Coquet to St Mary’s Marine Conservation Zone (MCZ): A study of a UK Subtidal mud feature (2019)

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Coquet to St Mary's Marine Conservation Zone (MCZ): A study of a UK Subtidal mud feature (2019)

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Foreword

Natural England commission a range of reports from external contractors to provide evidence and advice to assist us in delivering our duties. The views in this report are those of the authors and do not necessarily represent those of Natural England.

Background

Following designation, Natural England started a baseline monitoring programme across all marine protected areas.

This report was commissioned as part of an inshore benthic marine survey of the Coquet to St Mary’s MCZ.

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Abbreviations

aRPD	Apparent Redox Potential Discontinuity Depth
BACI	Before–After, Control–Impact
BSH	Broadscale Habitats
Cefas	Centre for Environment, Fisheries and Aquaculture Science
CP2	Charting Progress 2
Defra	Department for Environment, Food and Rural Affairs
DEM	Digital Elevation Model
EA	Environment Agency
EUNIS	European Nature Information System
FOCI	Feature of Conservation Interest
GES	Good Environmental Status
GMA	General Management Approach
IFCA	Inshore Fisheries and Conservation Authority
JNCC	Joint Nature Conservation Committee
MBES	Multibeam echosounder
MCZ	Marine Conservation Zone
MESH	Mapping European Seabed Habitats
MPA	Marine Protected Area
MPAG	Marine Protected Areas Group
MSFD	Marine Strategy Framework Directive
NE	Natural England
NIOZ	Koninklijk Nederlands Instituut voor Onderzoek der Zee
NIS	Non-Indigenous Species

NMBAQC	North-East Atlantic Marine Biological Analytical Quality Control Scheme
OSPAR	The Convention for the Protection of the Marine Environment of the North-East Atlantic
PSA	Particle Size Analysis
PSD	Particle Size Distribution
RV	Research Vessel
SAC	Special Area of Conservation
SACO	Supplementary Advice on Conservation Objectives
SNCB	Statutory Nature Conservation Body
SPI	Sediment Profile Imaging
Spp	Species
STR	SeaSpyder Telemetry
USBL	Ultra-short baseline
WoRMS	World Register of Marine Species

Glossary

Definitions signified by an asterisk (*) have been sourced from Natural England and JNCC Ecological Network Guidance (NE and JNCC, 2010).

Activity	A human action which may have an effect on the marine environment; e.g. fishing, energy production (Robinson, Rogers and Frid, 2008).*
Annex I Habitats	Habitats of conservation importance listed in Annex I of the EC Habitats Directive, for which Special Areas of Conservation (SAC) are designated.
Anthropogenic	Caused by humans or human activities; usually used in reference to environmental degradation.*
Assemblage	A collection of plants and/or animals characteristically associated with a particular environment that can be used as an indicator of that environment. The term has a neutral connotation and does not imply any specific relationship between the component organisms, whereas terms such as ‘community’ imply interactions (Allaby, 2015).
Backscatter	Multibeam echosounder returns used to measure sea floor ‘hardness’.
Bathymetry	The measurement of the underwater depth of oceans, seas and lakes.
Benthic	A description for animals, plants and habitats associated with the seabed. All plants and animals that live in, on or near the seabed are benthos (e.g. sponges, crabs, seagrass beds).*
Biotope	The physical habitat with its associated, distinctive biological communities. A biotope is the smallest unit of a habitat that can be delineated conveniently and is characterised by the community of plants and animals living there.*
Bioturbation	The reworking of sediments by living organisms.
Broadscale	Habitats which have been broadly categorised based on
Habitats	shared set of ecological requirements, aligning with level 3 of the EUNIS habitat classification. Examples of Broadscale Habitats are protected across the MCZ network.
Community	A general term applied to any grouping of populations of different organisms found living together in a particular environment; essentially the biotic component of an ecosystem.

	The organisms interact and give the community a structure (Allaby, 2015).
Conservation Objective	A statement of the nature conservation aspirations for the feature(s) of interest within a site, and an assessment of those human pressures likely to affect the feature(s).*
EC Habitats Directive	The EC Habitats Directive (Council Directive 92/43/EEC on the Conservation of natural habitats and of wild fauna and flora) requires Member States to take measures to maintain natural habitats and wild species of European importance at, or restore them to, favourable conservation status.
Epifauna	Fauna living on the seabed surface.
EUNIS	A European habitat classification system, covering all types of habitats from natural to artificial, terrestrial to freshwater and marine.*
Favourable Condition	When the ecological condition of a species or habitat is in line with the conservation objectives for that feature. The term ‘favourable’ encompasses a range of ecological conditions depending on the objectives for individual features.*
Feature	A species, habitat, geological or geomorphological entity for which an MPA is identified and managed.*
Feature Attributes	Ecological characteristics defined for each feature within site-specific Supplementary Advice on Conservation Objectives (SACO). Feature Attributes are monitored to determine whether condition is favourable.
Features of Conservation Importance (FOCI)	Habitats and species that are rare, threatened or declining in Secretary of State waters.*
General Management Approach (GMA)	The management approach required to achieve favourable condition at the site level; either maintain in, or recover to favourable condition.
Habitats of Conservation	Habitats that are rare, threatened, or declining in Secretary of State waters.*

Importance	
Impact	The consequence of pressures (e.g. habitat degradation) where a change occurs that is different to that expected under natural conditions (Robinson, Rogers and Frid, 2008).*
Infauna	Fauna living within the seabed sediment.
Joint Nature Conservation Committee (JNCC)	The statutory advisor to Government on UK and international nature conservation. Its specific remit in the marine environment ranges from 12 - 200 nautical miles offshore.
Kriging	A geostatistical technique which uses a limited set of sampled data points to estimate the value of a variable over a continuous spatial field.
Marine Strategy Framework Directive (MSFD)	The MSFD (EC Directive 2008/56/EC) aims to achieve Good Environmental Status (GES) of EU marine waters and to protect the resource base upon which marine-related economic and social activities depend.
Marine Conservation Zone (MCZ)	MPAs designated under the Marine and Coastal Access Act (2009). MCZs protect nationally important marine wildlife, habitats, geology and geomorphology, and can be designated anywhere in English and Welsh inshore and UK offshore waters.*
Marine Protected Area (MPA)	A generic term to cover all marine areas that are ‘A clearly defined geographical space, recognised, dedicated and managed, through legal or other effective means, to achieve the long-term conservation of nature with associated ecosystem services and cultural values’ (Dudley, 2008).*
Natura 2000	The EU network of nature protection areas (classified as Special Areas of Conservation and Special Protection Areas), established under the 1992 EC Habitats Directive.*
Natural England	The statutory conservation advisor to Government, with a remit for England out to 12 nautical miles offshore.
Non-indigenous	A species that has been introduced directly or indirectly by

Species	human agency (deliberately or otherwise) to an area where it has not occurred in historical times and which is separate from and lies outside the area where natural range extension could be expected (Eno *et al*., 1997).*
Pressure	The mechanism through which an activity has an effect on any part of the ecosystem (e.g. physical abrasion caused by trawling). Pressures can be physical, chemical or biological, and the same pressure can be caused by a number of different activities (Robinson, Rogers and Frid, 2008).*
Special Areas of Conservation	Protected sites designated under the European Habitats Directive for species and habitats of European importance, as listed in Annex I and II of the Directive.*
Species of Conservation Importance	Habitats and species that are rare, threatened or declining in Secretary of State waters.*
Supplementary Advice on Conservation Objectives (SACO)	Site-specific advice providing more detailed information on the ecological characteristics or 'attributes' of the site's designated feature(s). This advice is issued by Natural England and/or JNCC.
Taxon (plural taxa)	Any unit used in biological classification or taxonomy

Executive Summary

This report is one of a series of Marine Protected Area (MPA) characterisation and monitoring reports delivered to Defra by the Marine Protected Areas Group (MPAG). The purpose of the report series is to provide the necessary information to allow Defra to fulfil its MPA assessment and reporting obligations in relation to the UK Marine & Coastal Act (2009), the Oslo-Paris (OSPAR) Convention, and other relevant Directives (e.g., Marine Strategy Framework Directive). This report is focused on the Coquet to St Mary's Marine Conservation Zone (MCZ) and explores survey data collected within the site and surrounding areas in 2016.

Coquet to St Mary's MCZ is an inshore MPA located on the Northumberland coast in the northern North Sea. Thirteen Broadscale Habitats (BSHs) and two habitat Features of Conservation Importance (FOCI) are designated for protection at the site. Previous surveys suggest that an undesignated habitat FOCI, 'Sea-pen and burrowing megafauna communities', is also present within the MCZ. This report aims to confirm this possibility, describe the physical and biological conditions of the designated BSH 'A5.3 Subtidal mud' and provide information to guide effective monitoring of subtidal mud features at this site and throughout the MCZ network.

Burrowing megafauna were recorded throughout most of the MCZ survey area and in an area directly to the south that has been proposed (along with an area directly east of the MCZ) as a potential control site for before–after, control–impact (BACI) monitoring following the implementation of any MCZ management measures. Most records came from the observation of Norway lobster (*Nephrops norvegicus*) burrows in seafloor video footage, but at some stations records came from different species (usually the mud shrimp *Callianassa subterranea*) that were found in NIOZ corer samples. Despite *N. norvegicus* burrows being widespread throughout much of the surveyed area, burrow density exceeded the minimum requirement for classification of the habitat FOCI 'Sea-pen and burrowing megafauna communities' at only five stations: three inside the MCZ, one in the southern potential control area and one in the eastern potential control area. Where burrowing megafauna were present, they usually occurred in the absence of sea-pens; however, sea-pens and burrowing megafauna did co-occur at a group of stations in the centre of the MCZ survey area. Sea-pens were recorded only once in the southern potential control area and appeared absent from another potential control area to the east of the MCZ.

Within the MCZ, the BSH 'A5.3 Subtidal mud' was characterised by species that are common in muddy environments but appear tolerant of physical and/or organic anthropogenic disturbance (i.e. the brittle star *Amphiura filiformis* and polychaete *Peresiella clymenoides*) as well as other species that are usually considered to be typical of sandier or more gravelly sediments (the polychaete *Lumbrineris* spp. and bivalve *Chamelea striatula*). Diversity and composition of macrofauna varied in relation to mud content. Assemblages inhabiting 'A5.3 Subtidal mud' in the MCZ survey area were very similar to those in the southern potential control area, which reflects the similarity in sediment characteristics for the two areas. Assemblages inhabiting the sandier mud of the eastern potential control area

were relatively distinct from those within the MCZ. Therefore, the southern area appears to be the most suitable control site for BACI monitoring.

Data collected during the survey allowed the efficacy of different approaches to sampling 'A5.3 Subtidal mud' to be assessed. Macrofaunal sampling was conducted using both Day grabs and NIOZ corers (each ~0.1 m^2), which differed mainly in that the latter gear appeared more effective at sampling deep-burrowing organisms. This reflects the greater penetration depth of the NIOZ corer compared to the Day grab (~50 cm *vs* 10-15 cm). Analysis of triplicate macrofauna samples collected at a subset of stations indicated that a single sample of ~0.1 m^2 is unlikely to accurately represent macrofaunal community composition at a station (average within-station sample similarity ranged from 43% to 62%) but may be sufficient to capture broad spatial patterns in macrofaunal communities.

These findings are discussed and used to make recommendations for future monitoring of subtidal mud features in the Coquet to St Mary's MCZ and elsewhere.

1. Introduction

Coquet to St Mary's Marine Conservation Zone (MCZ) is part of a network of Marine Protected Areas designed to meet conservation objectives under the Marine and Coastal Access Act (2009), namely to halt the deterioration of the state of the UK's marine biodiversity and, where appropriate, promote recovery. These sites will also contribute to an ecologically coherent network of Marine Protected Areas (MPAs) across the North-east Atlantic agreed under the Oslo-Paris (OSPAR) Convention and other international commitments to which the UK is a signatory.

Under the UK Marine & Coastal Access Act (2009), Defra is required to provide a report to Parliament every six years that includes an assessment of the degree to which the conservation objectives set for MCZs are being achieved. To fulfil its obligations, Defra has directed the Statutory Nature Conservation Bodies (SNCBs) to carry out a programme of MPA monitoring. The SNCB responsible for inshore nature conservation (between 0 nm and 12 nm from the coast) is Natural England (NE) and the SNCB responsible for offshore nature conservation (between 12 nm and 200 nm from the coast) is the Joint Nature Conservation Committee (JNCC). Where possible, monitoring will also inform assessment of the status of the wider UK marine environment; for example, assessment of whether Good Environmental Status (GES) has been achieved, as required under Article 11 of the Marine Strategy Framework Directive (MSFD).

This report explores data acquired in 2016 from a dedicated survey of the 'A5.3 Subtidal mud' habitat in the Coquet to St Mary's MCZ and surrounding areas, which was designed primarily to verify the presence of the habitat Feature of Conservation Interest (FOCI) 'Sea-pen and burrowing megafauna communities'. The specific aims of the report are stated in section 1.3.3.

1.1 Site overview

Coquet to St Mary's MCZ is an inshore site located on the Northumberland coast in the northern North Sea (Figure 1). The site runs from Whitley Bay in the south to Alnmouth in the north and includes both St Mary's and Coquet Islands. The depth at the site ranges from 10 m above the mean low water mark to 30 m below at its seaward extent, covering a total area of 192 km^2. St Mary's Island is an existing voluntary marine reserve to protect the rocky reef and the abundant crabs and lobsters present, while Coquet Island has international importance due to the presence of breeding seabirds from late March to mid-September as well as being a foraging location for other birds throughout the year (Net Gain, 2011). Sightings of marine mammals at the site include the harbour porpoises, white beaked dolphins, grey seals, and minke, orca, and humpback whales. In recent years, both islands have been a haul out site for seals and their pups. The site is directly to the south of the Berwickshire and Northumberland Coast Special Area of Conservation (SAC) and is also neighboured by the Farnes East, North-East of Farnes Deep, and Swallow Sand MCZs (Figure 1).

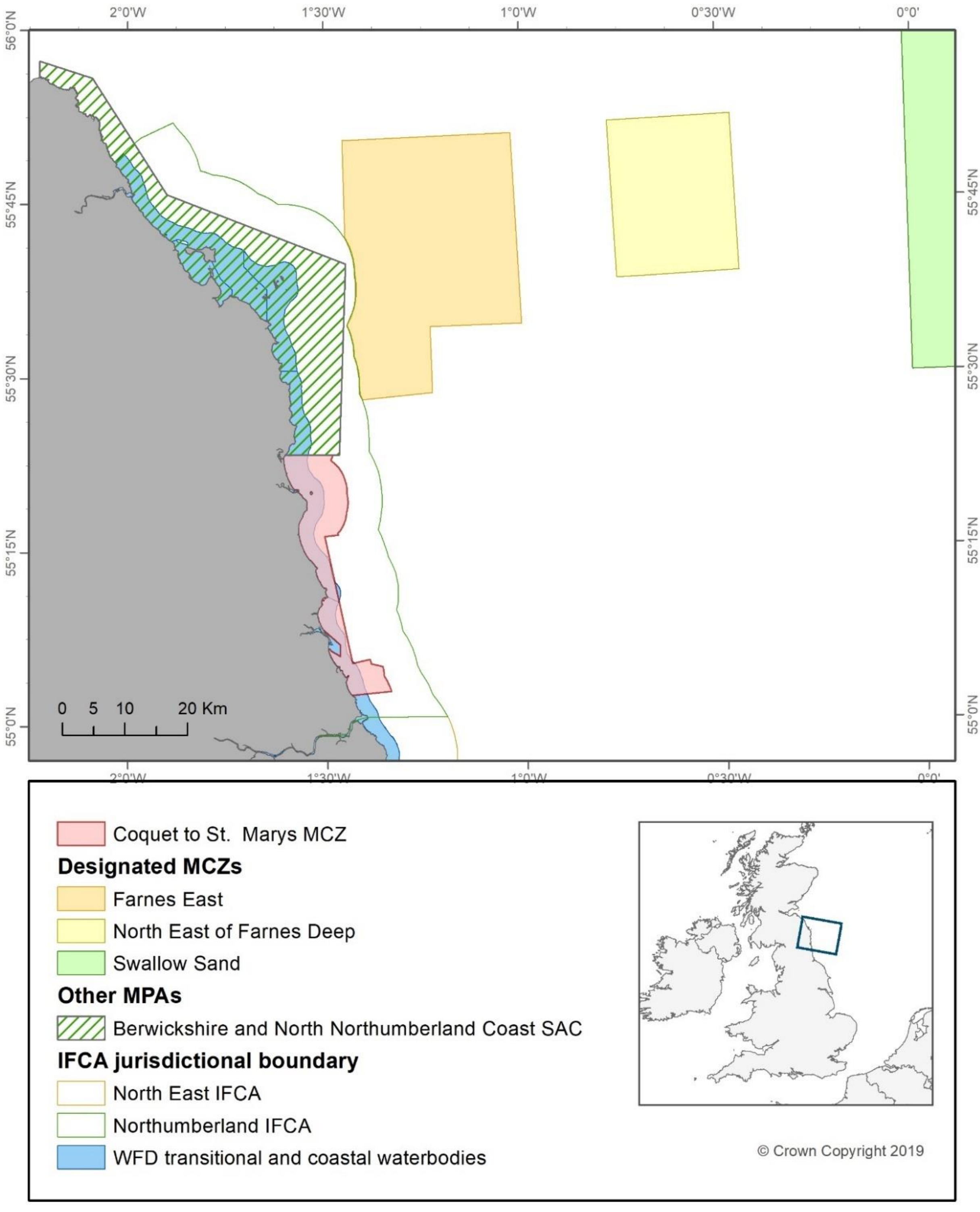

Figure 1. The location of the Coquet to St Mary's Marine Conservation Zone (MCZ) in relation to nearby MCZs and other Marine Protected Areas (MPAs) and jurisdictional boundaries.

Coquet to St Mary's MCZ was designated in January 2016 to protect a mosaic of rock and sedimentary habitats found both on the shoreline and on the seabed. Current designated features include 13 Broadscale Habitats (BSHs) and two habitat FOCI, 'Intertidal underboulder communities' and 'Peat and clay exposures' (

Table 1). A habitat verification survey in 2014 confirmed the presence of the BSH 'A5.3 Subtidal mud' and suggested the possible presence of another habitat FOCI that is not currently designated at the site, 'Sea-pen and burrowing megafauna communities'.

Table 1. The Coquet to St Marys MCZ site overview, including General Management Approach (GMA) for designated Broadscale Habitats (BSHs) and Features of Conservation Importance (FOCI) (© Natural England and Cefas 2022).

Charting Progress 2 Region[1]	Northern North Sea	
Spatial Area (km^2)	192	
Water Depth Range (m)	0-30	
Proposed Management Measures	No gear restrictions. Fishing activity and its interactions with designated features will be monitored and management measures put in place when necessary to achieve conservation objectives.	
BSHs present	**Designated**	**GMA**
A1.2 Moderate energy intertidal rock	✓	Maintain
A1.3 Low energy intertidal rock	✓	Maintain
A2.1 Intertidal coarse sediment	✓	Maintain
A2.2 Intertidal sand and muddy sand	✓	Maintain
A2.3 Intertidal mud	✓	Maintain
A2.4 Intertidal mixed sediments	✓	Maintain
A3.1 High energy infralittoral rock	✓	Maintain
A3.2 Moderate energy infralittoral rock	✓	Maintain
A4.2 Moderate energy circalittoral rock	✓	Maintain
A5.1 Subtidal coarse sediment	✓	Maintain
A5.2 Subtidal sand	✓	Maintain
A5.3 Subtidal mud*	✓	Maintain
A5.4 Subtidal mixed sediments	✓	Maintain
Habitat FOCI present	**Designated**	**GMA**
Intertidal underboulder communities	✓	Maintain
Peat and clay exposures	✓	Maintain

**** The characterisation survey reported on here focused on this designated feature and no other designated features.***

[1]http://webarchive.nationalarchives.gov.uk/20141203170558tf_/http://chartingprogress.defra.gov.uk/ [accessed 30/10/2019]

1.2 Existing data and habitat maps

Multibeam echosounder (MBES) bathymetry and backscatter data were collected at the Coquet to St Mary's MCZ between January and March 2014, followed by a ground truth survey carried out during July to September 2014. These data were collected by the Environment Agency on the CSV Humber Guardian survey vessel (Environment Agency, 2014; Godsell, 2014). The subtidal area was surveyed using a drop camera system at 95 stations, and a 0.1 m^2 mini-Hamon grab was deployed at 60 of these to collect sediment samples for Particle Size Distribution (PSD) and macrofaunal analyses. These data were used to produce a habitat map and a post-survey site report for Coquet to St Mary's MCZ (Fitzsimmons *et al*., 2015). Locations of video tows and grab samples, the BSHs inferred from data collected using each gear and the resulting BSH map are shown in Figure 2.

An existing published model based on kriged interpretation of surficial sediment composition (Stephens 2015; Stephens and Diesing, 2015) was used to predict spatial variation in % mud content within the MCZ and surrounding areas and compared against the results of the survey on which this report is based.

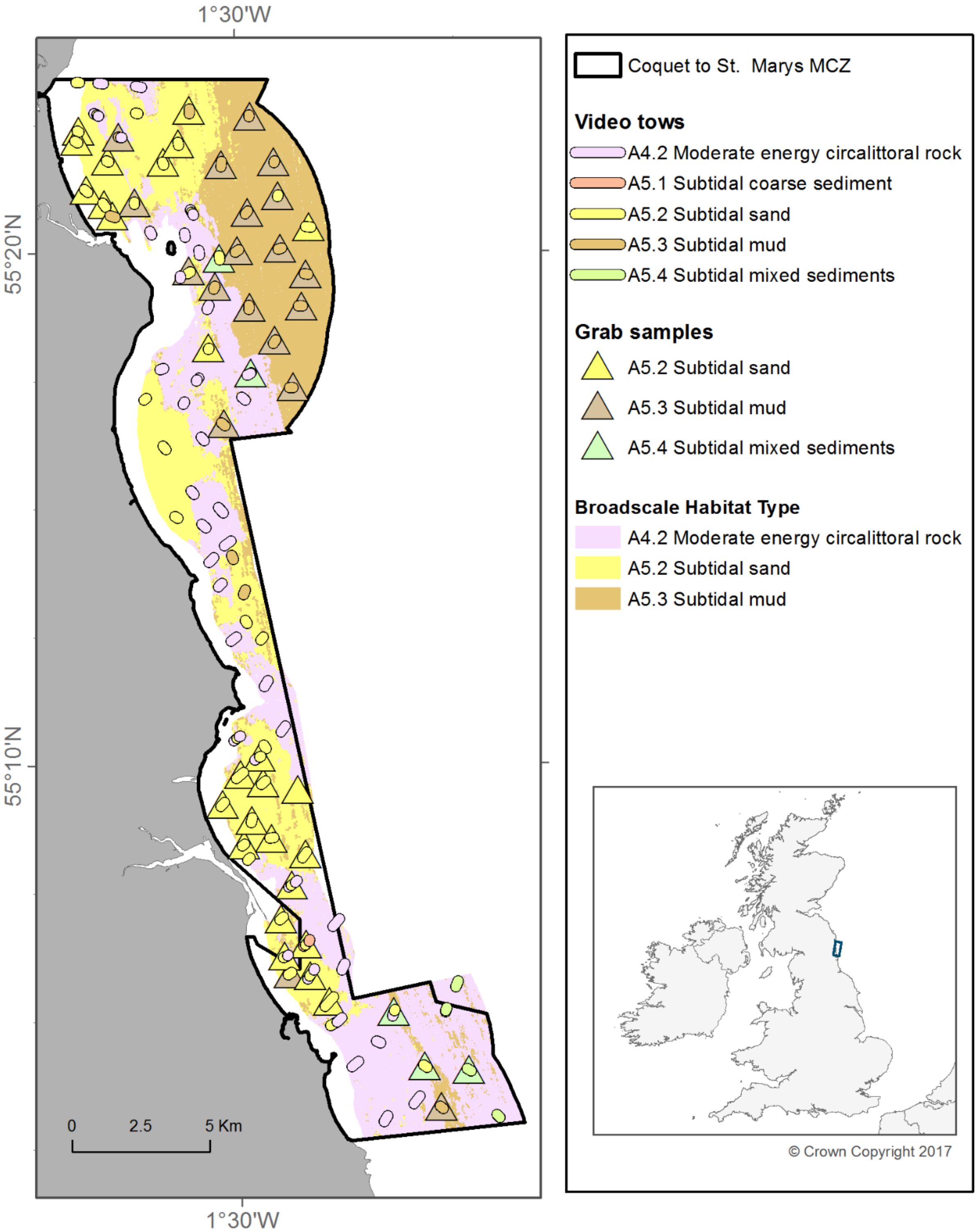

Figure 2. Locations of ground truth samples collected by the Environment Agency at the Coquet to St Mary's MCZ as part of the site verification process in 2014 (two years prior to the survey conducted for the present report) and the Broadscale Habitats (BSHs) inferred from these samples (© Natural England and Cefas 2022).

1.3 Aims and objectives

1.3.1 High-level conservation objectives

High-level, site-specific conservation objectives serve as benchmarks against which the efficacy of the General Management Approach (GMA) in achieving the conservation objectives (i.e. maintaining designated features at, or recovering them to, 'favourable condition') can be assessed and monitored.

As detailed in the Coquet to St Mary's MCZ designation order[2], the conservation objectives for the site are that the designated features:

a) So far as already in favourable condition, remain in such condition; and

b) So far as not already in favourable condition, be brought into such condition, and remain in such condition.

It should be noted that GMAs for the Coquet to St. Mary's MCZ have been applied based on an indirect or proxy assessment, as opposed to being based on empirical monitoring evidence (i.e. direct observations).

1.3.2 Definition of favourable condition

Favourable condition, with respect to a habitat feature, means that:

a) Its **extent and distribution** is stable or increasing;

b) Its **structures and functions**, including its quality, and the composition of its characteristic biological communities, are such as to ensure that it remains in a condition which is healthy and not deteriorating; and

c) Its natural **supporting processes** are unimpeded.

The extent of a habitat feature refers to the total area in the site occupied by the qualifying feature and must also include consideration of its distribution. A reduction in feature extent has the potential to alter the physical and biological functioning of sedimentary habitats (Elliott *et al*., 1998). The distribution of a habitat feature influences the component communities present and can contribute to the condition and resilience of the feature (JNCC, 2004).

Structure encompasses the physical components of a habitat type and the key and influential species present. Physical structure refers to topography, substrate composition and distribution. Physical structure can have a significant influence on the hydrodynamic regime operating at varying spatial scales in the marine environment, as well as influencing the presence and distribution of associated biological communities (Elliott *et al*. 1998). The

[2] http://www.legislation.gov.uk/ukmo/2016/3/pdfs/ukmo_20160003_en.pdf [accessed 30/10/2019]

function of habitat features includes processes such as: sediment reworking (e.g. through bioturbation) and habitat modification, primary and secondary production and recruitment dynamics. Habitat features rely on a range of supporting processes (e.g. hydrodynamic regime, water quality and sediment quality), which act to support their functioning as well as their resilience (e.g. the ability to recover following impact).

1.3.3 Report aims and objectives

In accordance with the aims of the 2016 survey of the Coquet to St Mary's MCZ and surrounding areas, this report investigates the presence of the undesignated habitat FOCI 'Sea-pen and burrowing megafauna communities' and describes the attributes of the designated BSH 'A5.3 Subtidal mud'. The results are used to develop recommendations for future monitoring of subtidal mud features within the Coquet to St Mary's MCZ and similar sites elsewhere.

The specific aims of this report are to:

i. Investigate the presence and map the spatial distribution of the habitat FOCI 'Sea-pen and burrowing megafauna communities' within the Coquet to St Mary's MCZ and surrounding areas;

ii. Describe the **structural** and (where possible) **functional** attributes (see section 1.3.4) of 'A5.3 Subtidal mud' within the MCZ and surrounding areas;

iii. Note observations of other BSHs, habitat FOCI or species FOCI recorded within the MCZ and surrounding areas;

iv. Present evidence relating to the presence of non-indigenous species (Descriptor 2) and marine litter (Descriptor 10), to satisfy requirements of the Marine Strategy Framework Directive (MSFD);

v. Provide practical recommendations for appropriate future monitoring approaches for 'A5.3 Subtidal mud'.

1.3.4 Feature attributes and supporting processes

To achieve Report Objective ii, the report presents evidence on several feature attributes and supporting processes, as defined in Supplementary Advice on Conservation Objectives (SACOs) developed by JNCC and Natural England (NE) for the designated 'A5.3 Subtidal mud' feature within the Coquet to St Mary's MCZ. The comprehensive nature of the attribute lists meant that focus had to be placed on a subset of attributes for which existing evidence was lacking. For example, the extent and distribution of 'A5.3 Subtidal mud' within the MCZ was investigated by a previous survey of the site (see section 1.2) and is therefore not described here. However, the presence of 'A5.3 Subtidal mud' outside the MCZ is investigated here to achieve Report Objective v (see sections 1.3.3 and 2.1). The feature attributes and supporting processes considered in this report, and the associated outputs, are presented in Table 2.

Table 2. Survey elements and report outputs aligned with the feature attributes and supporting processes identified at the Coquet to St Mary's MCZ (© Natural England and Cefas 2022).

Feature attributes	Features	Outputs
Attributes: Structure and function		
Sediment composition and distribution	A5.3 Subtidal mud	Maps of spatial variation in sediment composition
Presence and abundance of key structural and influential species Composition of component communities	A5.3 Subtidal mud	Maps of the habitat FOCI 'Sea-pen and burrowing megafauna communities' and its foundational species Analyses of benthic community composition
Supporting process:		
Energy/exposure Physico-chemical properties	Coquet to St Mary's Marine Conservation Zone	Tidal model Assessment of apparent redox potential discontinuity (aRPD) depth in burrowed substrate and related metrics

2 Methods

2.1 Survey design

The survey was carried out jointly by the Centre for Environment, Fisheries and Aquaculture Science (Cefas) and Natural England (NE) on the RV *Cefas Endeavour* (CEND0716) in April 2016 (Murray, 2016). An area in the northern part of the MCZ, where the presence of 'A5.3 Subtidal mud' is predicted in the habitat map (see Figure **2**), was surveyed, along with three areas immediately north, east, and south of the MCZ survey area (Figure 3). The areas to the south and east are potential 'control' areas for monitoring the effects of any future MCZ management measures on mud features. The northern area is located within the boundaries of the Berwickshire and Northumberland Coast SAC, where the BSH was predicted to be 'A5.3 Subtidal mud', and its inclusion within this report serves only to provide additional context to results. A total of 63 stations were sampled during the survey (Annex 1. Sampling Regime).

Ten of the stations were revisits to stations within the MCZ where video footage collected during the 2014 habitat verification survey provided evidence of burrowed mud (Fitzsimmons *et al*., 2015), therefore implying the presence of burrowing megafauna. A ten-minute camera tow (video footage and stills) was carried out at each of these stations to gather additional evidence on the presence of burrowed substrate. Three Day grab and NIOZ corer samples (each ~0.1 m^2) were also collected at all ten revisited stations to verify BSH type, identify the associated macrofauna, assess the sampling efficacy of the two gears and assess within-station variability in benthic communities. The NIOZ cylindrical box corer, developed by Koninklijk Nederlands Instituut voor Onderzoek der Zee (NIOZ), penetrates deeper into the sediment than the Day grab, meaning that it can reach organisms inhabiting deeper sediments and may therefore be more suitable for investigating the presence of burrowing megafauna. Lastly, at each revisit station, a transect of five 'hops' with a sediment profile imagery (SPI) camera was carried out to assess the biogeochemical conditions associated with burrowed mud within the MCZ.

A power analysis conducted prior to the survey indicated that 19 grab samples were needed to have an 80% chance of detecting a 20% change in macrofaunal taxon richness in 'A5.3 Subtidal mud' (Annex 2. Power analysis results). Therefore, a further nine stations within the MCZ that were predicted to be located on 'A5.3 Subtidal mud' were surveyed with a single Day grab sample (giving a total of 19 stations) to describe macrofaunal assemblages, confirm BSH type and provide a baseline for future monitoring. These stations were also surveyed using a ten-minute camera tow to gather evidence of burrowed substrate.

The area to the east of the MCZ was sampled to assess the extension of 'A5.3 Subtidal mud' beyond the Coquet to St Mary's MCZ boundary and determine this area's suitability as a 'control' for any before–after, control–impact (BACI) monitoring of future MCZ management measures (i.e. assess its similarity to the area surveyed within the MCZ in terms of physical and biological characteristics). Fourteen stations in the eastern potential control area were sampled using a single Day grab sample and 12 were surveyed using a ten-minute camera tow to describe macrofaunal assemblages, confirm BSH type and gather evidence of burrowed substrate. Weather conditions prevented the camera from being deployed at some stations. South of the MCZ in the other potential control area for BACI-type monitoring, 20 stations were surveyed with a single Day grab sample and a ten-minute camera tow, following the same rationale as applied to the eastern potential control area. NIOZ corer samples were also collected in triplicate within the southern potential control area at five stations where video footage showed evidence of burrowed mud. Finally, four stations north of the MCZ were surveyed using a single Day grab sample to confirm BSH type and describe the associated macrofaunal assemblages.

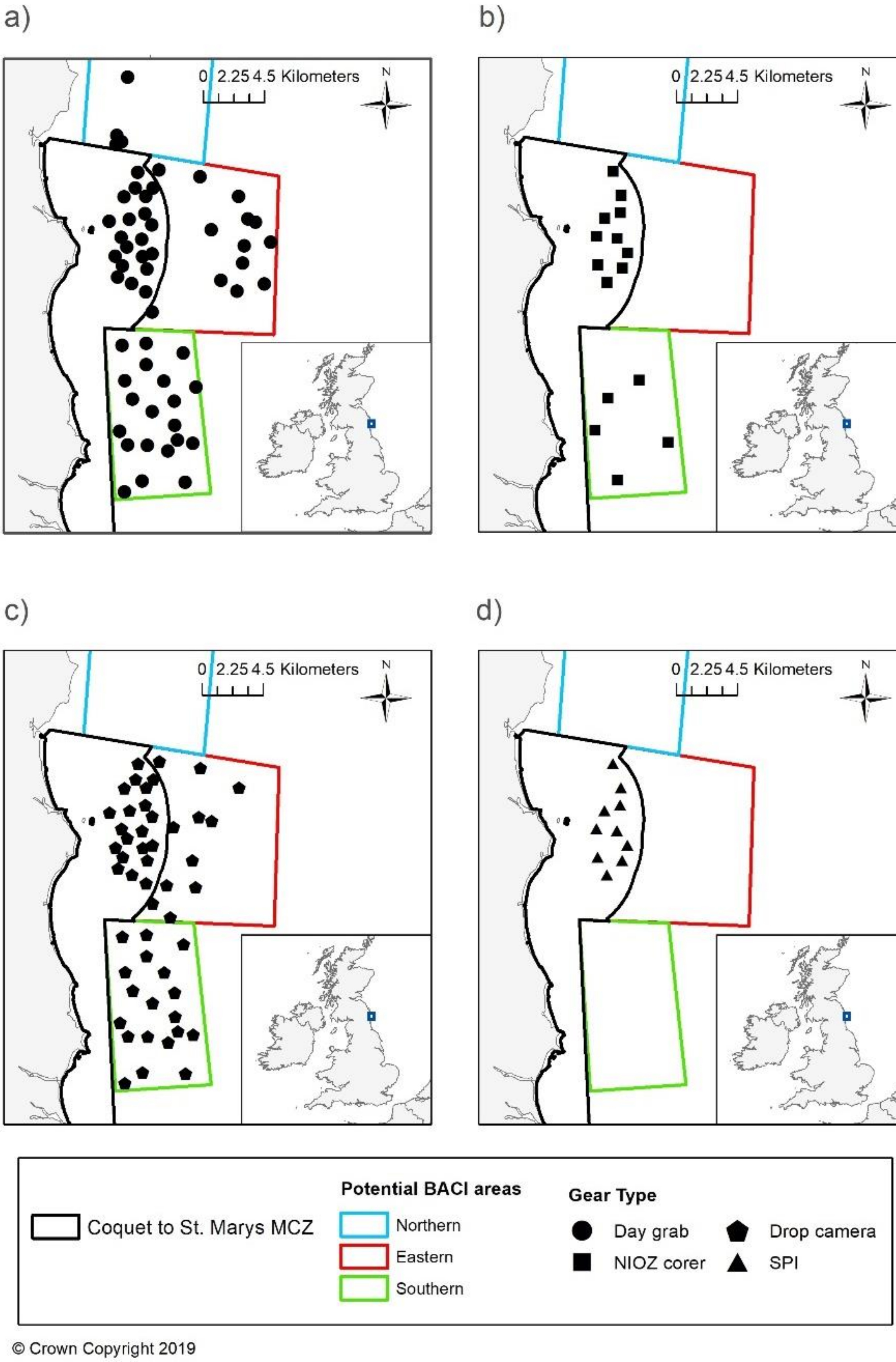

Figure 3. Locations of stations sampled to characterise the subtidal mud feature within and around the Coquet to St Mary's MCZ in 2016. Samples were collected using: a) Day grab, b) NIOZ corer, c) drop camera, and d) sediment profile imagery (SPI) camera.

2.2 Data acquisition and processing

2.2.1 Seabed imagery

Video footage and still images of the seafloor and epibiota were collected using a SeaSpyder Telemetry (STR) drop camera system according to the Mapping European Seabed Habitats (MESH) Recommended Operating Guidelines (Coggan *et al*., 2007). Ultra-short baseline (USBL) positioning was used to geo-reference the video footage and still images. Drop camera tows lasted a minimum of ten minutes, with the system being towed at c. 0.3 knots (c. 0.15 m s^{-1}) across a 100 m 'bullring' (i.e. a target circle) centred on the sampling station, producing transects of ~100 m length. Still images were captured at regular one-minute intervals and opportunistically if specific features of interest (e.g. sea-pens) were encountered. Video and still images were analysed by Envision Mapping Ltd. The collection of video footage and still imagery is described in detail in the survey report (Murray, 2016).

2.2.2 Sediment sampling

Sediment samples for particle size and benthic macrofauna analyses were collected using a ~0.1 m^2 Day grab (10-15 cm penetration depth) and a ~0.1 m^2 NIOZ standard box corer (c. 50 cm penetration depth). Note, however, that while the surface area of both sampling gears is approximately 0.1 m^2, the actual surface area resembles this much more accurately for the Day grab (0.096 m^2) than the NIOZ corer (0.078 m^2). Sub-samples for determining Particle Size Distribution were collected using a 3 cm diameter x 5 cm depth sub-core from each Day grab sample and a 5 cm diameter x 30 cm depth sub-core (sliced at 5 cm intervals down to 20 cm) from each NIOZ corer sample. This reduced sample surface area by 0.01 m^2 for the Day grab and 0.02 m^2 for the NIOZ corer. Sediment was placed in sealed containers and stored at -18°C for later analysis.

Sediment not extracted in the sub-sample was sieved over 1 mm mesh and photographed, with the retained animals fixed in 4% buffered formaldehyde. Macrofaunal samples from both the Day grab and the NIOZ corer were processed and identified by APEM Ltd. All individuals present in each sample were extracted, identified to the lowest taxonomic level possible, enumerated and weighed (blotted wet weight) to the nearest 0.1 mg following the recommendations of the National Marine Biological Analytical Quality Control (NMBAQC) scheme (Worsfold *et al*., 2010). Full details on the collection and processing of Day grab and NIOZ corer samples can be found in Murray (2016).

2.2.3 Sediment Profile Imagery

A Sediment Profile Imaging (SPI) camera was used to capture *in situ* vertical profile images across the sediment-water interface (Rhoads & Cande, 1971; Germano *et al*., 2011). These images were used primarily to assess the geochemical state of the sediment through the identification of the depth of the apparent redox potential discontinuity (aRPD) layer, a visual indication of the 'zone of mixing', along with other physico-chemical properties of the sediment (see section 2.3.3).

The SPI system, manufactured by Ocean Imaging Systems, consisted of a downward facing camera and a water-filled mirrored prism, which was driven down into the sediment as it landed on the seabed. This action triggers the camera system and two images were taken after a 15 second and 30 second delay. At each station the camera was 'hopped' a distance of 5 m, five times along a transect that ran through the centre of a 100 m 'bullring' centred on the sampling station, obtaining a total of 10 images. The system used a Nikon D100 digital camera (F10, 1/60th second, ISO400) with a 35 mm lens and self-contained strobe flash unit, which was downloaded upon recovery at each station. Example images are shown in Figure 4. The aRPD layer or 'zone of mixing' can be seen where the sediment changes colour from brown to black.

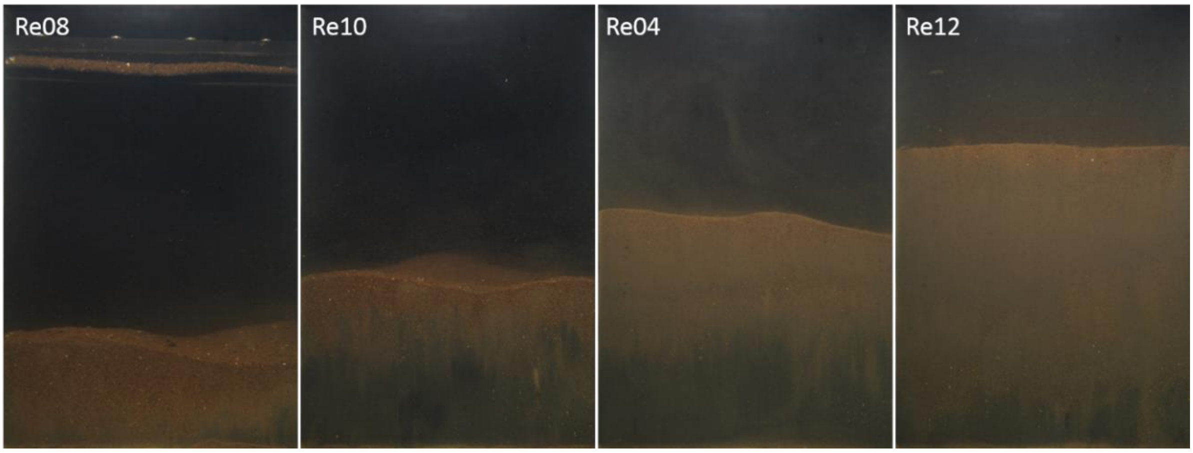

Figure 4. Images collected using the Sediment Profile Imaging (SPI) camera at four stations in the Coquet to St Marys MCZ in 2016. The depth of the apparent redox potential discontinuity (aRPD) layer – where the sediment turns from brown to black – ranges from 4.77 cm to 10.67 cm in these images. © Cefas and Natural England 2016

2.3 Data preparation and analysis

2.3.1 Tidal model production

Mean and maximum tidal current velocities (m s^{-1}) at the seabed and direction of flow at the peak of the flood tide were obtained from a high-resolution depth-averaged model of the North Sea. These data were used to model tidal energy at the site, thus contributing to Report Objective ii (section 1.3.3;

Table 2). The model was built with an unstructured triangular mesh, using the hydrodynamic software Telemac2D (v7p1). The model domain extends between 49.28°N – 60.69°N and 3.73°E – 9.57°W. The unstructured mesh was discretized with 340,000 nodes and 650,000 elements. The mesh has a resolution of 6 km around the open boundary, reducing to ~200 m along the coastline. Within the Coquet to St Mary's MCZ the resolution is refined further to 150 m. Bathymetry for the model was sourced from the Defra Digital Elevation Model (DEM) (Astrium, 2011). The resolution of the bathymetry dataset is one arc second (~30 m). The hydrodynamics are forced along the open boundaries using 11 tidal constituents (M2, S2, N2, K2, K1, O1, P1, Q1, M4, MS4 and MN4) from the OSU TPXO European Shelf 1/30° regional model (OSU Tidal Data Inversion) (Egbert and Erofeeva, 2002). After a spin-up period of 10 days, the model was run for 30 days to cover a full spring-neap cycle. The tidal model outputs were converted to raster as a 30 m cell size regular grid. The modelled mean and maximum tidal current velocities are calculated over the full spring-neap cycle, whereas the modelled peak flood and ebb directions are the instantaneous directions that occur at the timestamp of peak flood and ebb in relation to the centre of the Coquet to St Mary's MCZ.

2.3.2 Sediment Particle Size Distribution

Particle Size Analysis (PSA) of sediment samples was carried out by Cefas following the method set out by the NMBAQC (Mason, 2011) using combined sieves (>1 mm) and laser diffraction (<1 mm) to produce the full Particle Size Distribution (PSD) dataset. These data (half phi classes) were then grouped into the percentage contribution of gravel (>2 mm diameter), sand (0.063–2 mm) and mud (<0.063 mm), based on the classification proposed by Folk (1954), and used assess the sediment composition of 'A5.3 Subtidal mud' inside and outside the MCZ, thus contributing to Report Objective ii (section 1.3.3;

Table 2). This required that each sampling station was first assigned to one of four sedimentary BSHs, based on the proportions of gravel, sand and mud, using a modified version of the classification model produced during the MESH project (Long, 2006):

- ‘A5.1 Subtidal coarse sediment’
- ‘A5.2 Subtidal sand’
- ‘A5.3 Subtidal mud’
- ‘A5.4 Subtidal mixed sediments’

Video footage and stills were also used to identify BSHs potentially present at stations. The BSHs assigned were interpreted with caution, given the limited accuracy of this method and the precise definitions of BSHs with respect to proportions of gravel, sand and mud. As such, the results were mainly used to infer the level of small-scale spatial variability in the seabed habitat (not the exact BSHs that are present).

2.3.3 Physico-chemical properties of the sediment

Images collected using the SPI camera were analysed using a semi-automated Java macro within Image J (Version 1.64r) (Solan *et al*., 2004a). The parameters obtained from this analysis were: the penetration depth of the prism (for the determination of sediment packing), bed surface roughness (the difference in height of the highest and the lowest surface relief from across the sediment-water interface) and the apparent Redox Potential Discontinuity (aRPD) depth (i.e. the ‘zone of mixing’). These metrics were used to indicate the physico-chemical properties that underpin ecosystem functioning, thus contributing to Report Objective ii (section 1.3.3;

Table 2). Of particular note is aRPD depth, which reflects the surficial layer of oxygenated sediment and can be used, for example, as an indicator of relative habitat quality; the deeper the zone of mixing, the more oxygenated the sediment and the higher the quality of habitat (Teal *et al*., 2010).

2.3.4 Epifaunal data preparation

While seafloor imagery was collected at 50 stations, stills data were available for only 48 stations and video data were available for only 41. This is because the image quality was deemed too low for analysis at some stations due to turbidity. Consequently, video data were available for 16 stations within the MCZ, 12 stations in the eastern potential control area and 13 in the southern potential control area. Still imagery data were available for 19, 12 and 17 stations for the MCZ, eastern and southern areas respectively.

The primary purpose of collecting video footage and still images of the seafloor was to assess the presence and distribution of the habitat FOCI 'Sea-pen and burrowing megafauna communities' (Report Objective i; section 1.3.3). Therefore, taxa datasets derived from videos and stills were truncated to include only the species relevant to the identification of this feature; *Virgularia mirabilis, Nephrops norvegicus, Callianassa subterranea, Upogebia stellata and Goneplax rhomboides.* The other taxa recorded were not considered within the broader community analysis because video quality was consistently poor and the quality of still images was highly variable, making the available data unsuitable for quantitatively assessing the presence/density of most epifaunal taxa and broader epifaunal diversity. The lack of suitable epifaunal data is not likely to be of significant detriment to the objective of describing the structural attributes of 'A5.3 Subtidal mud' (Report Objective ii), because: 1) only a small proportion of the benthos lives on the sediment surface within the targeted habitat and would therefore be observable using seafloor imagery (even when image quality is high) and 2) the animals that live below the sediment surface (the infauna) typically dominate total abundance and biomass and can be effectively sampled and quantitatively assessed using other methods utilised for this survey (i.e. the Day grab and NIOZ corer).

In addition to data based on direct observations of sea-pens and burrowing megafaunal taxa, data on the number of *N. norvegicus* burrows (also obtained during analysis of video footage) were used to assess the spatial distribution of this species within the MCZ and surrounding areas. Burrow densities were estimated to determine whether the minimum density required for classification of the habitat FOCI 'Sea-pen and burrowing megafauna communities' (one individual per 10 m^2; JNCC, 2014) was reached. To calculate burrow densities, the number of observed burrows was divided by the transect area, based on the assumption that the area in view throughout the transect was 1 m^2, which is typically the average field of view over the duration of a tow with the drop camera.

If any non-designated habitat or species FOCI were observed in the seafloor imagery datasets prior to truncation, their distributions and abundances within the MCZ and surrounding areas were mapped (Report Objective iii).

2.3.5 Macrofaunal data preparation

Macrofaunal taxa recorded in the Day grab and NIOZ corer samples were checked for the application of consistent and up-to-date nomenclature using World Register of Marine Species (WoRMS) match taxa tool[3]. Any taxa recorded that are not benthic macrofauna (e.g. fish) were removed from the dataset. Juveniles were generally retained in the dataset and their densities (in terms of abundance and biomass) merged with those of adults of the same taxon. Only when juveniles were identified to a lower taxonomic resolution than adults were the juveniles removed from the dataset rather than having to reduce the taxonomic resolution of the adult records. In cases when it was not possible to determine whether one or more individuals of a taxon were present (e.g., with small colonial taxa) an abundance of '1' was assumed (*sensu* Callaway *et al*., 2016; Downie *et al.*, 2016). A full description and rationale for this truncation process is provided in (Annex 3. Macrofauna Data Truncation Protocol).

Following this procedure, macrofauna data were divided into four subsets for analysis:

1) The first subset consisted of data from all stations where three samples were collected using both a Day grab and NIOZ corer and where the BSH in all three samples was determined to be 'A5.3 Subtidal mud'. This allowed data to be statistically analysed in a balanced, fully crossed design (with respect to 'gear type' and 'station') and the relative sampling efficacy of the two gears to be determined. Specifically, the aim was to investigate whether gear penetration depth influences macrofauna samples. This information would help to inform gear selection for future monitoring surveys targeting mud features at Coquet to St. Mary's and other MCZs, thus contributing to Report Objective v (section 1.3.3). A total of 42 samples from seven stations (all within the MCZ) were included in this subset.

2) The second subset consisted of data from all stations where samples were collected in triplicate. The availability of multiple replicates per station allowed within-station community variability to be assessed, which in turn allowed the accuracy with which a single sample represents a macrofaunal community (relative to three samples) to be determined. This information was sought to help determine whether future monitoring might benefit from the collection of multiple replicates per station, thus contributing to Report Objective v. NIOZ corer samples were used rather than Day grab samples because 1) more stations were sampled in triplicate with the former gear, thus creating a larger sample size and 2) the NIOZ corer penetrates deeper into the sediment and so can reach any deep-burrowing organisms, making it at least as suitable as the Day grab for targeting the full macrofaunal community. A total of 45 samples from 15 stations (ten within the MCZ, five in the southern potential control area) were included in this subset.

3) The third subset consisted of data from every station sampled using a Day grab where the BSH was determined to be 'A5.3 Subtidal mud'. This allowed the structure of macrofaunal communities associated with this BSH within the MCZ to be described and

[3] http://www.marinespecies.org/aphia.php?p=match [accessed 30/10/2019]

compared to those in the surrounding area, thus contributing to Report Objective ii (section 1.3.3). This information was also sought to determine whether the benthos within the MCZ is representative of the wider region and to inform the selection of suitable control sites – areas outside the MCZ where the benthos is most similar to the benthos inside the MCZ – for potential future BACI-type monitoring surveys, thus contributing to Report Objective v. Day grab samples were used rather than NIOZ corer samples as most stations outside the MCZ were sampled only using the former gear, which is a standard gear type for such surveys. Because only one Day grab sample was collected from some stations, the second and third samples were dropped from all other stations to produce comparable data for each station. A total of 40 samples from 40 stations (16 within the MCZ, 15 in the southern area, five in the eastern area, and four in the northern area) were included in this subset.

4) The fourth subset consisted of data from every station at which the macrofaunal community was sampled using a Day grab. These data were used to analyse how benthic community composition varies in relation to both BSH type and fine-scale variation in sediment composition (e.g. % mud content), thus contributing to Report Objective ii. This in turn allowed the need to control for spatial variation in sediment characteristics, when monitoring ecological change inside and outside the MCZ, to be assessed, thus contributing Report Objective v. Day grab samples were used rather than NIOZ corer samples to maximise the sample size, spatial extent and the range of sediment compositions covered. As with 3), the second and third samples were dropped from stations where multiple samples were collected to produce comparable data for each station. A total of 56 samples from 56 stations (19 within the MCZ, 4 in the northern control area, 14 in the eastern control area, 19 in the southern control area) were included in this subset.

The distributions and abundances of any non-designated species FOCI recorded in these datasets were presented as described in section 2.3.4 (Report Objective iii).

2.3.6 Community data analysis

Sea-pen and burrowing megafauna communities

The first task of the primary survey objective was to investigate the presence of ‘Sea-pen and burrowing megafauna communities’ within the Coquet to St Mary’s MCZ (Report Objective i). Species present at the site that are of relevance to the identification of this habitat FOCI (see section 2.3.4) were identified using the information presented in Hughes (1998). Burrowing megafauna churn the sediment, which releases nutrients into the overlying water, mixes oxygen into the mud and in turn supports a wide diversity of life, while sea-pens are a conspicuous and fragile component of the epibenthos. The species that characterise this habitat FOCI are therefore also key structural and influential species within the ecosystem. Therefore, information on this FOCI and its foundational species also contributes to Report Objective ii (section 1.3.3;

Table 2).

To assess the spatial distribution of sea-pens and burrowing megafauna, i.e. to complete the primary survey objective (Report Objective i), maps were produced showing the locations where species were observed, the abundances at which they were recorded (typically using counts of individuals, but also burrows where possible), the gears with which they were sampled and the % mud content of the sediment in which they were found. This information was used to identify the locations where burrowing megafauna and sea-pens occurred either alone or together. Stations at which burrows were recorded at or above the density required to confirm the presence of 'Sea-pen and burrowing megafauna communities' according to JNCC guidelines (JNCC, 2014) were also noted.

Macrofaunal assemblages

Macrofaunal assemblages were analysed with respect to sampling gear type, within-station variability, location (inside *vs* outside the MCZ) and their relationships with sediment characteristics using four discrete datasets. The links between these sets of analyses and the Report Objectives are outlined in section 2.3.5. Differences between categories of a variable (e.g. Day grab and NIOZ corer) and relationships with quantitative variables (e.g. % mud content) were considered statistically significant when $p < 0.05$. Community data were transformed by ln(x+1), if necessary, to meet test assumptions. A suite of indices was used to reflect the density and diversity of macrofauna, each of which was selected to suit the data available for each section of the analysis. Note that although both sampling gears cover a surface area of approximately 0.1 m^2, the exact surface area sampled (including the removal of sediment sub-samples) is 0.095 m^2 for the Day grab and 0.076 m^2 for the NIOZ corer. This had to be corrected for when analysing abundance and biomass for the gear comparison and limited the approaches that could be used to compare diversity. A full description of the approach to each component of the macrofaunal community analysis is provided in Annex 4. Macrofaunal Community Analysis Methods All univariate analyses were conducted in R (version 3.4.1, R Core Team 2017) and multivariate analyses of assemblage composition were conducted in PRIMER (version 6; Clarke and Gorley, 2006).

2.3.7 Non-indigenous species

The untruncated taxa datasets derived from Day grabs, NIOZ corers, and seafloor imagery were cross-referenced against a list of 49 non-indigenous species (NIS) which have been selected for assessment of Good Environmental Status in British waters under MSFD Descriptor 2 (Stebbing *et al.,* 2014; Annex 5. Non-Indigenous Species (NIS)**Error! Reference source not found.**; Report Objective iv). The list includes two categories: species which are already known to be present within the assessment area (present) and species which are not yet thought to be present but have a perceived risk of introduction and impact (horizon). An additional list of taxa, which were identified as invasive in the 'Non-native marine species in British waters: a review and directory' (Eno *et al*., in 1997) was also used to cross reference against all taxa observed (Annex 5. Non-Indigenous Species (NIS).

2.3.8 Marine litter

During both sample collection and the processing of samples in the laboratory, the presence of marine litter was noted in accordance with the requirements of the MSFD Descriptor 10 (Report Objective iv).

Annex 4. Macrofaunal Community Analysis Methods

3 Results and Interpretation

3.1 Tidal model

The Coquet to St Mary's MCZ mainly experiences weak (< 0.5 m s^{-1}) tidal currents flowing on a north-south axis along the Northumbria coast (Figure 5). Average current velocity is relatively high in the northeast of the MCZ (which includes most of the MCZ survey area) and the north of the eastern potential control area (0.25–0.5 m s^{-1}) compared to the rest of the region (< 0.25 m s^{-1}). Currents within the MCZ and most of the surrounding areas reach a speed of up to 1.5 m s^{-1} during periods of maximum velocity (Figure 5). Currents reach a lower maximum velocity of 0.25–0.5 m s^{-1} in the south of the southern potential control area.

3.2 Physical characteristics of the sediment

Of the 79 sediment samples collected using a Day grab, 60 were classified as 'A5.3 Subtidal mud', 14 as 'A5.2 Subtidal sand', four as 'Subtidal mixed sediments', and one as 'A5.1 Subtidal coarse sediment'. Both mixed and coarse sediments were generally low in gravel content (< 20%); however, one station with mixed sediments had a gravel content of approximately 40% (Figure 6). Of the 45 sediment samples collected using a NIOZ corer, 39 were classified as 'A5.3 Subtidal mud' and six were classified as 'A5.2 Subtidal sand'. The BSH classification of each station based on Day grab and NIOZ corer samples is shown in Annex 6. Broadscale Habitats (BSHs) identified at each sampling station using different sampling methodologies

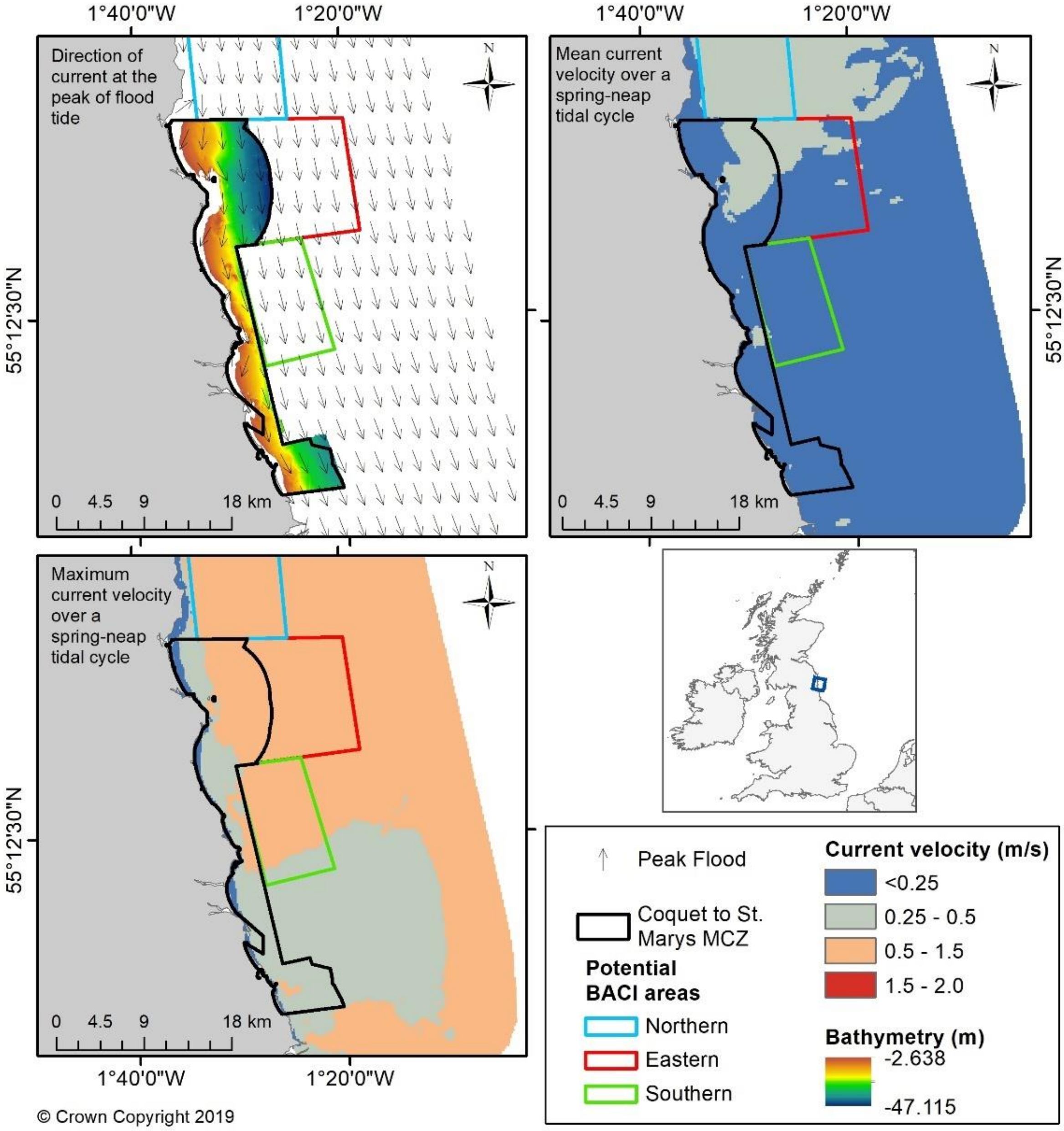

Figure 5. The direction and mean and maximum tidal current velocities in the Coquet to St Mary's MCZ and surrounding areas.

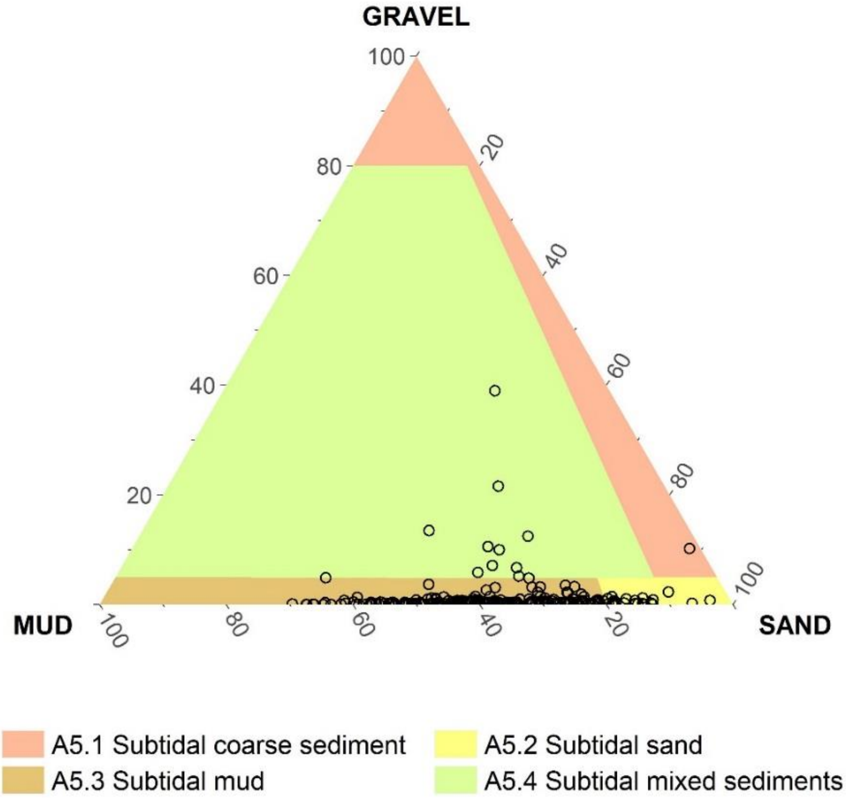

Figure 6. Classification of Particle Size Distribution (half phi) information for each sampling point (hollow black circles) into one of the Sedimentary Broadscale Habitats (coloured areas) plotted on a true scale subdivision of the Folk triangle into the simplified classification for UK SeaMap (Long, 2006; Folk, 1954) (© Natural England and Cefas 2022). Data on Particle Size Distribution are derived from 0.095 m^2 Day grab and 0.076 m^2 NIOZ corer (0-5 cm sediment depth) samples collected during a survey of the Coquet to St Mary's MCZ in 2016.

Particle Size Analysis (PSA) of Day grab samples verified the predicted general trend of decreasing % mud content with increasing distance offshore (i.e. into the eastern potential control area), with all samples with > 56 % mud content coming from within 5 km of the coast (Figure 7a & b). The area of high mud content in the southwest of the southern potential control area was also verified (Figure 7b). Surficial (0-5 cm) NIOZ corer samples showed similar spatial variation in mud content (Figure 7c); however, there were fewer offshore samples collected using this gear.

Stations located inside the MCZ were mainly classified as 'A5.2 Subtidal sand' based on the analysis of video footage; however, the other three sedimentary BSHs were also represented (Figure 7d). All four sedimentary BSHs were recorded in the southern potential control area, but most stations were classified as 'A5.3 Subtidal mud', whereas in the eastern potential control area there were no records of subtidal mud and the most commonly observed BSH was subtidal sand (Figure 7d). Video footage also indicated that habitat type was generally homogenous along transects within stations (Figure 7d). However, at one station inside the MCZ (Re12) the transect covered both subtidal mud and subtidal sand (Figure 7d) and at one station in the southern potential control area (GT34) there was a small area of rock along a transect that otherwise covered only subtidal mud (blue ring, Figure 7d).

BSH derived from PSA of Day grab samples was broadly consistent with that derived from video footage in the southern potential control area, with some exceptions (Figure 7e). In the eastern potential controlled area, most stations were classified as ‘A5.2 Subtidal sand’ based on Day grab samples, which was also the outcome of video analysis (Figure 7e). However, these two sampling methods were generally used at different stations in the eastern potential control area, and where both methods were used at the same station they indicated different BSHs (Figure 7e). Within the MCZ survey area, the dominant BSH based on Day grab samples was ‘A5.3 Subtidal mud’ (Figure 7e), which conflicts with the observations made from video footage and reflects the spatial pattern in % mud content that emerged from analysis of Day grab samples. Generally, where the two sampling methods produced inconsistent results, video footage indicated ‘coarser’ BSHs than were found when the sediment was sampled with a Day grab. Given the limited accuracy of visually assessing habitat type, we note that the BSHs inferred from video footage should be interpreted with caution (see sections 2.3.2 and 4.2.1).

When BSHs were determined using surficial (0-5 cm) NIOZ corer samples, stations were mainly classified as ‘A5.3 Subtidal mud’ (Figure 7f), which is to be expected given that this is the only BSH targeted by this gear. However, two stations located close to the MCZ eastern boundary were classified as ‘A5.2 Subtidal sand’ based on PSA analysis of NIOZ corer samples (Figure 7f), thus providing further support for the apparent decrease in mud content and increase in sand content with increasing distance offshore. These stations were classified as ‘A5.3 subtidal mud’ based on Day grab samples (Figure 7e).

At most stations, there was very little variation in Particle Size Distribution in relation to gear or sediment depth, and where there were clear differences (e.g. stations Re05 and Re12) the modes remained at the same size classes. In all cases, most mud within samples was in the silt fraction (2 µm-63 µm) rather than the clay fraction (< 2 µm). An example of the Particle Size Distribution (i.e. the % of each sediment grain size class) for all sediment samples collected using the both Day grab and NIOZ corer slices (0-5 cm depth, 5-10 cm, 10-15 cm, and 15-20 cm) is provided for station Re01 in Figure **8**. The full PSA distributions for all stations sampled using both gears are presented in Annex 7. Sediment particle size distributions of sampling stations within the Coquet to St Mary’s MCZ and surrounding areas

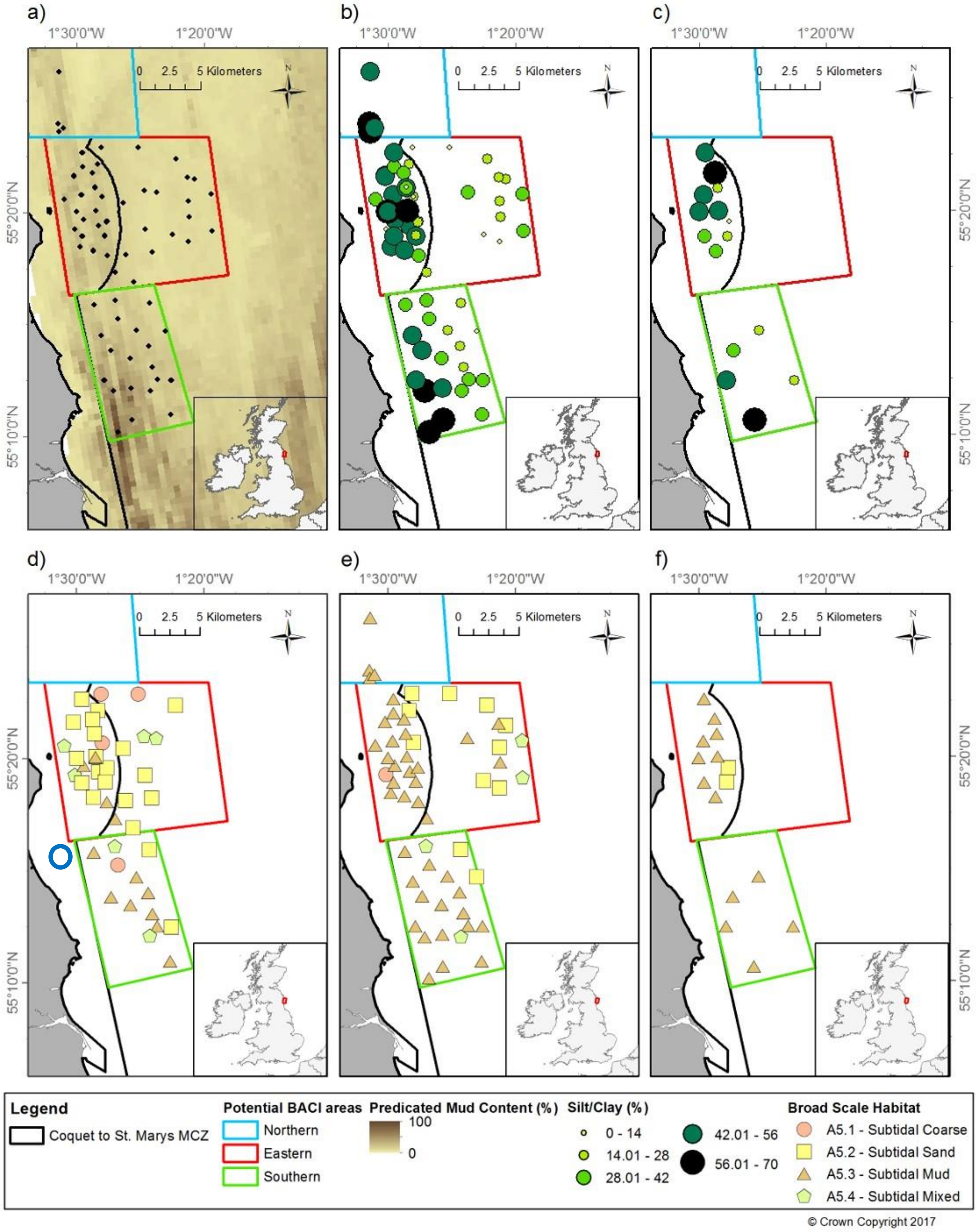

Figure 7. Maps of sediment type within and around the Coquet to St Mary's MCZ, showing: a) station locations in relation to the modelled % mud content (Stephens 2015; Stephens and Diesing, 2015), b) % mud content based on 0.095 m^2 Day grab samples, c) % mud content based on 0.076 m^2 NIOZ corer samples (0-5 cm sediment depth), d) BSH distribution from seafloor imagery (a small area of rock was observed at station GT34; blue ring), e) BSH distribution based on 0.095 m^2 Day grab samples, and f) BSH distribution based on 0.076 m^2 NIOZ corer samples (0-5 cm sediment depth (© Natural England and Cefas 2022).

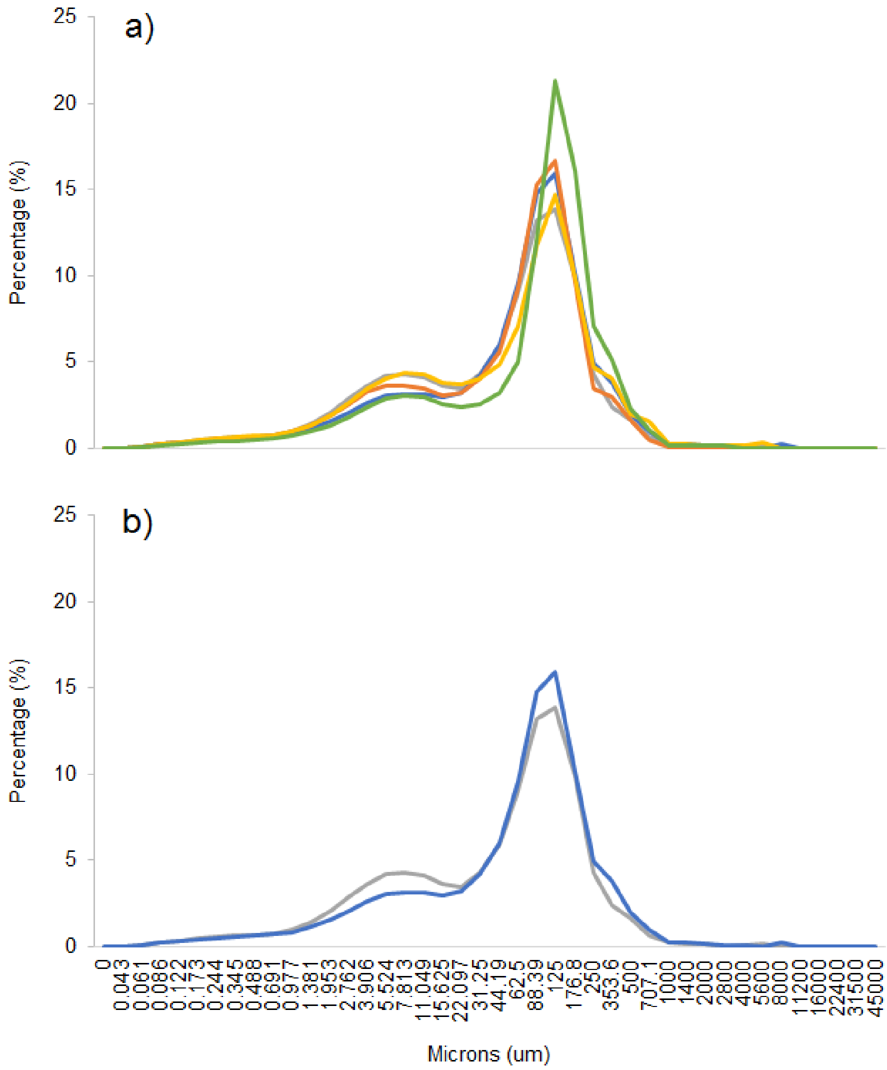

Figure 8. Particle size distributions for station Re01 within the Coquet to St Mary's MCZ in 2016 based on: a) 0.095 m^2 Day grab samples (■) and 0.076 m^2 NIOZ corer sample slices (0-5 cm ■, 5-10 cm ■, 10-15 cm ■, and 15-20 cm ■ sediment depth) and b) 0.095 m^2 Day grab samples (■) and 0.076 m^2 NIOZ corer samples for surface sediment only (0-5 cm ■) (© Natural England and Cefas 2022).

At some stations, there was a trend of sediments becoming muddier as depth increased, with mud content at 5-10 cm and/or 10-15 cm often higher than in surficial (0-5 cm) sediments (Figure 9). However, sediment often became less muddy at the greatest depth category considered (15-20 cm; Figure 9). The five stations containing the lowest overall mud content across the four depth categories were also some of the furthest offshore, reaffirming indications from Day grab samples and surficial NIOZ corer samples that mud content decreases with distance from the coast (Figure 7). There was generally little variation in mud content among samples from the same station compared to samples from different stations (Figure 9), suggesting that sediments tend to be somewhat homogenous at small spatial scales (i.e. individual stations) within the region.

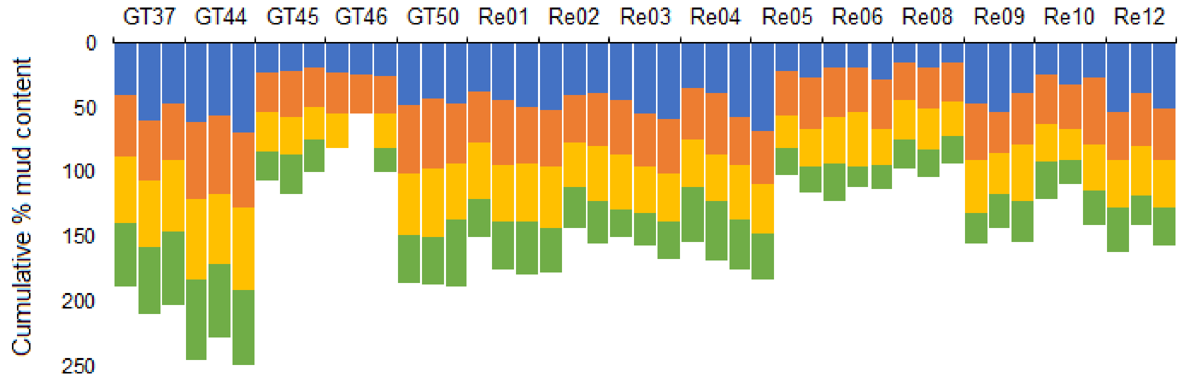

Figure 9. Cumulative % mud content of sediment from slices of replicate 0.076 m^2 NIOZ corer samples (0-5 cm ■, 5-10 cm ■, 10-15 cm ■, and 15-20 cm ■ sediment depth) collected from stations within the Coquet to St Mary's MCZ (Re01–Re12) and the southern potential control area (GT37–GT50) in 2016 (© Natural England and Cefas 2022).

Within the Coquet to St Mary's MCZ, the mud and sand contents estimated from Day grab samples were positively correlated with the mud and sand contents estimated from NIOZ corer (0-5 cm) samples, respectively (Figure 10a); however, the relationships were weak for both mud and sand (R^2 = 13%). The largest difference between sampling gears was at station Re05, where mud content was > 28% higher when based on the NIOZ corer (0-5 cm) samples than when based on the Day grab samples. The smallest difference was at station Re02, where there was < 1% difference in mud content between gears. All Day grab samples were classified as 'A5.3 Subtidal mud'. With the NIOZ corer (0-5 cm slice), stations Re06 and Re08 were classified as 'A5.2 Subtidal sand' and the rest were classified as 'A5.3 Subtidal mud'. However, there was more variation between gears in sedimentary habitat type when the Folk classification was used instead of the EUNIS classification, with three stations (Re05, Re09, and Re12) producing different classifications depending on gear type (Table 3).

The depth of the apparent redox potential discontinuity (aRPD) layer varied by < 3 cm (4.77–7.57 cm) throughout most of the MCZ, apart from at station Re12 where aRPD was > 3 cm deeper than observed at any other station (10.67 cm) (Table 3). There were no clear relationships between aRPD depth and % mud content for either Day grab samples or NIOZ corer (0-5 cm) samples (Figure 10b), suggesting that sediment biogeochemistry may not be strongly influenced by spatial variation in mud content within the MCZ. While penetration depth of the SPI prism had a clear positive relationship with % mud content (R^2 = 29% for both Day grab and NIOZ corer samples (Figure 10c), aRPD depth was nonetheless successfully measured at all stations, (Table 33) and therefore variation in aRPD depth in relation to mud content was not limited by penetration depth. Surface roughness of the sediment decreased with increasing % mud content (Figure 10d), suggesting that muddier, more consolidated sediments are not as compacted as the coarser sediments and therefore more susceptible to erosion, which is also implied by the greater penetration depth in muddy sediments.

Table 3. Summary of sediment profile imagery (SPI) analysis output, showing apparent redox potential discontinuity (aRPD) depth, penetration depth of the SPI prism, sediment surface roughness and associated particle size analysis (PSA) data from the Coquet to St Mary's MCZ in 2016 (© Natural England and Cefas 2022). Data on sediment composition are based on a single 0.095 m^2 Day grab sample and a single 0.076 m^2 NIOZ corer sample (0-5 cm sediment depth) from each station. For Folk classifications of habitat type, mS = muddy sand and sM = sandy mud.

	SPI (5 hop average)			NIOZ (0-5cm)					Day grab				
Station Code	aRPD (cm)	Penetration Depth (cm)	Surface Roughness (cm)	Folk Symbol	EUNIS Group	% Gravel	% Sand	% Mud	Folk Symbol	EUNIS Group	% Gravel	% Sand	% Mud
Re01	5.59	14.91	1.23	mS	mud and sandy mud	0.53	62.36	37.1	mS	mud and sandy mud	0.31	55.35	44.34
Re02	6.56	14.06	1.40	sM	mud and sandy mud	0.26	48.41	51.33	sM	mud and sandy mud	0.22	49.14	50.64
Re03	7.06	11.00	1.42	mS	mud and sandy mud	0.47	55.63	43.9	mS	mud and sandy mud	0.28	61.78	37.94
Re04	6.22	12.98	1.56	mS	mud and sandy mud	0.12	65.99	33.89	mS	mud and sandy mud	0.09	67.86	32.05
Re05	5.58	8.24	1.31	sM	mud and sandy mud	0.02	32.58	67.4	mS	mud and sandy mud	0.17	60.91	38.93
Re06	5.02	5.85	2.01	mS	sand and muddy sand	0.09	80.83	19.08	mS	mud and sandy mud	0.3	73.45	26.25
Re08	4.77	4.82	1.89	mS	sand and muddy sand	0.16	85.63	14.21	mS	mud and sandy mud	0.13	73.92	25.96
Re09	7.57	14.99	0.70	mS	mud and sandy mud	0.21	53.3	46.49	sM	mud and sandy mud	0.35	35.42	64.23
Re10	5.97	8.71	1.29	mS	mud and sandy mud	0.52	75.69	23.79	mS	mud and sandy mud	0.18	56.26	43.56
Re12	10.67	14.65	0.42	sM	mud and sandy mud	0.41	46.12	53.47	mS	mud and sandy mud	0.12	74.11	25.77

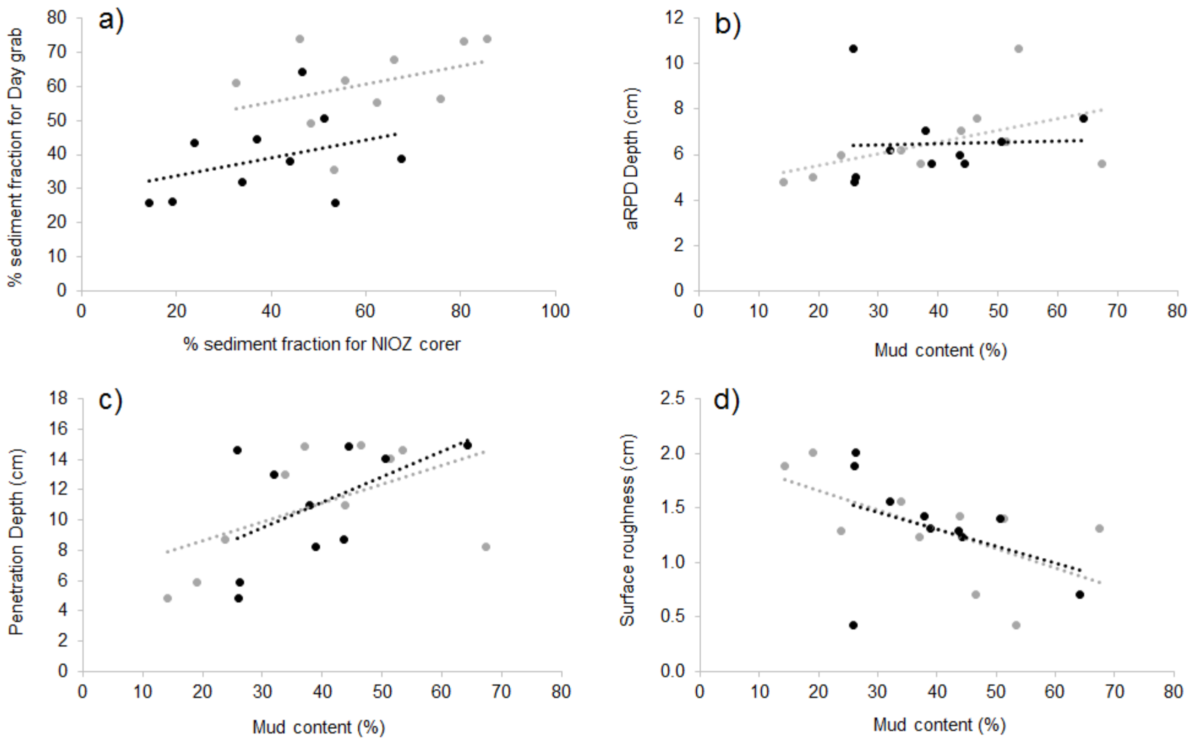

Figure 10. Relationships between physical properties of the sediment in the Coquet to St Mary's MCZ in 2016 (© Natural England and Cefas 2022), showing a) % mud (black ●) and % sand content (grey ●) in 0.095 m^2 Day grab vs 0.076 m^2 NIOZ corer (0-5 cm) samples at sites where sediment profile imaging was conducted, b) apparent redox potential discontinuity (aRPD) depth in relation to % mud content derived from 0.095 m^2 Day grab (black ●) and 0.076 m^2 NIOZ corer (0-5 cm) (grey ●) samples, c) penetration depth of the sediment profile imagery (SPI) prism in relation to % mud content derived from 0.095 m^2 Day grab (black ●) and 0.076 m^2 NIOZ corer (0-5 cm) (grey ●) samples and d) surface roughness in relation to % mud content derived from 0.095 m^2 Day grab (black ●) and 0.076 m^2 NIOZ corer (0-5 cm) (grey ●) samples.

3.3 Community composition and diversity

3.3.1 Sea-pen and burrowing megafauna communities

The key species with respect to the primary objective of this report (Report Objective i) are those used to identify the presence of the habitat FOCI 'Sea-pen and burrowing megafauna communities'. In the datasets analysed here, these species include the slender sea-pen *Virgularia mirabilis*, the Norway lobster *Nephrops norvegicus*, the mud shrimps *Callianassa subterranea* and *Upogebia stellata* and the angular crab *Goneplax rhomboides*. The stations where species were observed, the densities of individuals recorded at each station and the gears with which they were sampled are shown in Figure 11.

Sea-pens *V. mirabilis* were found mainly at four stations within the centre of the MCZ survey area (Figure 11a) and were typically observed using seafloor imagery (videos and stills) (e.g.,

Figure 12). Their abundances were particularly high at stations Re12 (56 individuals observed along the video transect) and GT71 (37 individuals). Two sea-pens were observed at Re10 (in still images only) and another two were found at station Re02 (in a NIOZ corer sample; no imagery data were collected at this station). *V. mirabilis* was also observed at one station outside the MCZ in the southern potential control area (GT37, five individuals in both the video footage and stills; Figure 11a). No sea-pens were observed in the eastern potential control area. There were also none observed in the northern area, but no imagery data were collected at the four northern stations.

N. norvegicus individuals and/or burrows were commonly observed in seafloor imagery (mainly video footage) throughout much of the MCZ survey area, the southern potential control area and the southwest corner of the eastern potential control area (Figure 11b). However, *Nephrops* burrows only occurred above the critical density for classification of the habitat FOCI 'Sea-pen and burrowing megafauna communities', (i.e. one individual per 10 m^2; JNCC, 2014), at three stations within the MCZ (Re04, GT71, and Re01), one in the southern area (GT37) and one station in the eastern area, which sits close to the MCZ boundary (GT26). *N. norvegicus* individuals were not recorded in Day grab or NIOZ corer samples at any of these stations. Sediment samples taken from each of these stations were classified as 'A5.3 Subtidal mud'; however, % mud was variable (20-55%) and it was noted during the inspection of video footage that the substrate at these stations appeared sandier than is typically associated with the 'Sea-pen and burrowing megafauna communities' FOCI. Where SPI imagery data were available for stations with *N. norvegicus* burrows above the critical density (Re01 and Re04), the depth of the aRPD layer ('zone of mixing') was not notably different in comparison to other stations (Table 3). Only at stations GT71 (inside the MCZ) and GT37 (in the southern potential control area) were *Nephrops* burrows observed both above the critical density and in association with sea-pens, though the latter is not an essential criterion for the identification of this FOCI (JNCC, 2014). *N. norvegicus* was not observed in the southwest part of the southern potential control area, where mud content was particularly high (Figure 11b). However, video data were not available for this region (i.e. stations GT43 and GT44) due to poor visibility. The stations where *N. norvegicus* burrows were observed in video footage, the areas in which stations are located, the associated BSHs and % mud content and the estimated burrow densities are shown in **Error! Reference source not found.**.

Of the other burrowing megafauna, *C. subterranea* was most commonly sampled in the MCZ using the NIOZ corer (1-2 individuals per grab), but individuals were also observed in the southern area in three NIOZ corer samples and one Day grab sample (Figure 11c). *U. stellata* was observed just once, in a Day grab sample, at the seaward extremity of the eastern potential control area (Figure 11d). One *G. rhomboides* individual was found in a NIOZ corer sample in the southern area (Figure 11e).

The stations where burrowing megafauna were observed, either alone or in association with sea-pens and whether these stations were classified as 'Sea-pen and burrowing megafauna communities' with respect to the critical *N. norvegicus* burrow density are shown in Figure 11f.

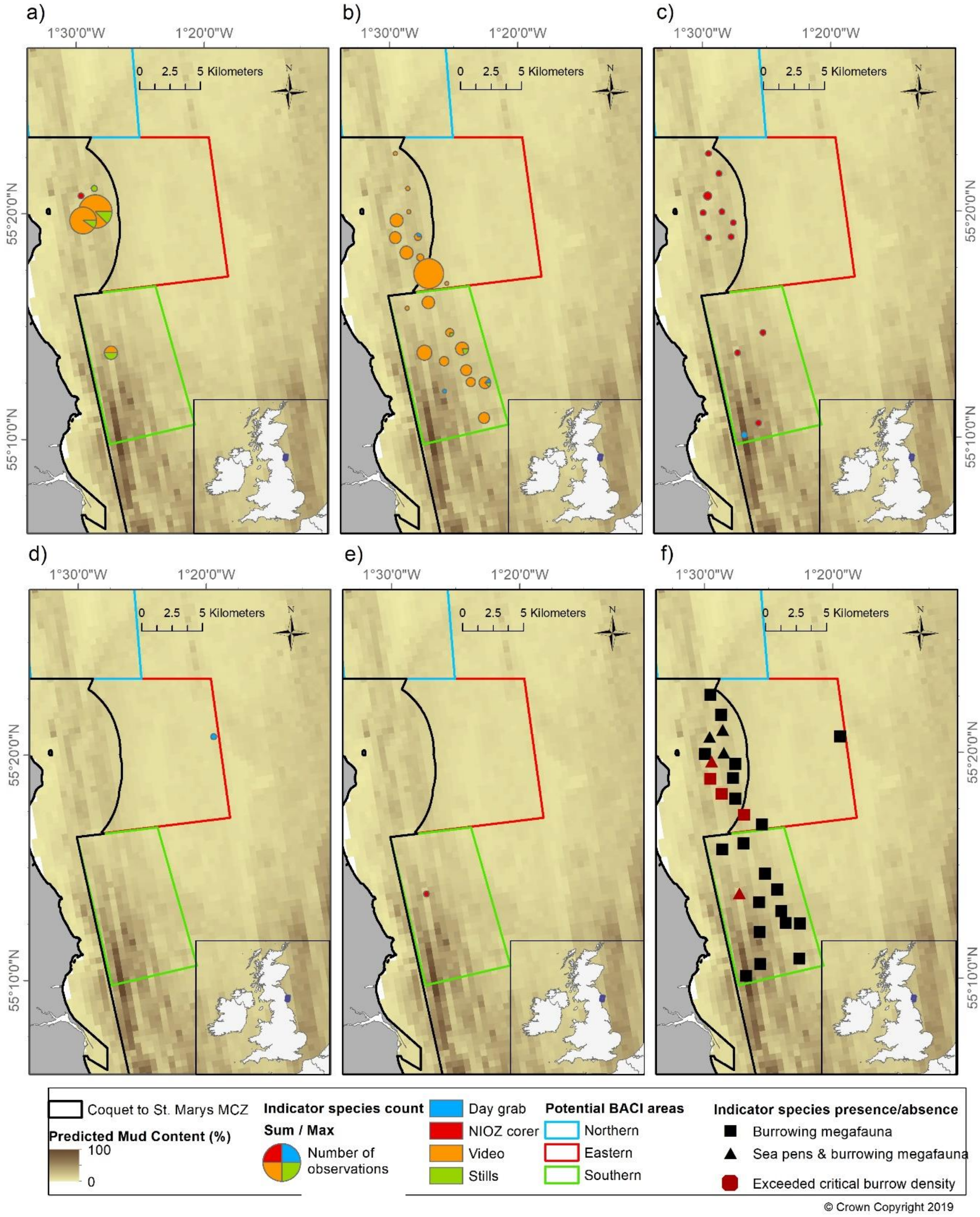

Figure 11. Species distributions, the gears with which species were sampled, and the proportional number of observations at each station (larger pie = more observations) for a) *Virgularia mirabilis* b) *Nephrops norvegicus* (records for video are based on burrow counts rather than the number of observed individuals) c) *Callianassa subterranea* d) *Upogebia stellata*, e) *Goneplax rhomboides* and f) all sea-pens and burrowing megafauna (presence/absence) throughout the region. In f), stations where *N. norvegicus* burrow density exceeded the critical burrow density of one individual per 10 m^2 are marked red. Modelled % mud content of the sediment (Stephens 2015; Stephens and Diesing, 2015) is also shown.

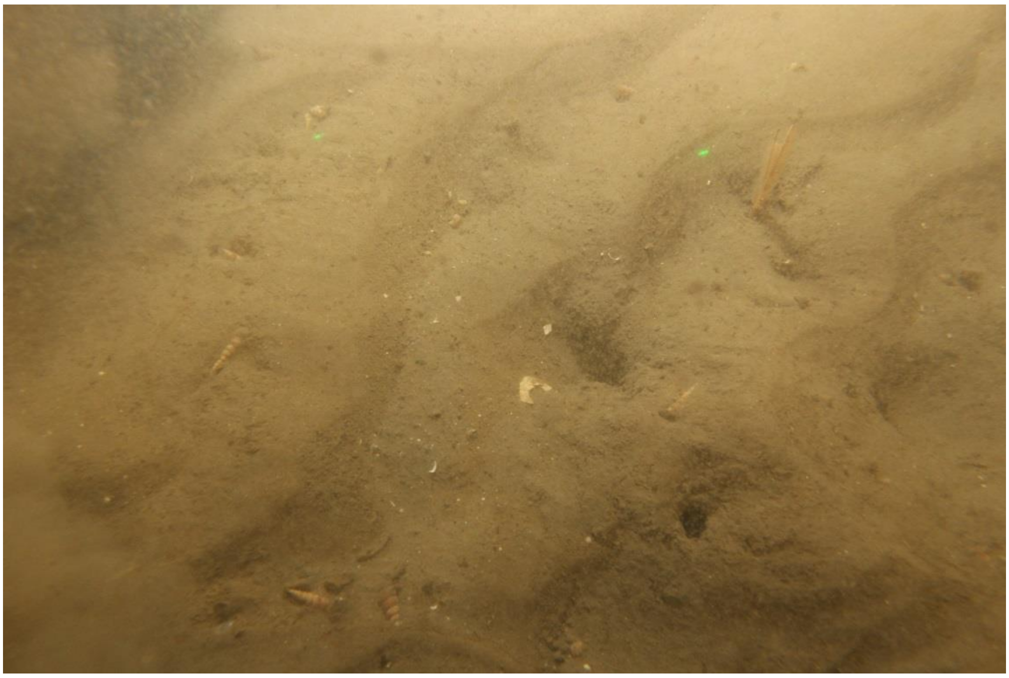

Figure 12. Example image of sea-pens (*Virgularia mirabilis*) and burrowed mud (*Nephrops norvegicus*) taken during a survey of the Coquet to St Mary's MCZ and surrounding areas in 2016. © Cefas and Natural England 2016.

Table 4. Stations where *Nephrops norvegicus* burrows were observed during video transects in 2016, the areas in which they were found (inside the MCZ and in eastern and southern potential control areas), the Broadscale Habitats (BSH) '(A5.2 Subtidal sand', 'A5.3 Subtidal mud' and 'A5.4 Subtidal mixed sediments') and associated % mud content (based on 0.095 m^2 Day grab samples), the number of burrows observed and the estimated burrow density (© Natural England and Cefas 2022). Stations above the dashed line had burrow densities that meet the criteria for classification of the habitat FOCI 'Sea-pen and burrowing megafauna communities' (JNCC, 2014). BSH was based on visual interpretation of seafloor imagery for station GT28* as no grab samples were collected from this station.

Station code	Area	BSH	% mud	Estimated transect area (m^2)	Number of burrows observed	Burrows per 10 m^2
GT26	Eastern	A5.3	20.97	96	51	5.3
GT37	Southern	A5.3	55.40	94	13	1.4
Re04	MCZ	A5.3	41.15	92	10	1.1
GT71	MCZ	A5.3	46.39	94	10	1.1
Re01	MCZ	A5.3	48.39	80	8	1.0
GT35	Southern	A5.4	37.37	96	9	0.9
GT39	Southern	A5.3	27.62	100	8	0.8
GT42	Southern	A5.3	22.79	92	7	0.8
GT38	Southern	A5.3	33.13	92	7	0.8
GT46	Southern	A5.3	37.63	106	6	0.6
GT41	Southern	A5.3	29.71	96	5	0.5
GT36	Southern	A5.3	35.50	102	5	0.5
GT45	Southern	A5.3	23.00	88	3	0.3
GT19	MCZ	A5.3	39.94	91	3	0.3
GT34	Southern	A5.3	40.30	36	1	0.3
Re06	MCZ	A5.3	31.50	94	2	0.2
Re10	MCZ	A5.3	30.78	63	1	0.2
GT28	Eastern	A5.2*	-	93	1	0.1
Re03	MCZ	A5.3	40.55	98	1	0.1
Re12	MCZ	A5.3	46.90	108	1	0.1

3.3.2 Macrofaunal communities

The sections below contain the results of analyses focused on benthic macrofaunal communities. The first section is a gear comparison, assessing the relative efficacy of the Day grab and NIOZ corer at sampling the benthos in 'A5.3 Subtidal mud'. The second assesses within-station variability in macrofaunal communities to determine how accurately a single sample represents the benthos at a location. The third section compares macrofaunal communities inside and outside the MCZ to determine whether the benthos within the MCZ is representative of that of the surrounding area and identify suitable control areas during potential BACI-type monitoring of the subtidal mud feature. The fourth section assesses how macrofaunal communities vary in relation to gradients in sediment components that determine Broadscale Habitat type (i.e. mud, sand, and gravel fractions) and other sediment properties that reflect habitat structure (sorting, skewness, and kurtosis).

Gear comparison

As this analysis was conducted to determine how a greater gear penetration depth affects macrofauna samples from 'A5.3 Subtidal mud' specifically (see section 2.3.5), stations where any samples were classified as a different BSH were excluded from the analysis (resulting in seven out of ten stations being used). All stations used in this analysis are located inside the MCZ. This analysis contributes to Report Objective v (see section 2.3.5, data subset 1).

Total abundance, biomass, and diversity

A total of 3606 individuals, 117 g of biomass and 131 taxa were recorded in 21 macrofauna samples collected from ‘A5.3 Subtidal mud’ using a Day grab, compared to 2456 individuals, 73 g of biomass and 132 taxa in 21 samples collected using the NIOZ corer. The univariate indices selected for the gear comparison (total abundance, total biomass, the predicted total number of taxa (*Smax*) and Gini-Simpson diversity) were those that are comparable between gears after correcting for their different sample surface areas (see ‘Gear comparison’ section of Annex 4. Macrofaunal Community Analysis Methods). Mean total abundance was slightly higher in Day grab samples than NIOZ corer samples, whereas Gini-Simpson diversity showed the opposite pattern (Table 5; Figure 13a and 13d). Mean total biomass and *Smax* were not significantly different between the two sampling gears (Table 5; Figure 13b and 13c).

Table 5. General linear model summary of variation in total abundance (N; individuals m^{-2}), total biomass (B; g m^{-2}, transformed by ln(x+1)), predicted total number of taxa (*Smax*) and Gini-Simpson diversity of macrofauna samples in relation to sampling station and sampling gear (0.095 m^2 Day grab samples vs 0.076 m^2 NIOZ corer samples) at the Coquet to St Mary’s MCZ in 2016 (© Natural England and Cefas 2022). Significant results (p < 0.05) are highlighted in bold.

	Total N			Total B			*Smax*			Gini-Simpson		
	d.f.	F	*p*	d.f.	F	*p*	d.f.	F	*p*	d.f.	F	*p*
Station	6,34	1.10	0.381	6,34	1.71	0.150	6,6	1.31	0.376	6,34	3.37	**0.010**
Gear	1,34	4.29	**0.046**	1,34	3.02	0.091	1,6	0.93	0.371	1,34	6.15	**0.018**

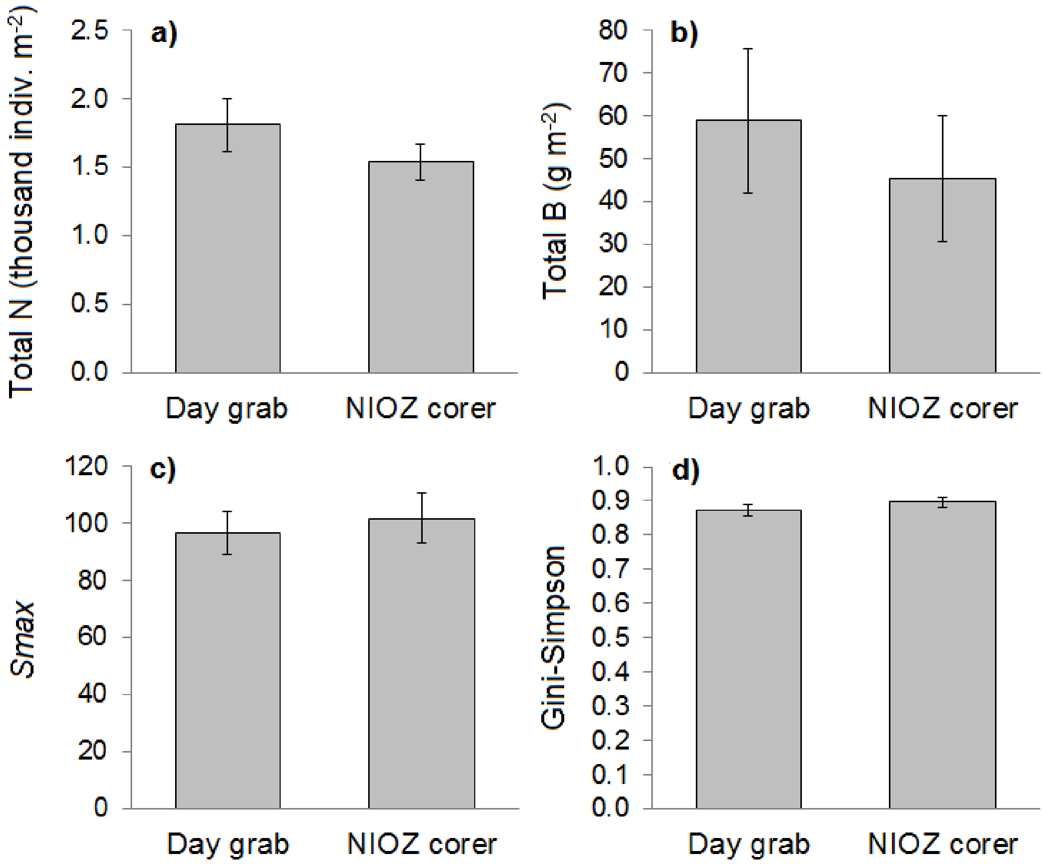

Figure 13. Mean and 95% confidence intervals of: a) total abundance (N; individuals m^{-2}), b) total biomass (B; g m^{-2}), c) predicted total number of taxa (*Smax*) and d) Gini-Simpson diversity of macrofauna based on 0.095 m^2 Day grab samples and 0.076 m^2 NIOZ corer samples (n = 21) collected at the Coquet to St Mary's MCZ in 2016 (© Natural England and Cefas 2022).

When species accumulation curves were produced for each gear using station-level data (i.e. the number of taxa pooled across all three samples at a station) to predict the total number of taxa in 'A5.3 Subtidal mud' within the MCZ, the Day grab and NIOZ corer produced almost identical results, with *Smax* estimated at 153.04 and 153.31 respectively. The average number of taxa recorded by each gear as the number of sample stations increased (in absolute terms and as a proportion of *Smax*) is shown in Table 6.

Table 6. Michaelis-Menton model output showing the number of taxa recorded as the number of sampling stations increases within the Coquet to St Mary's MCZ (© Natural England and Cefas 2022). Results are shown for 0.095 m^2 Day grab samples and 0.076 m^2 NIOZ corer samples, with taxa numbers presented in absolute terms and as a proportion of the total number of taxa predicted for the MCZ (*Smax*). *Smax* was 153.03 for the Day grab and 153.31 for the NIOZ corer.

Number of stations	Number of taxa		% of *Smax*	
	Day grab	NIOZ corer	Day grab	NIOZ corer
1	63.5	63.2	41.5	41.2
2	89.8	89.5	58.7	58.4
3	104.1	104.0	68.1	67.8
4	113.2	113.1	74.0	73.7
5	119.4	119.3	78.0	77.8
6	123.9	123.9	81.0	80.8
7	127.4	127.4	83.3	83.1

Community composition (abundance-based)

When the Bray-Curtis similarity index was based on abundance (with taxa abundances transformed by ln (x+1)), the same taxa characterised samples collected using the Day grab and NIOZ corer, with the brittle star *Amphiura filiformis*, the polychaetes *Lumbrineris* spp. and *Peresiella clymenoides* and the bivalve *Thyasira flexuosa* contributing ~25% of sample similarity for each gear type. Average sample similarity for the full community was 60% for the Day grab and 56% for the NIOZ corer.

Although Day grab and NIOZ corer samples were mainly characterised by the same taxa, assemblages sampled using the two gears could be separated statistically (Analysis of Similarity (ANOSIM): R = 0.296, p = 0.004). These differences were small however, as illustrated by the high-level of overlap between gears in a two-dimension ordination of community composition (Figure 14a). Of the 97 taxa that contributed 90% of between gear dissimilarity, 54 were recorded in higher abundances by the Day grab and 43 were recorded in higher abundances by the NIOZ corer. The major contributors to between gear dissimilarity were the polychaetes *Galathowenia oculata*, *Podarkeopsis capensis* and *Scoloplos (Scoloplos) armiger*, the nemertean *Cerebratulus* spp., the gastropod *Cylichna cylindracea* and the bivalve *Kurtiella bidentata*; however, these taxa together contributed just 10% of overall sample dissimilarity. *P. capensis* was recorded in higher abundances by the NIOZ corer, whereas the other species were recorded in higher abundances by the Day grab.

Community composition (biomass-based)

Day grab and NIOZ corer samples were also characterised by the same taxa when the Bray-Curtis similarity index was based on biomass; however, these taxa differed from those that characterised samples based on abundance. The gastropod *Turritella communis*, the sea cucumber *Leptopentacta elongata* and the bivalve *Chamelea striatula* were among the major contributors to sample similarity. *Lumbrineris* spp. was the only taxon among the top four contributors to sample similarity for both biomass-based and abundance-based assessments of community composition. Together, these taxa contributed 56% of sample similarity for both gear types. Overall sample similarity based on biomass was 51% for the Day grab and 39% for the NIOZ corer. Biomass-based community composition was not significantly different between Day grab and NIOZ corer samples (ANOSIM: R = 0.021, p = 0.396; Figure 14b).

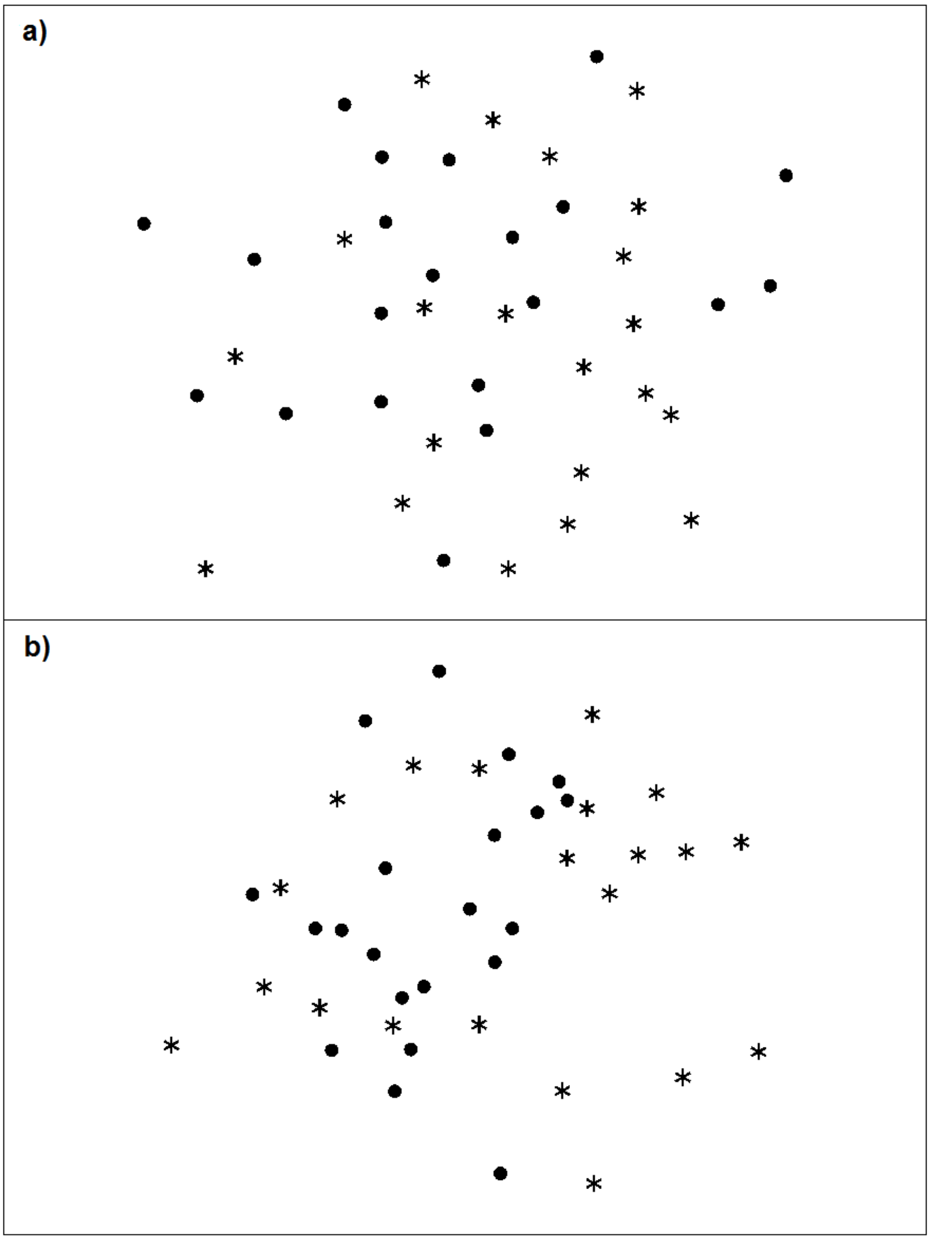

Figure 14. Non-metric multidimensional scaling ordinations of macrofaunal taxa composition derived from 0.095 m² Day grab samples (circles ●) and 0.076 m² NIOZ corer samples (stars ✱) at the Coquet to St. Mary's MCZ in 2016 (© Natural England and Cefas 2022). Ordinations are based on Bray-Curtis similarity of a) taxa abundances and b) taxa biomasses. Abundances and biomasses were transformed by ln (x+1) prior to calculating Bray-Curtis similarity of samples. Two-dimensional stress = 0.30 for a) and 0.23 for b).

Deep-burrowing organisms

A total of 15 taxa were identified as deep-burrowing organisms. Eleven of these were recorded in higher abundances (individuals m^{-2}) by the NIOZ corer, including all taxa that live mainly at depths greater than 10 cm (i.e. spend little time in surface sediments) (

Table 7). Three such taxa (the prawn *Callianassa subterranea*, the sipunculid *Golfingia (Golfingia) elongata* and the bivalve *Mya truncata*) were recorded only in NIOZ corer samples. The sea-pen *Virgularia mirabilis* was also only recorded using the NIOZ corer. This species typically protrudes above the seafloor but can withdraw deep into its burrow when disturbed. Of the taxa that were recorded in higher abundances using the Day grab (the polychaetes *Abyssoninoe hibernica*, *Glycera alba*, *Scalibregma inflatum* and *Scoloplos (Scoloplos) armiger*), all four commonly inhabit surface sediments as well as being capable of burrowing to depth (

Table 7).

Table 7. Abundances of deep-burrowing taxa in 0.095 m^2 Day grab samples and 0.076 m^2 NIOZ corer samples (n = 21 for each gear) at the Coquet to St. Mary's MCZ in 2016 (© Natural England and Cefas 2022).

Taxon	Life zone	Day grab		NIOZ corer	
		count	indiv. m^{-2}	count	indiv. m^{-2}
Abyssoninoe hibernica	Shallow to > 10 cm	1	0.5	0	0.0
Callianassa subterranea	Usually > 10 cm	0	0.0	7	4.4
Enteropneusta	Shallow to > 10 cm	9	4.5	19	11.9
Glycera alba	Shallow to > 10 cm	30	15.0	18	11.3
Glycera unicornis	Shallow to > 10 cm	8	4.0	11	6.9
Golfingia (Golfingia) elongata	Usually > 10 cm	0	0.0	1	0.6
Leptopentacta elongata	Surface to > 10 cm	35	17.5	32	20.1
Leptosynapta	Shallow to > 10 cm	7	3.5	6	3.8
Mya truncata	Usually > 10 cm	0	0.0	2	1.3
Ophelina acuminata	Shallow to > 10 cm	1	0.5	2	1.3
Scalibregma inflatum	Shallow to > 10 cm	5	2.5	1	0.6
Scoloplos (Scoloplos) armiger	Shallow to > 10 cm	35	17.5	16	10.0
Thracia convexa	Usually > 10 cm	6	3.0	5	3.1
Thracia phaseolina	Usually > 10 cm	3	1.5	3	1.9
Virgularia mirabilis	Surface to > 10 cm	0	0.0	2	1.3

Within-station variability

Assessment of within-station variability in macrofauna was conducted using NIOZ corer samples because more stations were sampled in triplicate using this gear than using the Day grab (15 vs 10); all stations sampled in triplicate with the Day grab samples were also sampled using the NIOZ corer; and the NIOZ corer penetrates deeper into the sediment and will therefore be at least as effective as the Day grab at capturing the full macrofaunal community (as indicated by the assessment of deep-burrowing organisms in the section above). Moreover, analysis of macrofaunal diversity and composition showed only minor differences between the two gears. Therefore, NIOZ corer results can be considered as applicable to both gears. This analysis contributes to Report Objective v (see section 2.3.5, data subset 2).

Total abundance, biomass, and diversity

Mean total abundance of macrofauna ranged from 706 individuals m^{-2} at station GT44 to 2425 individuals m^{-2} at station Re06 (Table 8). These two stations also had the lowest and highest mean total biomass, at 16 g m^{-2} and 119 g m^{-2} respectively (Table **8**). Likewise, station GT44 had the lowest taxon richness (23 taxa per sample; Table **8**) and Margalef diversity (5.6; Table 8), while station Re06 had the highest values for both of these indices (47 and 9.7 respectively; Table 8).

Total abundance generally showed a degree of consistency among samples collected from the same station, with the coefficient of variation (CV) low (< 25%) at 13 stations and medium (25-49%) at the remaining two (Table 8). Total biomass showed greater within-station variability, with most stations having a medium to high (25-100%) CV, one station having a low CV and another having a very high (> 100%) CV (Table 8). Taxon richness showed similar patterns to total abundance, with CV low at 13 stations and medium at two (Table 8). Variability in Margalef diversity was low for all stations (Table 8).

There was generally a higher level of within-station variability in sediment characteristics than in macrofaunal communities and there were no clear shared patterns of variability between biological and physical variables (Table 8). However, % mud content showed the least variability (CV was low for 12 stations) and the stations where CV was medium or high for % mud content were associated with a high or very high CV in total biomass.

Table 8. Mean total abundance (N; individuals m^{-2}), total biomass (B; g m^{-2}), taxon richness (taxa per 0.076 m^2 sample), Margalef diversity of macrofauna, and % mud content, sorting, skewness, and kurtosis coefficients of the sediment for triplicate 0.076 m^2 NIOZ corer samples collected from stations within the Coquet to St. Mary's MCZ (Re01–Re12) and southern potential control area (GT37–GT50) in 2016 (© Natural England and Cefas 2022). Stations with a low (< 25%) coefficient of variation (CV) are in green, medium (25-49%) CV are in blue, high (50-100%) CV are in purple, and very high (> 100%) CV are in red.

Station	Macrofaunal community				Sediment characteristics			
	Total N	Total B	Taxon richness	Margalef	% mud	Sorting	Skewness	Kurtosis
GT37	1092	35	35	7.7	49	328	11.3	229
GT44	706	16	23	5.6	62	269	15.1	323
GT45	1123	58	36	7.8	21	427	7.0	95
GT46	1504	63	47	9.7	24	751	6.5	61
GT50	2118	72	42	8.1	45	167	8.8	156
Re01	1693	42	38	7.7	44	293	10.9	186
Re02	1303	35	35	7.4	43	204	9.9	168
Re03	1518	19	34	6.9	52	679	10.0	140
Re04	1474	61	41	8.6	43	206	7.7	132
Re05	1588	27	42	8.6	38	216	11.8	249
Re06	2425	119	41	7.8	22	252	6.5	98
Re08	1496	107	37	7.7	16	203	5.3	78
Re09	1640	99	39	8.0	46	228	8.8	123
Re10	1425	18	35	7.3	27	427	12.6	217
Re12	1557	36	35	7.2	47	300	8.4	129

Species accumulation curves indicated that on average, a single sample contained 51-61% of the taxa recorded in all three samples at a station and 27-42% of the estimated total number of taxa at a station (*Smax*) (Table 9). Therefore, while a single sample underestimated the number of taxa by up to 50% compared to three samples, the underestimation was by a similar amount for each station (≤ 10% variation among stations), suggesting that a single sample per station may be sufficient to produce comparable data for taxon richness. Variability across stations was slightly greater however, when considering the difference between the number of taxa in a single sample compared to *Smax* (≤ 15% variation among stations).

Table 9. The average number of macrofaunal taxa in one, two, and three 0.076 m^2 NIOZ corer samples collected at each station within the Coquet to St. Mary's MCZ (Re01–Re12) and southern potential control area (GT37–GT50) in 2016, the predicted total number of taxa at each station (*Smax*), and the number of taxa recorded in the first sample as a percentage of the number in three samples and as a percentage of *Smax* (© Natural England and Cefas 2022).

	Number of Taxa				1 sample vs 3 samples	1 sample vs *Smax*
	1 sample	2 samples	3 samples	*Smax*		
GT37	35	51	61	96	57%	36%
GT44	23	36	45	84	51%	27%
GT45	36	52	64	105	56%	34%
GT46	47	71	87	150	54%	31%
GT50	42	59	71	106	59%	40%
Re01	38	56	67	106	57%	36%
Re02	35	51	63	104	56%	34%
Re03	34	49	59	92	58%	37%
Re04	41	58	69	102	59%	40%
Re05	42	61	73	114	58%	37%
Re06	41	60	71	111	58%	37%
Re08	37	55	68	114	54%	32%
Re09	39	57	70	113	56%	35%
Re10	35	53	64	107	55%	33%
Re12	35	49	57	81	61%	43%

Community composition (abundance-based)

When Bray-Curtis similarity was based on abundance, the average similarity of samples collected from the same station ranged from 43% at station GT44 to 62% at station GT50 (Table 10). Thirteen of fifteen stations had an average within-station sample similarity of < 60%, indicating that a single sample is unlikely to accurately capture abundance-based community composition at most stations. SIMPROF showed that samples separated into six clusters that were significantly different at $p < 0.05$ (Figure 15). Most samples occurred within the same cluster as others from the same station, with the exceptions of samples from stations GT46 and Re05 (Figure 15). This suggests that, while there is a degree of within-station variability among samples, a single sample will usually be sufficient to determine whether stations are different in terms of abundance-based community composition.

Table 10. The average Bray-Curtis similarity (%) of macrofaunal community composition, based on ln(x+1) transformed taxa abundances and biomasses, in triplicate 0.076 m^2 NIOZ corer samples from stations within the Coquet to St. Mary's MCZ (Re01–Re12) and southern potential control area (GT37–GT50) in 2016 (© Natural England and Cefas 2022).

	GT37	GT44	GT45	GT46	GT50	Re01	Re02	Re03	Re04	Re05	Re06	Re08	Re09	Re10	Re12
Abundance	53	43	53	48	62	58	53	60	55	54	59	53	58	55	58
Biomass	32	11	22	30	36	65	21	22	17	24	49	57	68	36	39

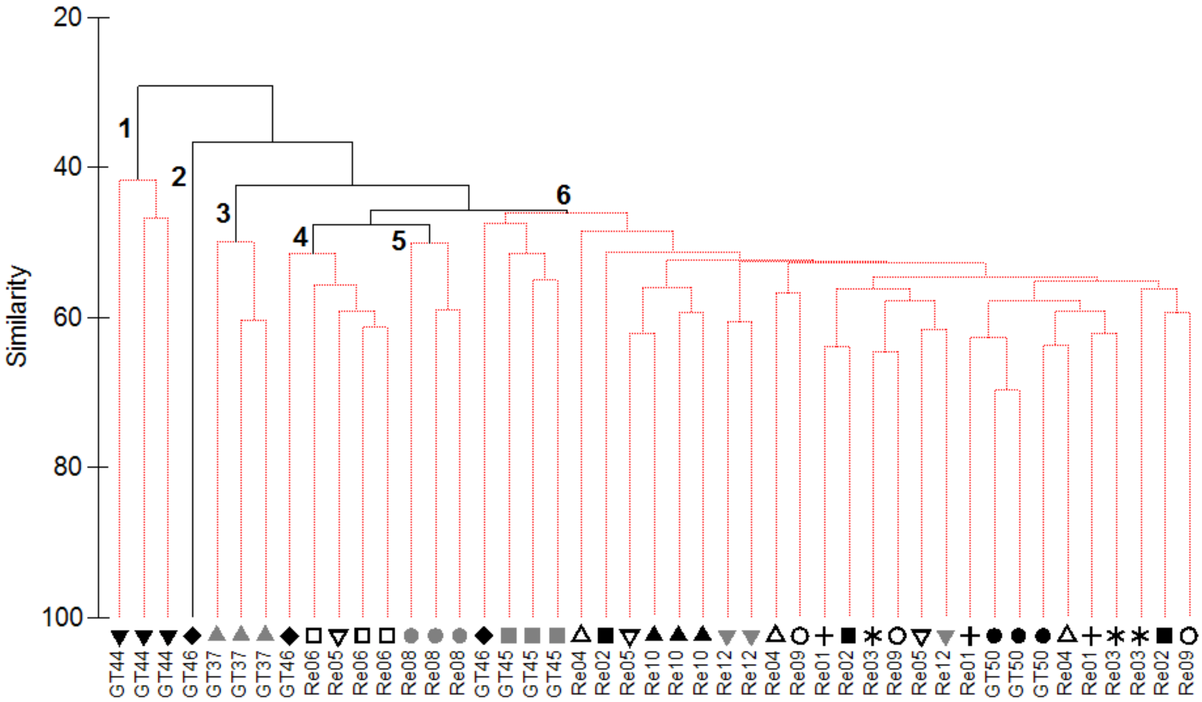

Figure 15. Dendrogram of macrofaunal community composition, based on ln(x+1) transformed taxa abundances, of all three 0.076 m2 NIOZ corer samples collected from each station within the Coquet to St. Mary's MCZ (Re01–Re12) and southern potential control area (GT37–GT50) in 2016 (© Natural England and Cefas 2022). Distinct clusters (significantly different at $p < 0.05$) are separated by black branches, labelled 1-6. Samples within the same cluster (not significantly different from each other) are separated by red branches.

Community composition (biomass-based)

When the Bray-Curtis similarity index was based on biomass, the average similarity of samples collected from the same station ranged from 11% at station GT44 to 68% at station Re09 (Table 10). Thirteen of fifteen stations had an average within-station sample similarity of up to 60% and eleven of up to 40%, indicating that a single sample is likely to give a highly inaccurate representation of biomass-based community composition at most stations. SIMPROF showed that samples divided into five clusters that were significantly different at $p < 0.05$ (Figure 16). Eight stations (i.e. over 50%) had samples that were distributed across different clusters (Figure 16), indicating that a single sample cannot be reliably used to determine whether stations are different in terms of biomass-based community composition.

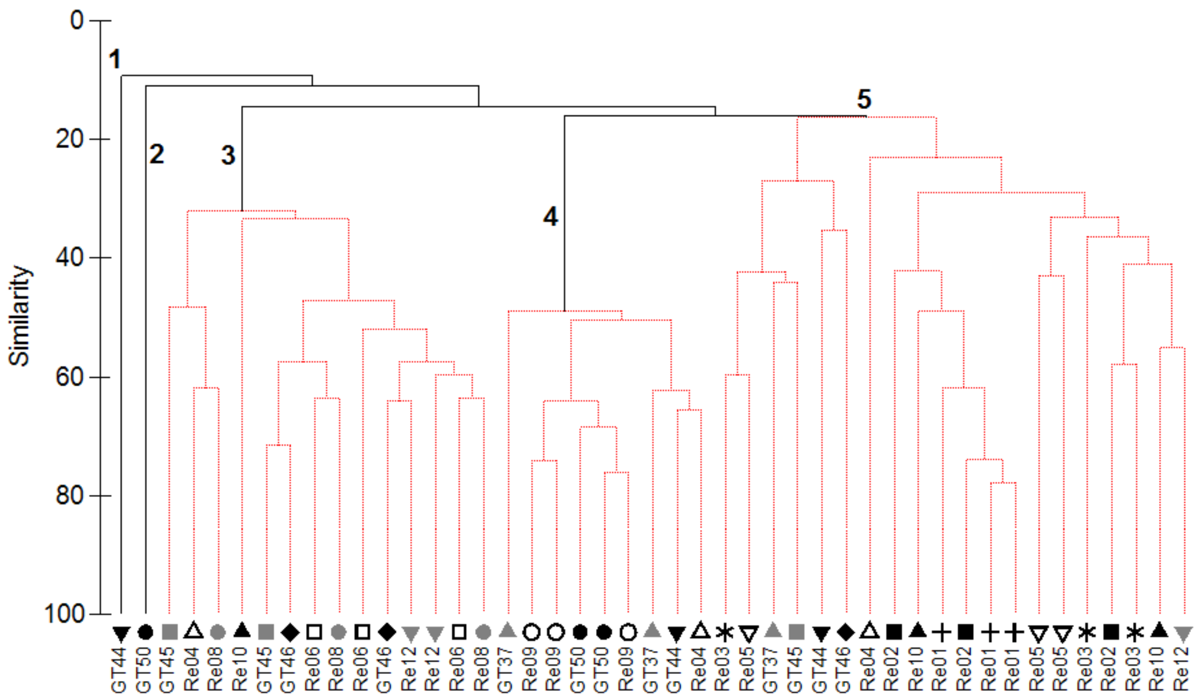

Figure 16. Dendrogram of macrofaunal community composition, based on ln(x+1) transformed taxa biomasses, of all three 0.076 m^2 NIOZ corer samples collected from each station within the Coquet to St. Mary's MCZ (Re01–Re12) and southern potential control area (GT37–GT50) in 2016 (© Natural England and Cefas 2022). Distinct clusters (significantly different at *p* < 0.05) are separated by black branches, labelled 1-5. Samples within the same cluster (not significantly different from each other) are separated by red branches.

Inside vs outside MCZ

Macrofaunal communities inhabiting 'A5.3 Subtidal mud' located inside the Coquet to St Mary's MCZ were compared to those in three areas outside the MCZ, i.e. two potential BACI control areas immediately south and east of the MCZ and an area to the north of the MCZ within the Berwickshire and Northumberland Coast SAC. Comparisons were made using Day grab samples, as NIOZ corer samples were collected mainly inside the MCZ, thus preventing comparisons using data derived from this gear. As the results of the gear comparison show that the Day grab and NIOZ corer produce similar data for macrofaunal diversity and composition, the results of this section are expected to apply to both gears. This analysis contributes to Report Objectives ii & v (see section 2.3.5, data subset 3).

Total abundance, biomass, and diversity

Macrofaunal communities in ‘A5.3 Subtidal mud’ inside the MCZ did not differ significantly from those outside the MCZ for the univariate biodiversity indices considered (Table 11; Figure 17). This in part reflects high variability among samples collected from the same area (Figure 17). Mean total abundance inside the MCZ was more similar to the adjacent northern and southern areas than the eastern area (Figure 17a). In contrast, mean total biomass inside the MCZ was most similar to the eastern area, but biomass was highly variable both within and across all areas (Figure 17b). Communities inside the MCZ and in the adjacent southern area were highly alike in terms of mean taxon richness and Margalef diversity (Figure 17c and d).

Table 11. General linear model summary of variation in total abundance (N; individuals m^{-2}), total biomass (B; g m^{-2}, transformed by ln(x+1)), taxon richness (taxa per 0.095 m^2 Day grab sample) and Margalef diversity of macrofauna inhabiting ‘A5.3 Subtidal mud’ inside the Coquet to St Mary’s MCZ and in three areas adjacent to the MCZ (north, east and south of the MCZ survey area) in 2016 (© Natural England and Cefas 2022). Significant results (*p* < 0.05) are highlighted in bold.

Total N			Total B			Richness			Margalef		
d.f.	F	*p*	d.f.	F	*p*	d.f.	F	*P*	d.f.	F	*p*
3,36	1.30	0.096	3,36	0.77	0.517	3,36	2.16	0.110	3,36	2.28	0.096

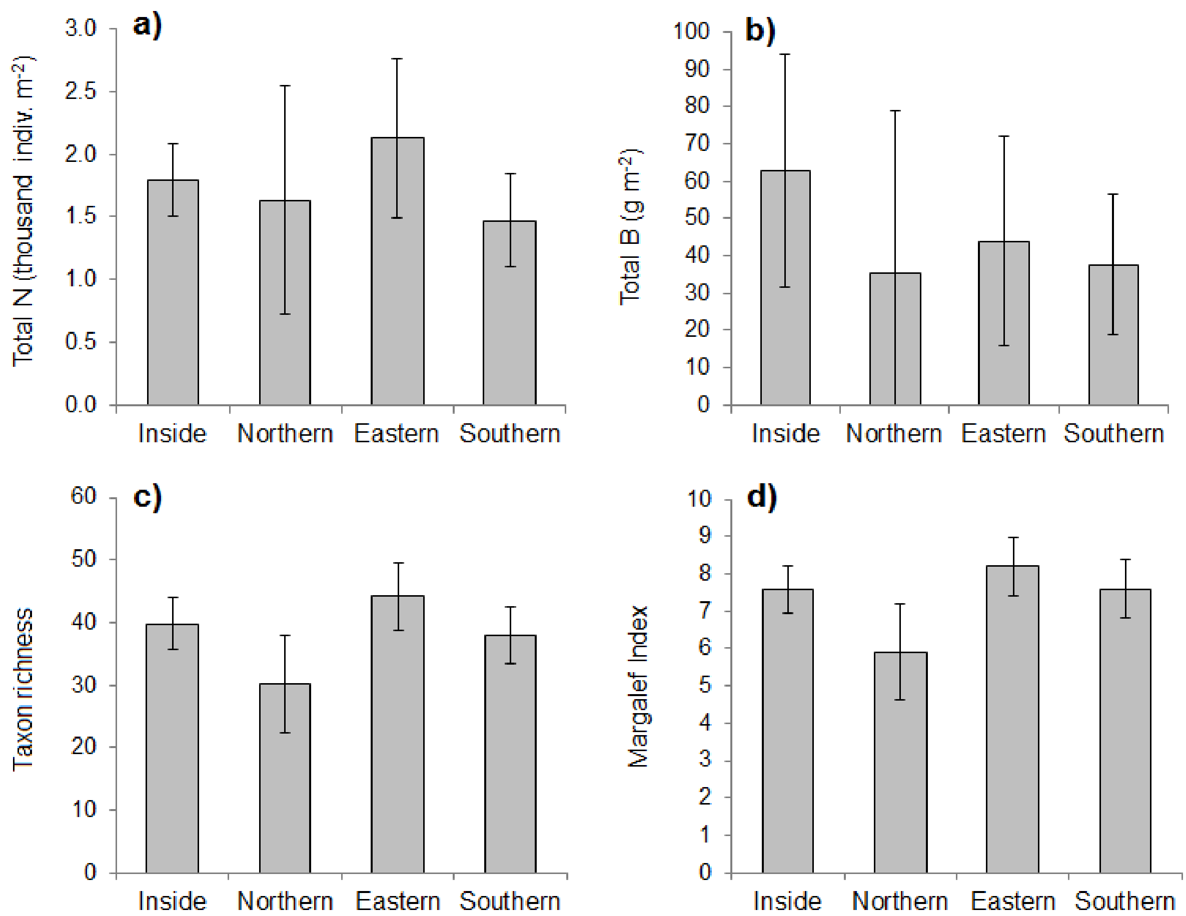

Figure 17. Mean and 95% confidence intervals of: a) total abundance (N; individuals m^{-2}), b) total biomass (B; g m^{-2}), c) taxon richness (taxa per 0.095 m^2 Day grab sample) and d) Margalef diversity of macrofauna inhabiting 'A5.3 Subtidal mud' inside the Coquet to St Mary's MCZ (n = 16) and in adjacent areas to the north (n = 5), east (n = 4) and south (n = 13) of the MCZ in 2016 (© Natural England and Cefas 2022).

Community composition (abundance-based)

Macrofaunal communities inhabiting subtidal mud inside the MCZ were characterised by the polychaetes *Lumbrineris* spp. and *Peresiella clymenoides*, the brittle star *Amphiura filiformis* and the bivalve *Chamelea striatula*, which contributed 27% of sample similarity. These taxa were also the four most abundant within the community. Average sample similarity for the full community was 52%.

The composition of macrofauna in subtidal mud inside the MCZ differed significantly from macrofauna in subtidal mud in each of the three areas outside the MCZ (ANOSIM: Global R = 0.278, $p < 0.001$; Figure 18). However, the difference was largest in relation to the northern area (R = 0.551, average dissimilarity = 58%) and smallest in relation to the southern area (R = 0.165, average dissimilarity = 53%). The taxa that distinguished communities inside the MCZ from those outside the MCZ were highly variable across the surveyed areas, with *P. clymenoides* the only consistent major contributor to between-area dissimilarity. In all cases, this species occurred in higher abundances inside the MCZ. Despite the differences in macrofaunal community composition inside and outside the MCZ, all four survey areas were characterised and numerically dominated by *Lumbrineris* spp. and *A. filiformis*.

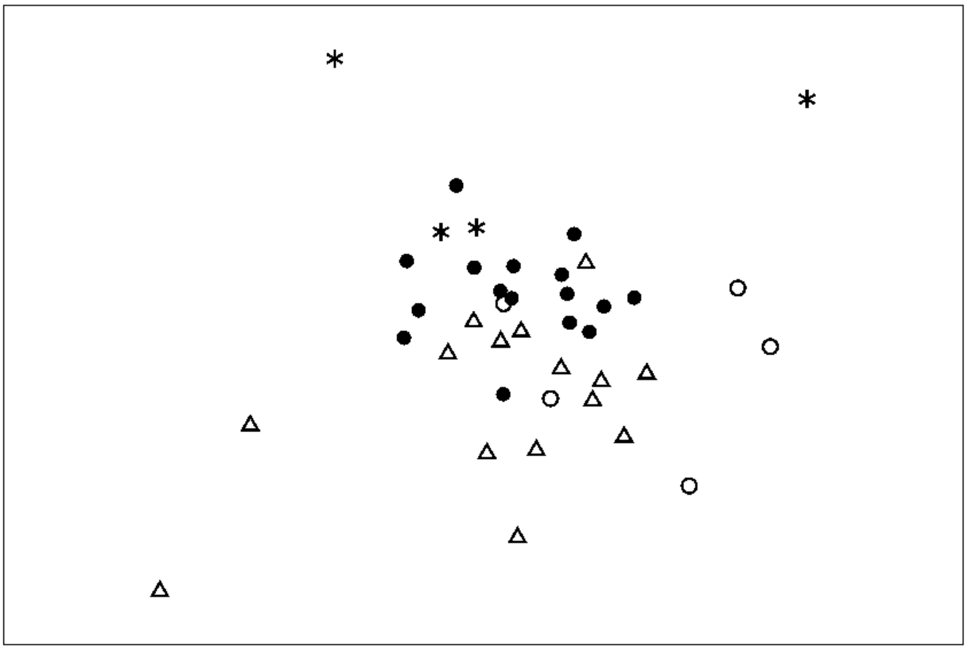

Figure 18. Non-metric multidimensional scaling ordination of macrofaunal community composition in 'A5.3 Subtidal mud' at stations located inside the Coquet to St Mary's MCZ (solid circles ●) and adjacent areas to the north (stars ✱), east (hollow circles ○) and west (hollow triangles △) in 2016 (© Natural England and Cefas 2022). The ordination is based on Bray-Curtis similarity of ln(x+1) transformed taxa abundances in 0.095 m^2 Day grab samples. Two-dimensional stress = 0.20.

Influence of sediment composition

An analysis of the relationships between macrofaunal communities and sediment composition was conducted using Day grab samples. A small number of stations were sampled using the NIOZ corer compared to the Day grab (15 vs 56), all of which targeted burrowed mud and therefore these data were not suitable for assessing variation in macrofaunal assemblages across the range of sediment compositions within the surveyed areas. As the results of the gear comparison show that the Day grab and NIOZ corer produce similar data for macrofaunal diversity and composition, the results of this section are expected to apply to both gears. This analysis contributes to Report Objectives ii & v (see section 2.3.5, data subset 4).

Total abundance, biomass, and diversity

Macrofaunal communities in 'A5.3 Subtidal mud' did not differ significantly from those in 'A5.2 Subtidal sand' or 'A5.4 Subtidal mixed sediments' in terms of total abundance or total biomass (Table 12; Figure 19a and b). Macrofaunal taxon richness and Margalef diversity in subtidal mud were also not significantly different from subtidal mixed sediments, but were significantly higher than subtidal sand (Table 12; Figure 19c and d). Total abundance, taxon richness and Margalef diversity were all highly variable in subtidal mixed sediments (Figure 19a, c, and d), which may be due to the small number of samples collected in this BSH (n=4) compared to subtidal sand (n=11) and subtidal mud (n=40) or the broader range of sediment compositions that are categorised as this BSH (Figure 6). ‘A5.1 Subtidal coarse sediment’ wasn’t included in the analysis as only one sediment sample was classified as this BSH. However, the values of all biotic indices for the single subtidal coarse sediment sample were lower than the means for subtidal mud.

Table 12. General linear model summary of differences in the total abundance (N; individuals m^{-2}), total biomass (B; g m^{-2}), taxon richness (taxa per 0.095 m^2 Day grab sample) and Margalef diversity of macrofaunal communities inhabiting ‘A5.3 Subtidal mud’ compared to those inhabiting ‘A5.2 Subtidal sand’ and ‘A5.4 Subtidal mixed sediments’ within the Coquet to St Mary’s MCZ and adjacent areas in 2016 (© Natural England and Cefas 2022). All biotic indices were transformed by ln(x+1) prior to analysis. Significant results (*p* < 0.05) are highlighted in bold.

	Total N (indiv. m^{-2})			Total B (g m^{-2})			Taxon richness			Margalef		
	d.f.	F	*p*	d.f.	F	*p*	d.f.	F	*p*	d.f.	F	*p*
	2,52	2.44	0.097	2,52	1.50	0.232	2,52	4.49	**0.016**	2,52	4.70	**0.013**
A5.3 vs *A5.2*			0.392			0.094			**0.035**			**0.018**
A5.3 vs *A5.4*			0.064			0.599			0.086			0.142

Analysis of how biotic indices vary in relation to fine-scale variation in sediment properties indicated that total biomass, taxon richness and Margalef diversity all followed significant unimodal trends with increasing % mud content (**Error! Reference source not found.**; Figure 20a, c, and e). The same trends were also observed in relation to % sand content (Figure 20b, d, and f); however, sand content was removed as an explanatory variable from the model due to its strong, negative correlation with mud content (R^2 = 98%). Total biomass, taxon richness and Margalef diversity all peaked when mud content was 30–40% and sand content was 60–70% (Figure 20), which falls within the range of particle size distributions of the BSH ‘A5.3 Subtidal mud’ (Figure 6). In contrast to the other biotic indices, total abundance was not significantly related to mud content (**Error! Reference source not f ound.**). No biotic indices were significantly related to any of the other sediment properties considered (% gravel content, sorting, skewness and kurtosis) (**Error! Reference source n ot found.**).

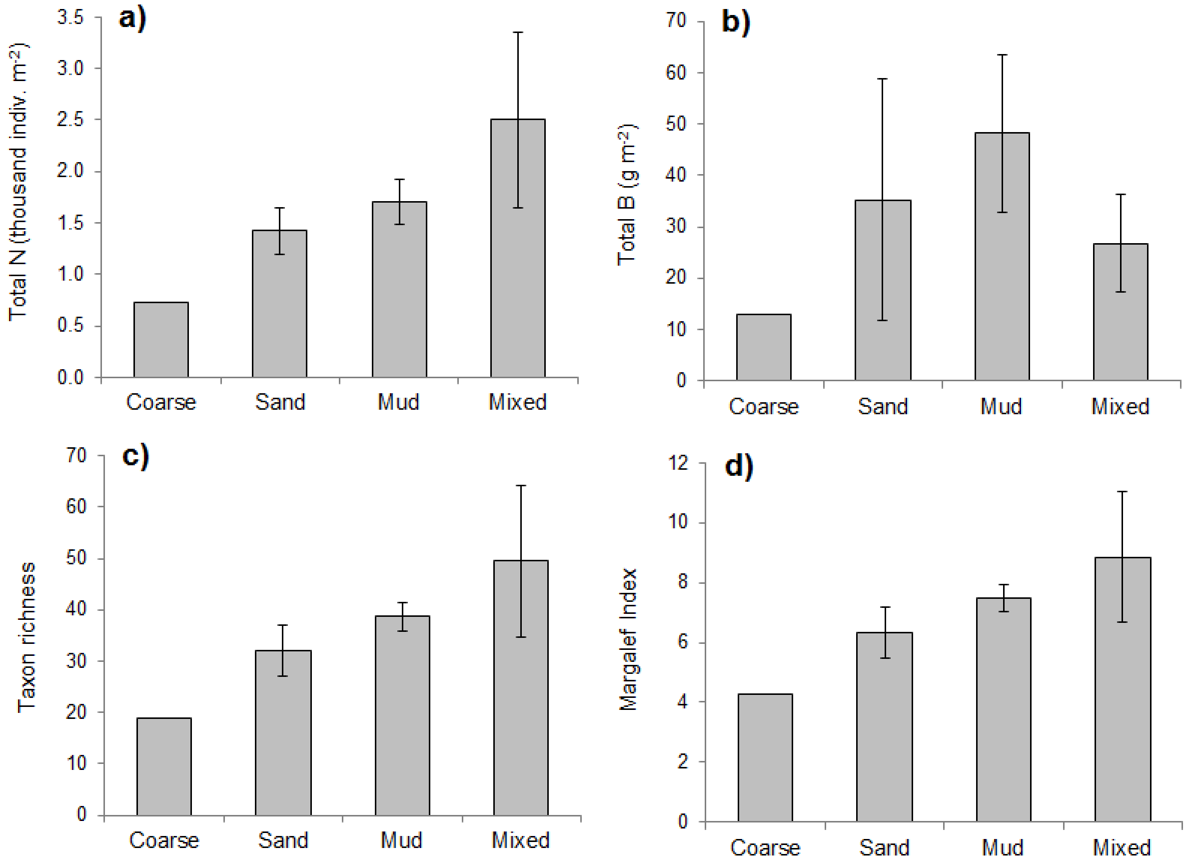

Figure 19. Mean and 95% confidence intervals of: a) total abundance (N; individuals m^{-2}), b) total biomass (B; g m^{-2}), c) taxon richness (taxa per 0.095 m^2 Day grab sample) and d) Margalef diversity of macrofauna inhabiting 'A5.1 Subtidal coarse sediment' (n=1), 'A5.2 Subtidal sand' (n=11), 'A5.3 Subtidal mud' (n=40) and 'A5.4 Subtidal mixed sediments' (n=4) within the Coquet to St Mary's MCZ and adjacent areas in 2016 (© Natural England and Cefas 2022).

Table 13. Generalised additive model summary of variation in total abundance (N; ln(individuals m^{-2} + 1)), total biomass (B; ln(g m^{-2} + 1)), taxon richness (taxa per 0.095 m^2 Day grab sample) and Margalef diversity of macrofaunal communities within the Coquet to St Mary's MCZ and surrounding areas in relation to sediment properties (© Natural England and Cefas 2022). Significant results (*p* < 0.05) are highlighted in bold. edf = estimated degrees of freedom.

Total N			Total B			Richness			Margalef		
edf	F	*p*	edf	F	*p*	edf	F	*p*	edf	F	*p*
Mud content											
1.68	0.59	0.509	2.09	2.70	**0.045**	2.88	5.93	**0.002**	2.98	6.34	**0.000**
Gravel content											
1.00	1.52	0.225	1.00	0.29	0.591	6.96	2.14	0.060	4.46	1.78	0.136
Sorting											
1.00	0.12	0.729	1.00	0.28	0.597	1.00	2.06	0.160	4.41	2.26	0.229
Skewness											
1.00	1.09	0.302	2.91	1.11	0.385	6.24	1.70	0.159	6.19	1.25	0.322
Kurtosis											
4.90	4.89	0.065	4.03	0.47	0.725	4.12	1.11	0.384	1.00	1.35	0.252

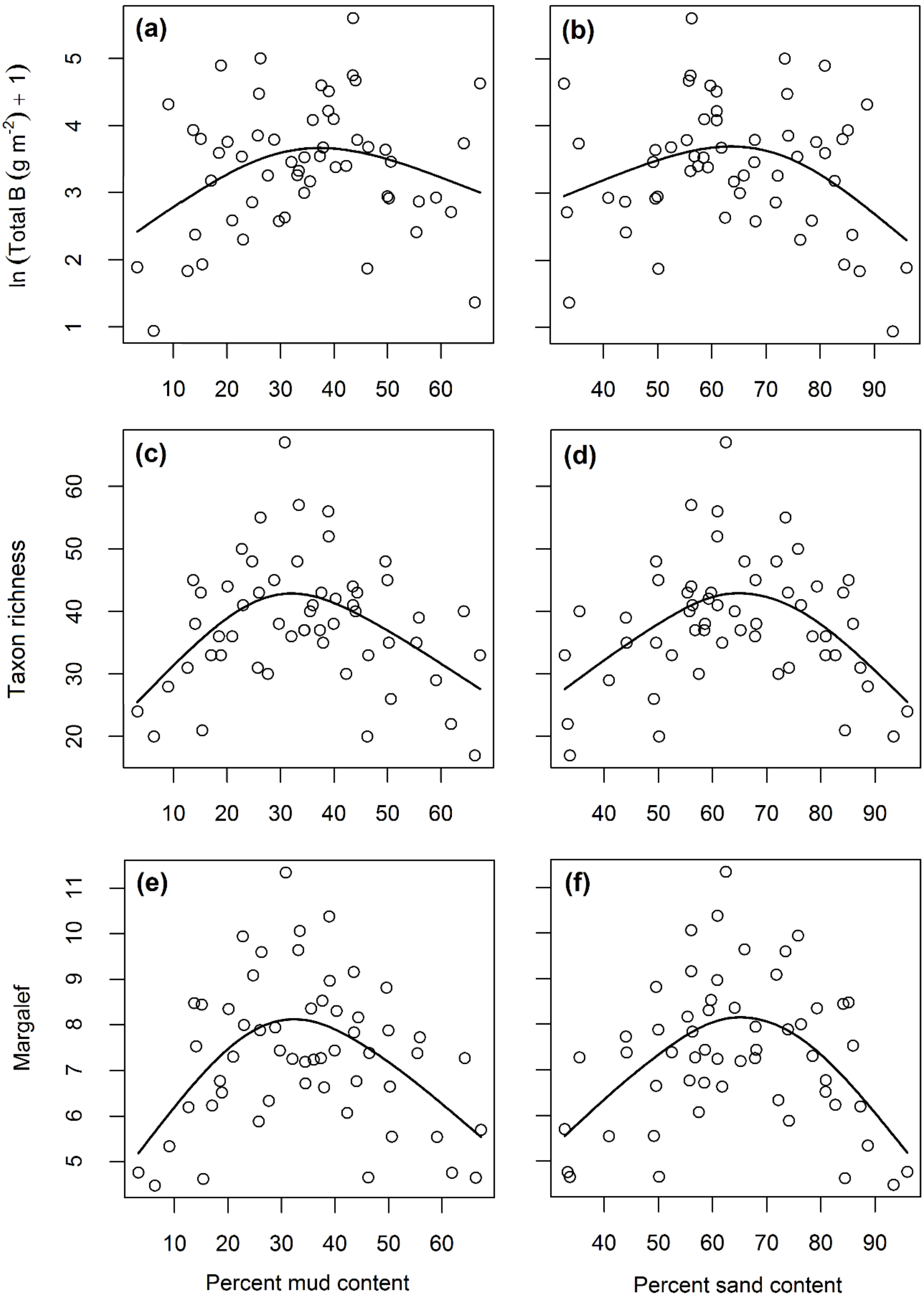

Figure 20. Variation in (a,b) total biomass (B; ln(g m^{-2} + 1)), (c,d) taxon richness (taxa per 0.095 m^2 Day grab sample) and (e,f) Margalef diversity of macrofauna in relation to mud content (a,c,e) and sand content (b,d,f) within the Coquet to St Mary's MCZ and surrounding areas in 2016 (© Natural England and Cefas 2022).

Community composition (abundance-based)

Macrofaunal communities in ‘A5.3 Subtidal mud’ were significantly different from those in ‘A5.2 Subtidal sand’, ‘A5.4 Subtidal mixed sediments’ and ‘A5.1 Subtidal coarse sediment’ in terms of abundance-based community composition (ANOSIM: Global R = 0.507, $p < 0.001$; Figure 21). The difference between subtidal mud and other BSHs was greatest for subtidal coarse sediment (R = 0.843, average dissimilarity = 72%), followed by subtidal mixed sediments (R = 0.563, average dissimilarity = 66%) and subtidal sand (R = 0.415, average dissimilarity = 63%). The taxa that distinguished subtidal mud communities from those in other BSHs varied from habitat to habitat. The polychaete *Peresiella clymenoides*, the bivalve *Thyasira flexuosa* and the aplacaphoran *Chaetoderma nitidum* distinguished mud from both sand and coarse sediment. All three of these species occurred in relatively high densities in mud and were absent from the single coarse sediment sample. The sea urchin *Echinocyamus pusillus* and bivalve *Abra prismatica* were among the distinguishing taxa that occurred in higher densities in sand, while the polychaetes *Glycera lapidum* and *Terebellides* spp. occurred in higher densities in coarse sediment. Various molluscs distinguished mud from mixed sediments (the bivalves *Kurtiella bidentata*, *Chamelea striatula*, *Dosinia* spp. and the gastropod *Cylichna cylindracea*), all of which occurred in higher densities in mud. However, the reef-forming polychaete *Sabellaria spinulosa* was also among the major contributors to community dissimilarity and occurred in higher densities in mixed sediments.

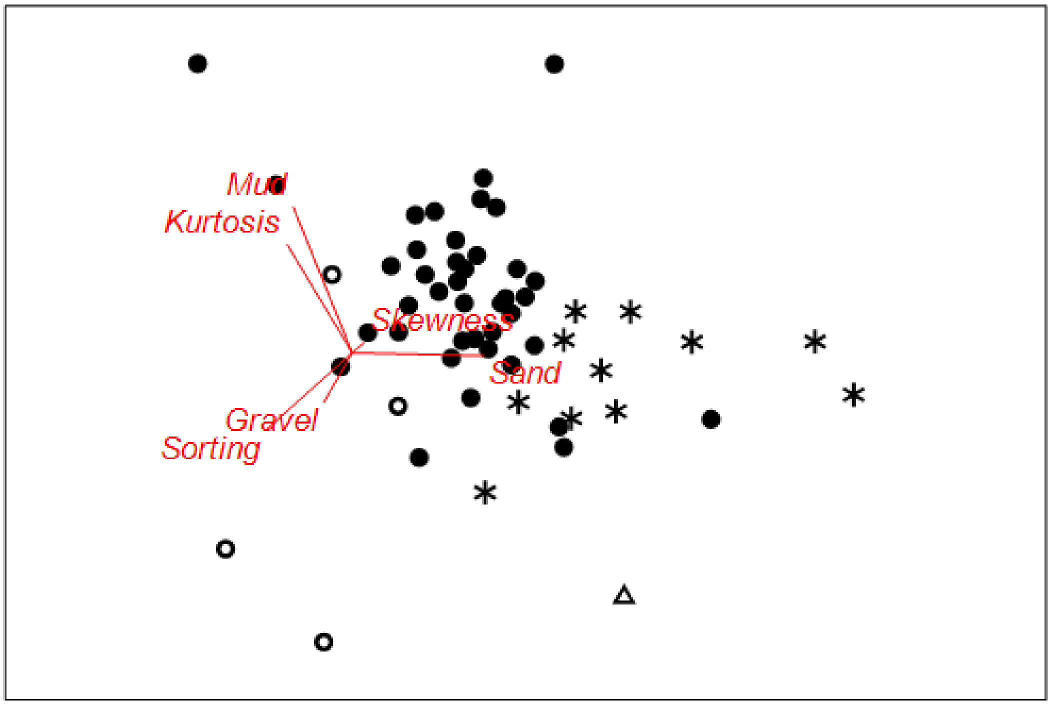

Figure 21. Non-metric multidimensional scaling ordination of macrofaunal community composition in 'A5.3 Subtidal mud' (solid circles; ●), A5.2 Subtidal sand' (stars; ✳), 'A5.4 Subtidal mixed sediments' (hollow circles; ○) and 'A5.1 Subtidal coarse sediment' (hollow triangles; △) at stations located in the Coquet to St Mary's MCZ and adjacent areas in 2016 (© Natural England and Cefas 2022). The ordination is based on Bray-Curtis similarity of ln(x+1) transformed taxa abundances in 0.095 m^2 Day grab samples. Sediment properties vectors are overlaid. Two-dimensional stress = 0.18.

Macrofaunal community composition (across the full range of sedimentary BSHs) was significantly correlated with sediment properties (RELATE: Rho = 0.320, $p < 0.001$). BEST indicated that variation in community composition was best explained by sand and gravel contents (Rho = 0.578). Unsurprisingly, given the strong correlation between sand and mud contents, the second-ranked model consisted of mud and gravel and had a very similar correlation coefficient to the former (Rho = 0.575). The fourth-ranked model was the first to contain a sediment property other than mud, sand or gravel contents. This model contained kurtosis in addition to these three variables (Rho = 0.548). Variation in community composition in relation to sediment properties is depicted in Figure 21.

SIMPROF identified nine distinct community clusters that were significantly different at $p < 0.05$ (Figure 22). The taxa that characterised each cluster are shown in Annex 8. Cluster analysis of macrofaunal community composition. Communities in inshore waters tended to cluster together and were distributed across the MCZ and the southern potential control area (e.g. clusters f and g, Figure 23). Communities further offshore in the sandier eastern potential control area also clustered together (e.g. a and d, Figure 23). However, some clusters were distributed across inshore and offshore areas (e.g. e and i, Figure 23). Two stations in the particularly muddy southwest region of the southern potential control area also clustered together (cluster b, Figure 23).

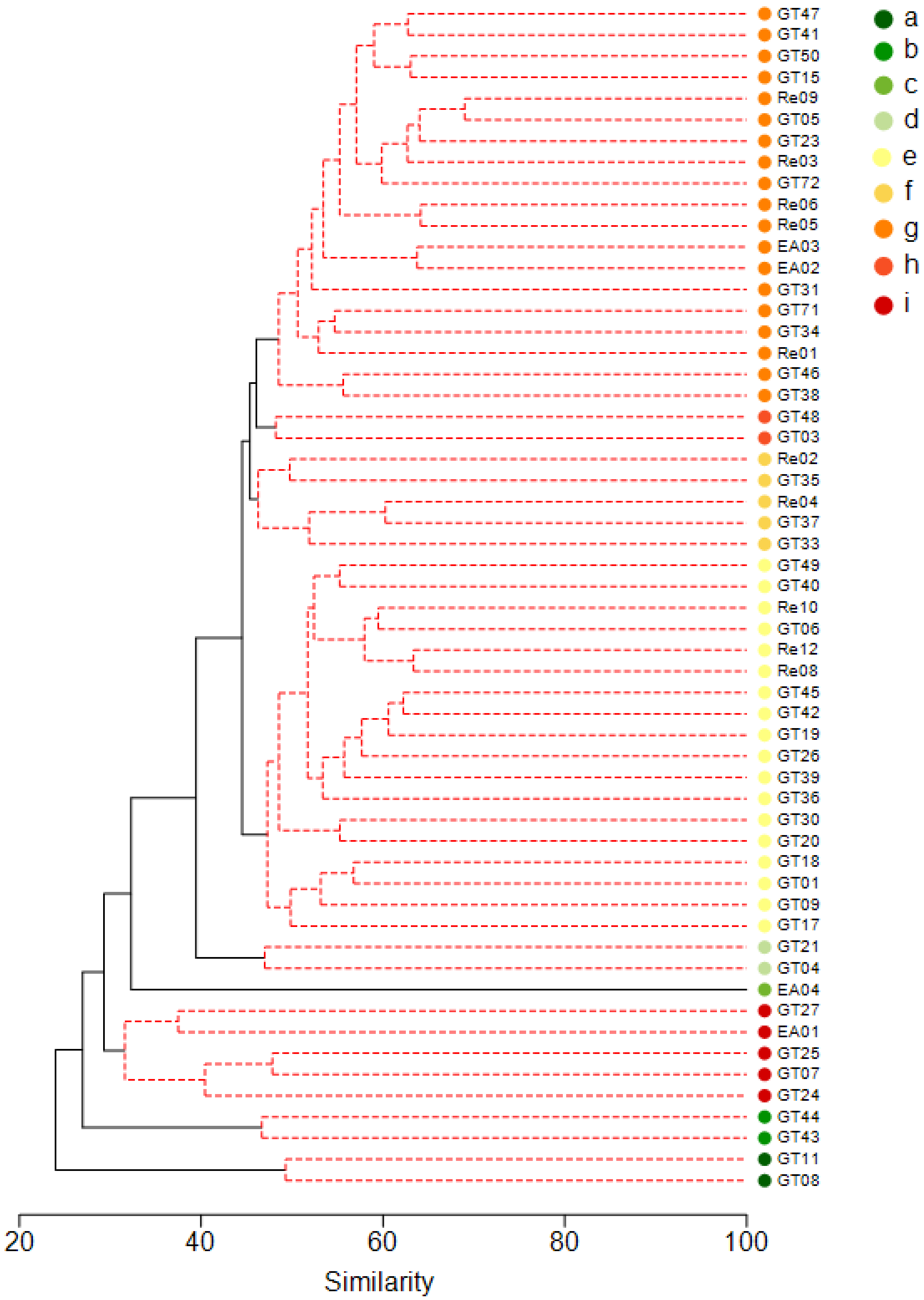

Figure 22. Dendrogram of macrofaunal community composition, based on Bray-Curtis similarity of ln(x+1) transformed taxa abundances in 0.095 m^2 Day grab samples, within the Coquet to St Mary's MCZ and surrounding areas in 2016 (© Natural England and Cefas 2022). Stations separated by black lines are significantly different (p < 0.05); stations separated by red dashed lines are not significantly different (p > 0.05).

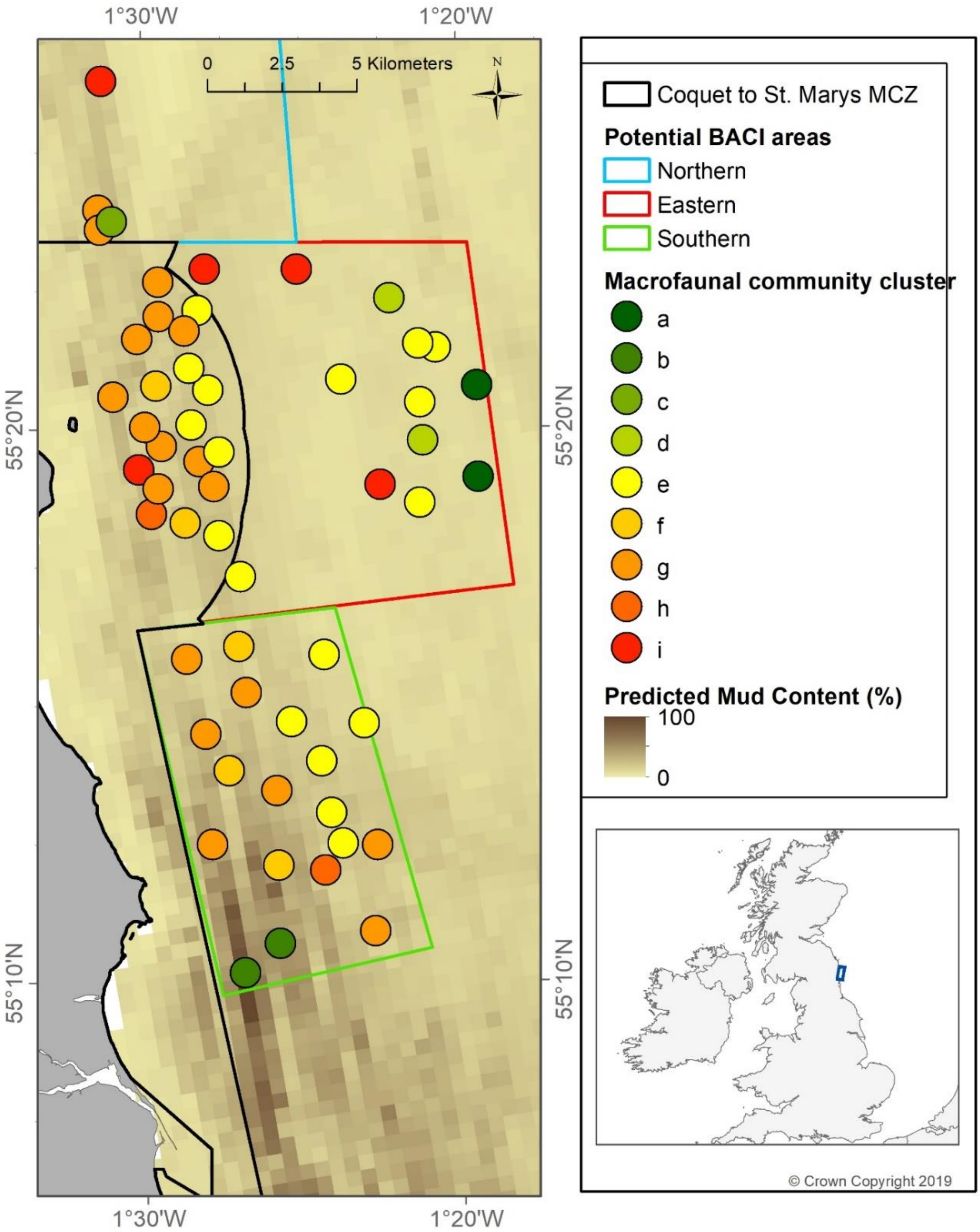

Figure 23. Clusters in macrofaunal community composition, based on Bray-Curtis similarity of ln(x+1) transformed taxa abundances in 0.095 m^2 Day grab samples, within the Coquet to St Mary's MCZ and surrounding areas in 2016. Clusters are significantly different at p < 0.05.

3.4 Other Features of Conservation Importance (FOCI)

3.4.1 Ocean quahog (*Arctica islandica*)

A species FOCI not designated at the Coquet to St Mary's MCZ, the ocean quahog (*Arctica islandica*), was recorded at 17 of 56 stations (30%) sampled using a Day grab (

Figure **24**). Inside the MCZ, *A. islandica* was recorded at seven of 20 stations (35%), compared to two of 13 stations in the eastern potential control area (15%), six of 19 in the southern potential area (32%), and two of four in the northern area (50%). When present in Day grab samples, this species was recorded at an abundance of one or two individuals per grab.

A. islandica was also recorded at four of 15 stations (27%) sampled using a NIOZ corer, two inside the MCZ and two in the southern area (Figure 24), at a density of one individual per grab. These were the only two areas sampled using the NIOZ corer. Of the 41 stations sampled using the drop camera, two *A. islandica* individuals were observed (via syphons) at one station in the eastern area (

Figure **24**).

3.4.2 Ross worm (*Sabellaria spinulosa*)

The Ross worm, *Sabellaria spinulosa*, is not a species FOCI but does constitute a habitat FOCI when it occurs in aggregations dense enough to form reefs. This species was found in Day grab samples at densities ranging from 10 to 285 individuals m^{-2}, which is substantially lower than the density that implies low 'reefiness' (~500 individuals m^{-2}; Hendrick and Foster-Smith, 2006). Nevertheless, *S. spinulosa* was characteristic of faunal cluster a (Annex 8), on the seaward limit of the eastern potential control area (Figure 23), and was relatively abundant in 'A5.4 Subtidal mixed sediments' compared to 'A5.3 Subtidal mud' (see section 3.3.2). All records of *S. spinulosa* were from outside the MCZ, although individuals of this species have previously been recorded inside the MCZ (Fitzsimmons *et al*., 2015).

As seafloor imagery data are not available for the stations where *S. spinulosa* abundances were highest in Day grabs, this method cannot be used to further inspect the possibility in the areas where they appear most likely based on available evidence. It should be noted, however, that the survey was not designed to target *S. spinulosa* reef. As such, the findings neither confirm nor disconfirm the presence of this feature in the Coquet to St Mary's MCZ and surrounding areas.

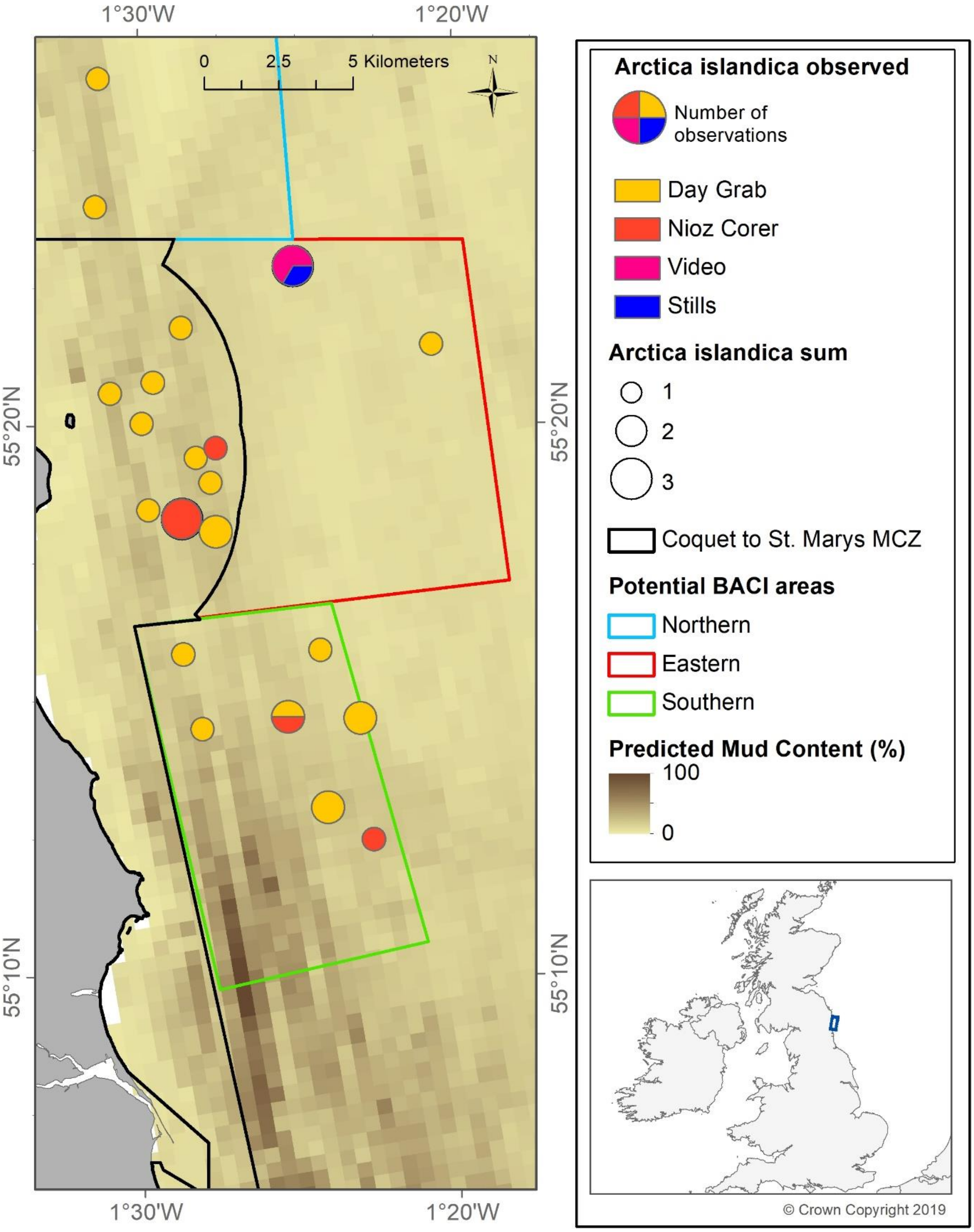

Figure 24. Distribution of the non-designated species FOCI *Arctica islandica* within the Coquet to St Mary's MCZ and surrounding areas in 2016, the gears with which it was sampled, and the number of observations at each station.

3.5 Non-indigenous species

No species that have been selected for assessment of Good Environmental Status in British waters under MSFD Descriptor 2 (Stebbing *et al*., 2014; Annex 5. Non-Indigenous Species (NIS)) were recorded in the Day grab, NIOZ corer, or marine imagery datasets during the survey. However, one species that is not included in MSFD Descriptor 2, but is on an additional list of NIS in British waters (Eno *et al*., 1997; Annex 5. Non-Indigenous Species (NIS)), the Australian barnacle *Austrominius modestus*, was recorded at station GT45 in the southern potential control area (Figure 25). Nine individuals of this species were recorded in a single Day grab sample in which the sediment was classified as 'A5.3 Subtidal mud'.

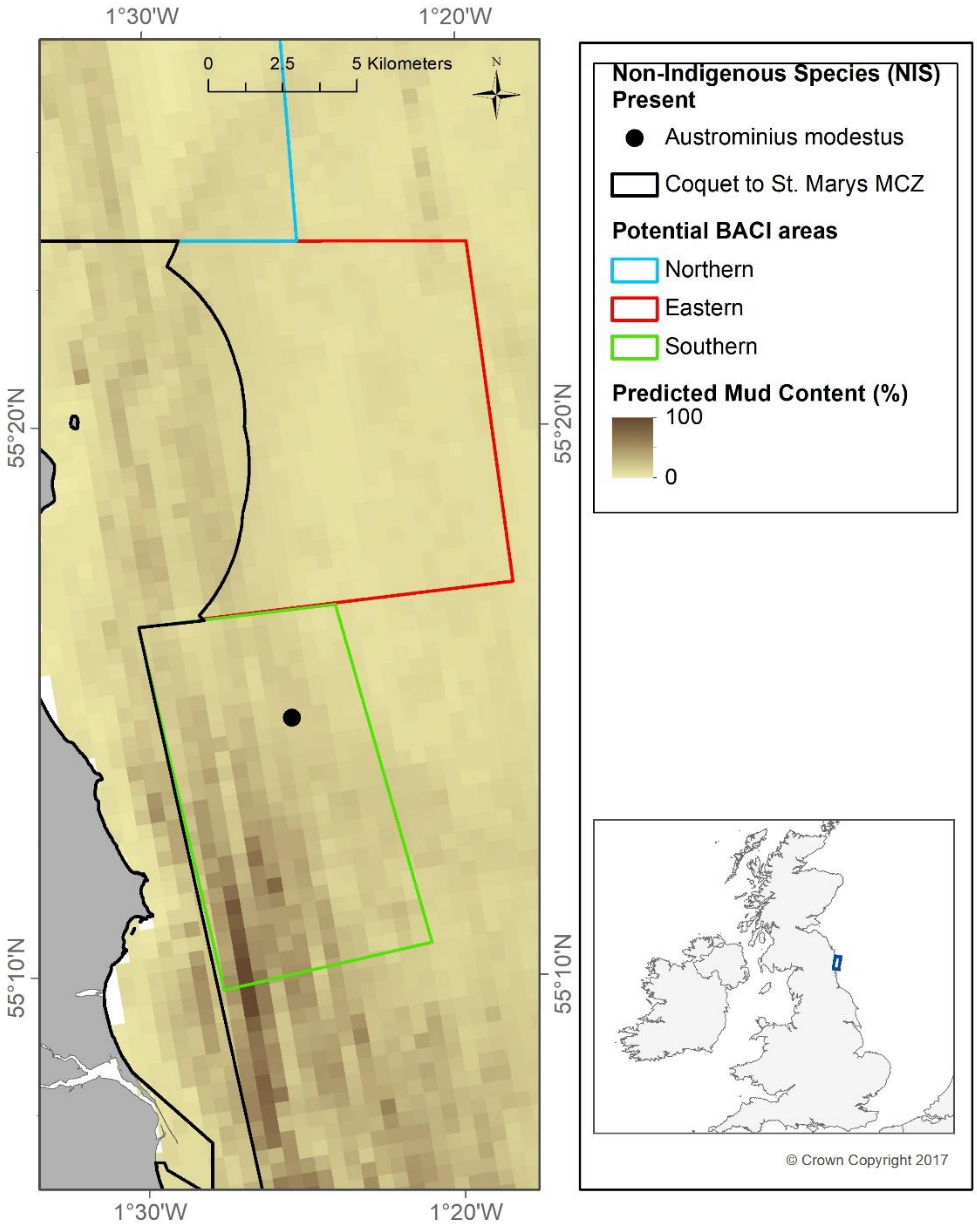

Figure 25. The location at which the non-indigenous species *Austrominus modestus* was recorded in a 0.095 m^2 Day grab sample within the potential control area to the south of Coquet to St Mary's MCZ in 2016.

3.6 Marine litter

Macrolitter (>5mm) was recorded on two instances, at GT07 with the drop camera and at GT43 in a Day grab sample (Figure 26b and d). All other litter presence was noted during the processing of faunal samples of Day grab and NIOZ core sediment samples and is presumed to be microlitter (<5mm). Microlitter was ubiquitous throughout the site; its presence being recorded in 72% of the Day grab samples and 100% of the NIOZ samples (Figure 26a and c).

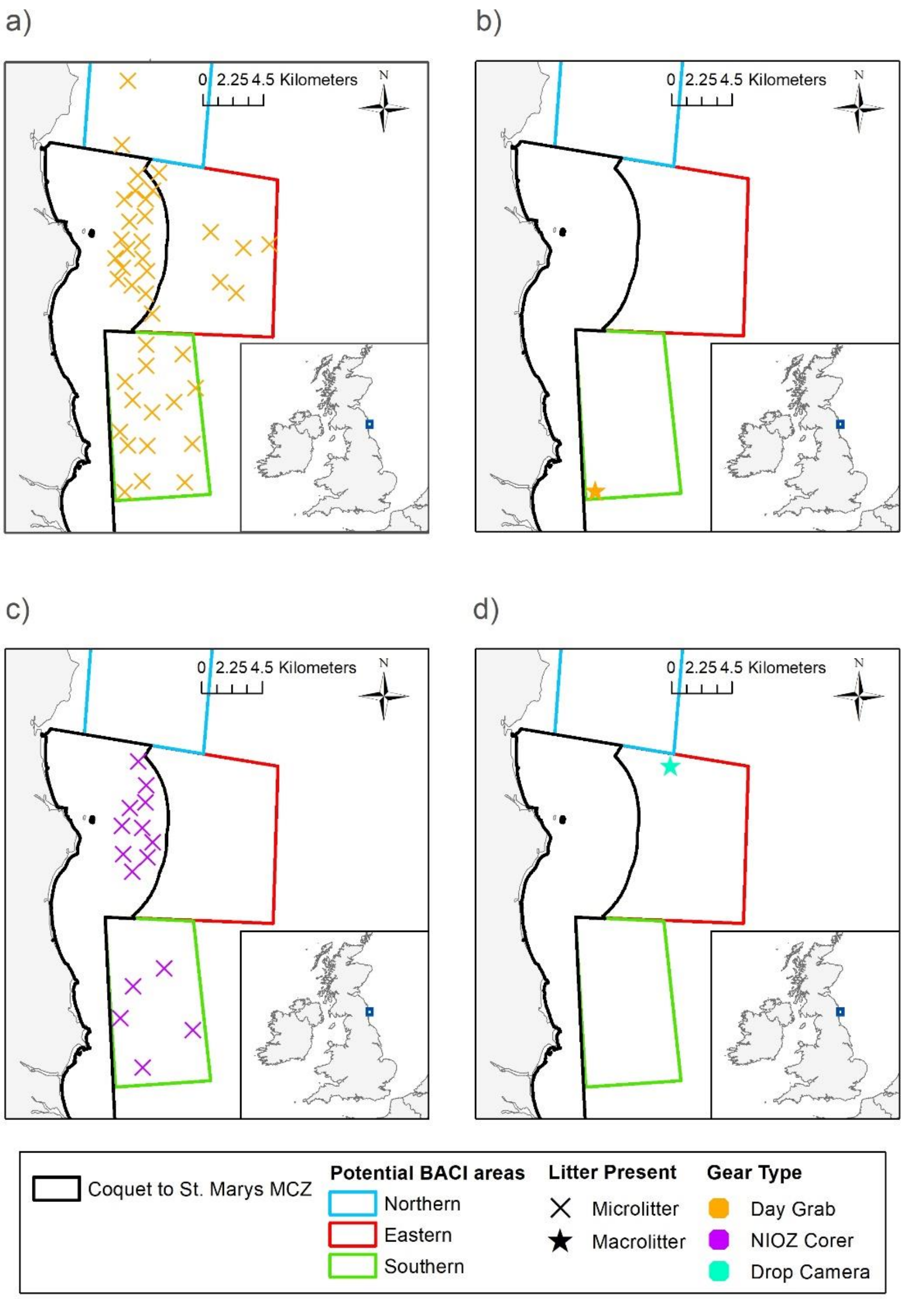

Figure 26: Microlitter (< 5mm) and Macrolitter (> 5mm) observed in a) & b) 0.095 m^2 Day grab samples, c) 0.076 m^2 NIOZ core samples and d) drop camera footage collected at the Coquet to St Mary's MCZ and surrounding areas in 2016.

4. Discussion

This report has achieved its primary objective by confirming the presence of the Habitat Feature of Conservation Importance (FOCI) 'Sea-pen and burrowing megafauna communities' within the Coquet to St Mary's Marine Conservation Zone (MCZ) and mapping its known spatial distribution. The secondary objective of describing the physical and biological characteristics of the designated Broadscale Habitat (BSH) 'A5.3 Subtidal mud' within the MCZ and surrounding areas was also achieved. A species FOCI not currently designated at the site, *Arctica islandica*, was recorded within the MCZ; a non-native species, *Austrominius modestus*, was recorded in an area to the south of the MCZ; and a widespread distribution of microlitter was observed throughout the MCZ and surrounding areas, constituting the successful completion of the third and fourth objectives of the report. The following sections discuss the evidence pertaining to the primary and secondary objectives and provide monitoring recommendations for the designated features reported on here (Report Objective v).

4.1 Sea-pen and burrowing megafauna communities

Based on the evidence presented here, burrowing megafauna appear to be distributed throughout the MCZ survey area and the southern potential control area, as well as the southwest corner of the eastern potential control area (between the MCZ and southern potential control areas; Figure 11f). Burrow densities of *Nephrops norvegicus,* however, only exceeded the critical threshold for classification of the habitat FOCI 'Sea-pen and burrowing megafauna communities' (one individual per 10 m^2) at five stations – three inside the MCZ (Re04, GT71, and Re01), one in southern area (GT37) and one in the eastern area (GT26). It should be noted that the burrow density at another station in the southern potential control area (GT35) was also not far below the critical threshold (**Error! Reference source not found.**). Given the assumptions made when calculating the video transect area to estimate burrow density (see section 2.3.4), coupled with the poor visibility in the video footage, data from this station could be considered as inconclusive with respect to the presence of this FOCI.

N. norvegicus (individuals or burrows) were not observed in the southwest corner of the southern potential control area (Figure 11b), where the sediment is relatively muddy and presence might therefore be expected. The only megafaunal taxon recorded in this area was *Callianassa subterranea* (Figure 11c). However, the apparent absence of *N. norvegicus* may be attributable to video data being unavailable for these stations due to very poor visibility in the footage. *N. norvegicus* may therefore have been present within this area but went undetected as the sampling method with which this species is most commonly observed could not be used.

N. norvegicus were also not observed throughout most of the eastern potential control area (Figure 11b). This result is more likely to reflect a true absence of this species, partly because video footage was available for all stations surveyed with the drop camera within this area (Figure 3), but also because the BSH throughout most of this area was ‘A5.2 Subtidal sand’, which differs quite substantially from the fine mud habitat that burrowing megafauna are usually associated with. That said, stations with *N. norvegicus* burrow densities above the critical threshold also had sandier sediment than is typically associated with ‘Sea-pen and burrowing megafauna communities’ but were nonetheless classified as ‘A5.3 Subtidal mud’. Moreover, the station with the highest *N. norvegicus* burrow densities (GT26), immediately adjacent to the MCZ boundary in the eastern area, had a mud content of just 21% (i.e. ‘sandy mud’), whereas the other stations with burrow densities above the critical threshold had mud contents in the range of 40-55% (**Error! Reference source not found.**). While these observations are surprising, records of burrowing megafauna in sandy mud are not unheard of and not a disqualifying observation with respect to ‘Sea-pen and burrowing megafauna communities’ classification (JNCC, 2014).

The JNCC definition of ‘Sea-pen and burrowing megafauna communities’ allows for the presence or absence of sea-pens (JNCC, 2014). Nevertheless, sea-pens were observed in association with megafauna at some stations, with their populations concentrated mainly in the centre of the MCZ survey area (Figure 11a). Sea-pens were only recorded outside the MCZ at one station (GT37) in the southern area. This station is located north of the patch with high mud content (Figure 11a), where the presence of sea-pens might be expected given their habitat preferences. However, as with burrowing megafauna, the apparent absence of sea-pens from this highly muddy area may be attributable to the lack of video data. There was no evidence of sea-pens in the eastern potential control area despite the availability of video data. This also mirrors the pattern observed for burrowing megafauna (except for the single *Upogebia stellata* individual observed at the eastern extremity of the eastern potential control area), which again is unsurprising given the relatively sandy sediment in this area (Figure 7) and the habitat preferences of sea-pens.

Given the distribution of stations with *N. norvegicus* burrow density above the critical threshold, it appears that the habitat FOCI ‘Sea-pen and burrowing megafauna communities’ is present from the southern section of the MCZ survey area through to the southwestern corner of the eastern potential control area (Figure 11f). It also seems likely that this habitat FOCI extends over large parts of the southern potential control area, but the lack of video imagery data from the southwest of the area (where the habitat type is most suitable for *N. norvegicus*) means that this could not be confirmed.

4.2 Broadscale Habitats (BSH)

4.2.1 Physical characteristics of the sediment

In accordance with the observed distribution of sea-pens and burrowing megafauna, the ‘A5.3 Subtidal mud’ feature was found across most of the MCZ survey area and southern potential control area, based on the results of PSA (Figure 7e and f). However, analysis of seafloor imagery suggested that sediments within the MCZ survey area were mainly ‘A5.2 Subtidal sand’ (Figure 7d). As BSH type was typically observed to be homogenous along video transects, this suggests that it is unlikely that grab samples happened to be extracted from relatively muddy patches within an otherwise sandy area. It seems more likely that %

mud content was underestimated when analysing video footage by eye and that this led to the erroneous identification of subtidal sand in an area that is correctly classified as subtidal mud. Nevertheless, the video footage does provide an indication of the small-scale spatial homogeneity of sediment type both within the MCZ and in surrounding areas and is therefore useful in this regard.

Sediment samples collected from the same station using Day grabs and NIOZ corers (0-5 cm sediment depth) were typically classified as the same BSH; however, inconsistent results were obtained at two stations close to the seaward boundary of the MCZ ('A5.3 Subtidal mud' for the Day grab and 'A5.2 Subtidal sand' for the NIOZ corer; Figure 7e-f). This may be due to a trend of decreasing mud content and increasing sand content with increasing distance offshore, thus leading to a transitional zone between the two BSHs in the area where these two stations are located. Indeed, one of the NIOZ corer samples (Re06) was very close to being classified as 'A5.3 Subtidal mud' (19%, with 20% being the lower limit for classification of this BSH), although the other was less close (14%) (Table 3). A more detailed analysis of Particle Size Distribution indicates that conclusions about the physical characteristics of the sediment at a station are largely unaffected by the gear used (Day grab vs NIOZ corer) and the depth to which sediment is extracted (0-5 cm, 5-10 cm, 10-15 cm, and 15-20 cm slices of NIOZ corer samples) (Figure 8). The specific gear used to collect samples for PSA and infer sediment characteristics may therefore be of low importance at this site.

Across stations, the physical characteristics of the sediment within the MCZ survey area were largely the same as those in the southern potential control area, even when considering a finer level of detail than BSH classification. In both areas, most of the mud content was from the silt fraction (2-63 µm) rather than clay fraction (< 2 µm), which implies that the mud is low in cohesiveness (compared to mud habitats consisting mostly of clay) and may therefore be easily eroded by natural or anthropogenic disturbance (e.g. demersal fishing). Indeed, the low surface roughness and deep penetration depth of the sediment profile imaging (SPI) camera in muddier sediments within the MCZ (Figure 10c and d) suggests a higher likelihood of erosion, which appears to be supported by the relatively low mud content in surficial sediments (0-5 cm) compared to deeper sediments (5-15 cm) at various stations (Figure 9). If the seafloor within the MCZ experiences little natural disturbance, which may be the case given the weak tidal currents at the site (Figure 5), then high erodibility may mean that even low levels of fishing pressure have large impacts on sediments and their associated benthic assemblages. However, if sediments are often naturally disturbed (e.g. by storms), then fishing pressure may have little additional physical and ecological impacts. The similarity in the physical characteristics of the sediment in the MCZ and proposed southern control area will allow such impacts of anthropogenic disturbance to be tested using BACI-type monitoring if a fishery closure is implemented. As maximum current velocity is particularly low in the south of the proposed southern control area (Figure 5), such monitoring may benefit from focusing on the central or northern parts of this area. In contrast, the different physical characteristics of the sediment in the eastern potential control area (i.e. higher sand content) make it unsuitable as a control for monitoring future changes within the MCZ.

Sediment composition was largely consistent within the MCZ survey area (i.e. the BSH was ‘A5.3 Subtidal mud’, with the mud fraction consisting mainly of silt), but there was notable spatial variation in the depth of the apparent redox potential discontinuity (aRPD) layer (~4 to 11 cm; Table 3). This, in turn, implies spatial variation in biogeochemical cycling, including organic matter decomposition and nutrient regeneration (Teal *et al*., 2010). The shallowest aRPD depths were recorded at the two stations where the BSH were classified as ‘A5.2 Subtidal sand’ based on NIOZ corer samples, which could be considered unusual given that sand typically has greater porosity than mud, thus making it more permeable to overlying water containing dissolved oxygen (Huettel *et al*., 2014). However, active sediment reworking by burrowing megafauna aerates the sediment to great depths (Hughes *et al*., 1998) and preference for muddy sediments by burrowing megafauna may contribute to the relatively shallow aRPD at stations with the highest sand content. The functioning (i.e. geochemical cycling) of the subtidal mud feature within the MCZ may therefore be shaped, in part, by the presence and distribution of the habitat FOCI ‘Sea-pen and burrowing megafauna communities’. However, it should also be noted that of the ten stations where aRPD depth was measured (each of which showed evidence of burrowed mud), the ‘zone of mixing’ was not especially deep at those where *N. norvegicus* burrows were above the critical density. Therefore, *N. norvegicus* density may not have a dominant influence over biogeochemical cycling as reflected by aRPD depth, possibly because of the sporadic distribution of burrows and the low likelihood of penetrating a burrow with the SPI prism. The capacity of the whole community to rework and aerate the sediment at any point in space may be more important in this regard (Solan *et al*., 2004b; Morys *et al*., 2017, Wrede *et al*., 2018).

4.2.2 Community composition and diversity

Macrofaunal assemblages inhabiting the BSH ‘A5.3 Subtidal mud’ within the Coquet to St Mary’s MCZ were characterised by the polychaetes *Lumbrineris* spp. and *Peresiella clymenoides*, the brittle star *Amphiura filiformis* and the bivalve *Chamelea striatula*. *A. filiformis* is commonly found in muddy sediments and there is some evidence that amphiurid brittle stars benefit from disturbance by demersal fishing, possibly due to reduced densities of their predators such as *N. norvegicus* (Sköld *et al.*, 2018). Therefore, if this characteristic species declines in abundance following the implementation of any future fisheries management measures within the MCZ, this change should not be assumed to be a negative outcome. Similarly, *P. clymenoides* is a capitellid, a group of opportunistic worms that often respond positively to organic enrichment (e.g. through eutrophication; Pearson and Rosenberg, 1978). The high mud content within much of the MCZ is likely to be associated with high organic matter content (Ellingsen, 2002) and *P. clymenoides* could be abundant in these conditions irrespective of anthropogenic eutrophication. Nevertheless, any decline in the population of this characteristic species should also not be assumed to be undesirable. The remaining two of the top four characteristic taxa, *Lumbrineris* spp. and *C. striatula*, have been found to characterise communities elsewhere (e.g. Hily *et al.*, 2008) but are typically found in sand (or even coarser sediments in the case of *Lumbineris* spp.). It is therefore unclear why these species characterise subtidal mud assemblages in the

Coquet to St Mary's MCZ, but it seems likely that they can tolerate a wide range of physical conditions.

As with the physical characteristics of the sediment, macrofaunal communities inhabiting 'A5.3 Subtidal mud' in the southern potential control area were very similar to those within the MCZ. None of the univariate biodiversity measures considered differed significantly between these two areas, with taxon richness and Margalef diversity being almost identical (Figure 17c and d), while differences in community composition were statistically significant but small. The southern area therefore appears to be a suitable control for BACI-type monitoring, both with respect to physical and biological characteristics of the subtidal mud feature.

Total biomass, taxon richness and Margalef diversity varied significantly in relation to mud content, peaking at around 30-40% (Figure 20). Both the RELATE analysis and the distribution of community clusters in relation to % mud content (Figure 23) suggest that the benthos is influenced by variability in this sediment component even when variability is constrained within the limits of the BSH 'A5.3 Subtidal mud'. This suggests that future monitoring may benefit from considering stations with different % mud contents separately (and grouping together stations with similar mud contents) or including mud content as a covariate in statistical analyses of change over time. While communities in the eastern potential control area were not significantly different from those within the MCZ in terms of the univariate biodiversity indices, the dissimilar community composition within this relatively sandy area indicates that this would not be a suitable control to use within BACI-type monitoring.

The data from the survey on which this report is based allowed the suitability of different sampling approaches to be assessed. Both the Day grab and the NIOZ corer were used to sample macrofauna and the two gear types produced slightly different results. After correcting for the difference in surface area sampled by each gear, more individuals were recorded with the Day grab than with the NIOZ corer on average (Figure 13a), which is surprising given that the NIOZ corer penetrates deeper into the sediment and is therefore able to reach a greater proportion of the full macrofaunal assemblage. Indeed, more deep-burrowing organisms were recorded in NIOZ corer samples (

Table 7), so the explanation for this slight discrepancy in total abundance between gears is not clear. However, the predicted total number of taxa per station (*Smax*) and total biomass did not differ between gear types (Figure 13b,c), whereas the Gini-Simpson index was greater when based on Day grab samples (Figure 13d). Moreover, the predicted total number of taxa in 'A5.3 Subtidal mud' throughout the MCZ was the same for both gears (153 taxa). Therefore, neither gear was clearly more effective than the other at sampling macrofaunal communities.

Analysis of the three NIOZ corer replicates per station indicated that a single sample is unlikely to accurately represent the macrofaunal community at a location, with average within-station similarity in abundance-based community composition ranging from 43% to 62%. This variability is likely to introduce substantial noise if change over time is assessed by collecting only one grab sample per station. Nevertheless, macrofaunal samples from the same station clustered together in most cases, suggesting that a single sample will usually be sufficient for assessing spatial variation in the benthos in an area such as an MCZ. When macrofaunal community composition was based on biomass, within-station variability was higher and replicates from the same station were often distributed across different clusters, suggesting that biomass-based community composition might not be a useful aspect of the community to consider when tracking change over time or assessing spatial patterns in the benthos, unless many grab samples are collected per station.

5. Recommendations for future monitoring

- Burrowing megafauna were most often identified based on seafloor video footage (mainly through the observation of *Nephrops norvegicus* burrows). This method should therefore be used in future monitoring surveys of the habitat FOCI 'Sea-pen and burrowing megafauna communities'.
    - Landing the camera frame on the seabed or deploying a camera sledge will allow video footage to be captured at a more stable height above the seafloor than is usually possible with a drop camera hovered above the seafloor. This would maintain a more stable sample area (field of view) and allow *N. norvegicus* burrow densities to be more accurately estimated. Assessments of whether the critical density needed to confirm the presence of 'Sea-pen and burrowing megafauna communities' has been exceeded (one individual per 10 m^2; JNCC, 2014) would therefore be more reliable.
    - Turbidity resulted in poor seafloor image quality, with *N. norvegicus* burrows indistinguishable at some stations, while the variability in quality across stations prevented a broader analysis of epifaunal assemblages. Where possible, survey planning should attempt to minimise the risk of turbidity impacting image quality, in terms of both hydrodynamic conditions and equipment used. A freshwater lens camera may be more effective in sub-optimal visibility than a standard camera.
- At some stations, the presence of burrowing megafauna was confirmed based solely on the presence of organisms recorded in NIOZ corer samples (usually *Callianassa subterranea*). This was the case even at stations where the drop camera was deployed. The NIOZ corer should therefore be used alongside video footage, where possible, when targeting burrowing megafauna.
- *N. norvegicus* individuals were not recorded in NIOZ corer (or Day grab) samples at any of the stations where *N. norvegicus* burrows were observed above the critical density in seafloor imagery. Therefore, if an aim of any future survey is to confirm the presence of this species by direct sampling, many replicates may be required at stations where there is evidence of burrowed mud just for a single individual to be recorded. Baited creels or camera traps could be considered for future surveys to achieve this aim.
- As the NIOZ corer penetrates deeper into the sediment than benthic grabs and retains an intact sample, this gear should also be given preference when the aim is to assess the vertical profile of sediment characteristics in subtidal mud. Nevertheless, the results of this survey indicate little difference in sediment characteristics in relation to gear type (NIOZ corer vs Day grab) and sediment depth. Therefore, Day grab samples may be sufficient to monitor the physical characteristics of 'A5.3 Subtidal mud' within the Coquet to St Mary's MCZ and surrounding areas if it is not possible to use the NIOZ corer.

- Neither the NIOZ corer nor the Day grab was clearly more effective at sampling macrofauna communities in ‘A5.3 Subtidal mud’. Unless targeting burrowing megafauna (which make up a small proportion of the community), either gear could be used to monitor the condition of this BSH with respect to its associated benthic assemblages.
- A set of fixed ‘sentinel’ monitoring stations could be selected to monitor macrofaunal communities in ‘A5.3 Subtidal mud’.
    - The locations of stations should be selected so that together they cover the range of physical (e.g. % mud content) and biological conditions observed within subtidal mud.
    - Multiple replicates should be collected at each station to account for the high-level of within-station variability in macrofaunal communities (average sample similarity for abundance-based community composition ranged from 43% to 62%).
    - By capturing within-station variability and eliminating the effects of variation among stations (due to spatial variation in sediment characteristics and other environmental characteristics) this will increase the statistical power to detect temporal change.
- The southern potential control area should be used for any future before–after, control–impact (BACI) monitoring of the Coquet to St Mary’s MCZ due to its similarity to the MCZ survey area in terms of the physical and biological characteristics of subtidal mud. The eastern potential control area is less suitable. Given that macrofaunal assemblages respond to variation in mud content and that such variation occurs within and across the MCZ and southern areas, this variable should be controlled, ideally through experimental design (e.g. by selecting stations inside and outside the MCZ where mud content is similar) but potentially using statistical methods if required (e.g. by including mud content as a covariate in analyses if samples unexpectedly have different mud contents). Other potentially influential environmental variables should also be controlled, where possible. For example, the particularly low maximum current velocity in the south of the southern potential control area suggests that surveys for BACI monitoring might best be focused on central and northern parts of this area, where maximum current velocity is comparable to that of the MCZ survey area. Information on baseline fishing activity in the MCZ and southern potential control area would also be needed to interpret change over time if management measures are applied.
- Taxa that currently characterise the subtidal mud habitat within the Coquet to St Mary’s MCZ have been shown to respond positively to anthropogenic disturbances (e.g., *Amphiura filiformis* and *Peresiella clymenoides*), while others are thought to be typical of habitats with coarser sediments (e.g. *Lumbrineris* spp. and *Chamelea striatula*). Any future declines in the populations of these characteristic taxa should therefore not be assumed to indicate a deterioration to the condition of the subtidal mud habitat.

- Indices that respond predictably to environmental stress may be a more suitable way of assessing changes in condition. There is some evidence that the Margalef Index might be useful as a general indicator of physical, organic, and chemical disturbance (van Loon *et al*., 2018). Indices based on specific suites of life-history traits may reveal more specific anthropogenic effects on the ecosystem, e.g. reductions in large and long-lived taxa in response to trawling (Tillin *et al.* 2006; van Denderen *et al.*, 2015; Rijnsdorp *et al*., 2018). Similar trait-based indices may also be useful for monitoring likely changes to ecological processes (e.g. sediment reworking and aeration) and associated ecosystem functions (e.g. Solan *et al*., 2004b; Morys *et al*., 2017; Wrede *et al*., 2018).

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Annex 1. Sampling Regime

Sampling regime for the survey of the Coquet to St Mary's MCZ and surrounding areas in 2016 (© Natural England and Cefas 2022). Ticks indicate the stations at which each gear was deployed and the numbers in brackets indicate the number of replicate samples collected. Each station sampled using the drop camera was done so with a single tow. *The macrofaunal sample for station GT32 was not successfully preserved and therefore this station is not included in any community data analyses.

Station code	Area	Day grab	Drop camera	NIOZ corer	SPI
EA_01	Northern	✓(1)	-	-	-
EA_02	Northern	✓(1)	-	-	-
EA_03	Northern	✓(1)	-	-	-
EA_04	Northern	✓(1)	-	-	-
GT01	Eastern	✓(1)	-	-	-
GT02	Eastern	-	✓	-	-
GT03	MCZ	✓(1)	✓	-	-
GT04	Eastern	✓(1)	✓	-	-
GT05	MCZ	✓(2)	✓	-	-
GT06	MCZ	✓(2)	✓	-	-
GT07	Eastern	✓(1)	✓	-	-
GT08	Eastern	✓(1)	-	-	-
GT09	Eastern	✓(1)	-	-	-
GT10	Eastern	-	✓	-	-
GT11	Eastern	✓(1)	-	-	-
GT13	Eastern	-	✓	-	-
GT15	MCZ	✓(1)	✓	-	-
GT16	Eastern	-	✓	-	-
GT17	MCZ	✓(1)	✓	-	-
GT18	Eastern	✓(1)	-	-	-
GT19	MCZ	✓(1)	✓	-	-
GT20	Eastern	✓(1)	-	-	-
GT21	Eastern	✓(1)	-	-	-
GT22	Eastern	-	✓	-	-
GT23	Eastern	✓(2)	✓	-	-
GT24	Eastern	✓(1)	-	-	-
GT25	Eastern	✓(1)	✓	-	-
GT26	Eastern	✓(1)	✓	-	-
GT27	MCZ	✓(1)	✓	-	-
GT28	Eastern	-	✓	-	-
GT30	Eastern	✓(1)	✓	-	-
GT31	Southern	✓(1)	✓	-	-
GT32	Southern	✓(1)*	✓	-	-
GT33	Southern	✓(1)	✓	-	-
GT34	Southern	✓(1)	✓	-	-
GT35	Southern	✓(1)	✓	-	-
GT36	Southern	✓(1)	✓	-	-
GT37	Southern	✓(1)	✓	✓(3)	-

Station code	Area	Day grab	Drop camera	NIOZ corer	SPI
GT38	Southern	✓(1)	✓	-	-
GT39	Southern	✓(1)	✓	-	-
GT40	Southern	✓(1)	-	-	-
GT41	Southern	✓(1)	✓	-	-
GT42	Southern	✓(1)	✓	-	-
GT43	Southern	✓(1)	✓	-	-
GT44	Southern	✓(1)	✓	✓(3)	-
GT45	Southern	✓(1)	✓	✓(3)	-
GT46	Southern	✓(1)	✓	✓(3)	-
GT47	Southern	✓(1)	✓	-	-
GT48	Southern	✓(1)	✓	-	-
GT49	Southern	✓(1)	✓	-	-
GT50	Southern	✓(1)	✓	✓(3)	-
GT71	MCZ	✓(1)	✓	-	-
GT72	MCZ	✓(1)	✓	-	-
Re01	MCZ	✓(3)	✓	✓(3)	✓(5)
Re02	MCZ	✓(3)	✓	✓(3)	✓(5)
Re03	MCZ	✓(3)	✓	✓(3)	✓(5)
Re04	MCZ	✓(3)	✓	✓(3)	✓(5)
Re05	MCZ	✓(3)	✓	✓(3)	✓(5)
Re06	MCZ	✓(3)	✓	✓(3)	✓(5)
Re08	MCZ	✓(3)	✓	✓(3)	✓(5)
Re09	MCZ	✓(3)	✓	✓(3)	✓(5)
Re10	MCZ	✓(3)	✓	✓(3)	✓(5)
Re12	MCZ	✓(3)	✓	✓(3)	✓(5)

Annex 2. Power analysis results

Power analysis of grab sample data collected from 'A5.3 Subtidal mud' in the Coquet to St. Mary's MCZ in 2014 shows that 19 grab samples would provide an 80% chance of detecting 20% change in infaunal species richness (see bold) (© Natural England and Cefas 2022). Power analysis was conducted by Natural England.

ALL DATA INCLUDING OUTLIERS					HIGHEST & LOWEST OUTLIERS REMOVED				
% Dif from mean	**Difference**	**Sample Size**	**Target Power**	**Actual Power**	**% Dif from mean**	**Difference**	**Sample Size**	**Target Power**	**Actual Power**
10	3.7	330	0.9	0.90061	10	3.8	95	0.9	0.901554
10	3.7	247	0.8	0.801242	10	3.8	71	0.8	0.800968
10	3.7	194	0.7	0.700393	10	3.8	56	0.7	0.700739
20	7.4	84	0.9	0.903228	20	7.6	25	0.9	0.907295
20	7.4	63	0.8	0.804502	**20**	**7.6**	**19**	**0.8**	**0.811522**
20	7.4	50	0.7	0.707162	20	7.6	15	0.7	0.70769
30	11.1	38	0.9	0.904072	30	11.2	12	0.9	0.904844
30	11.1	29	0.8	0.810637	30	11.2	10	0.8	0.839871
30	11.1	23	0.7	0.711356	30	11.2	8	0.7	0.738597

Annex 3. Macrofauna Data Truncation Protocol

Raw taxon abundance and biomass matrices can often contain entries that include the same taxa recorded differently, erroneously or differentiated according to unorthodox, subjective criteria. Therefore, ahead of analysis, data should be checked and truncated to ensure that each row represents a legitimate taxon and that they are consistently recorded within the dataset. An artificially inflated taxon list (i.e. one that has not had spurious entries removed) risks distorting the interpretation of pattern contained within the sampled assemblage.

It is often the case that some taxa must be merged to a level in the taxonomic hierarchy that is higher than the level at which they were identified. In such situations, a compromise must be reached between the level of information lost by discarding recorded detail on a taxon's identity and the potential for error in analyses, results and interpretation if that detail is retained.

Details of the data preparation and truncation protocols applied to the infaunal datasets acquired at the Coquet to St Mary's MCZ ahead of the analyses reported here are provided below:

- Where there are records of one named species together with records of members of the same genus (but the latter not identified to species level) the entries are merged, and the resulting entry retains only the name of the genus.
- Taxa are often assigned as 'juveniles' during the identification stage with little evidence for their actual reproductive natural history (except for some well-studied molluscs and commercial species). Many truncation methods involve the removal of all 'juveniles'. However, a decision must be made on whether removal of all juveniles from the dataset is appropriate or whether they should be combined with the adults of the same species where present. For the macrofaunal data collected at the Coquet to St Mary's MCZ, if 'juvenile' records were recorded at the same taxonomic level as 'adult' records then the two records were combined, whereas if juveniles were recorded at a higher taxonomic level than adults then the 'juvenile' records were removed to avoid having to reduce the taxonomic resolution of the 'adult' records.
- Records of meiofauna (i.e. nematodes) were removed.
- Records of fish species were removed.

Annex 4. Macrofaunal Community Analysis Methods

Gear comparison

To examine the effect that the greater penetration depth of the NIOZ corer has on the composition of the macrofauna samples from 'A5.3 Subtidal mud' compared to those collected using the Day grab, it is important that any other differences between the two gears are controlled for. Both gears cover a surface area of approximately 0.1 m^2; however, the exact surface area sampled is 0.096 m^2 for the Day grab and 0.078 m^2 for the NIOZ corer. Moreover, the corers used to take sediment sub-samples for PSA were not the same for each gear (0.001 m^2 for the Day grab, 0.002 m^2 for the NIOZ corer). Therefore, macrofauna samples collected using the two gears are not quantitatively comparable without the application of corrections. To mitigate the extraneous differences between the two gears, the abundances and biomasses of taxa in each sample were standardised to values per square metre by dividing by 0.095 and 0.076 for the Day grab and NIOZ corer, respectively. This standardised abundance and biomass data to individuals and grams per square metre, respectively.

As there is no basis to assume any specific relationship between sampled area and the total number of macrofaunal taxa (taxon richness), simple corrections like those used for abundance and biomass cannot be used to standardise taxon richness across samples with different surface areas. Therefore, the three samples collected at each station were used to create species accumulation curves for each gear in PRIMER (version 6; Clarke and Gorley, 2006). From these curves, the total number of taxa at each station (*Smax*) was predicted using the Michaelis-Menten richness estimator (Soberón and Llorente, 1993), thus producing comparable values for taxon richness across the two gear types. Additionally, species accumulation curves were created for each gear using station-level data (i.e. the number of taxa in the three pooled samples at a station) to determine whether they converge on the same *Smax*. Moreover, as all samples used for the gear comparison were collected from inside the MCZ, the station-level species accumulation curve was used to estimate the proportion of the total number of taxa within the MCZ that are recorded as the number of sample stations (each sampled in triplicate) increases.

Diversity of macrofauna was also represented using the Gini-Simpson index. This index reflects both the number of taxa present and the evenness with which total abundance is distributed across taxa. A higher value indicates more taxa and/or a more even spread of abundances across taxa, although the index is mainly responsive to changes in evenness (i.e. it is only slightly responsive to changes in the number of rare taxa). The Gini-Simpson index is preferable to other diversity indices for the gear comparison analysis as it is not affected by the size of the sample area (Clarke and Warwick, 2001). It is also easy to interpret, denoting the probability that any two individuals randomly selected from a sample will belong to different taxa.

Differences in the total abundance, total biomass, *Smax* and Gini-Simpson diversity of macrofauna samples extracted using different gears (Day grabs vs NIOZ corers) were analysed using general linear models in R (version 3.4.1, R Core Team 2017). 'Station' was also included as an explanatory variable in the model to account for any between-station variability in communities before testing for differences between sampling gears. That is, the analysis was conducted using two fully crossed factors, ‘gear’ (two levels; one for each gear type) and ‘station’ (seven levels; one for each station that met the criteria in section 2.3.5), with three replicates for each combination of levels across factors. Assumptions of homoscedasticity (i.e. the variance in the response variables is the same across the range of categories/values for the explanatory variable) and normality of residuals were checked by inspection of plots of residuals against fits and normal quantile plots, respectively. Data were transformed by ln(x+1), if necessary, to meet test assumptions. Sums of squares were calculated using the Type II approach. That is, the relationship that the response variable has with each explanatory variable was tested after accounting for its relationship with the other explanatory variable(s). Differences between gear types or among stations were considered significant when $p < 0.05$.

Variation in macrofaunal community composition was analysed in PRIMER (v6). Bray-Curtis similarities of samples were calculated with taxa densities weighted by numerical abundance and by biomass. Data were transformed by ln(x+1) prior to analysis to down weigh the influence of dominant taxa and allow any variation in less abundant taxa to be detected. Non-metric multidimensional scaling (nMDS) ordinations were used to depict variation in the composition of samples collected using the two sampling gears. Differences between samples collected using the two gear types were then tested in ANOSIM, with $p < 0.05$ considered as a significant difference. SIMPER was used to indicate average sample similarity and reveal the taxa that were major contributors to sample similarity (i.e. that characterised samples) for each gear type. If samples differed significantly between gears, SIMPER was used to determine which taxa were the major contributors to sample dissimilarity and whether they were recorded in higher densities by the Day grab or NIOZ corer.

A biological trait matrix compiled by Cefas was used to identify macrofaunal taxa within samples that are known to burrow to depths of greater than 10 cm, as these are the organisms most likely to be ineffectively sampled by the Day grab (10-15 cm penetration depth) compared to the NIOZ corer (~50 cm penetration depth). The abundance of each of these taxa was averaged across the 21 samples collected using each gear type and compared in terms of the mean number of individuals per square metre.

Within-station variability

This section of the analysis used data collected from NIOZ cores only, for the reasons stated in sections 2.3.5 and 3.3.2. Total abundance and biomass values were converted to densities per square metre, as described above in the ‘Gear comparison’ section of this Annex. As all samples used to assess within-station variability were collected using the same gear, taxon richness was simply taken as the number of taxa recorded in a sample (i.e. no standardisation was required). The Margalef Index was also used to indicate macrofaunal diversity in these samples. This index is calculated by subtracting one from the total number of taxa and dividing by the natural log of total abundance, thus indicating taxon richness relative to the number of individuals present. The actual recorded abundances were used in this calculation rather than abundances converted to individuals per square metre to retain the real richness to abundance ratio.

The coefficient of variation (standard deviation/mean x 100; CV) was used to provide a standardised measure of within-station variability for each index at each station. The level of variability was assessed with reference to four ranges: < 25% = low, 25-49% = medium, 50-100% = high, and > 100% = very high. These ranges are somewhat arbitrary, but nevertheless allow the relative level of within-station variability to be assessed for the variables considered. The same approach was used to assess the level of within-station variability in the physical habitat with respect to the % mud content, sorting, skewness and kurtosis of the sediment. Any shared patterns of variability between biological and physical variables were noted.

To assess how the number of taxa changes as the number of replicate samples at a station increases, and therefore determine the degree to which a single sample is likely to be representative of the macrofaunal diversity at a station, species accumulation curves were created in PRIMER (v6). The total number of taxa at each station was predicted using the Michaelis-Menten model (*Smax*). The average number of taxa in one sample at each station was then expressed in terms of a percentage of the number of taxa in all three samples and as a percentage of *Smax*. These percentages were then compared across stations and the level of consistency noted.

Within-station variability in community composition was analysed in PRIMER (v6). As with the gear comparison analyses, Bray-Curtis similarities of samples were calculated with taxa densities weighted both by abundance and by biomass. Data were transformed by ln(x+1) prior to analysis to down weigh the influence of dominant taxa and thus allow any variation in less abundant taxa to be detected. SIMPER was used to calculate the average similarity of samples at each station and therefore determine the degree to which a single sample is likely to accurately represent community composition at the station. SIMPROF was then used to perform a cluster analysis and determine which samples (across all stations) were significantly different from others at $p < 0.05$. If all samples from a station fell within the same cluster, then this was taken to suggest that collecting single samples from stations will likely provide a reliable means of assessing spatial clustering in benthic macrofauna. If samples from the same station fell within different clusters, then this was taken to suggest that collecting single samples is unlikely to allow macrofauna communities at different stations to be reliably distinguished.

Inside *vs* outside the MCZ4

This section of the analysis used data collected from Day grabs only, for the reasons explained in sections 2.3.5 and 3.3.2. General linear models were used to test whether subtidal mud-associated macrofaunal communities sampled inside the MCZ were different from those sampled in the three areas outside the MCZ (to the north, east and south of the MCZ survey area) in terms of total abundance, total biomass, taxon richness and Margalef diversity. These indices were calculated in the same way as described in the 'Within-station variability' section of this Annex, except abundances and biomasses of taxa were converted to numbers per square metre using the conversion necessary for Day grab samples rather than NIOZ corer samples. Assumptions of homoscedasticity and normality of residuals were checked by inspection of plots of residuals against fits and normal quantile plots, respectively. Data were transformed, analysed and the significance of results interpreted using the same procedure described in the ‘Gear comparison’ section of this Annex.

Macrofaunal community composition inside and outside the MCZ was assessed using PRIMER (v6). The same procedure described above in the ‘Gear comparison’ section of this Annex was used to compare communities in different areas, except here Bray-Curtis similarity was based only on abundances, as analysis of within-station variability indicated that a single sample is unlikely to reliably distinguish stations in terms of biomass-weighted community composition (section 3.3.2).

Relation to sediment characteristics

This section of the analysis used data collected from Day grabs only, for the reasons explained in sections 2.3.5 and 3.3.2. General linear models were used to test how the total abundance, total biomass, taxon richness and Margalef diversity of macrofauna in ‘A5.3 Subtidal mud’ differs from that in the other sedimentary BSHs recorded during the survey (‘A5.2 Subtidal sand’ and ‘A5.4 Subtidal mixed sediments’). The BSH ‘A5.1 Subtidal coarse sediment’ was not included in statistical analyses as it was recorded at just one station and therefore had no replication. Biodiversity indices were calculated in the same way described in the ‘Within-station variability’ section of this Annex, except abundances and biomasses were converted to densities per square metre using the conversion necessary for Day grab samples rather than NIOZ corer samples.

Relationships between biodiversity indices and quantitative sediment properties (percent mud, sand and gravel contents, particle sorting, particle skewness and particle kurtosis) were analysed using generalised additive models (GAMs) in the *mgcv* package in R (v 3.4.1). In GAMs, relationships between explanatory and response variables can be ‘smoothed’, whereby the shapes of trends are estimated using regression splines rather than assumed to have a specific form (e.g. linear) (Lin and Zhang, 1999). Relationships between biodiversity indices and all sediment properties were fitted in this way. Data were transformed, analysed and the significance of results interpreted using the same procedure described in the ‘Gear comparison’ section of this Annex.

Macrofaunal community composition in ‘A5.3 Subtidal mud’ was assessed and compared to other sedimentary BSHs using PRIMER (v6). The same procedure described in the ‘Gear comparison’ section of this Annex was used to compare communities in different BSHs, except here Bray-Curtis similarity was weighted using only abundances, as analysis of within-station variability indicated that a single sample is unlikely to reliably distinguish stations based on biomass-weighted community composition (section 3.2.2).

RELATE was then used to determine whether variation in macrofaunal community composition was significantly correlated (Spearman Rank) with the quantitative sediment properties listed above and BEST was used to determine which combination of these variables best explained variation in macrofaunal community composition. Finally, SIMPROF was used in association with cluster analyses determine which stations were significantly different from others ($p < 0.05$) in terms of macrofaunal community composition. The resulting clusters were mapped and inspected for any apparent spatial patterns in relation to % mud content to identify any associations between community composition and the physical environment across the surveyed areas. SIMPER was used to determine which taxa characterised each cluster.

Annex 5. Non-Indigenous Species (NIS)

Taxa listed as non-indigenous species (present and horizon) which have been selected for assessment of Good Environmental Status in GB waters under MSFD Descriptor 2 (*Stebbing et al*., 2014).

Species name	List	Species name	List
Acartia (Acanthacartia) tonsa	Present	*Alexandrium catenella*	Horizon
Amphibalanus amphitrite	Present	*Amphibalanus reticulatus*	Horizon
Asterocarpa humilis	Present	*Asterias amurensis*	Horizon
Bonnemaisonia hamifera	Present	*Caulerpa racemosa*	Horizon
Caprella mutica	Present	*Caulerpa taxifolia*	Horizon
Crassostrea angulata	Present	*Celtodoryx ciocalyptoides*	Horizon
Crassostrea gigas	Present	*Chama sp.*	Horizon
Crepidula fornicata	Present	*Dendostrea frons*	Horizon
Diadumene lineata	Present	*Gracilaria vermiculophylla*	Horizon
Didemnum vexillum	Present	*Hemigrapsus penicillatus*	Horizon
Dyspanopeus sayi	Present	*Hemigrapsus sanguineus*	Horizon
Ensis directus	Present	*Hemigrapsus takanoi*	Horizon
Eriocheir sinensis	Present	*Megabalanus coccopoma*	Horizon
Ficopomatus enigmaticus	Present	*Megabalanus zebra*	Horizon
Grateloupia doryphora	Present	*Mizuhopecten yessoensis*	Horizon
Grateloupia turuturu	Present	*Mnemiopsis leidyi*	Horizon
Hesperibalanus fallax	Present	*Ocenebra inornata*	Horizon
Heterosigma akashiwo	Present	*Paralithodes camtschaticus*	Horizon
Homarus americanus	Present	*Polysiphonia subtilissima*	Horizon
Rapana venosa	Present	*Pseudochattonella verruculosa*	Horizon
Sargassum muticum	Present	*Rhopilema nomadica*	Horizon
Schizoporella japonica	Present	*Telmatogeton japonicus*	Horizon
Spartina townsendii var. anglica	Present		
Styela clava	Present		
Undaria pinnatifida	Present		
Urosalpinx cinerea	Present		
Watersipora subatra	Present		

Additional taxa listed as non-indigenous species in the JNCC 'Non-native marine species in British waters: a review and directory' report by Eno *et al*. (1997) which have not been selected for assessment of Good Environmental Status in GB waters under MSFD Descriptor 2.

Species name (1997)	Updated name (2017)
Thalassiosira punctigera	
Thalassiosira tealata	
Coscinodiscus wailesii	
Odontella sinensis	
Pleurosigma simonsenii	
Grateloupia doryphora	
Grateloupia filicina var. luxurians	*Grateloupia subpectinata*
Pikea californica	
Agardhiella subulata	
Solieria chordalis	
Antithamnionella spirographidis	
Antithamnionella ternifolia	
Polysiphonia harveyi	*Neosiphonia harveyi*
Colpomenia peregrine	
Codium fragile subsp. atlanticum	
Codium fragile subsp. tomentosoides	*Codium fragile* subsp. *atlanticum*
Gonionemus vertens	
Clavopsella navis	*Pachycordyle navis*
Anguillicoloides crassus	
Goniadella gracilis	
Marenzelleria viridis	
Clymenella torquata	
Hydroides dianthus	
Hydroides ezoensis	
Janua brasiliensis	
Pileolaria berkeleyana	
Ammothea hilgendorfi	
Elminius modestus	*Austrominius modestus*
Eusarsiella zostericola	
Corophium sextonae	
Rhithropanopeus harrissii	
Potamopyrgus antipodarum	
Tiostrea lutaria	*Tiostrea chilensis*

Mercenaria mercenaria	
Petricola pholadiformis	
Mya arenaria	

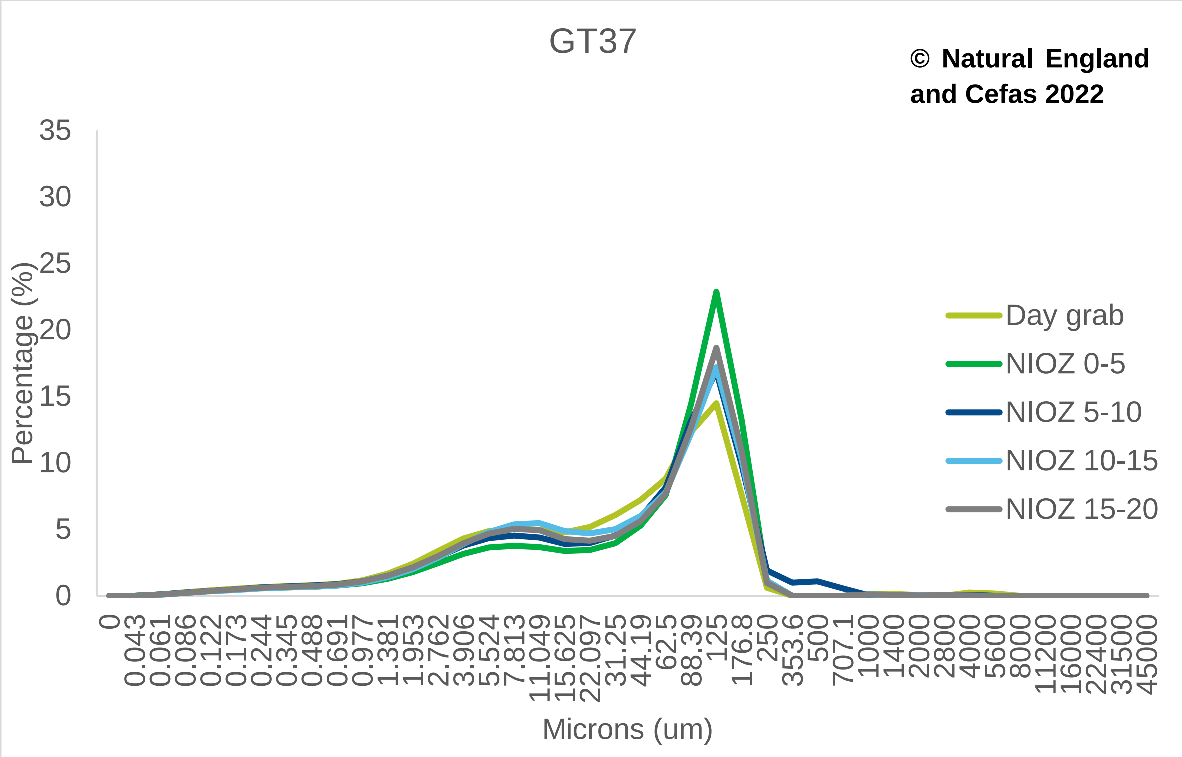
Annex 6. Broadscale Habitats (BSHs) identified at each sampling station using different sampling methodologies

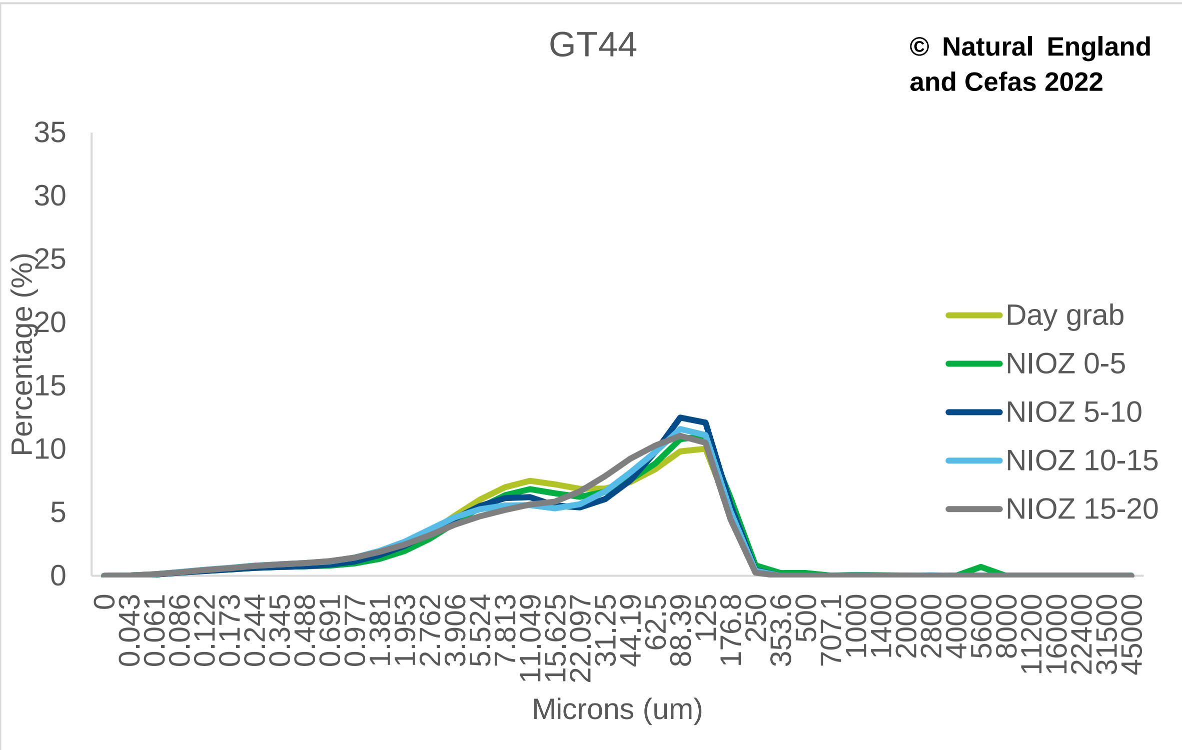
Broadscale Habitats (BSHs) at stations sampled during a survey of the Coquet to St Mary's MCZ and surrounding areas in 2016 based on data collected using different sampling methodologies. (© Natural England and Cefas 2022). A5.1 = 'Subtidal coarse sediment', A5.2 = 'Subtidal sand', A5.3 = 'Subtidal mud' and A5.4 = 'Subtidal mixed sediments'.

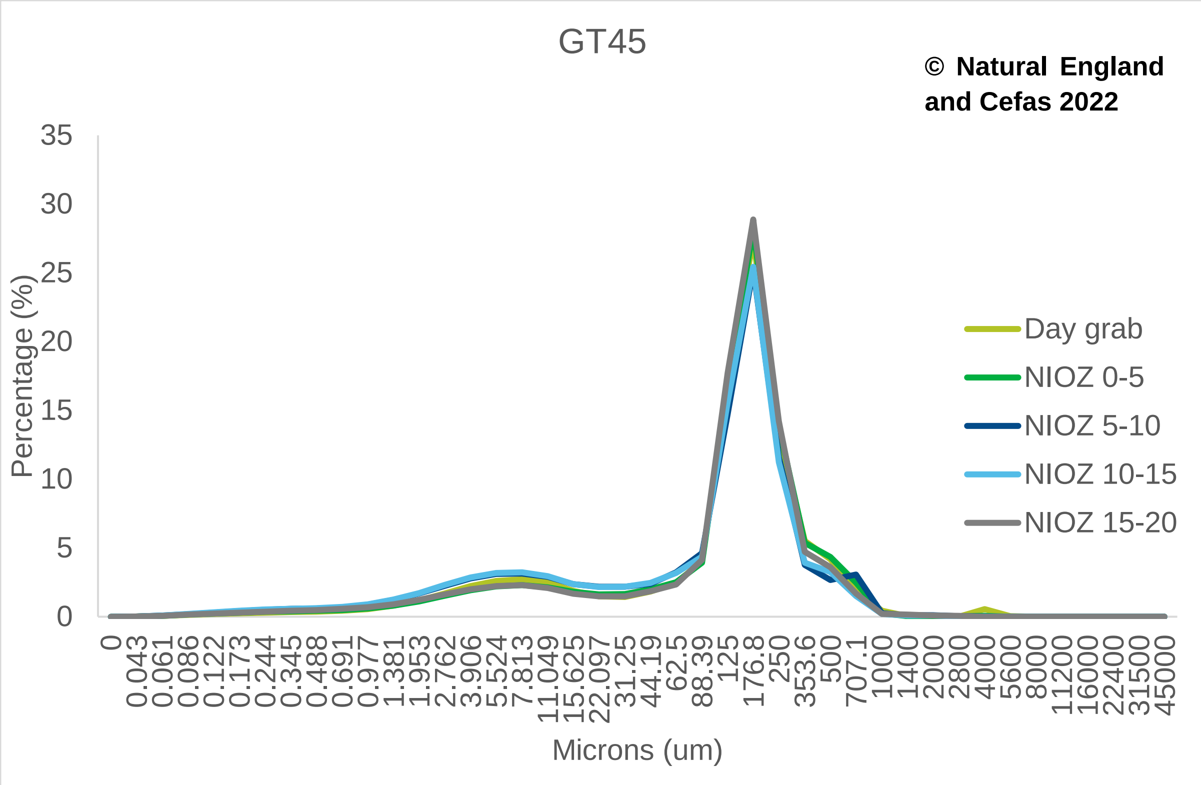
Station code	Area	Day grab	Drop camera		NIOZ corer (0-5 cm)
			Video	Stills	
EA_01	Northern	A5.3	-	-	-
EA_02	Northern	A5.3	-	-	-
EA_03	Northern	A5.3	-	-	-
EA_04	Northern	A5.3	-	-	-
GT01	Eastern	A5.2	-	-	-
GT02	Eastern	-	A5.2	A5.2	-
GT03	MCZ	A5.3	-	A5.2	-
GT04	Eastern	A5.2	A5.2	A5.1/A5.2	-
GT05	MCZ	A5.3	A5.2	A5.1/A5.2/A5.4	-
GT06	MCZ	A5.2	A5.1	A5.2	-
GT07	Eastern	A5.2	A5.1	A5.1/A5.2	-
GT08	Eastern	A5.4	-	-	-
GT09	Eastern	A5.3	-	-	-
GT10	Eastern	-	A5.4	A5.4	-
GT11	Eastern	A5.4	-	-	-
GT13	Eastern	-	A5.2	A5.2	-
GT15	MCZ	A5.3	A5.2	A5.2	-
GT16	Eastern	-	A5.2	A5.2	-
GT17	MCZ	A5.2	A5.2	A5.1/A5.2	-
GT18	Eastern	A5.2	-	-	-
GT19	MCZ	A5.3	A5.3	A5.3	-
GT20	Eastern	A5.2	-	-	-
GT21	Eastern	A5.3	-	-	-
GT22	Eastern	-	A5.2	A5.2	-
GT23	Eastern	A5.3	A5.4	A5.3/A5.4	-
GT24	Eastern	A5.2	-	-	-
GT25	Eastern	A5.2	A5.1	A5.1/A5.2	-
GT26	Eastern	A5.3	A5.3	A5.2/A5.3	-
GT27	MCZ	A5.1	A5.4	A5.4	-
GT28	Eastern	-	A5.2	A5.3	-
GT30	Eastern	A5.3	A5.4	A5.4	-
GT31	Southern	A5.3	-	A5.3	-
GT32	Southern	A5.3	-	A5.3	-
GT33	Southern	A5.3	-	A5.3	-
GT34	Southern	A5.3	A5.2/A4.3	A5.2/A4.3	-

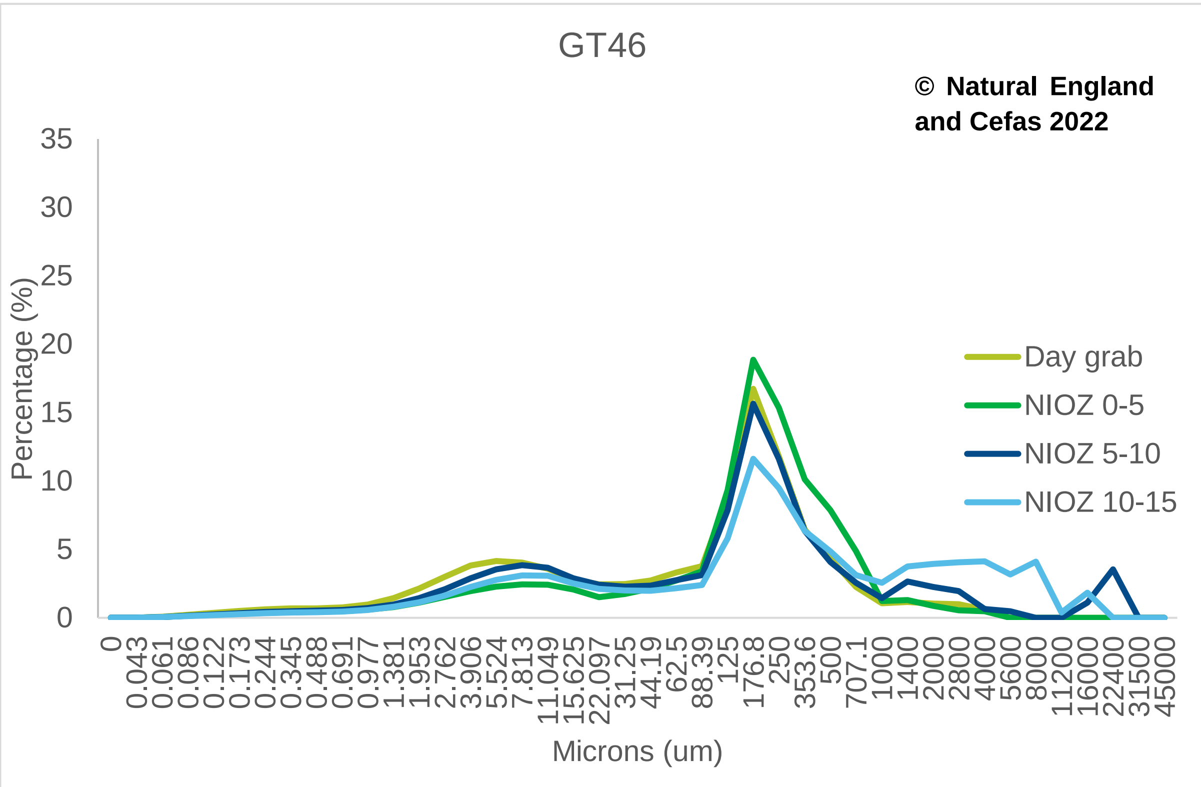
Station code	Area	Day grab	Drop camera		NIOZ corer (0-5 cm)
			Video	Stills	
GT35	Southern	A5.4	A5.4	A5.3/A5.4	-
GT36	Southern	A5.3	A5.3	A5.3	-
GT37	Southern	A5.3	A5.3	A5.3	A5.3
GT38	Southern	A5.3	A5.3	A5.3	-
GT39	Southern	A5.3	A5.3	A5.3	-
GT40	Southern	A5.2	-	-	-
GT41	Southern	A5.3	A5.3	A5.4/A5.3	-
GT42	Southern	A5.3	A5.3	A5.3	-
GT43	Southern	A5.3	-	-	-
GT44	Southern	A5.3	-	A5.3	A5.3
GT45	Southern	A5.3	A5.3	A5.3	A5.2/A5.3
GT46	Southern	A5.3	A5.2	A5.2	A5.3
GT47	Southern	A5.3	A5.1	A5.4	-
GT48	Southern	A5.4	A5.4	A5.2/A5.1	-
GT49	Southern	A5.2	A5.2	A5.2	-
GT50	Southern	A5.3	-	-	A5.3
GT71	MCZ	A5.3	A5.3	A5.3	-
GT72	MCZ	A5.3	-	A5.2	-
Re01	MCZ	A5.3	A5.2	A5.2	A5.3
Re02	MCZ	A5.3	-	A5.2	A5.3
Re03	MCZ	A5.3	A5.2	A5.2	A5.3
Re04	MCZ	A5.3	A5.2	A5.2	A5.3
Re05	MCZ	A5.3	A5.2	A5.2	A5.3
Re06	MCZ	A5.2/A5.3	A5.2	A5.2	A5.2/A5.3
Re08	MCZ	A5.3	A5.2	A5.2	A5.2
Re09	MCZ	A5.3	A5.2	A5.2	A5.3
Re10	MCZ	A5.2/A5.3	A5.2	A5.2	A5.3
Re12	MCZ	A5.3	A5.2	A5.3	A5.3

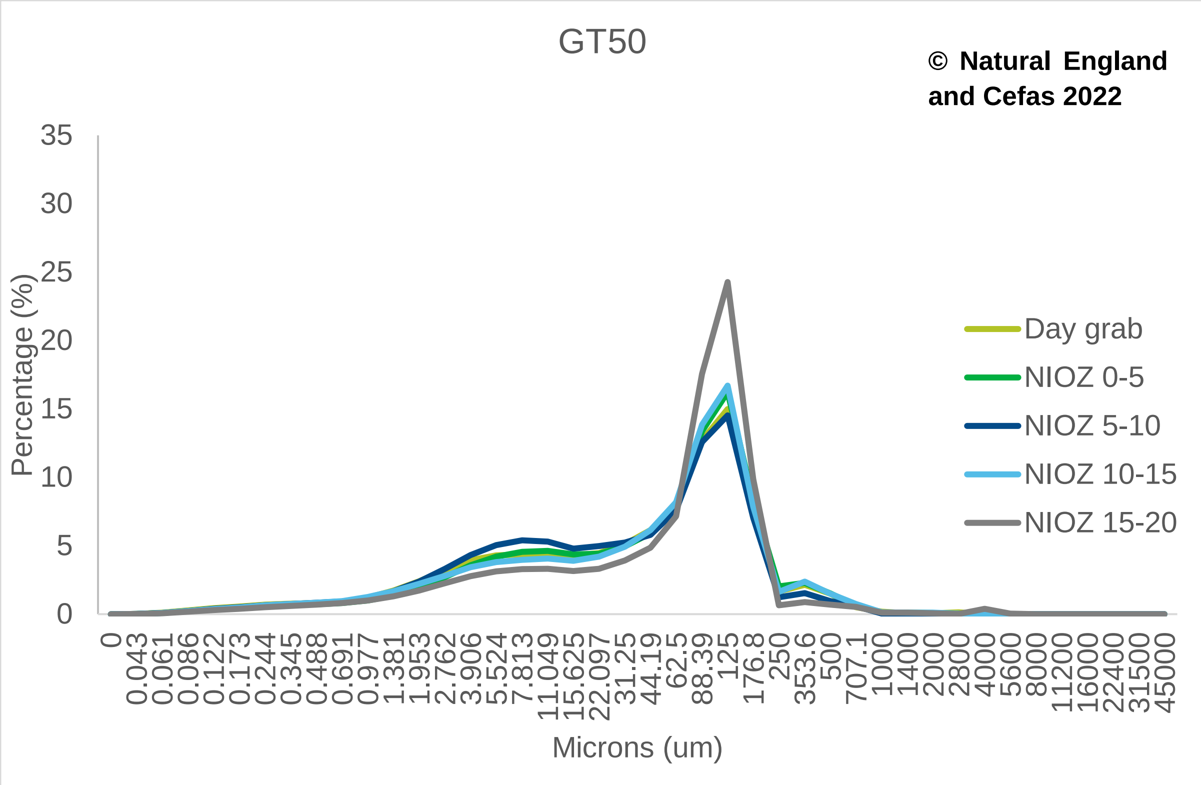
Annex 7. Sediment particle size distributions of sampling stations within the Coquet to St Mary’s MCZ and surrounding areas

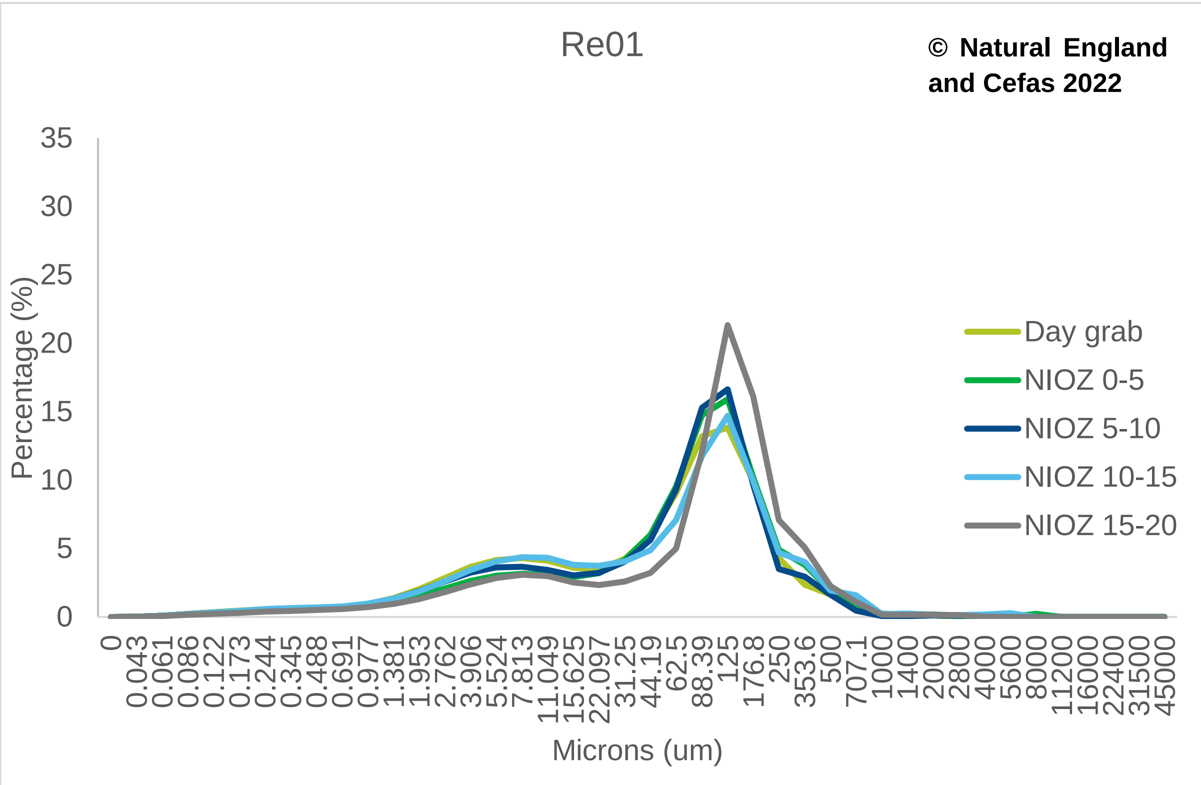


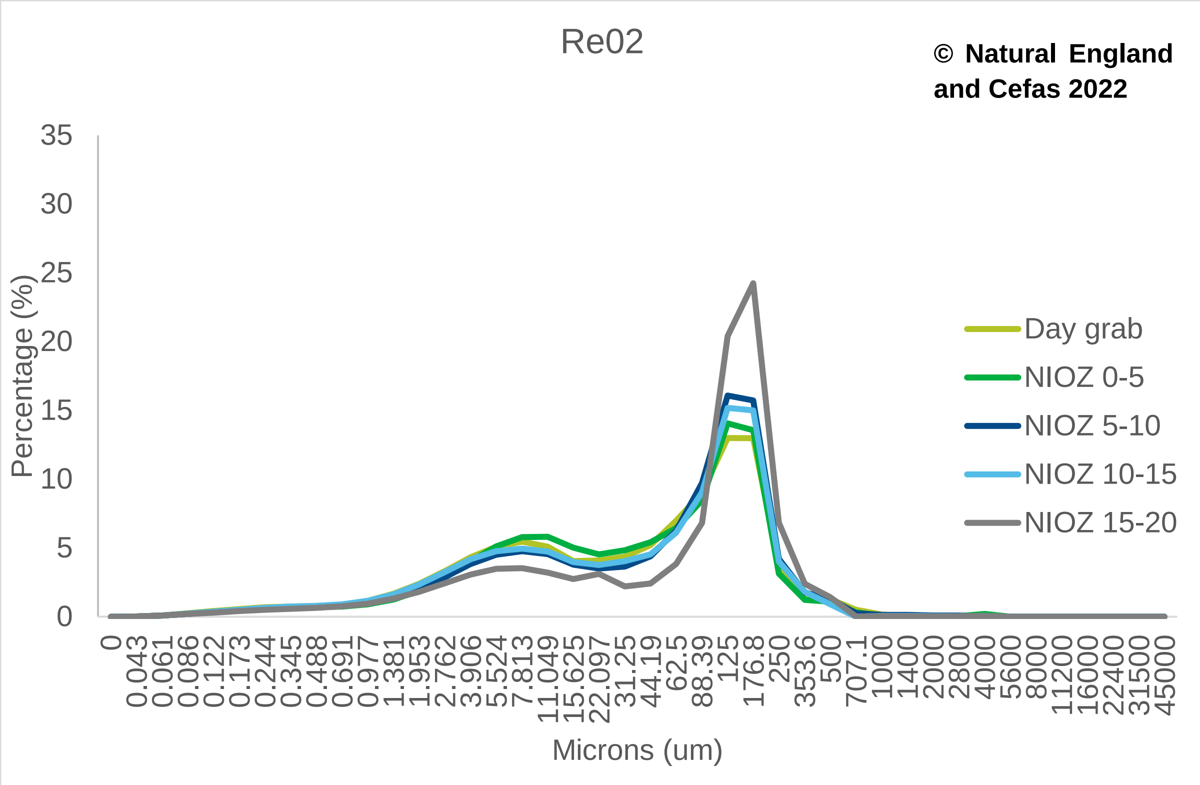


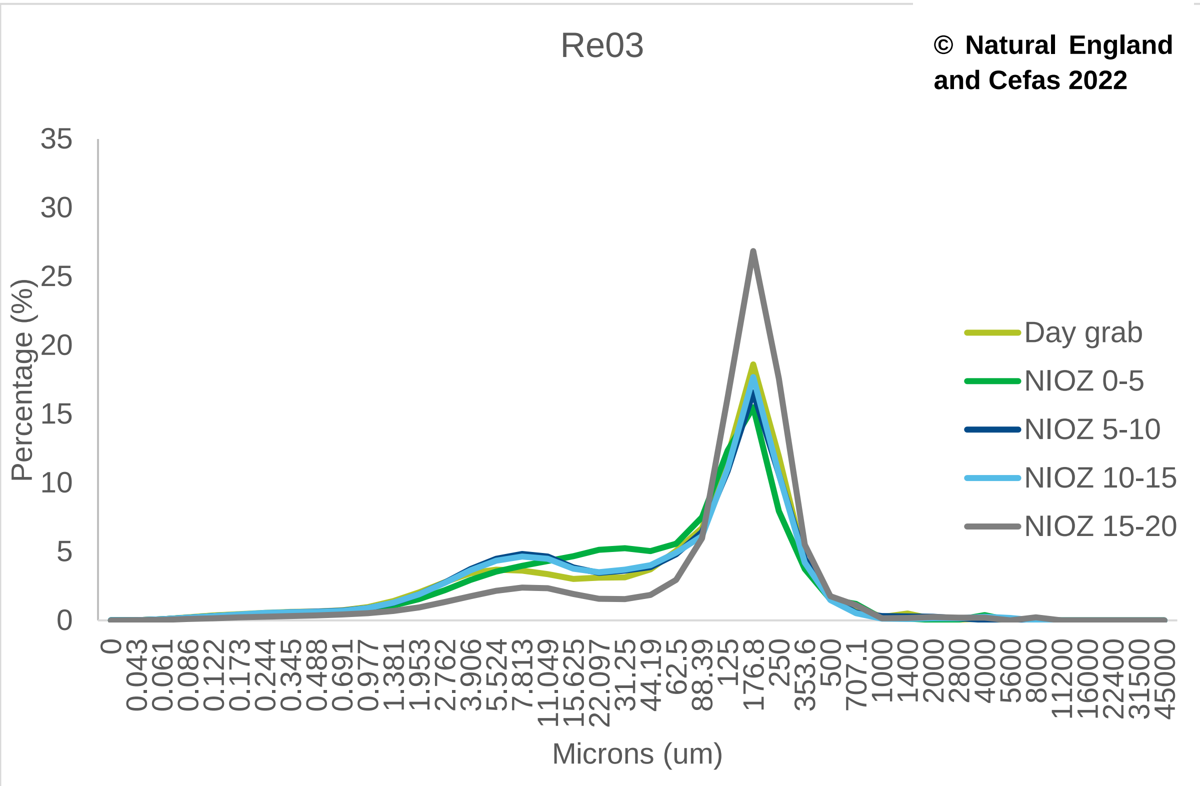

GT45
© Natural England and Cefas 2022
Percentage (%)
35
30
25
20
15
10
5
0
Day grab
NIOZ 0-5
NIOZ 5-10
NIOZ 10-15
NIOZ 15-20
0
0.043
0.061
0.086
0.122
0.173
0.244
0.345
0.488
0.691
0.977
1.381
1.953
2.762
3.906
5.524
7.813
11.049
15.625
22.097
31.25
44.19
62.5
88.39
125
176.8
250
353.6
500
707.1
1000
1400
2000
2800
4000
5600
8000
11200
16000
22400
31500
45000
Microns (um)


GT46
© Natural England and Cefas 2022
Percentage (%)
35
30
25
20
15
10
5
0
Day grab
NIOZ 0-5
NIOZ 5-10
NIOZ 10-15
0
0.043
0.061
0.086
0.122
0.173
0.244
0.345
0.488
0.691
0.977
1.381
1.953
2.762
3.906
5.524
7.813
11.049
15.625
22.097
31.25
44.19
62.5
88.39
125
176.8
250
353.6
500
707.1
1000
1400
2000
2800
4000
5600
8000
11200
16000
22400
31500
45000
Microns (um)


GT50
© Natural England and Cefas 2022
Percentage (%)
35
30
25
20
15
10
5
0
Day grab
NIOZ 0-5
NIOZ 5-10
NIOZ 10-15
NIOZ 15-20
0
0.043
0.061
0.086
0.122
0.173
0.244
0.345
0.488
0.691
0.977
1.381
1.953
2.762
3.906
5.524
7.813
11.049
15.625
22.097
31.25
44.19
62.5
88.39
125
176.8
250
353.6
500
707.1
1000
1400
2000
2800
4000
5600
8000
11200
16000
22400
31500
45000
Microns (um)


Re01
© Natural England and Cefas 2022
Percentage (%)
35
30
25
20
15
10
5
0
Day grab
NIOZ 0-5
NIOZ 5-10
NIOZ 10-15
NIOZ 15-20
0
0.043
0.061
0.086
0.122
0.173
0.244
0.345
0.488
0.691
0.977
1.381
1.953
2.762
3.906
5.524
7.813
11.049
15.625
22.097
31.25
44.19
62.5
88.39
125
176.8
250
353.6
500
707.1
1000
1400
2000
2800
4000
5600
8000
11200
16000
22400
31500
45000
Microns (um)


Re02
© Natural England and Cefas 2022
Percentage (%)
35
30
25
20
15
10
5
0
Day grab
NIOZ 0-5
NIOZ 5-10
NIOZ 10-15
NIOZ 15-20
0
0.043
0.061
0.086
0.122
0.173
0.244
0.345
0.488
0.691
0.977
1.381
1.953
2.762
3.906
5.524
7.813
11.049
15.625
22.097
31.25
44.19
62.5
88.39
125
176.8
250
353.6
500
707.1
1000
1400
2000
2800
4000
5600
8000
11200
16000
22400
31500
45000
Microns (um)


Re03
© Natural England and Cefas 2022
Percentage (%)
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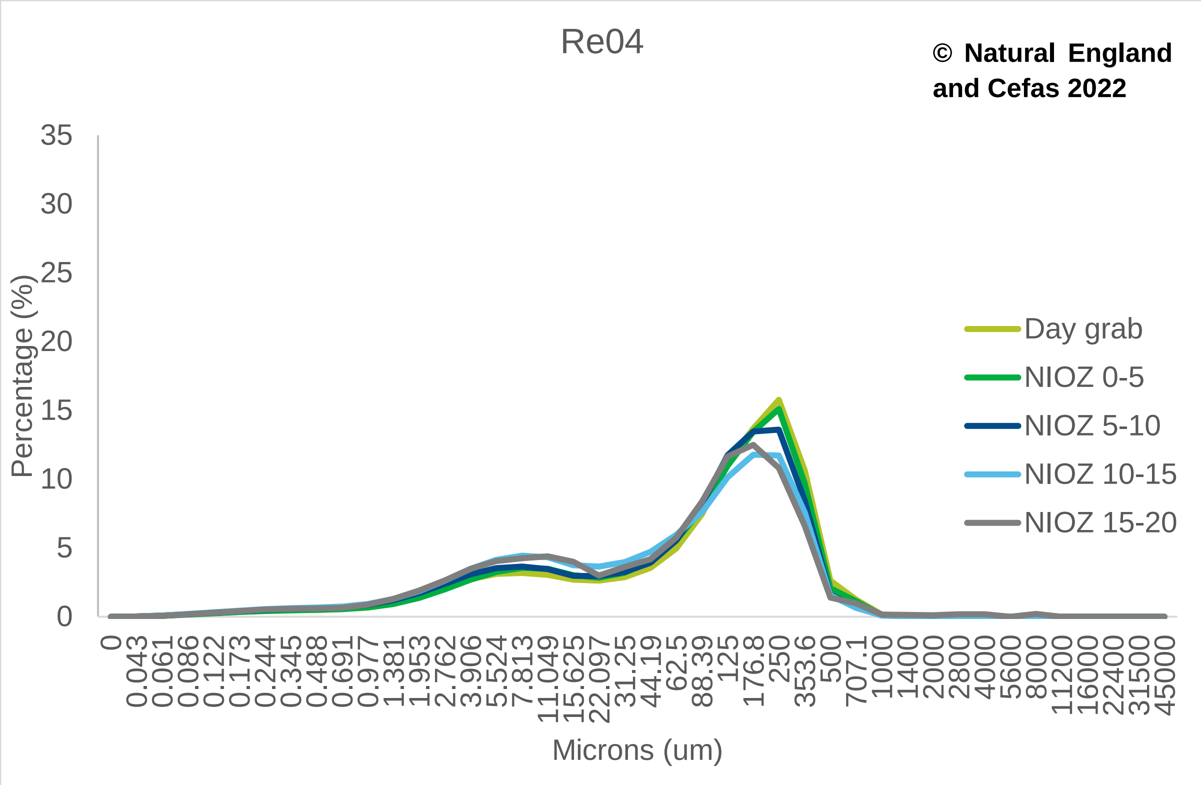
30
25
20
15
10
5
0
Day grab
NIOZ 0-5
NIOZ 5-10
NIOZ 10-15
NIOZ 15-20
0
0.043
0.061
0.086
0.122
0.173
0.244
0.345
0.488
0.691
0.977
1.381
1.953
2.762
3.906
5.524
7.813
11.049
15.625
22.097
31.25
44.19
62.5
88.39
125
176.8
250
353.6
500
707.1
1000
1400
2000
2800
4000
5600
8000
11200
16000
22400
31500
45000
Microns (um)


Re04
© Natural England and Cefas 2022
Percentage (%)
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30
25
20
15
10
5
0
Day grab
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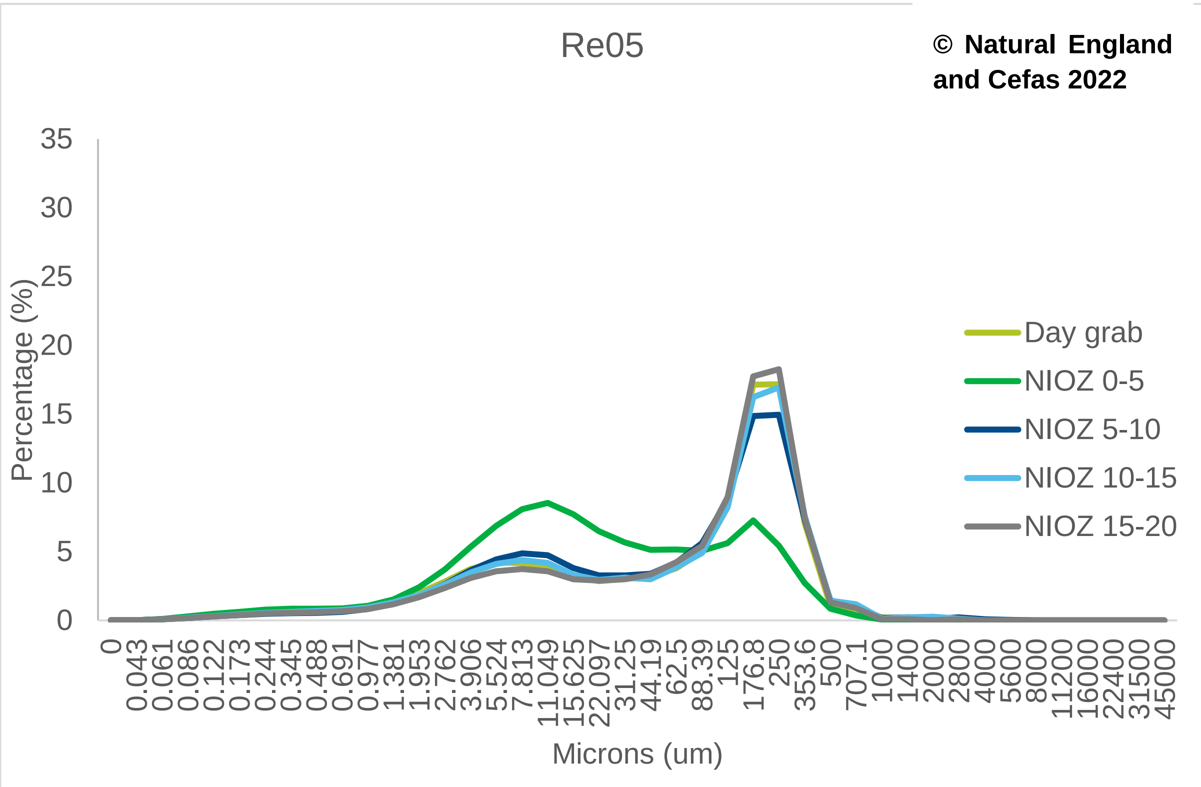
NIOZ 5-10
NIOZ 10-15
NIOZ 15-20
0
0.043
0.061
0.086
0.122
0.173
0.244
0.345
0.488
0.691
0.977
1.381
1.953
2.762
3.906
5.524
7.813
11.049
15.625
22.097
31.25
44.19
62.5
88.39
125
176.8
250
353.6
500
707.1
1000
1400
2000
2800
4000
5600
8000
11200
16000
22400
31500
45000
Microns (um)


Re05
© Natural England and Cefas 2022
Percentage (%)
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30
25
20
15
10
5
0
Day grab
NIOZ 0-5
NIOZ 5-10
NIOZ 10-15
NIOZ 15-20
0
0.043
0.061
0.086
0.122
0.173
0.244
0.345
0.488
0.691
0.977
1.381
1.953
2.762
3.906
5.524
7.813
11.049
15.625
22.097
31.25
44.19
62.5
88.39
125
176.8
250
353.6
500
707.1
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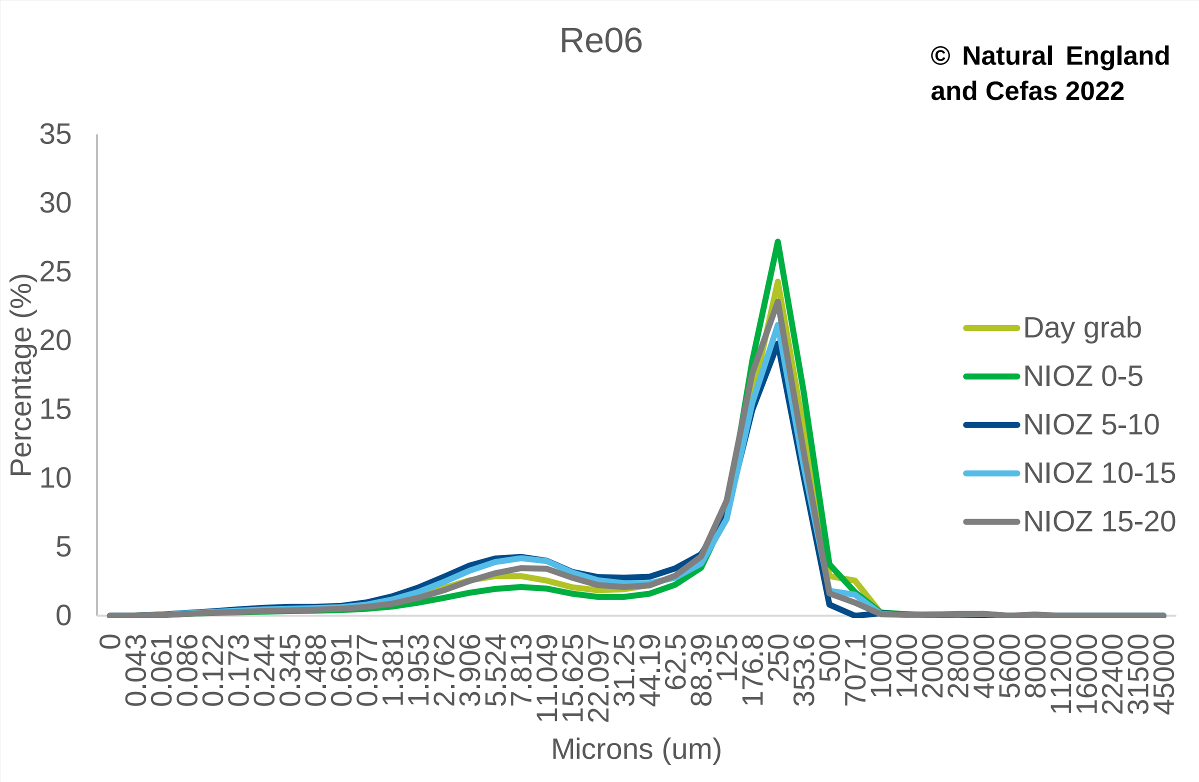
1400
2000
2800
4000
5600
8000
11200
16000
22400
31500
45000
Microns (um)



Re06
© Natural England and Cefas 2022
Percentage (%)
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25
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5
0
Day grab
NIOZ 0-5
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NIOZ 10-15
NIOZ 15-20
0
0.043
0.061
0.086
0.122
0.173
0.244
0.345
0.488
0.691
0.977
1.381
1.953
2.762
3.906
5.524
7.813
11.049
15.625
22.097
31.25
44.19
62.5
88.39
125
176.8
250
353.6
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707.1
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4000
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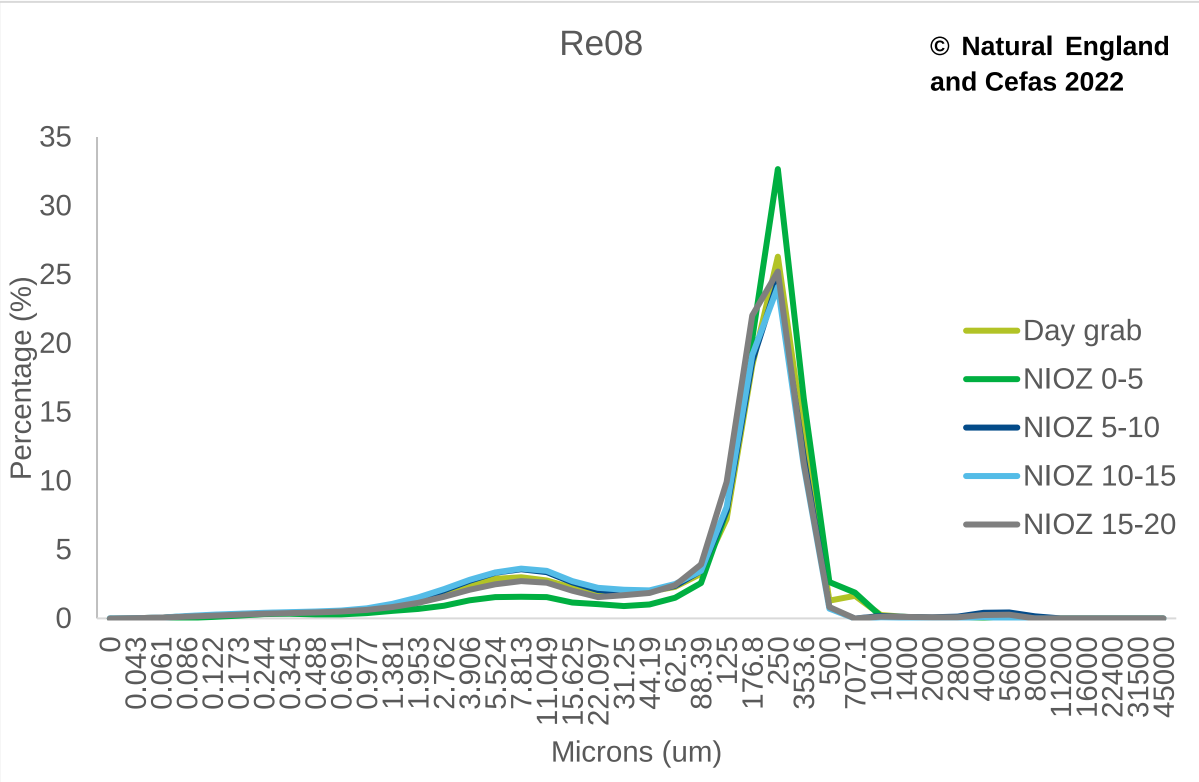
8000
11200
16000
22400
31500
45000
Microns (um)



Re08
© Natural England and Cefas 2022
Percentage (%)
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30
25
20
15
10
5
0
Day grab
NIOZ 0-5
NIOZ 5-10
NIOZ 10-15
NIOZ 15-20
0
0.043
0.061
0.086
0.122
0.173
0.244
0.345
0.488
0.691
0.977
1.381
1.953
2.762
3.906
5.524
7.813
11.049
15.625
22.097
31.25
44.19
62.5
88.39
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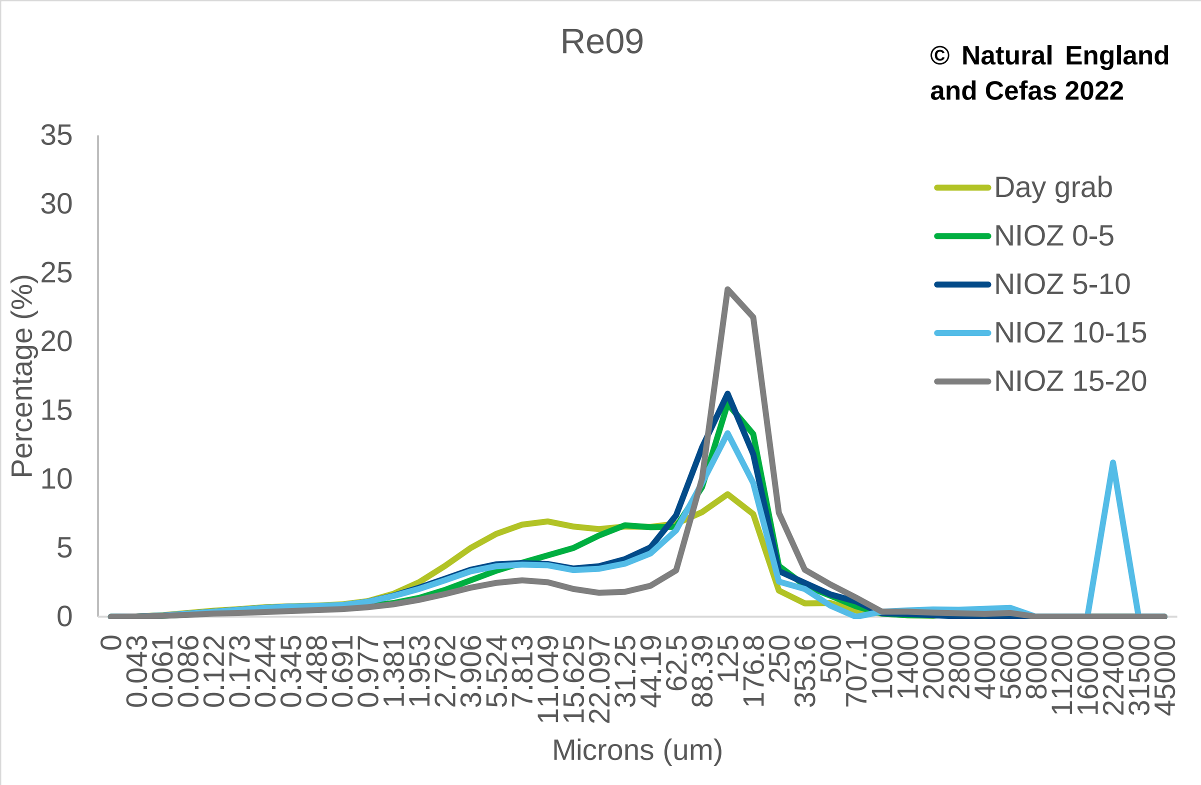
176.8
250
353.6
500
707.1
1000
1400
2000
2800
4000
5600
8000
11200
16000
22400
31500
45000
Microns (um)


Re09
© Natural England and Cefas 2022
Percentage (%)
35
30
25
20
15
10
5
0
Day grab
NIOZ 0-5
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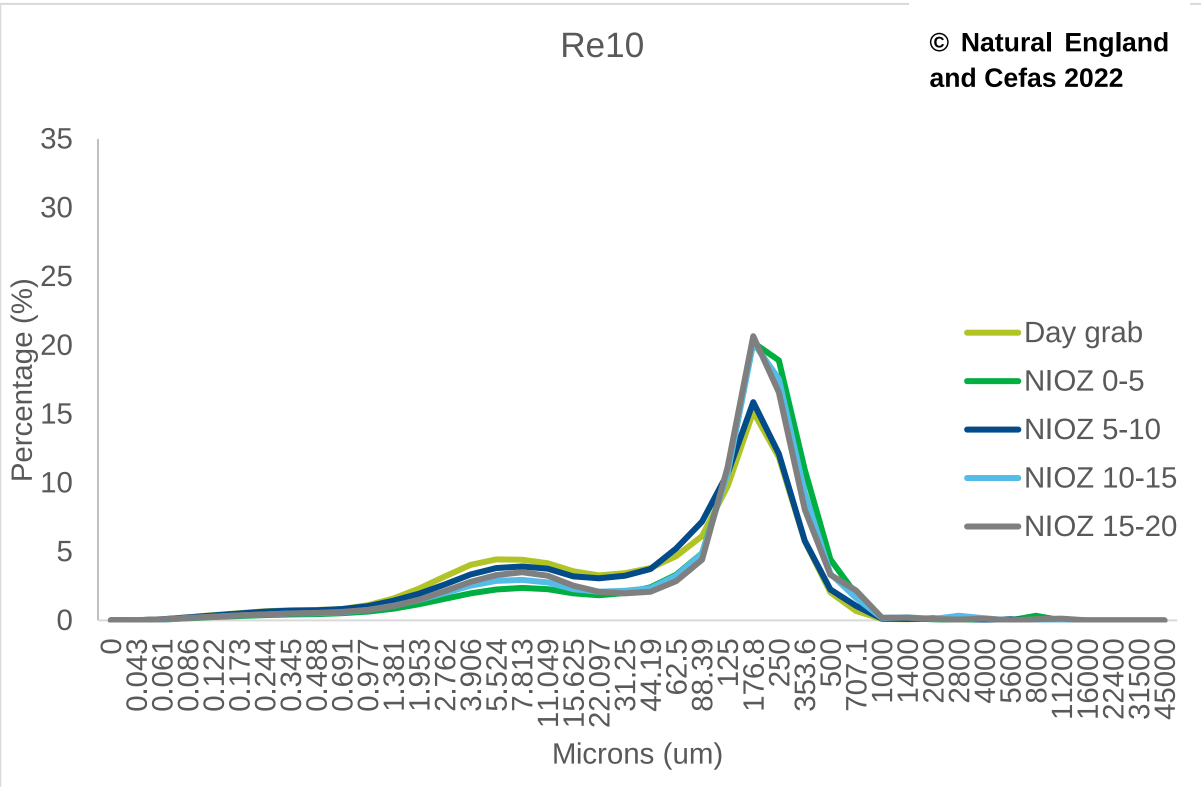
NIOZ 10-15
NIOZ 15-20
0
0.043
0.061
0.086
0.122
0.173
0.244
0.345
0.488
0.691
0.977
1.381
1.953
2.762
3.906
5.524
7.813
11.049
15.625
22.097
31.25
44.19
62.5
88.39
125
176.8
250
353.6
500
707.1
1000
1400
2000
2800
4000
5600
8000
11200
16000
22400
31500
45000
Microns (um)


Re10
© Natural England and Cefas 2022
Percentage (%)
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30
25
20
15
10
5
0
Day grab
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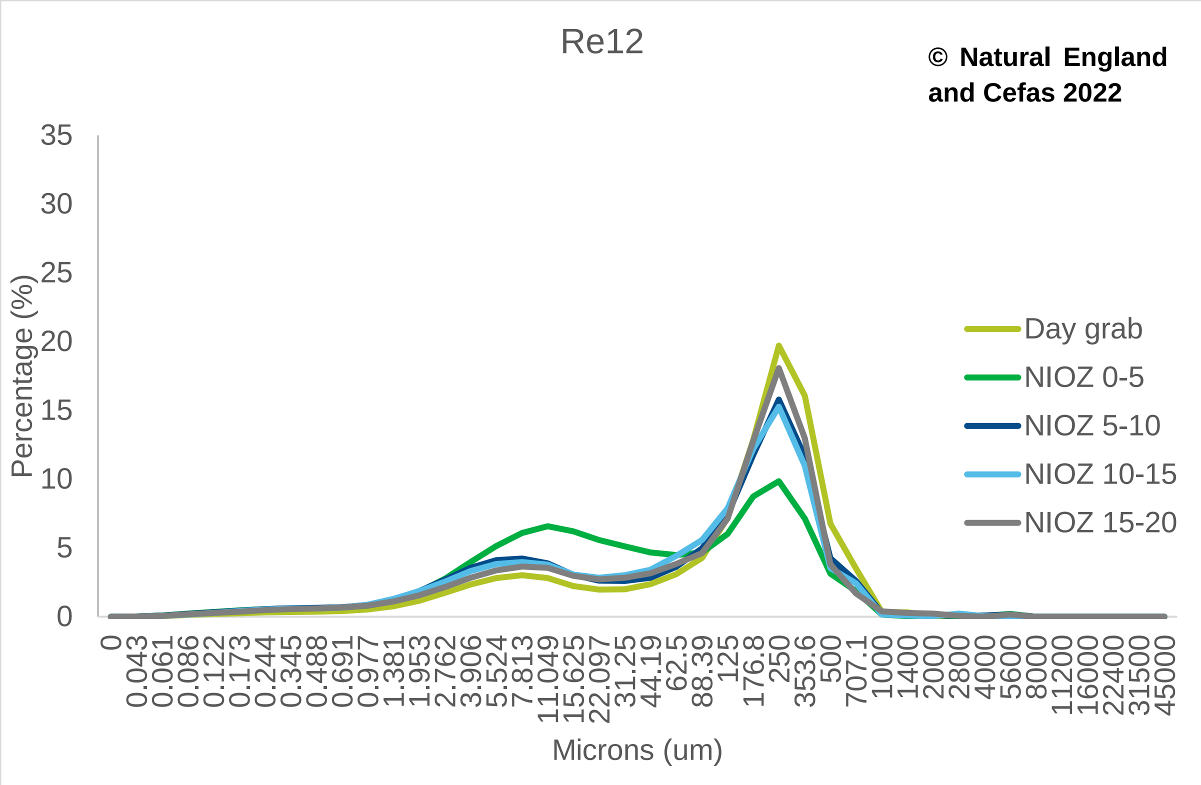
NIOZ 10-15
NIOZ 15-20
0
0.043
0.061
0.086
0.122
0.173
0.244
0.345
0.488
0.691
0.977
1.381
1.953
2.762
3.906
5.524
7.813
11.049
15.625
22.097
31.25
44.19
62.5
88.39
125
176.8
250
353.6
500
707.1
1000
1400
2000
2800
4000
5600
8000
11200
16000
22400
31500
45000
Microns (um)


Re12
© Natural England and Cefas 2022
Percentage (%)
35
30
25
20
15
10
5
0
Day grab
NIOZ 0-5
NIOZ 5-10
NIOZ 10-15
NIOZ 15-20
0
0.043
0.061
0.086
0.122
0.173
0.244
0.345
0.488
0.691
0.977
1.381
1.953
2.762
3.906
5.524
7.813
11.049
15.625
22.097
31.25
44.19
62.5
88.39
125
176.8
250
353.6
500
707.1
1000
1400
2000
2800
4000
5600
8000
11200
16000
22400
31500
45000
Microns (um)

Annex 8. Cluster analysis of macrofaunal community composition

Taxa that made the greatest contributions to the internal similarity of macrofaunal clusters observed in the Coquet to St Mary's MCZ and surrounding areas (© Natural England and Cefas 2022). When a cluster consisted of just one sample, the numerically dominant taxa are listed as the main taxa.

Cluster	Characteristic taxa	Internal similarity
a	*Lumbrineris* spp. *Sabellaria spinulosa* *Ampelisca diadema* *Terebellides* spp. *Galathowenia oculata*	49.26
b	*Spiophanes kroyeri* *Leucon nasica* *Cylichna cylindracea* *Diplocirrus glaucus* *Eunereis longissima*	46.65
c	*Amphiura filiformis* *Chamelea striatula* *Chaetoderma nitidum* *Lumbrineris* spp. *Nephtys* spp.	-
d	*Amphiura filiformis* *Galathowenia oculata* *Lumbrineris* spp. *Terebellides* spp. *Owenia* spp.	46.96
e	*Amphiura filiformis* *Lumbrineris* spp. *Echinocyamus pusillus* *Dosinia* spp. *Galathowenia oculata*	50.43
f	*Lumbrineris* spp. *Amphiura filiformis* *Thyasira flexuosa* *Nephtys* spp. *Turritella communis*	49.12
g	*Amphiura filiformis* *Lumbrineris* spp. *Kurtiella bidentata*	53.18

Cluster	Characteristic taxa	Internal similarity
	Peresiella clymenoides	
	Thyasira flexuosa	
h	*Lumbrineris* spp. *Amphiura filiformis* *Kurtiella bidentata* *Peresiella clymenoides* *Ampelisca tenuicornis*	48.19
i	*Amphiura filiformis* *Lumbrineris* spp. *Dosinia spp.* *Echinocyamus pusillus* *Abra prismatica*	35.58

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