across the Lady Shoal survey area.

Station	S	N	d	J'	H'	1- λ
ST001	25	655	3.393	0.684	2.144	0.845
ST002	28	474	3.895	0.743	2.391	0.860
ST003	40	877	4.722	0.651	2.275	0.849
ST004	79	3411	7.745	0.513	2.135	0.787
ST005	54	2274	5.693	0.448	1.704	0.672
ST006	60	541	8.104	0.623	2.460	0.798
ST007	78	6151	7.221	0.424	1.762	0.696
ST010	67	3152	6.827	0.445	1.790	0.608
ST011	70	2400	7.195	0.462	1.868	0.720
ST012	52	3154	5.710	0.288	1.107	0.361
ST013	38	360	5.946	0.656	2.350	0.797
ST014	59	1781	7.081	0.533	2.127	0.791
ST015	35	575	4.721	0.525	1.802	0.673
ST016	65	1083	7.728	0.518	2.077	0.754
ST017	28	827	4.019	0.299	0.995	0.355

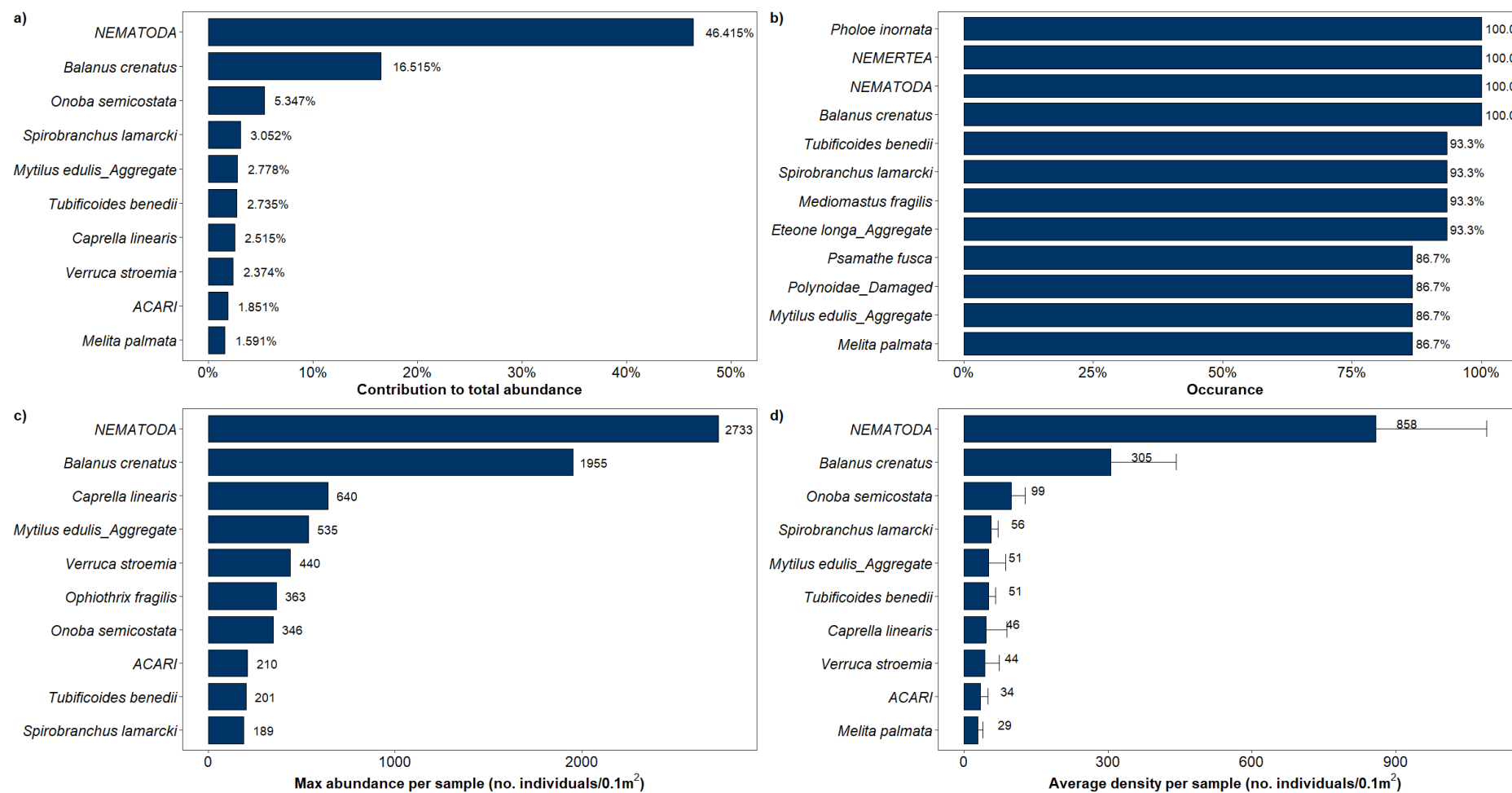


Figure 17 Percentage contributions of the top 10 macrobenthic taxa to total abundance (a) and occurrence (b) from samples collected across the Lady Shoal survey area. Also shown are the maximum densities of the top 10 taxa per sample (c) and average densities of the top 10 taxa per sample (d).

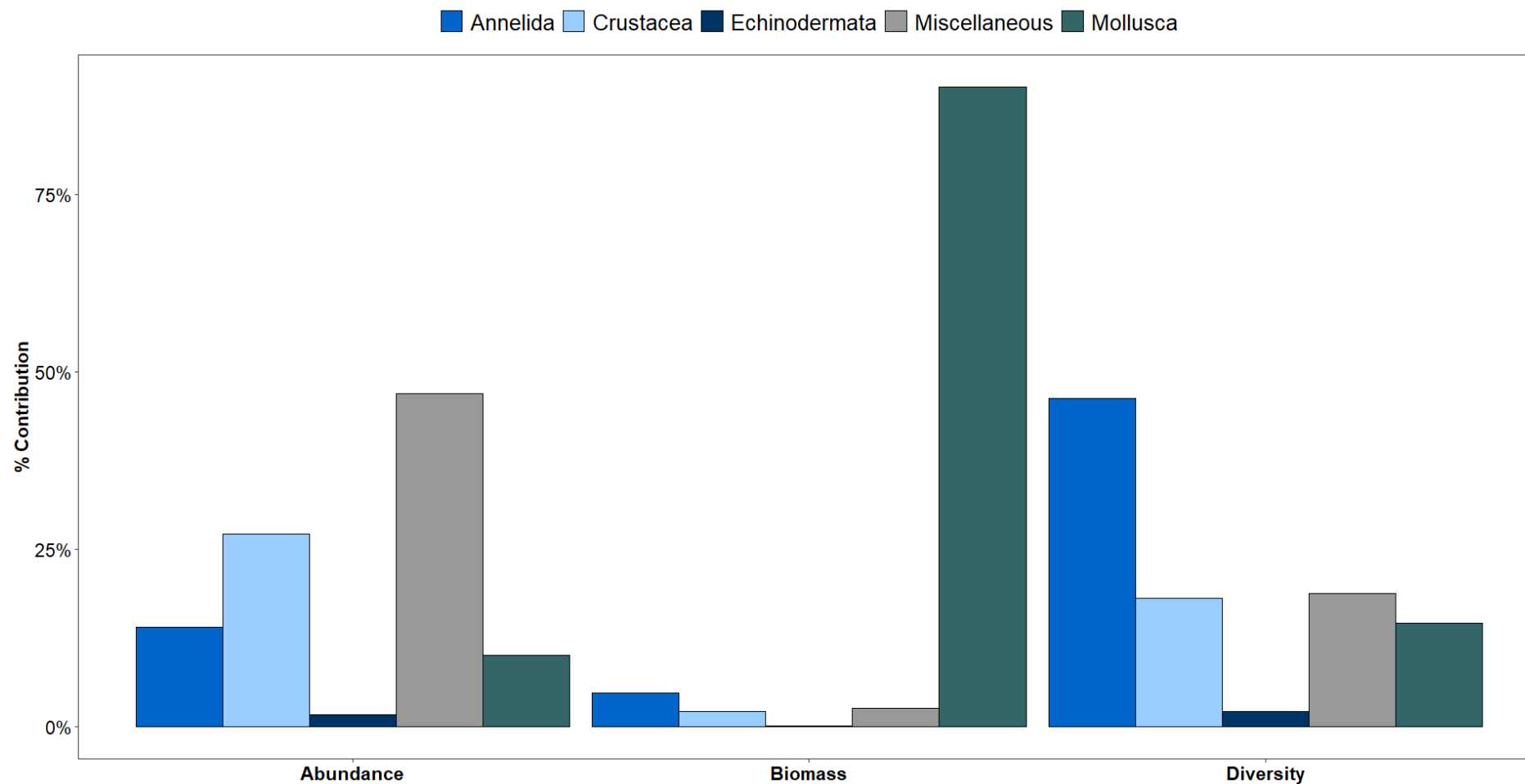


Figure 18 Relative contribution of the major taxonomic groups to the total abundance, diversity and biomass of the macrobenthos sampled across the Lady Shoal survey area. To note that biomass measurements include juveniles.

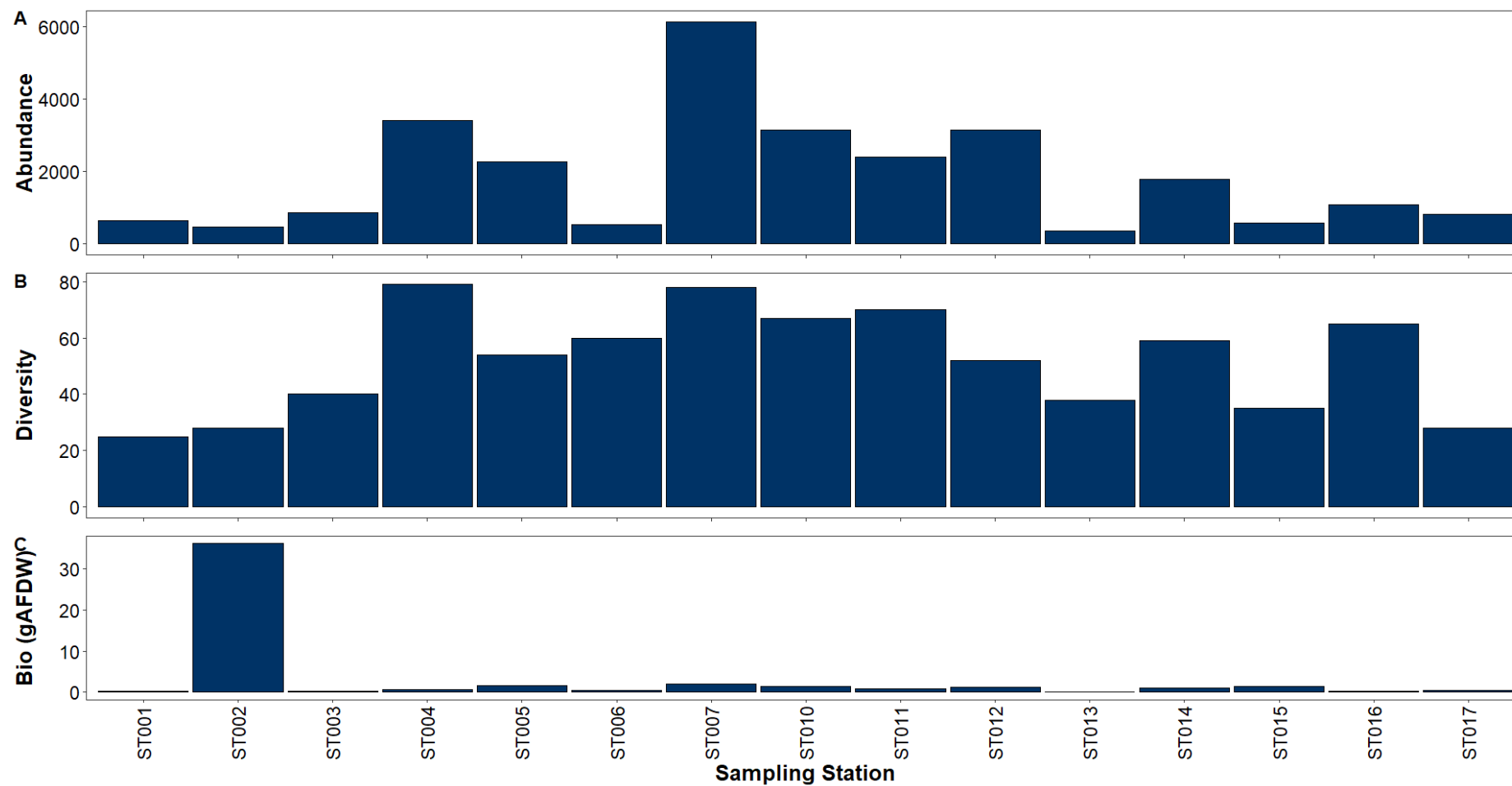


Figure 19 A) Abundance, B) diversity, and C) biomass per station (N=15) across the Lady Shoal survey area.

6.3.2. Macrobenthic Groups

Multivariate analysis was undertaken on the square-root transformed macrobenthic grab abundance data to identify spatial distribution patterns in the macrobenthic assemblages across the survey area and identify characterising taxa present.

Cluster analysis of the macrobenthic data was performed on a Bray-Curtis similarity matrix to analyse the spatial similarities in macrobenthic communities recorded across all sampled stations. The dendrogram resulting from the cluster analysis and associated Type 1 SIMPROF similarity (similarity profile routine) permutation test of all nodes within the dendrogram, identified four statistically significant groups ($p < 0.05$) and three outlier stations that did not belong to any group. A dendrogram resulting from the cluster analysis and associated Type 1 SIMPROF permutation test are provided in Appendix XII.

To visualise the relationships between the sampled macrobenthic assemblages, a non-metric multi-dimensional scaling (nMDS) plot was generated on the abundance data (Figure 20). The nMDS represents the relationships between the communities sampled, based on the distance between sample (station) points. The stress value of the nMDS ordination plot (0.11) indicates that the two-dimensional plot provides a good representation of the similarity between stations. In general, the degree of clustering of intra-group sample points demonstrates the level of within group similarity (e.g. points within Macrobenthic Group A), whilst the degree of overlap of inter-group sample points is indicative of the level of similarity between different macrobenthic groups.

SIMPER (similarity percentage analysis) was used to identify the key taxa contributing to the within group similarity of the macrobenthic group recognised; the full SIMPER results are provided in Appendix XIII. The spatial distribution of these macrobenthic groups is presented in Figure 21.

Macrobenthic Group A – 5 stations belonged to this group: (ST005, ST007, ST010, ST011, and ST012). These stations were characterised by the presence of Nematodes, several barnacle species (*B. crenatus*, *Verruca stroemia*, and *Austrominius modestus*), the sea snails *O. semicostata* and *O. aculeus*, numerous polychaete species (*Spirobranchus lamarcki*, *Pholoe inornata*, *Eulalia ornata*, *Dipolydora coeca* and *Myrianida* spp.), *M. edulis*, the oligochaete *Tubificoides benedii*, and Nemerteans, contributing to about 71% of the group average similarity of 68.84%.

Macrobenthic Group B – Three stations belonged to this group: ST001, ST002, and ST003, all located in Survey Area B. These stations were characterised by the presence of the oligochaete species *T. benedii* and *T. insularis*, Nematodes, the polychaete *Mediomastus fragilis*, the amphipod species *Melita palmata* and *Gammarus salinus*, and the barnacle *B. crenatus*, all together contributing to about 72% of the group average similarity of 63.25%.

Macrobenthic Group C – Two stations belonged to this group: ST0013 and ST0015. These stations were characterised by the presence of Nematodes, numerous polychaete species (*S. lamarcki*, *Psamathe fusca*, *Dipolydora coeca*, *M. fragilis* and *Capitella* spp.), the amphipod *M. palmata*, the oligochaete *T. benedii*, and the barnacle *B. crenatus*, contributing to about 73 % of the group average similarity of 58.68%,

Macrobenthic Group D – Two stations belonged to this group: ST0014 and ST0016. These stations were characterised by Nematodes, the sea snails *O. semicostata* and *O. aculeus*, numerous polychaete species (*S. lamarcki*, *Psamathe fusca*, *P. inornata*, *Eteone longa*, *Scoloplos armiger*, *Cirriformia tentaculata* and *M. fragilis*), the barnacle *B. crenatus*, the gastropod *Steromphala cineraria*, the oligochaete *T. benedii*, the brittlestar *Amphipholis squamata*, and Nemerteans, contributing to about 72 % of the group average similarity of 57.33%.

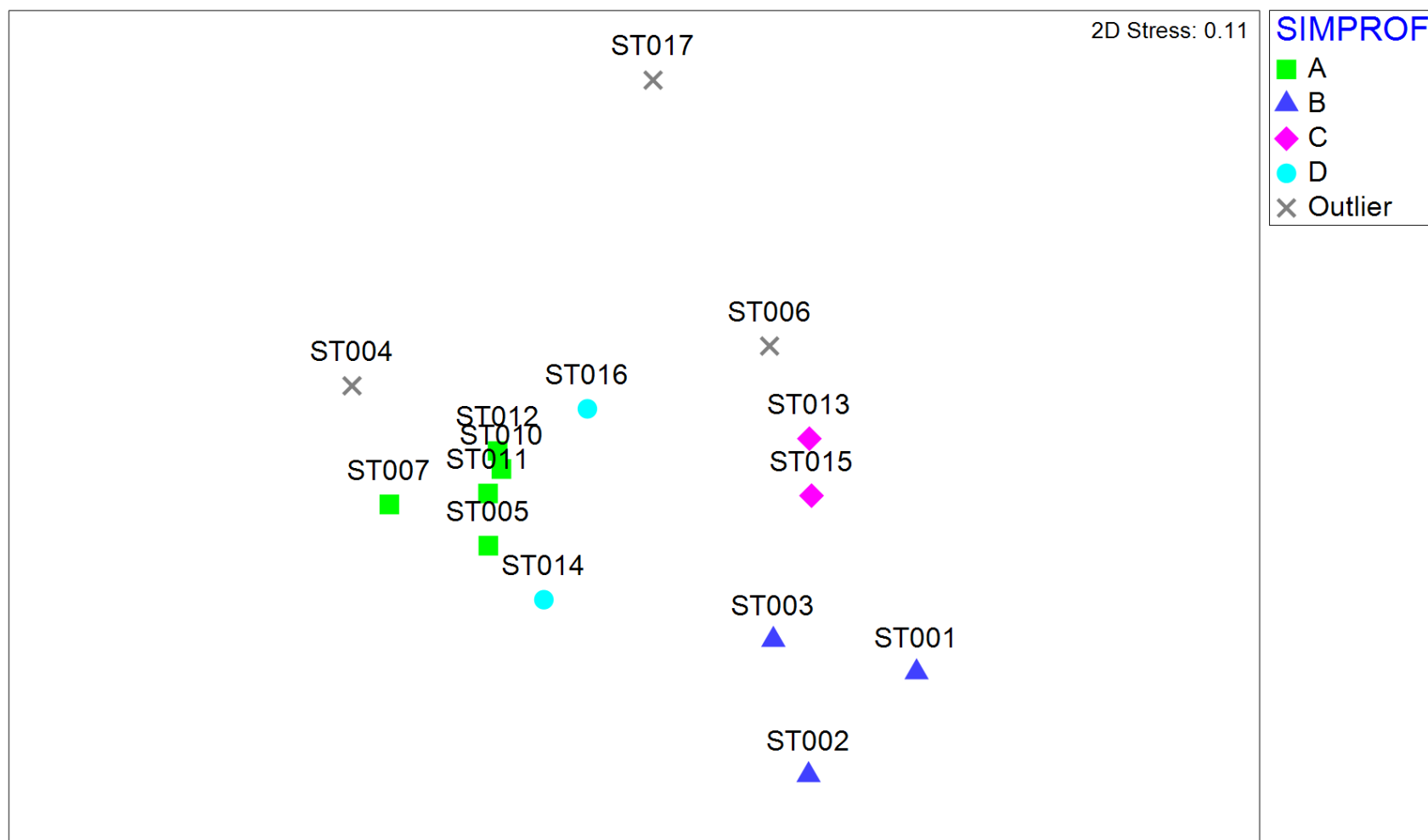


Figure 20 Two-dimensional nMDS ordination of macrobenthic communities at the stations across the Lady Shoal survey area based on square root transformed and Bray-Curtis similarity abundance data.

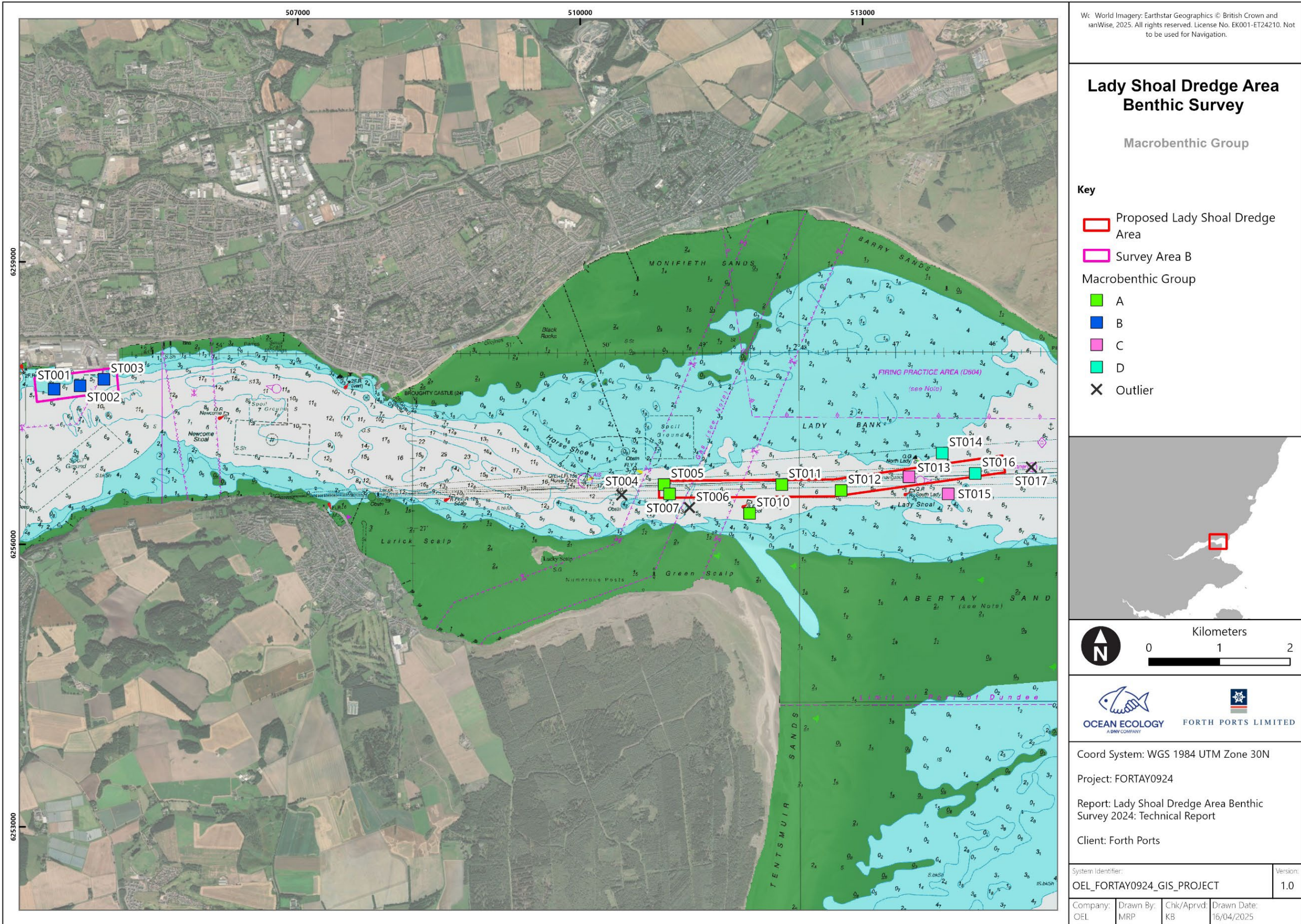


Figure 21 Spatial distribution of macrobenthic groups across the Lady Shoal survey area.

6.3.3. Juveniles of Mytilidae

Large numbers of juvenile mussels (Mytilidae) were recorded across the 12 stations within the proposed Lady Shoal dredge area, ranging from 184 to 9,257 individuals per station (Figure 22 and Table 9). Juvenile mussels were also recorded at the three stations (ST001, ST002 and ST003) within Survey Area B (separate to the primary dredge area) but in lower abundance, ranging from 13 to 132 individuals per station (Table 9). Interestingly, lower numbers of juveniles were recorded in grab samples (ST002, ST003 and ST014 in Figure 22) where mussel beds were primarily observed in the DDC imagery (TR002, TR003 and TR015 in Figure 8 and Figure 10). Nevertheless, stations with the highest juvenile mussel abundance were within or in close vicinity to known blue mussel beds from available data sources (Figure 3), particularly to the east of the survey area.

Table 9 Abundance of juveniles Mytilidae across the survey area.

Area	Station	Abundance of juveniles Mytilidae
Survey Area B	ST001	61
	ST002	13
	ST003	132
Lady Shoal Dredge Area	ST004	2,752
	ST005	8,371
	ST006	1,290
	ST007	9,357
	ST010	7,060
	ST011	4,593
	ST012	8,317
	ST013	379
	ST014	3,372
	ST015	184
	ST016	608
	ST017	2,278

6.3.4. Notable Taxa

Nine notable taxa were recorded across the survey area including a mix of economically important species, species included in the OSPAR list of Threatened and/or Declining Species and Habitats and INNS (Table 10).

To note that the Ross worm *S. spinulosa* is protected only when occurring in reef form which was not the case for the samples analysed where the highest number of *S. spinulosa* was counted at station ST004 or a total of 15 individuals.

Blue mussels are of economic importance and qualify as a PMF habitat where forming beds but they are not a PMF species on their own.

Table 10 List of notable taxa recorded across the survey areas.

Taxa	Common Name	Status	Total Number
<i>Alitta virens</i>	King ragworm	Economically Important Species	4
<i>Austrominius modestus</i>		Invasive & Non-Native	145
<i>Cancer pagurus</i>	Edible crab	Economically Important Species	6
<i>Carcinus maenas</i>	Green shore crab	Economically Important Species	41
<i>Mya arenaria</i>		Invasive & Non-Native	4
<i>Mytilus edulis</i>	Blue mussel	Economically Important Species	770
<i>Nucella lapillus</i>	Dog whelk	OSPAR Listed	5
<i>Sabellaria spinulosa</i>	Ross Worm	OSPAR Listed (in reef form)	30
Veneridae		Economically Important Species	508

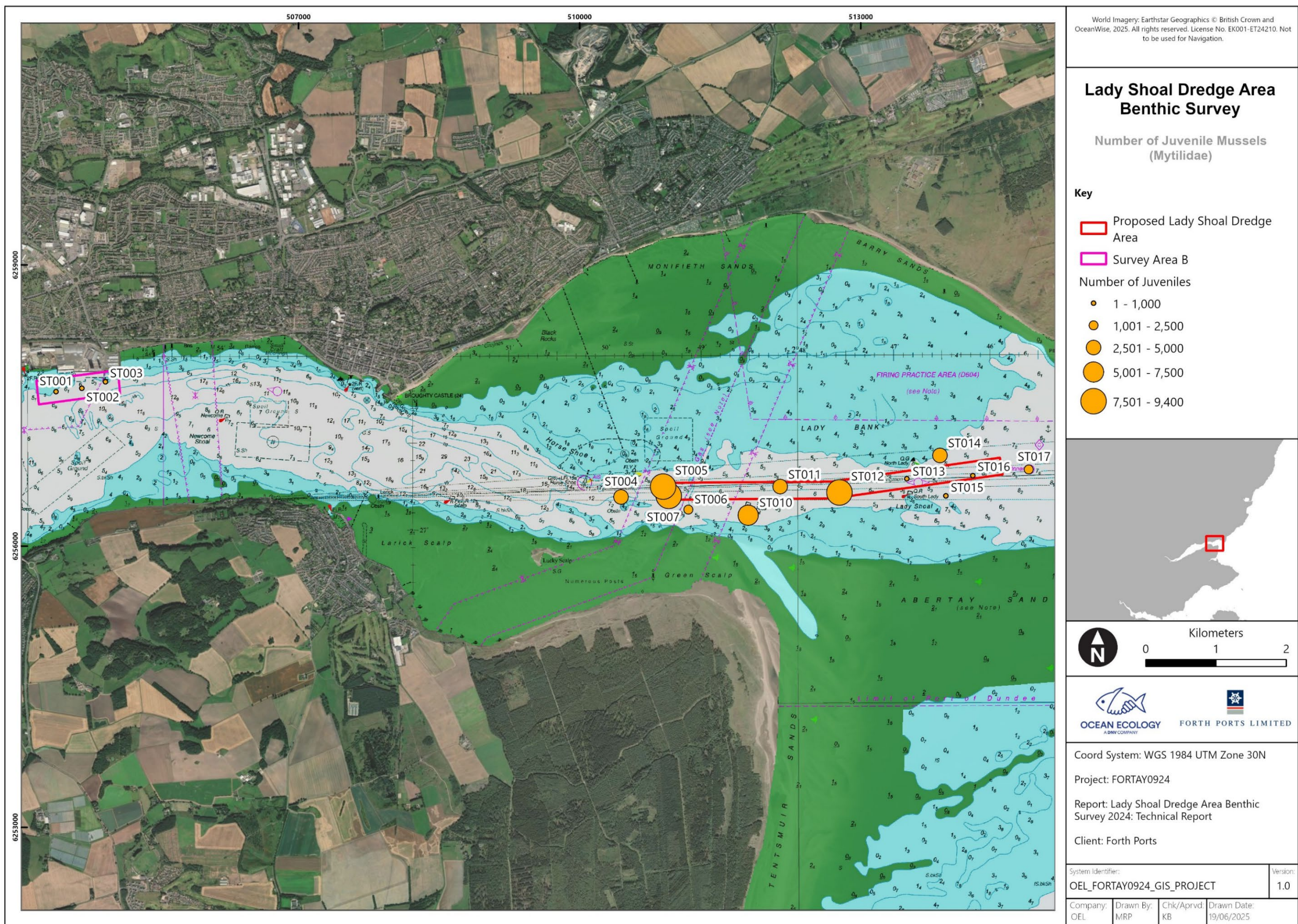


Figure 22 Spatial distribution of juvenile mussels (Family Mytilidae) across the Lady Shoal survey area.

6.3.5. Biotope Assignment

For each of the Macrobenthic Groups determined using cluster analysis, biotopes and habitats were assigned in consideration of JNCC guidance based upon their faunal and physical characteristics (Parry 2019).

Macrobenthic multivariate analysis did not show any pattern in the community composition that could be used to assign a biotope due to insufficient characterising species and therefore all stations were assigned to their corresponding EUNIS level 4 classification based on sediment and seabed imagery analyses. Most stations were assigned to MC42 'Atlantic circalittoral mixed sediment' based on PSD data except ST005, which was assigned to MC32 'Atlantic circalittoral coarse sediment'. Note that MC3 'Circalittoral coarse sediment' was observed in most of the DDC imagery but there is considerable overlap with MC42 Atlantic circalittoral mixed sediment.

7. Discussion

Across the survey area, six EUNIS classifications were identified in the seabed imagery, including three EUNIS Level 4 habitat complexes: MC124 'Faunal communities on variable salinity Atlantic circalittoral rock', MC32 'Atlantic circalittoral coarse sediment', MC42 'Atlantic circalittoral mixed sediments', and three EUNIS Level 5 biotopes: MC1241 'Cushion sponges and hydroids on turbid tide-swept sheltered Atlantic circalittoral rock', MC3211 '*Pomatoceros triqueter* with barnacles and bryozoan crusts on Atlantic circalittoral unstable cobbles and pebbles', and MC2235 '*Mytilus edulis* beds on Atlantic circalittoral sediment'.

The trends in epifaunal abundance and diversity observed across the survey area indicated a relationship between habitat type and community composition. The highest overall abundance of epifauna along TR015 coincides with the presence of the PMF habitat 'Blue mussel bed' which are known to provide structural complexity and refuge for various species. This suggests that mussel beds may function as hotspots of biological activity, supporting dense aggregations of epifauna. The presence of PMF 'Blue mussel beds' raises questions about the classification criteria for *Mytilus* beds versus Annex I biogenic reefs. While *Mytilus* beds were observed, their classification as biogenic reefs depend on additional factors such as persistence, density, and structural integrity. Further investigation is necessary to determine whether these mussel beds contribute to reef formation over time.

Similarly, the highest species diversity was observed along TR004 which covered geogenic reef habitats, indicating that these areas might support a broader range of ecological niches due to the structural complexity of the substrate. The presence of reef habitats likely enhances microhabitat availability, leading to higher species richness compared to more uniform sedimentary environments. However, stony reef was found to be patchy rather than continuous across most of the proposed Lady Shoal dredging area, due to the sparse presence of cobbles and boulders within a larger extent of coarse sediments, which could affect the reef's ability to sustain a diverse epifaunal community and consolidate the substrate, hence the low confidence in the Annex I reef assignment. To note that the Firth of Tay and Eden Estuary SAC is not designated for the protection of Annex I reef but for the protection of Annex I estuaries, sandbanks, mudflats and sandflats.

Two INNS were also recorded across the survey area with the Darwin's barnacle *A. modestus* occurring in 9 of the 15 grab stations for a total of 145 individuals counted and a maximum abundance of 58 individuals at station ST005. The other INNS was the sand-gaper *M. arenaria* which was only found at station ST014 for a total of four individuals. While not much is known about the invasion history of the sand-gaper besides the fact that this species is native of North America and that is now well established and naturalised in the UK, a more detailed record exists for the Darwin's barnacle. This species is native of Australasia and was first recorded in the UK in 1945 at the Chichester Harbour.

It is posited that it was first introduced during the Second World War as *A. modestus* was attached to the hulls of merchant and warships. Once introduced, it spread rapidly competing for space with the UK native barnacles (Eno et al. 1997).

The presence of mixed sediments across the survey area suggested a dynamic environment influenced by tidal currents and wave action. The variation in sediment composition across stations indicated that different benthic communities may be supported based on substrate stability. More stable mixed sediments could provide suitable conditions for sessile and encrusting organisms, whereas coarser, more mobile substrates might favour opportunistic species adapted to dynamic conditions.

It is also worth noting that the findings from the seabed imagery analysis generally aligned with existing EUSeaMap broad-scale predictive habitat mapping, which indicated the presence of the EUNIS habitat MC2235 '*Mytilus edulis* beds on Atlantic circalittoral sediment'. This predictive mapping supports the findings from the imagery analysis, where *Mytilus* beds were indeed observed. However, the PMF distribution noted in the EUSeaMap and in the JNCC PMF habitat mapping suggests extensive mussel beds to the west of the Lady Shoal dredge area. While individual mussels were recorded at several locations across the survey area, mussel beds were only observed at three transects: TR002 and TR003 in Survey Area B, and TR015 to the east of the survey area. Of these, the only location outside the Port of Dundee where mussel beds met the criteria for PMF habitat was TR015

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