West of Walney Marine Conservation Zone (MCZ) Monitoring Report 2018

Marine Protected Area (MPA) Monitoring Programme

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Peter Mitchell, Paul McIlwaine, Riccardo Arosio, Oliver Hogg, David Clare (Cefas)

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Natural England Project manager

James Highfield

Contractor

Cefas

Authors

Peter Mitchell (Cefas)

Paul McIlwaine (Cefas)

Riccardo Arosio (Cefas)

Oliver Hogg (Cefas)

David Clare (Cefas)

Further information

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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.

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Abbreviations

AIC	Akaike Information Criterion
ANOSIM	Analysis of Similarities
AOI	Area of Interest
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
GAMM	Generalised Additive Mixed Model
GES	Good Environmental Status
GMA	General Management Approach
HOCI	Habitats of Conservation Importance
IECS	Institute of Estuarine and Coastal Studies
IFCA	Inshore Fisheries and Conservation Authority
JNCC	Joint Nature Conservation Committee
NMBAQC	North East Atlantic Marine Biological Analytical Quality Control Scheme
MBES	Multibeam echosounder
MCZ	Marine Conservation Zone
MESH	Mapping European Seabed Habitat
MMO	Marine Management Organisation
MPA	Marine Protected Area
MPAG	Marine Protected Areas Group
MSFD	Marine Strategy Framework Directive
NE	Natural England
NIS	Non-Indigenous Species
OSPAR	The Convention for the Protection of the Marine Environment of the North-East Atlantic

OWF	Offshore windfarm
PAH	Polycyclic Aromatic Hydrocarbons
PCB	Polychlorinated Biphenyl
PSA	Particle Size Analysis
PSD	Particle Size Distribution
SAC	Special Area of Conservation
SACFOR	Superabundant, Abundant, Common, Frequent, Occasional, Rare
SACO	Supplementary Advice on Conservation Objectives
s.d.	Standard Deviation
SIMPER	Similarity Percentages Analysis
SIMPROF	Similarity Profile Analysis
SNCB	Statutory Nature Conservation Body
SOCI	Species of Conservation Importance
TBT	Tributyltin

Glossary

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

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).
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.*
Broadscale Habitats	Habitats which have been broadly categorised based on a 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).*
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	When the ecological condition of a species or habitat is in line

Condition	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 Importance (HOCI)	Habitats that are rare, threatened, or declining in Secretary of State waters.*
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.
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).*
Natural England	The statutory conservation advisor to Government, with a remit for England out to 12 nautical miles offshore.
Non-indigenous Species	A species that has been introduced directly or indirectly by 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 *et al*., 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 (SOCI)	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.

Executive Summary

This report is one of a series of Marine Protected Area (MPA) 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 obligations in relation to MPA assessment and reporting, in relation to current policy instruments, including the Oslo-Paris (OSPAR) Convention, the UK Marine and Coastal Access Act (2009) and Community Directives (e.g. the Habitats and Birds Directives and the Marine Strategy Framework Directive).

The Statutory Nature Conservation Bodies (SNCB) responsible for nature conservation offshore (between 0 nm and 12 nm from the coast) is Natural England (NE). NE utilise evidence gathered by targeted environmental and ecological surveys and site-specific MPA reports in conjunction with other available evidence (e.g. activities, pressures, historical data, survey data collected from other organisations or data collected to meet different obligations). These data are collectively used by SNCBs to make assessments of the condition of designated features within sites, to inform and maintain up to date site-specific conservation advice and produce advice on operations and management measures for anthropogenic activities occurring within the site. This report, as a stand-alone document, **does not** therefore aim to assess the condition of the designated features or provide advice on management of anthropogenic activities occurring within the site. Anthropogenic pressures and their interaction with the data reported on here are considered by SNCBs at a later stage as part of condition assessment and management advice for this site.

This report includes recommendations which inform continual improvement and development of sample acquisition, analysis and data interpretation for future survey and reporting. Site and feature specific indicator metrics are not currently defined for this site. Potential indicators, where identified, will be evaluated and considered for inclusion in recommendations for future reporting.

This monitoring report is informed by data acquired during a dedicated survey carried out at West of Walney MCZ in 2018 and by NE and Bangor University in 2016, which will form part of the ongoing time series data and evidence for this MPA.

West of Walney MCZ is an inshore site located off the coast of Walney Island, Cumbria within the ‘Irish Sea’ Charting Progress Area. The MCZ was designated in 2016 to protect the habitat Feature of Conservation Importance (FOCI) ‘Sea-Pen and Burrowing Megafauna Communities’ and the Broadscale Habitats (BSHs) ‘Subtidal sand’ and ‘Subtidal mud’. This report provides a characterisation of the BSHs present in a northern section of the MCZ in the form of an updated habitat map and assessment of the infauna communities. The report also presents additional evidence on the presence and distribution of the FOCI within that region.

For the area surveyed, the site is predominantly ‘Subtidal mud’ with a gradual coarsening of the sediment to the east, where ‘Subtidal sand’ is also present. There

was some evidence of differences in infauna communities between the two BSHs with measures of diversity and total biomass generally higher in ‘Subtidal sand’ samples. However, a reliable characterisation of the BSH ‘Subtidal sand’ was not possible due to the limited number of samples of this class.

While the survey was not designed to sample the habitat FOCI ‘Sea-Pen and Burrowing Megafauna Communities’ by confirming the presence of species which are thought to be indicative of this FOCI, this report provides evidence of the presence of ‘Sea-Pen and Burrowing Megafauna Communities’. All samples that were classified as ‘Subtidal mud’ were observed to have species indicative of this habitat FOCI, suggesting this habitat is widespread across the area of interest. However, monitoring the health and changes in extent of these communities would require additional surveys that specifically target the epifauna of the seabed.

A comparison of infauna assemblages collected from different sampling gears indicated that the larger Box core identified a more diverse and abundant assemblage than the Day grab. The Day grab sampled the same dominant taxa but in fewer numbers, while the Box core collected more burrowing species. These findings need to be considered for planning of future monitoring of the MCZ. Further recommendations for monitoring have been outlined within the report.

1 Introduction

West of Walney MCZ is part of a network of sites designed to meet conservation objectives under the Marine and Coastal Access Act (2009). These sites will also contribute to an ecologically coherent network of Marine Protected Areas (MPAs) across the north-east Atlantic, as agreed under the OSPAR Convention and other international commitments to which the UK is a signatory.

Under the Marine and 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. In order to fulfil obligations of reporting on the condition of sites and features and advising on their management, the SNCBs require data to be collected through a programme of MPA monitoring. The SNCB responsible for nature conservation inshore (up to 12 nm from the coast) is Natural England (NE) and the SNCB responsible for nature conservation offshore (between 12 nm and 200 nm from the coast) is the Joint Nature Conservation Committee (JNCC). Where possible, this 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 monitoring report primarily explores data acquired from the first dedicated monitoring survey of West of Walney MCZ, which will form the initial point in a monitoring time series against which feature condition can be assessed in the future. The specific aims of the report are discussed in more detail in section 1.2.

1.1 Site overview

The West of Walney MCZ covers an area of 388 km^2 of seabed off the coast of Walney Island, Cumbria (Figure 1; Table 1). West of Walney MCZ was recommended as an MCZ by the 'Irish Sea Conservation Zones' regional stakeholder group project and was designated in 2016 due to the presence of 'Subtidal mud', 'Subtidal Sand' and 'Sea-Pen and Burrowing Megafauna Communities'[1].

At the time of designation this site filled a gap in the network for 'Subtidal mud' and 'Sea-Pen and Burrowing Megafauna Communities'. Neither of these features were sufficiently protected in the region by the network of MPAs at the time of designation of this site.

The site is within the jurisdictional area of both the North Western Inshore Fisheries Conservation Authority (IFCA) and the Marine Management Organisation (MMO), as it straddles both the six and 12 nm territorial limits. The site falls within the wider

[1] http://www.legislation.gov.uk/ukmo/2016/22/contents/created [accessed 04/06/20]

‘Charting Progress 2’ (CP2) area ‘Irish Sea’. The MCZ partially overlaps the northern tip of the Liverpool Bay / Bae Lerpwl Special Protection Area (SPA) (Figure 1). The MCZ is also neighboured by the Morecambe Bay and Duddon Estuary SPA and Morecambe Bay Special Area of Conservation (SAC) to the east, the Drigg Coast SAC and Cumbria Coast MCZ to the north and the Shell Flat and Lune Deep SAC and Fylde MCZ to the south.

In the south of the West of Walney MCZ are two established offshore wind farms (OWF), the Ormonde Wind Farm and Walney Wind Farm, with a third, West of Duddon Sands Wind Farm, under development.

The site ranges in depth from 15-33 m below sea level (chart datum). The seabed mud in this site is an important habitat for many animals like worms, cockles, urchins and sea cucumbers. Other larger animals, such as mud shrimps and fish, live within this habitat and burrow into the mud. This creates networks of burrows which shelter smaller creatures like worms and brittle stars. The mud also provides a habitat for Sea-Pens, which are tall and bioluminescent organisms, which live in groups and get their name because they look like quill pens.

The sand on the seabed is also an important habitat. At first glance, sand may appear desert like, but close inspection can reveal flat fish and sand eels camouflaged on the surface of the sand, and worms living within it.

Due to the large extent of the West of Walney MCZ and time and financial constraints, it was not possible to sample the entire site in 2018. Therefore, priority was given to sampling a section of the MCZ north of the Walney Wind Farm, where previous survey effort was most limited. This priority area for sampling also coincided with a section that was partially covered by acoustic data. This report presents analysis of the data collected in this northern priority area.

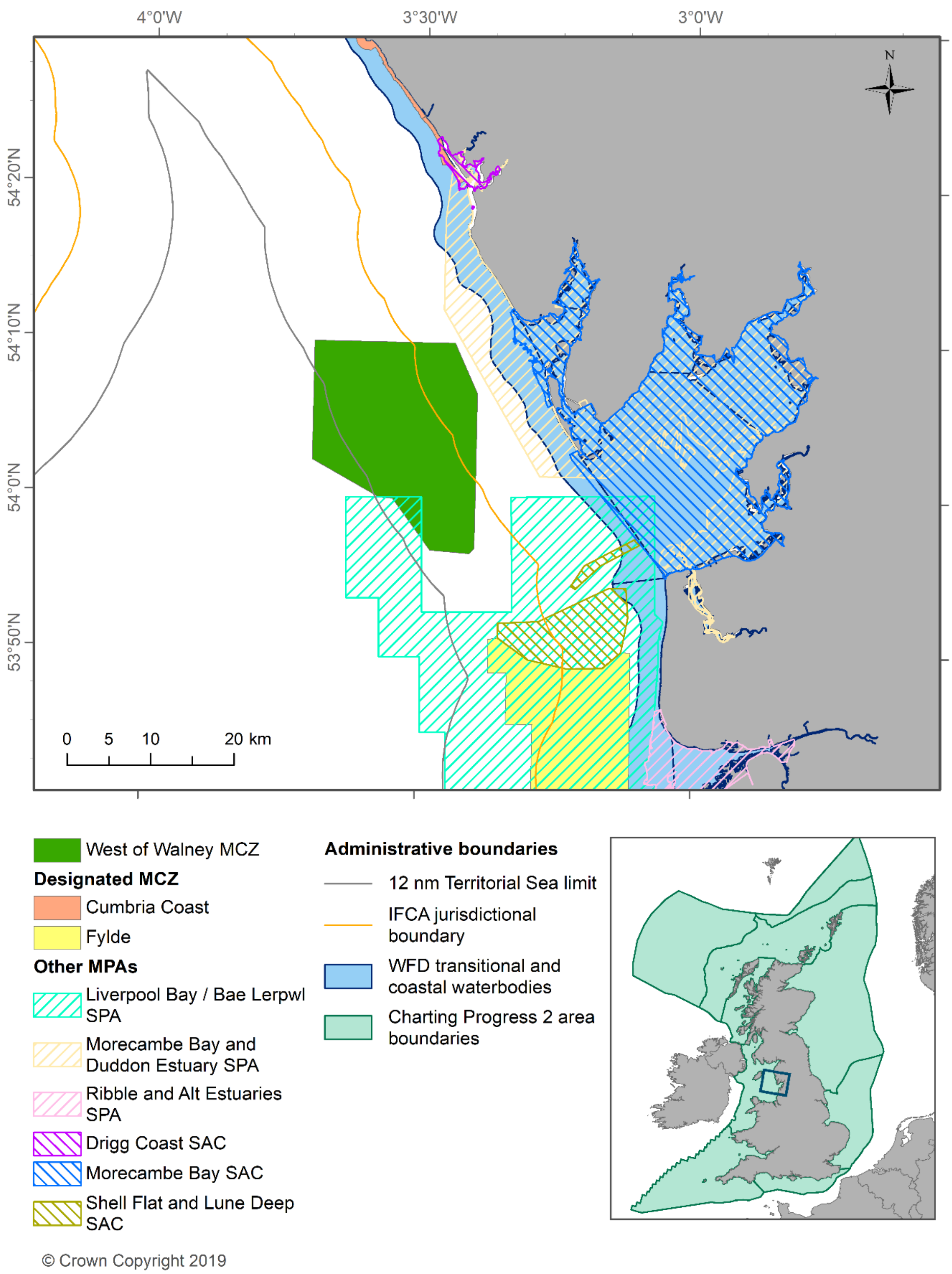


Figure 1. Location of the West of Walney MCZ in the context of MPAs and management jurisdictions proximal to the site.

Table 1. West of Walney MCZ site overview (© Natural England and Cefas 2022).

Charting Progress 2 Region[2]	Irish Sea
Spatial Area (km²)	388
Water Depth Range (m)	15 - 33
Broadscale Habitat (BSH) Features Present and designated	Subtidal sand and mud
Habitat FOCI Present	Sea – Pen and Burrowing Megafauna Communities
Species FOCI Present	NA

1.2 Existing data and habitat maps

Acoustic data were collected in the north of the MCZ as part of a wind farm development project between 2009 and 2013 (Figure 2). Based on approximately 150 m line spacing multibeam echosounder (MBES) bathymetry and backscatter data were acquired in a north-south orientation for the area north of Walney Wind Farm development. A limited number of east-west MBES transects were also collected with a 1000 m line spacing. This resulted in approximately 66 % acoustic data coverage in the east of the survey region which was shallower and ~100 % acoustic data coverage in the west of the survey region which was deeper. Data were available at a 1 m by 1 m grid cell size for both bathymetry and backscatter. The MBES backscatter data in the west of the site was not available for analysis. It is not known why the backscatter layer does not cover the same extent as the bathymetry as this was collected and provided as a processed layer from a commercial organisation separate to the MPA programme.

[2] http://webarchive.nationalarchives.gov.uk/20141203170558tf_/http://chartingprogress.defra.gov.uk/ [accessed 04/06/20]

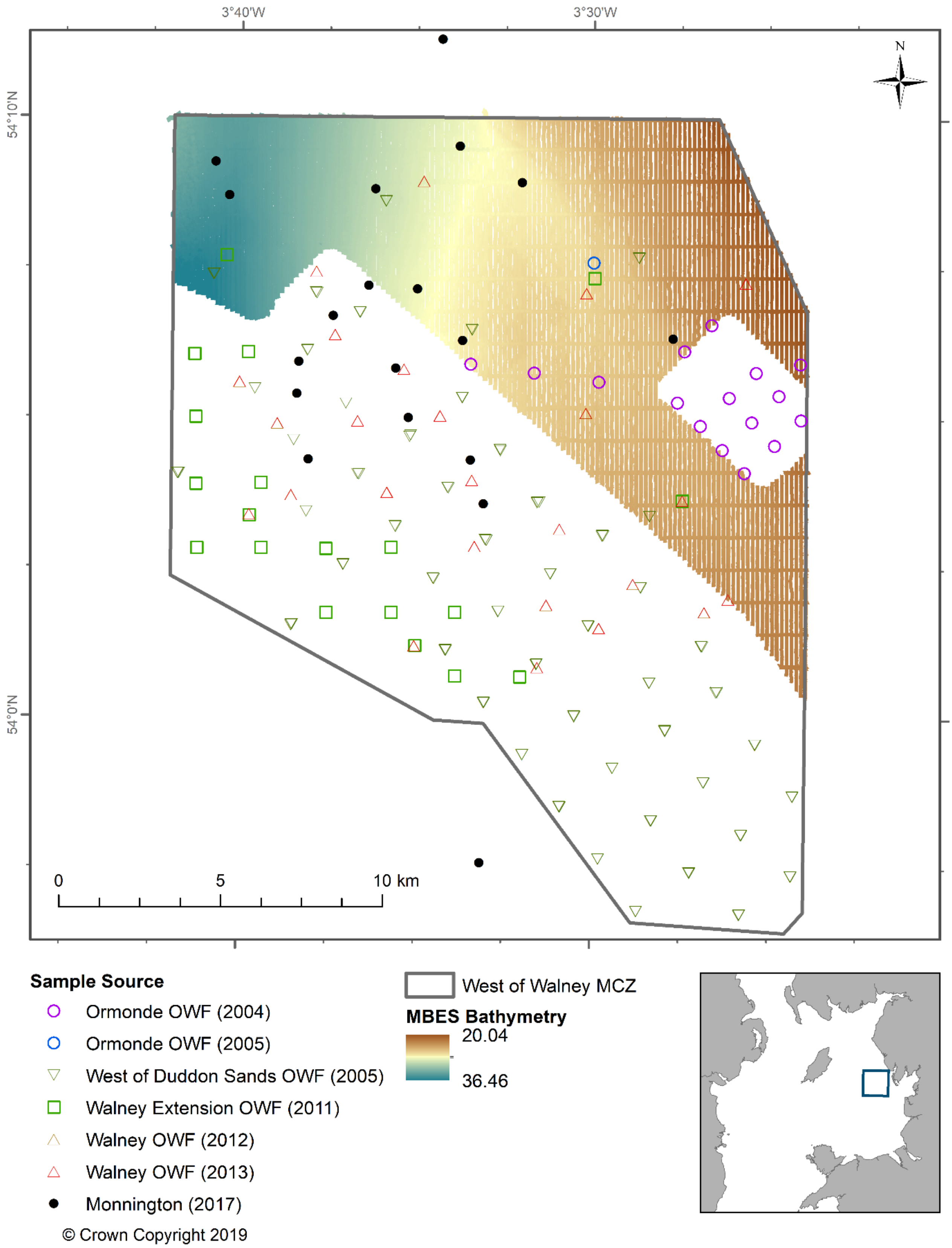


Figure 2. Location of ground truth samples collected within the West of Walney MCZ prior to the 2018 baseline data collection.

Pre-existing seabed sediment sample data within the West of Walney MCZ are available from a number of surveys that were collected over a number of years as part of OWF development projects and the MPA programme (Figure 2). A total of 313 sediment grab samples have been collected within the West of Walney MCZ between 2004 and 2013 as part of windfarm baseline benthic surveys, environmental impact assessments and cable route planning. These were associated with the Ormonde, Walney, Walney Extension and West of Duddon Sands OWF.

As part of the baseline condition assessment of the West of Walney MCZ Bangor University and NE conducted a groundtruthing survey in August 2016 (Monnington, 2017). From the 20 sampling stations visited during that survey 17 were located within the MCZ. Each station was sampled with three Box Cores, five Day grabs, two 2 m beam trawls and one towed video sled. Day grab samples were used for Particle Size Analysis (PSA) and infauna derived from Box Cores. Epifauna was assessed from the 2 m beam trawl and video sled.

To date no site-specific habitat map has been generated based on acoustic data and ground truth samples acquired within this area of the West of Walney MCZ.

1.3 Aims and objectives

High-level conservation objectives

High-level site-specific conservation objectives serve as benchmarks against which to monitor and assess the efficacy of management measures in maintaining a designated feature in, or restoring it to, ‘favourable condition’.

As detailed in West of Walney MCZ designation order[1], 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.

Definition of favourable condition

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

a) Its extent and distribution are 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 sediment habitat types (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, sediment 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 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).

Report aims and objectives

The primary aim of this monitoring report is to explore and describe the attributes of the designated features within West of Walney MCZ, to enable future assessment and monitoring of feature condition. The results presented will be used to develop recommendations for future monitoring, including the operational testing of specific metrics which may indicate whether the condition of the feature has been maintained, is improving or is in decline.

The broad objectives of this monitoring report are provided below:

1) Provide a description of the **extent[3], distribution, structural** and **functional** attributes of the designated features within the site (see Table 2 for more detail), to enable subsequent condition monitoring and assessment;

2) Present any available evidence on the supporting processes of the designated features of the site;

3) Note observations of any habitat or species FOCI not covered by Designation Order as features of the site;

[3] Note that where current habitat maps are not available, extent will be described within the limits of available data.

4) Present evidence relating to non-indigenous species (NIS) (Descriptor 2) and marine litter (Descriptor 10), to satisfy requirements of the MSFD;

5) Record any anthropogenic activities or pressures encountered during the dedicated monitoring survey;

6) Provide practical recommendations for appropriate future monitoring approaches for the designated features (e.g., metric selection, survey design, data collection approaches) with a discussion of their requirements;

7) Provide a comparison of previously used sampling gears to identify the most suitable approach for future monitoring.

Reporting sub-objectives (objective 1)

To achieve report objective 1, reporting sub-objectives will be addressed to provide evidence for Feature Attributes and supporting processes (as defined in SACOs developed by JNCC and NE for West of Walney MCZ [4]). It was not possible to address all Feature Attributes in the 2018 evidence gathering survey design, given the comprehensive nature of the attribute lists for each feature. The Feature Attributes were therefore rationalised according to SNCB priorities, resulting in a smaller sub-set.

The list of reporting sub-objectives for selected Feature Attributes (and supporting processes) of the designated features is presented in Table 2, alongside the generated outputs for each.

[4] https://designatedsites.naturalengland.org.uk/Marine/MarineSiteDetail.aspx?SiteCode=UKMCZ0045&SiteName=west%20of%20walney&countyCode=&responsiblePerson=&SeaArea=&IFCAArea= [accessed 04/06/20]

Table 2. Reporting sub-objectives addressed to achieve report objective 1 for Feature Attributes of West of Walney MCZ (© Natural England and Cefas 2022).

Reporting sub-objective	Feature Attribute*	Features	Output
Generate a habitat map to determine the extent of BSH and habitat FOCI within the MCZ.	**Extent and distribution**	Subtidal sand Subtidal mud Sea-Pen and Burrowing Megafauna Communities	Section 3.1.2 Updated habitat map. Figure 7
Discuss the composition and distribution of sediments across the MCZ, with reference to the BSH classes and habitat map.	**Sediment composition and distribution**	Subtidal sand Subtidal mud	Section 3.2. Particle size analysis.
Conduct multivariate analysis of infaunal: - Identify patterns in biological assemblages; - Map assigned biotopes; - Describe variance in biological assemblage structure within and between BSH and habitat FOCI; - Identify key structural and influential species; - Identify any potential indicator taxa.	**Presence and spatial distribution of biological communities** **Presence and abundance of key structural and influential species** **Species composition of component communities**	Subtidal sand Subtidal mud	Section 3.4. Infaunal community analysis (2018 Day grabs).
Map the location and abundance of NIS, as listed by the Great Britain Non-native Species Secretariat and under MSFD Descriptor 2 (Annex 1.)	**Non-indigenous species (NIS)**	Entire MCZ	Section 3.8. Non-indigenous species.
Generate a tidal model for the site.	**Supporting processes: energy and exposure**	Entire MCZ	Section 3.1.1. Hydrodynamics:

Reporting sub-objective	Feature Attribute*	Features	Output
Wherever possible identify possible mobility of sediments in different areas of site based on tidal model.	**Supporting processes: sediment movement and hydrodynamic regime**	Entire MCZ	energy and exposure.

* As defined in SACO for the West of Walney MCZ.

https://designatedsites.naturalengland.org.uk/Marine/MarineSiteDetail.aspx?SiteCode=UKMCZ0045&SiteName=west%20of%20walney&countyCode=&responsiblePerson=&SeaArea=&IFCAArea= [Accessed 04/06/2020]

2 Methods

2.1 Survey design

This report combines and analyses the data from the 2016 survey (Monnington, 2017) and 2018 MPA surveys. The 2016 survey collected replicate Day grab samples (n = 5) from 20 stations (A-T) within the West of Walney MCZ. In addition, 19 of these stations (A-S) were sampled, in triplicate, using a 0.25 m^2 Box corer.

In January 2018 a dedicated monitoring survey was conducted at the West of Walney MCZ onboard the *Mersey Guardian*. The survey focussed on collecting samples to assess the extent, distribution and structure of designated features in the MCZ north of the Walney Wind Farm (objective 1; section 1.3.3 and 1.3.4). Data in the north of the MCZ were previously limited, so the survey was designed to provide as much information as possible on the distribution of BSHs in this area. Sampling station locations were positioned based on a stratified design that considered available depth and backscatter data derived from MBES acoustics (Figure 3). Stations were positioned at a safe distance from any undersea cables or wind farm installations.

A total of 100 0.1 m^2 Day grab samples were collected within this northern area and were analysed for PSA and infauna present (Figure 3). Day grabs were chosen to allow comparison with historical data collected by the wind farm developments and Bangor University.

To provide evidence on anthropogenic pressures within the MCZ (objective 5; section 1.3.3), five stations were also sampled for sediment contaminant analysis (tributyltin (TBT), polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs) and heavy metals).

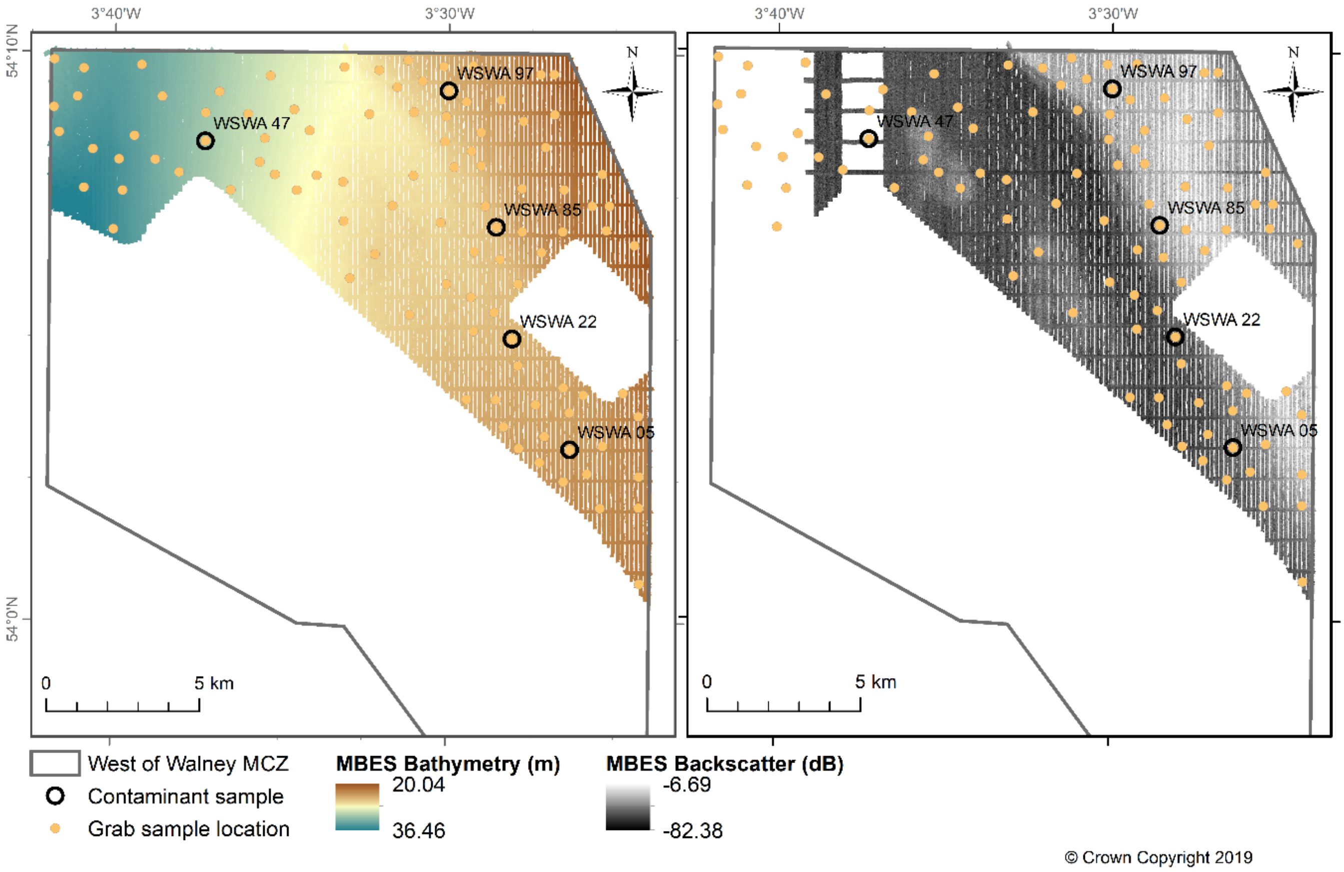


Figure 3. Location of ground truth samples collected at West of Walney MCZ in 2018. The left pane presents the MBES bathymetry data previously collected within the MCZ. The right pane presents the MBES backscatter data previously collected within the MCZ.

2.2 Data acquisition and processing

Grab sampling

Seabed sediment samples for particle size distribution (PSD) analyses and benthic infauna analyses were collected using a 0.1 m^2 Day grab or (2016 only) a 0.25 m^2 Box corer. Sampling positions were recorded (fixed) when the gear contacted the seabed.

A 50-250 ml sub-sample was taken from each grab sample in 2018, and the first three replicates at each station in 2016 and stored at -20°C prior to determining the PSD. Sediment samples were processed following the recommended methodology of the North East Atlantic Marine Biological Analytical Quality Control (NMBAQC) scheme (Mason, 2011). The less than 1 mm sediment fraction was analysed using laser diffraction and the greater than 1 mm fraction was dried, sieved and weighed at 0.5 phi (ϕ) intervals. Sediment distribution data were merged and used to classify samples into sediment BSHs.

The faunal fraction was sieved over a 1 mm mesh, photographed, then fixed in buffered 4 % formaldehyde. Faunal samples were processed to extract all fauna present in each sample. Fauna were identified to the lowest taxonomic level possible, enumerated and weighed (blotted wet weight) to the nearest 0.0001 g following the recommendations of the NMBAQC scheme (Worsfold *et al*., 2010). A sub-set of taxa, considered to be burrowing megafauna, were individually measured to estimate 'size' (width, length, biomass, carapace length). Anthropogenic material was also noted during the infaunal processing by recording the abundance of marine litter present in each sediment sample.

Additional environmental data

During the 2018 survey, five stations were also sampled for sediment contaminant analysis (heavy metals, PAHs, polychlorinated biphenyls and tributyltin) using the Day grab, alongside a comparative infauna and PSA sample (Figure 3). Surface scrapes to a maximum depth of 1 cm were collected from each sample to assess the presence of contaminants within the recently deposited sediment, and the remaining material was then discarded. See Annex 2 for a full list of the analytes assessed.

2.3 Data preparation and analysis

Hydrodynamic modelling

To assess the level of exposure experienced by designated features (report objective 2; see section 1.3.3), mean tidal current velocities (m s^{-1}) at the seabed and wave velocities were obtained from regional datasets published in Mitchell *et al.,* (2019). The currents data were derived from a depth-averaged model of the north-west European continental shelf and has been built using an unstructured triangular mesh using the hydrodynamic software Telemac2D (v7p1). Bathymetry for the model was sourced from the Defra Digital Elevation Model (DEM) (Astrium, 2011). The unstructured mesh was discretised with 382373 nodes and has an approximate resolution of approximately 1 km in the east and 2 km in the west of the MCZ. This was then interpolated to a resolution of ~200 m. While it would be possible to refine the tidal currents model to a more detailed spatial scale within the MCZ, it was determined that, due to the limited variation within the site, this would have little value for understanding the dynamics of the site.

Peak orbital velocity of waves at the seabed were derived from a European continental shelf model of peak wave height and period from 2001-2010. This model had a grid spacing of approximately 11 km. Peak wave height and period were then interpolated to a resolution of ~200 m and then using the method of Soulsby (1997), the peak wave height and period were combined with available depth data.

Sediment particle size distribution

Sediment PSD data (0.5 ϕ intervals) were grouped into the percentage contribution of gravel (> 2 mm diameter), sand (0.063–2 mm) and mud (< 0.063 mm) derived from the classification proposed by Folk (1954). In addition, samples were assigned to one of four sediment BSHs using a modified version of the classification model produced during the MESH project (Long, 2006). The 0.5 ϕ distributions and the percentage contributions of each sediment component (gravel, sand and mud) at each station were assessed to support the understanding of the composition and distribution of sediments around the West of Walney MCZ (report objective 1; see section 1.3.3 and Table 2).

Habitat map

In order to map the distribution of the BSHs within the West of Walney MCZ (report objective 1; see section 1.3.3 and Table 2), a semi-automated modelling approach was considered. This typically involves applying a machine learning algorithm to find patterns in the relationship between the predictor variables (i.e. acoustic data and associated derivatives) and the response variables (i.e. substrate type from ground truth samples). Once developed, the machine learning model can be applied to the rest of the site to predict the most likely sediment type based on the values of predictor variables in that location. Mapping substrate type in this way is common in the marine environment (Brown *et al.,* 2011), and within the MPA programme. However, in order to develop a reliable prediction, the substrate classes need to be acoustically different with regards to the morphology (derived from bathymetry) or the backscatter intensity, which can reflect grainsize and other properties (McGonigle and Collier, 2014). For the area of interest in the West of Walney MCZ the bathymetry is shallower in the east than the west but is essentially featureless. From the surveyed bathymetry, the finer scale variation in depth was a result of artefacts from the data collection, that were more prominent than any changes to the seabed morphology (Figure 4). This rendered the typical suite of morphological classifiers, such as slope, bathymetric position index, rugosity and others, as being of no use as predictors of seabed substrate. In order to minimise noise in the backscatter and bathymetry layers, these two layers were initially smoothed by calculating the focal mean based on a 100 m radius. This also had the effect of filling in the areas of no data with the mean values from the surrounding survey lines.

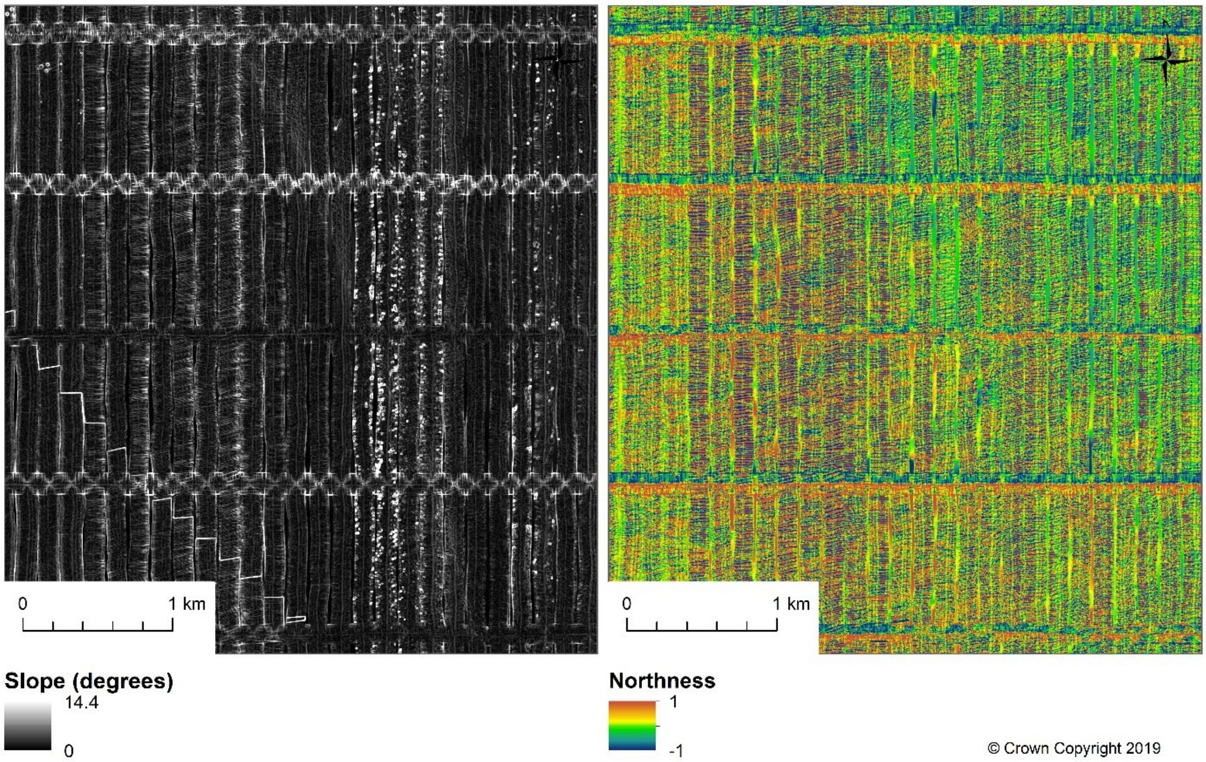


Figure 4. Examples of noise in the original bathymetry data at West of Walney MCZ. The left pane shows the slope and the right pane shows northness, both of which have been calculated from the bathymetry layer.

Of the samples collected in 2016 and 2018 within the area of interest, only 106 samples coincided with both the MBES backscatter and bathymetry data, of which 96 were determined to be 'Subtidal mud' and ten were determined to be 'Subtidal sand'. To explore the relationship between EUNIS BSH and the acoustic data, for each sediment sample location the backscatter and bathymetry values were extracted. Based on a bivariate plot of backscatter and bathymetry, no clear relationship between BSH and backscatter was observed (Figure 5). There is some indication that 'Subtidal sand' is the predominant Broadscale Habitat type in the shallower areas of the site. Although the number of samples was insufficient to allow for statistical analysis, a manual depth-based classification marginally improved the agreement between habitat map and ground truth samples (Figure 5). The area < 21.95 m has therefore been classified as 'Subtidal sand' (discussed further in section 4.2.1).

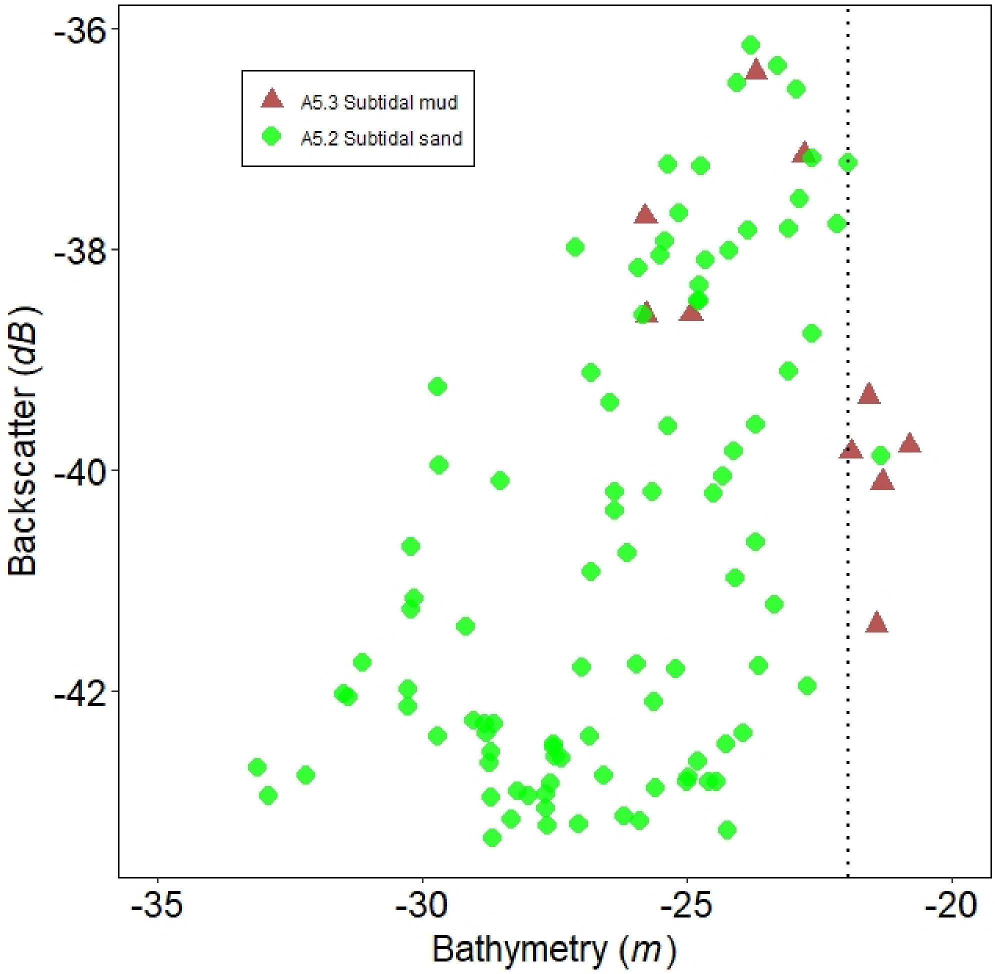


Figure 5. Bivariate plot of the acoustic values of bathymetry and backscatter at West of Walney MCZ, with the points grouped based on BSH type (manual depth classification indicated by dotted line at -21.95 m) (© Natural England and Cefas 2022). Sediment samples are derived from the 2018 EA survey and Mornington (2017).

The technical quality of the updated habitat map was assessed using the MESH Confidence Assessment Tool[5], originally developed by an international consortium of marine scientists working on the MESH project. This tool considers the provenance of the data used to make a biotope/habitat map, including the techniques and technology used to characterise the physical and biological environment and the expertise of the people who had made the map. In its original implementation, it was used to make an auditable judgement of the confidence that could be placed in a range of existing, local biotope maps that had been developed using different techniques and data inputs, but were to be used in compiling a full coverage map for north-west Europe.

[5] https://www.emodnet-seabedhabitats.eu/media/1667/step4_guidance_confidenceassessment.pdf [Accessed 05/06/2020]

Subsequent to the MESH project, the confidence assessment tool has been applied to provide a benchmark score that reflects the technical quality of newly developed habitat/biotope maps. Both physical and biological survey data are required to achieve the top score of 100 but, as the current MCZ exercise requires the mapping of Broadscale physical Habitats not biotopes, it excludes the need for biological data. In the absence of biological data, the maximum score attainable for a purely physical map is 88.

Infaunal data preparation

Infaunal samples were processed by the Institute of Estuarine and Coastal Studies (IECS) to extract and identify all infauna greater than 1 mm, present in each sample, and subsequently audited by APEM Ltd. to ensure accurate extraction and consistent identification. Plastic litter fragments were also extracted, and their abundance recorded. Infauna were identified to the lowest taxonomic level possible, enumerated and weighed (blotted wet weight) to the nearest 0.0001 g following the recommendations of the NMBAQC invertebrate scheme component (Worsfold *et al.,* 2010; Worsfold and Hall, 2017). Benthic infaunal data were checked to ensure consistent nomenclature and identification policies. Discrepancies were resolved using expert judgement following the truncation steps presented in Annex 3. Invalid taxa were removed from the data set, while the presence of colonial taxa or fragments of taxa, at the species level, were changed to a numeric value of one and their abundance and biomass records combined. Records labelled as ‘juvenile’ were combined with ‘adults’ of the same genus/species/family, when present, e.g. *Callianassa subterranea* juvenile.

Biological data analysis

Infaunal community analysis

The composition and variation within biological assemblages associated with each BSH was assessed using the infaunal (Day grab) samples (report objective 1; see section 1.3.3 and Table 2). Using data currently available, the analysis of infaunal communities will also provide practical recommendations (where possible) for future monitoring (report objective 6; see section 1.3.3).

Highly variable taxon counts were down-weighted in the infaunal matrices using a dispersion weighting (Clarke *et al.,* 2006) and Bray-Curtis similarity matrices were produced from the square root-transformed data for both samples and variables (Clarke and Warwick, 1994).

Infaunal and epifaunal assemblages were assigned based on the non-hierarchical 'k R Clustering' method, whereby the optimum number of groups within the data set was determined using the Analysis of Similarities (ANOSIM) R statistic to provide a value for k-group division and the Similarity Profile (SIMPROF) algorithm to test whether a suitable number of groups had been reached (Min:2- Max:20) (Clarke *et al.,* 2016). The choice of non-hierarchical clustering enables samples to be reallocated at latter points in the analysis without becoming isolated as similarity measures are developed during algorithm computation.

Several metrics were generated using the DIVERSE routine in Primer v7 for each sample:

- Number of Taxa (*S*): the number of taxa present in a sample;
- Abundance (*N*): the total number of individuals of enumerable taxa. Colonial taxa are recorded as present and subsequently assigned an abundance of 1;
- Total biomass (*g*): the summed mass of all enumerable taxa; blotted wet weight;
- Margalef's richness (d) $\frac{(S-1)}{\ln(N)}$: a measure of the number of species present for a given number of individuals. The higher the index, the greater the diversity;
- Shannon-Wiener index (H'): measures the uncertainty in predicting the identity of the next species in a sample with high values indicating a high biodiversity;
- Pielou's evenness (J') $\frac{H'}{\ln(S)}$: where H' is the Shannon Weiner diversity; shows how evenly the individuals in a sample are distributed. J' is a range of zero to one. The less dominance of a taxa in the sample, the higher J' is;

- Simpson's index (1-λ): a measure of the probability of choosing two individuals from a sample that are different species. (0 = minimum diversity;1 = maximum diversity).

Sampling gear comparison

In order to identify any impact of choosing to monitor the West of Walney MCZ with either the 0.25 m^2 Box corer or the 0.1 m^2 Day grab, and to address report objective 7 in section 1.3.3, the infaunal abundance data collected in 2016 was used to generate species richness (*S*) for each sample acquired.

Species richness was modelled using a Poisson distribution. Generalised Additive Mixed Models (GAMMs) (Wood, 2006), fitted in the R package 'mgcv', were used to test the difference in *S* between the two gears. Use of GAMMs allowed us to model spatial variation by fitting 'station' as a random effect, thus potentially removing any spatial correlation, and to statistically control for the effects of variation in sediment penetration. The sediment penetration term was smoothed, using the penalised regression splines with up to four degrees of freedom, to avoid having to make assumptions about the form of their relationships with the response variable. The model fitted took the form:

$$\log(\mu) = \alpha + \text{gear} + s\,(\text{sediment penetration}) + \text{station} \quad (1)$$

where μ is the Poisson mean of the response when modelling richness, α is an intercept parameter, s (sediment penetration) is a smooth function of how deep the gear sampled into the seabed and station is a random effect, such that station~n (0, σ2). The means (bootstrapped and fitted within each gear type) were plotted with their associated 95 % confidence intervals using 'ggplot2' and 'rcompanion' packages.

The Akaike Information Criterion (AIC) was used to determine if inclusion of penetration depth aided in the model, with a lower AIC value identifying the most parsimonious model.

Penetration was used *in lieu* of sediment volume, which was not recorded during deck operations, as it is reasonably expected that the two are highly correlated as a consequence of the parallel form of the box corer.

Delineating species FOCI and habitat FOCI

Infaunal species were cross-referenced against lists of species FOCI and habitat FOCI-defining taxa (report objective 3, see section 1.3.3).

The habitat FOCI 'Sea-Pen and Burrowing Megafauna Communities' was designated for protection within the West of Walney MCZ. To confirm the extent of this habitat FOCI, some form of sampling to assess area of coverage of the communities present would be required within the site. Data from sampling such as seabed imagery and epifaunal trawl catch data were not available for inclusion in this report and therefore a tentative assessment of the presence of the 'Sea-Pen and Burrowing Megafauna Communities' habitat FOCI was made using the sediment and infaunal datasets collected in 2016 and 2018. The scale of the observation from point sediment samples would result in an assessment of extent that may not be reliable and as such, the absence in this report of the 'Sea-Pen and Burrowing Megafauna Communities' FOCI from a station does not preclude it from occurring there. With only sediment samples available to tentatively indicate sites of 'Sea-Pen and Burrowing Megafauna Communities' this was deemed insufficient information to assess the coverage of this FOCI. Therefore, the point locations were classified as indicative of the habitat FOCI present, and no inference was made regarding the spatial distribution of the 'Sea-Pen and Burrowing Megafauna Communities' FOCI. This is discussed further in section 4.5.

Species were identified as comprising 'Sea-Pen and Burrowing Megafauna Communities' based on inclusion in the list of species linked through Table 4 of the JNCC definition development paper[6].

For the West of Walney MCZ datasets this included the presence of species in three classifications:

- Circalittoral fine mud (SS.SMu.CFiMu);
- Circalittoral sandy mud (SS.SMu.CSaMu);
- Burrowing megafauna and *Maxmuelleria lankesteri* in circalittoral mud (SS.SMu.CFiMu.MegMax).

All stations surveyed in 2016 and 2018 had representation from those species listed in the three classifications above. A full list is presented in Annex 4.

Additionally, expert judgement was used to select taxa which may reasonably be described as, or associated with, the habitat FOCI 'Sea-Pen and Burrowing Megafauna Communities' (Figures C and D in Annex 4). The list of taxa which are characteristic of the three classifications contained many instances of taxa which are ubiquitous in the north-east Atlantic and not specifically associated with the 'Sea-Pen and Burrowing Megafauna Communities' FOCI e.g. *Pagurus bernhardus*, Nemertea, *Phoronis* spp.

JNCC guidance (Hiscock, 1996) suggests that taxa 'should be present in sufficient numbers to be identified as at least 'Frequent' on the 'Superabundant, Abundant, Common, Frequent, Occasional, Rare (SACFOR) scale' for the habitat to be considered present. This equates to one to nine individuals, in the 1 - 3 cm size range, per 1 m^2. Individual sizes were not available for all taxa. Therefore, when one individual from the list was present in any of the sediment samples, and that taxa is

[6] http://jncc.defra.gov.uk/pdf/Advice_Document_MudHabitats_FOCIdefinitions_v1.0.pdf. [Accessed 04/06/20]

known to grow to the 1 – 3 cm size range, the habitat FOCI ‘Sea-Pen and Burrowing Megafauna Communities’ was attributed to that station.

Non-indigenous species

The infaunal and epifaunal taxon lists generated from the infaunal samples were cross-referenced against lists of NIS which have been selected for assessment of GES in UK waters, under MSFD Descriptor 2, and identified as significant by the UK Non-Native Species Secretariat (objective 4; see section 1.3.3). These taxa are listed in Annex 1.

Marine litter

Items of litter found in the 2016 and 2018 grab samples were identified according to the categories in Annex 5 and a map was produced showing the number of samples recorded in each grab (objective 4; see section 1.3.3).

3 Results

3.1 Benthic and environmental overview

Hydrodynamics: energy and exposure

Based on modelled data, the West of Walney MCZ has a weak hydrodymic regime at the seabed that has low spatial variation (Figure 6). The tidal currents in the area are weak (< 0.5 m/s) and while the peak orbital velocity of waves at the seabed are slightly stronger (although still < 1 m/s) in the east, this decreases to the west where it is deeper. Based on the MBES bathymetry data there is no evidence of moving bedforms and the low wave and current velocities do not support the presence of mobile sediments.

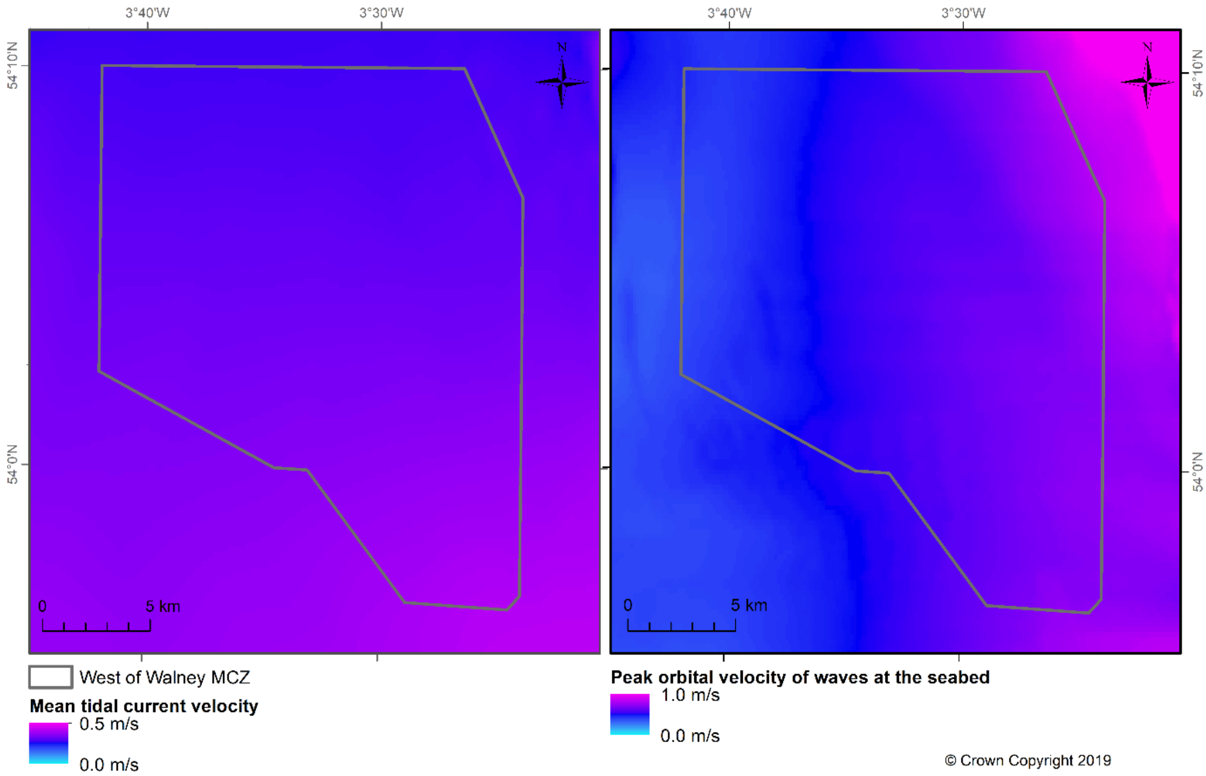


Figure 6. Modelled hydrodynamic data for the West of Walney MCZ. The left pane presents the tidal current speed data. The right pane presents the peak orbital velocity of waves at the seabed. Both datasets are derived from Mitchell *et al.,* (2019).

Updated habitat map

The BSH map was created based on analysis of the MBES bathymetry and the 2016 and 2018 sediment sample PSA data. The sediment samples show that seabed within the MCZ comprised of ‘Subtidal sand’ and ‘Subtidal mud’. Based on the available data, the northern section of the West of Walney MCZ has predominantly been mapped as ‘Subtidal mud’ (Figure 7). The site becomes shallower in the east, and in that area ‘Subtidal sand’ is also present. The delineation of the boundary between these two substrata should not be considered as absolute, as the change in grain size is gradational. No bedforms or changes in seabed morphology were evident in the areas where MBES data were present. Biological information, such as faunal distributions, abundance and biomass, were not used to inform the BSH classification.

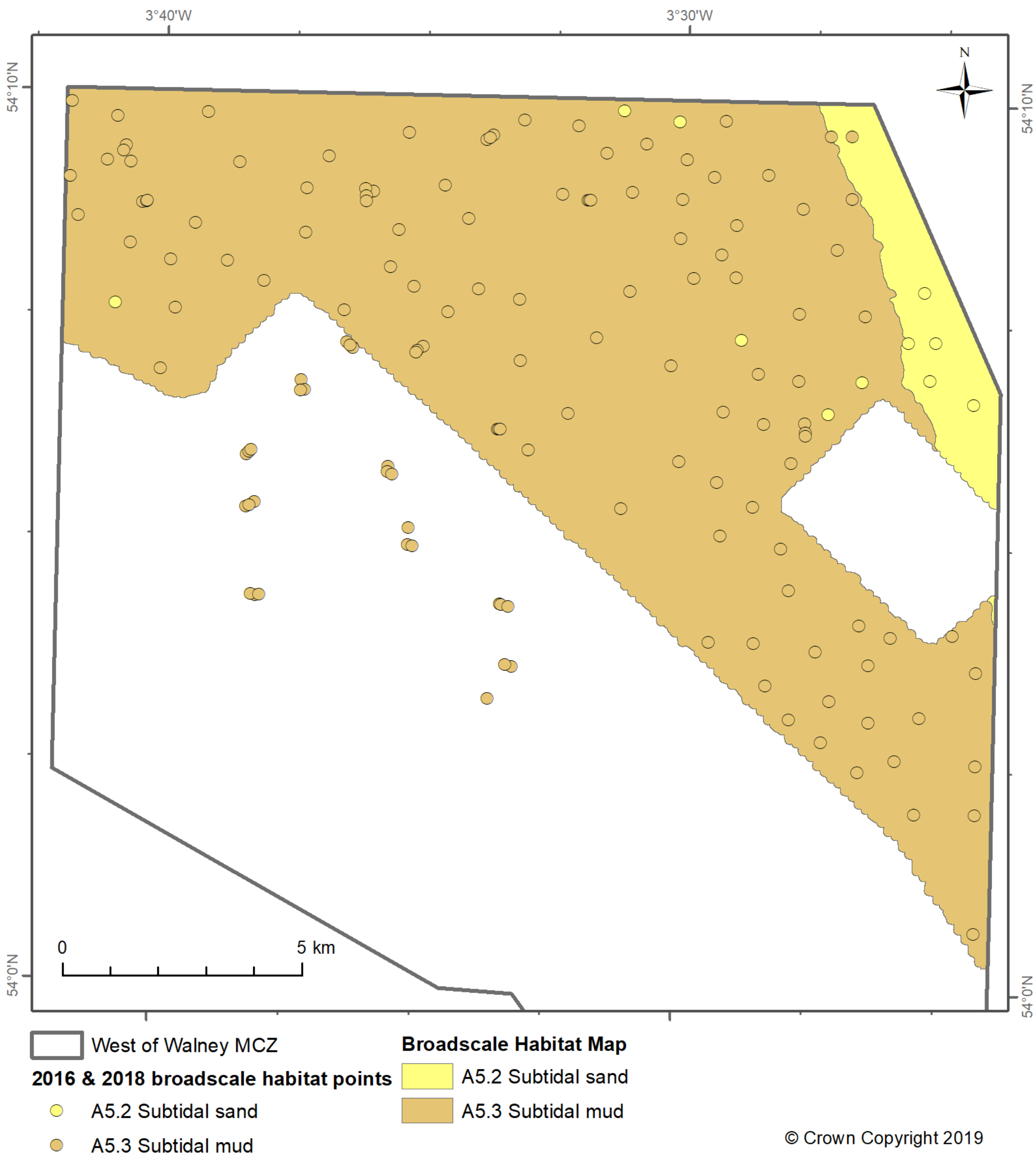


Figure 7. Updated BSH map and classified ground truth samples. Crown Copyright 2019.

Based on the MESH Confidence Assessment Tool, the updated habitat map attained two levels of map confidence which differed based on the underlying data (Figure 8). Higher confidence (74/100) was attributed to areas where both MBES bathymetry and backscatter were available, and lower confidence (70/100) was assigned to those thin strips of no data between survey lines and areas where MBES backscatter data were not available. These scores are considered good, given that the maximum possible score for a map based on purely physical data is 88.

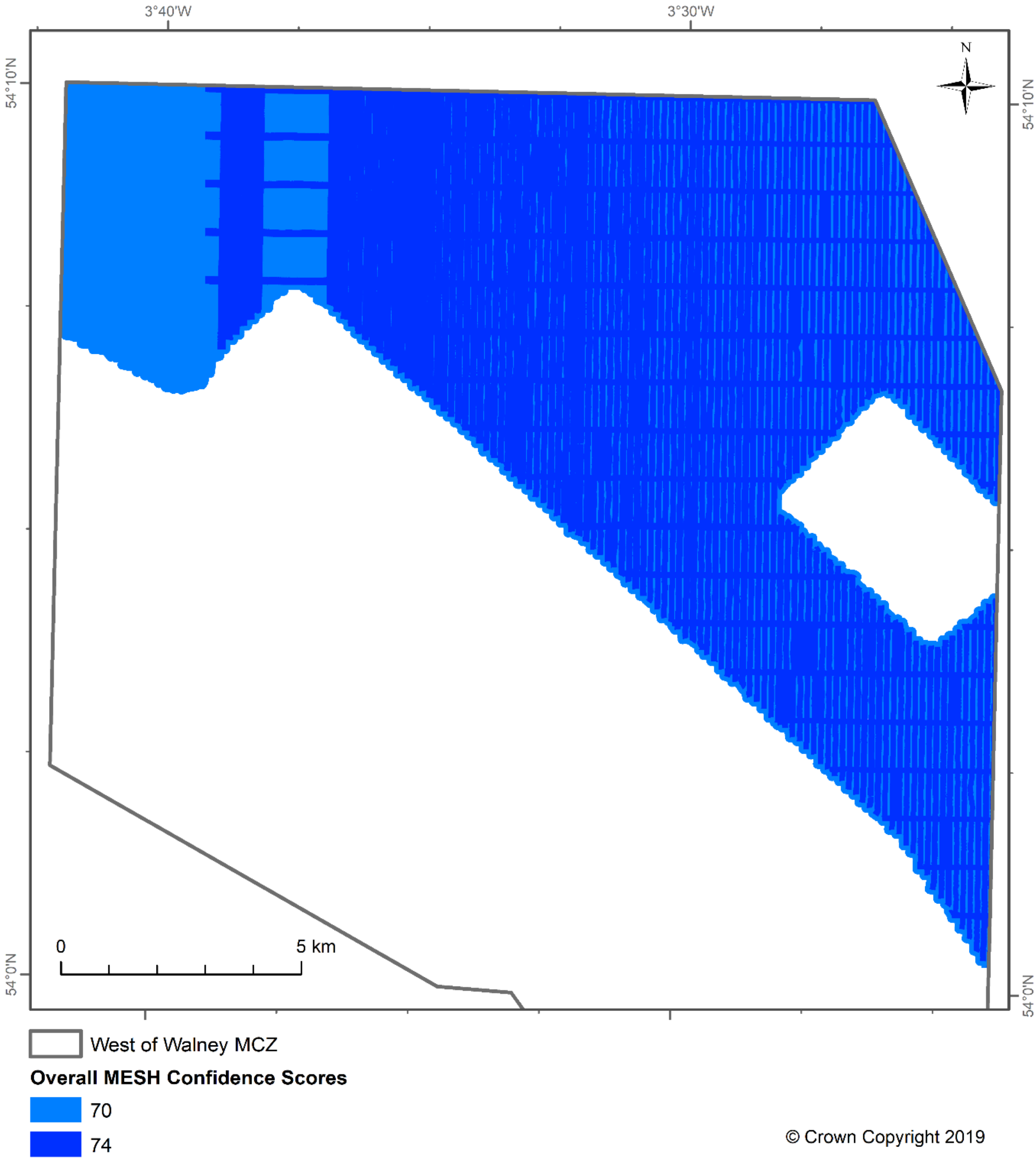


Figure 8. Overall MESH confidence score for the updated BSH map at West of Walney MCZ. Crown Copyright 2019.

3.2 Particle size analysis

Sediment composition (based on the percentage of mud, sand and gravel) and the resulting BSH for each of the 2016 and 2018 sediment samples are presented in

Figure 9 and Figure 10 respectively. The proportions of mud, sand and gravel are represented spatially in Figure 11 (right pane). The BSH classes are derived from a simplification of the Folk triangle (Folk, 1954). By classifying the proportions of mud, sand and gravel using the Folk 16 classification scheme it is clear that there is some spatial sorting of grain size that is not evident from the EUNIS level 3 definitions for BSH classes (Figure 11, left pane).

Analysis of the 2018 sediment samples illustrates that all samples were generally composed of sand and mud. A negligible gravel fraction was found in 64 samples and ranged from 0.01 - 2.82 %, with only six samples having greater than 1 % gravel contribution. The sand and mud fractions ranged between 8.8 – 99.7 % and 0 – 91.24 % respectively.

Analysis of the 2016 Day grab sediment samples demonstrated a similar pattern of minimal gravel fractions and a predominance of sands and mud (Table 3).

In general, the coarser sediments (i.e. sand, muddy sand and (gravelly) muddy sand) appear to be associated with higher backscatter reflectance in the east, and the finer sediments (i.e. mud and sandy mud) are generally located in the west of the area covered. There is also an increased percentage of sand in the samples to the west of the MCZ, where backscatter data were not available for analysis (Figure 11, right pane).

Table 3. Percentage contribution of gravel, sand and mud (range and mean values) of sediment samples collected in 2016 (Day grab) and 2018 (Day grab) from the northern region of the West of Walney MCZ (© Natural England and Cefas 2022).

Survey year	Sampling gear	Percentage contribution (%)		Mean
2016	Day grab	Gravel	0 - 0.43	0
		Sand	23.3 - 65.4	34.3
		Mud	34.4 - 76.7	65.7
2018	Day grab	Gravel	0 - 2.82	0.2
		Sand	8.8 - 99.7	49.1
		Mud	0 - 91.2	50.7

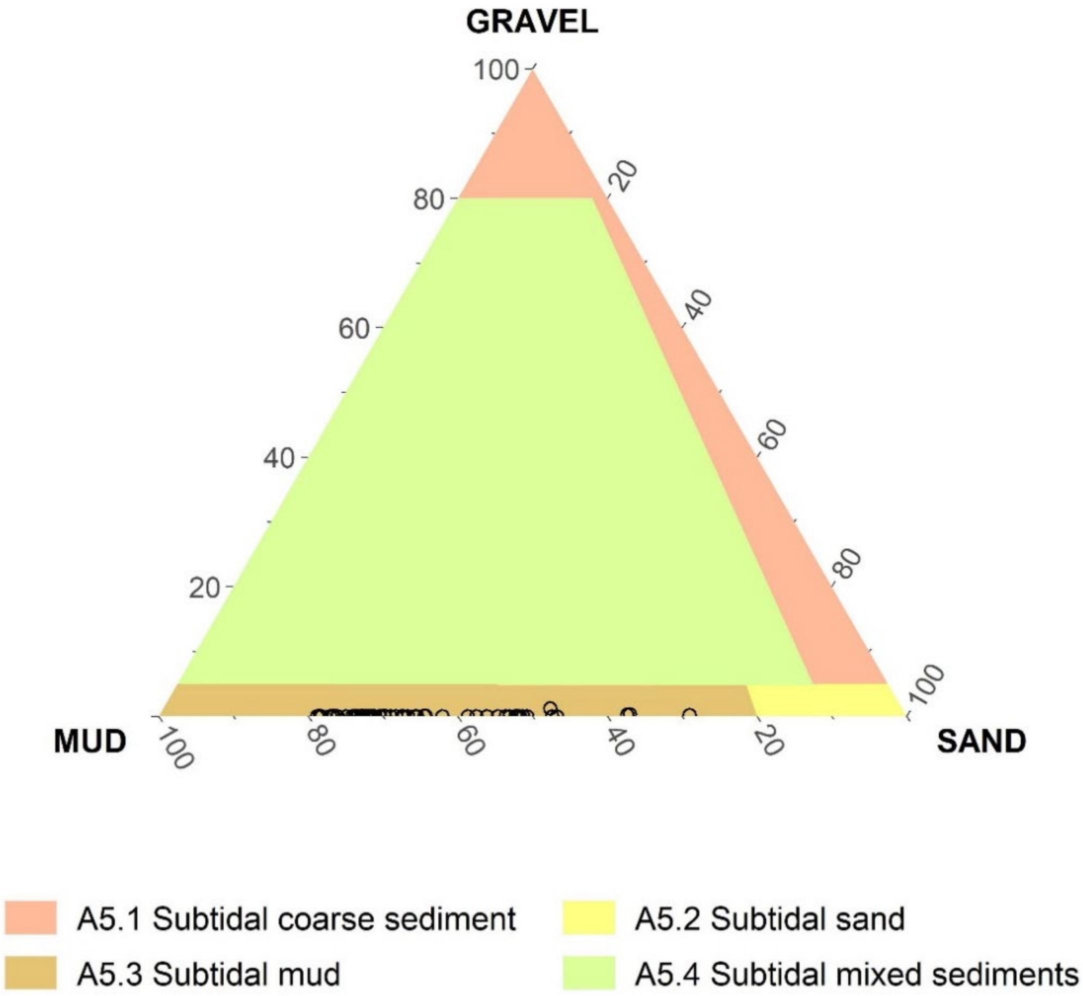


Figure 9. Classification of PSD (half ϕ) information for each sampling point from 2016 (Day grab) at West of Walney MCZ into one of the sedimentary BSHs (coloured areas) plotted on a true scale subdivision of the Folk triangle into the simplified classification for UKSeaMap (Long, 2006; Folk, 1954) (© Natural England and Cefas 2022).

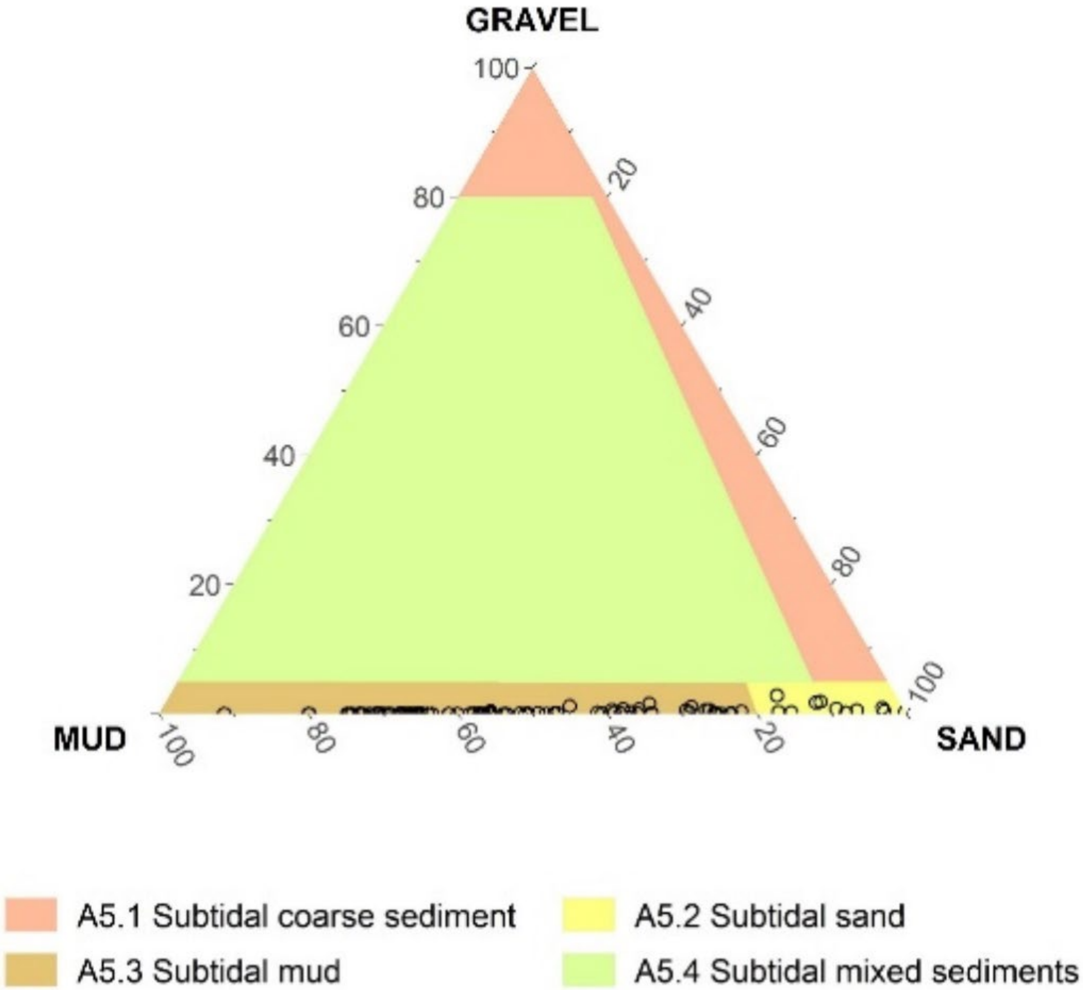


Figure 10. Classification of PSD (half ɸ) information for each sampling point from 2018 (Day grab) at West of Walney MCZ into one of the sedimentary BSHs (coloured areas) plotted on a true scale subdivision of the Folk triangle into the simplified classification for UKSeaMap (Long, 2006; Folk, 1954) (© Natural England and Cefas 2022).

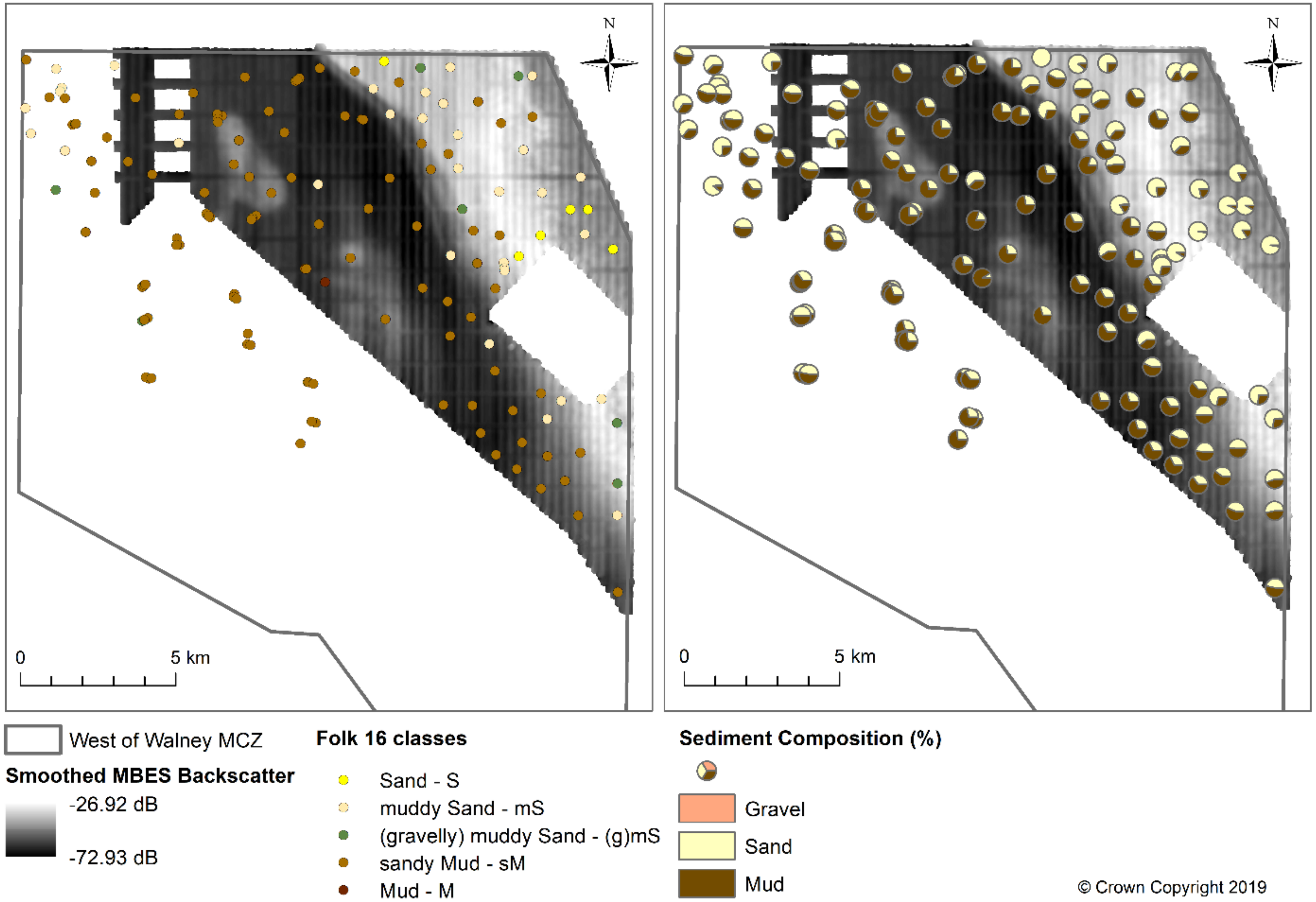

N
0
5 km
N
0
5 km
West of Walney MCZ
Smoothed MBES Backscatter
-26.92 dB
-72.93 dB
Folk 16 classes
Sand - S
muddy Sand - mS
(gravelly) muddy Sand - (g)mS
sandy Mud - sM
Mud - M
Sediment Composition (%)
Gravel
Sand
Mud
© Crown Copyright 2019

Figure 11. Spatial distribution of PSA results from the 2016 and 2018 sediment samples at West of Walney MCZ. The left pane presents the samples classified to the Folk 16 (Folk, 1954) classification scheme, overlaid on the smoothed MBES backscatter data. The right pane presents the percentage sediment compositions of the samples as pie charts overlaid on the smoothed MBES backscatter data.

3.3 Broadscale Habitats

These results present the combined data from the 2016 and 2018 grab samples collected within the northern area of interest (n = 116). The majority of the samples were classified as ‘Subtidal mud’ and 11 of the 2018 samples were classified as ‘Subtidal sand’ (Table 4) and the majority of the area surveyed was designated as ‘Subtidal mud’ (Table 5). It is not appropriate to make a direct comparison between the areas delineated as either subtidal sediment feature in this report to the areas of subtidal sediment features given in the original site assessment document as the figures given below are only for the section of the site subjected to targeted sample collection and analysis detailed in this report.

Table 4. The classification of sediment samples collected during the 2016 and 2018 surveys at West of Walney MCZ within and outside the area of interest (AOI) (© Natural England and Cefas 2022).

Sedimentary BSH	2016 Day grab – PSA and infauna		2018 Day grab – PSA and infauna
	Within AOI	Outside AOI	Within AOI
Subtidal sand	0	0	11
Subtidal mud	27	27	89

Table 5. Extent of BSH features at West of Walney MCZ within the area of interest subjected to targeted sampling and analysis to produce the updated habitat map (© Natural England and Cefas 2022).

Sedimentary BSH	Extent
Subtidal sand	10.85 km^2
Subtidal mud	152.47 km^2

Subtidal sand

A total of 11 sediment samples were classified as the BSH ‘Subtidal sand’ (2018 survey) (Figure 7). The contribution of particles categorised as ‘sand’ (i.e. less than 2 mm and greater than 0.0063 mm) for these 11 samples ranged from 81.3 – 99.7 %. The mean contribution of gravel, sand and mud and their associated confidence intervals are presented in Figure 12. The mean contribution of each half ϕ particle size for samples classified as ‘Subtidal sand’ is shown in Figure 13 and Annex 6 shows the individual line plots.

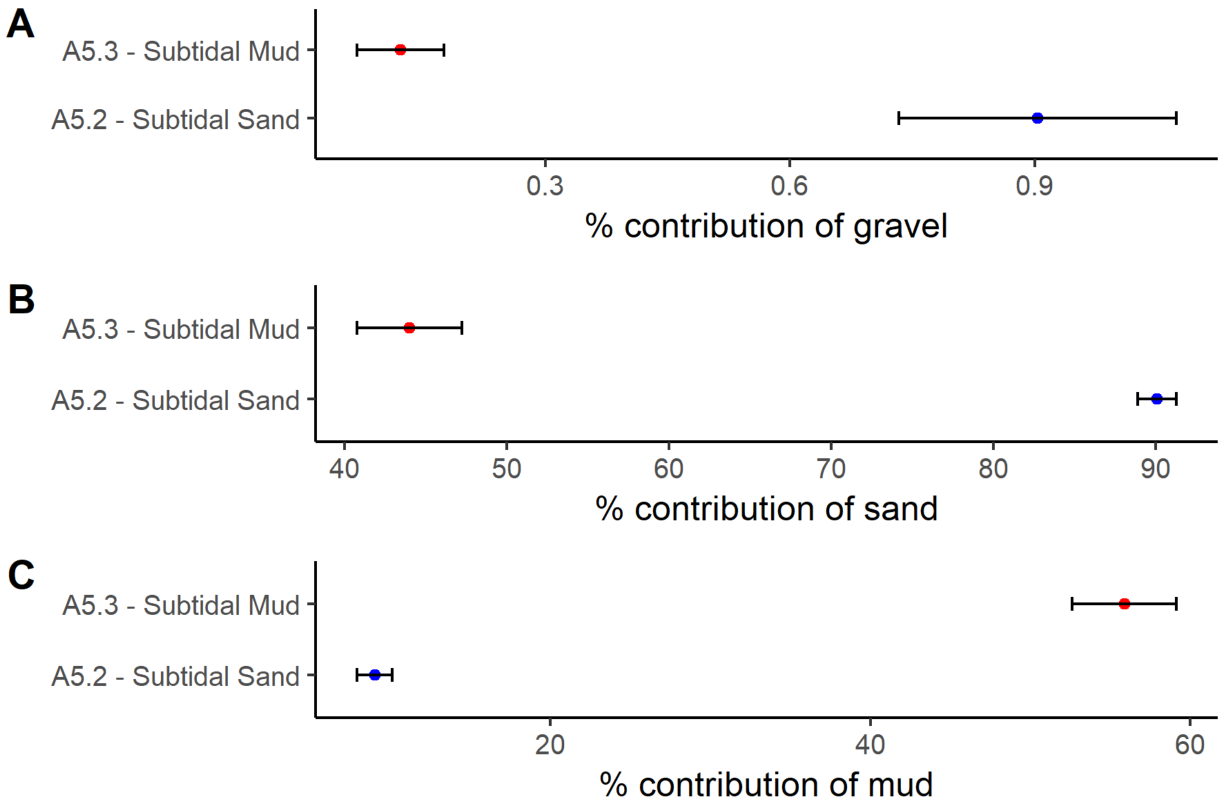


Figure 12. Percentage contribution of A) gravel, B) sand and C) mud showing the 95 % confidence intervals around the mean values, for the BSHs 'Subtidal sand' (blue) and 'Subtidal mud' (red) at West of Walney MCZ in 2018 (© Natural England and Cefas 2022).

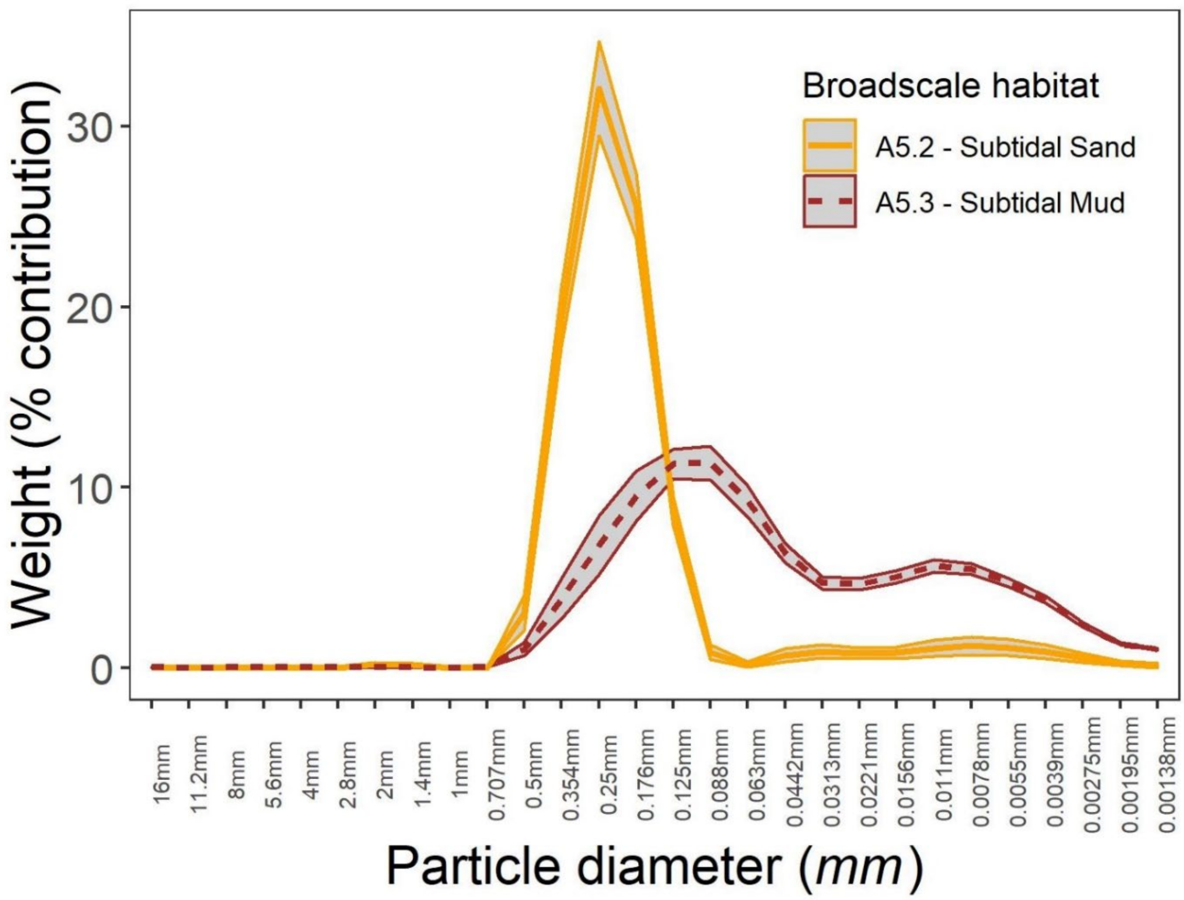


Figure 13. Mean (with shaded 95% confidence interval) percentage contribution of each half ϕ sediment class for the BSH 'Subtidal sand' (solid orange) and 'Subtidal mud' (dashed brown) at West of Walney MCZ in 2018 (© Natural England and Cefas 2022).

Subtidal mud

A total of 89 sediment samples collected during the 2018 survey were classified as the BSH 'Subtidal mud'. The contribution of particles categorised as 'mud' (i.e. less than 0.0063 mm) for these 89 samples ranged from 21.9 – 91.2 %. The mean contribution of gravel, sand and mud and their associated confidence intervals are presented in Figure 12. The mean contribution of each half ϕ particle size for samples classified as 'Subtidal mud' is shown in Figure 13.

All Day grab samples collected in 2016 were classified as 'Subtidal mud' (Figure 9).

3.4 Infaunal community analysis (2018 Day grabs)

A total of 159 taxon records remained following truncation of the infaunal abundance data set from the 100 Day grab samples collected in January 2018. This included 70 annelid (segmented worms) taxa, 32 arthropod taxa, 26 molluscan taxa, ten cnidarian taxa and eight echinoderm taxa. Other phyla (*n* = 7) accounted for the remaining 8.2 % of the total number of taxa. A table summarising the abundance and biomass values for the most dominant taxa is presented in Annex 7.

The most abundant taxa overall were the horseshoe worm *Phoronis* spp. (occurring in 91 samples at an average of nine individuals per sample) and the bivalves *Corbula gibba* (occurring in 89 samples at an average of seven individuals per sample) and *Nucula nitidosa* (occurring in 75 samples at an average of three individuals per sample). Although the most numerically dominant taxa (1920 individuals recorded accounting for 7.7 % of the total biomass of the site), the brittle star *Amphiura filiformis* was only present in approximately half of the samples collected (52). Common taxa, occurring in >60 % samples were (in descending order of percentage occurrence) *Chamelea striatula* (Mollusca), *Nephtys incisa* (Annelida), *Magelona alleni* (Annelida) and *Lovenella clausa* (Cnidaria).

Nine taxa accounted for 80 % of the total biomass at the site. *Turitella communis* (Gastropod mollusc) accounted for the largest proportion of the total biomass (21 %) with 89 individuals occurring in 17 samples. Individuals of the bivalve mollusc *Acanthocardia echinata* occurred singly in only two samples yet accounted for 18.5 % of the total biomass.

Of the metrics assessed, several varied significantly between 'Subtidal sand' and 'Subtidal mud' (Figure 14). However, there were too few 'Subtidal sand' samples to allow reliable interpretation of any comparisons. Measures of diversity (*d, H', 1-λ, S*) and the total biomass (*g*) were generally higher in samples classified as 'Subtidal sand' (Table 6). The abundance of individuals (*N*) and the measure of evenness (*J'*) were not significantly different between BSHs. A limited number of samples were classified as 'Subtidal sand' (n = 11) and these were predominantly located in the east of the survey area, although not exclusively so (Figure 7). Figures showing notable differences in the distribution of the richness and diversity measures are provided in Annex 8.

The number of taxa (*S*) collected from samples in the centre of the area surveyed appears to be lower than that in the shallower eastern region and the deepest area to the west. This pattern is also true when reviewing the total number of individuals (*N*) and Margalef's diversity index (*d*). Pielou's evenness (*J'*) is generally high across the site. There are small regions in the south-east of the surveyed area where samples display low *J'* values and are dominated by a few taxa. Diversity (*H'* & 1-λ) was generally high, however, diversity as assessed using the Shannon-Wiener measure was typically lower at some central stations and to the south-east. Simpsons diversity was also low, predominantly in the south-east region. Few stations with noticeably high biomass values were spread across the site, with no discernible pattern in their distribution.

The spatial distribution of these metrics appears to be associated with the marked change in backscatter values in the north-east of the site, shown in Figure 11 and Annex 9.

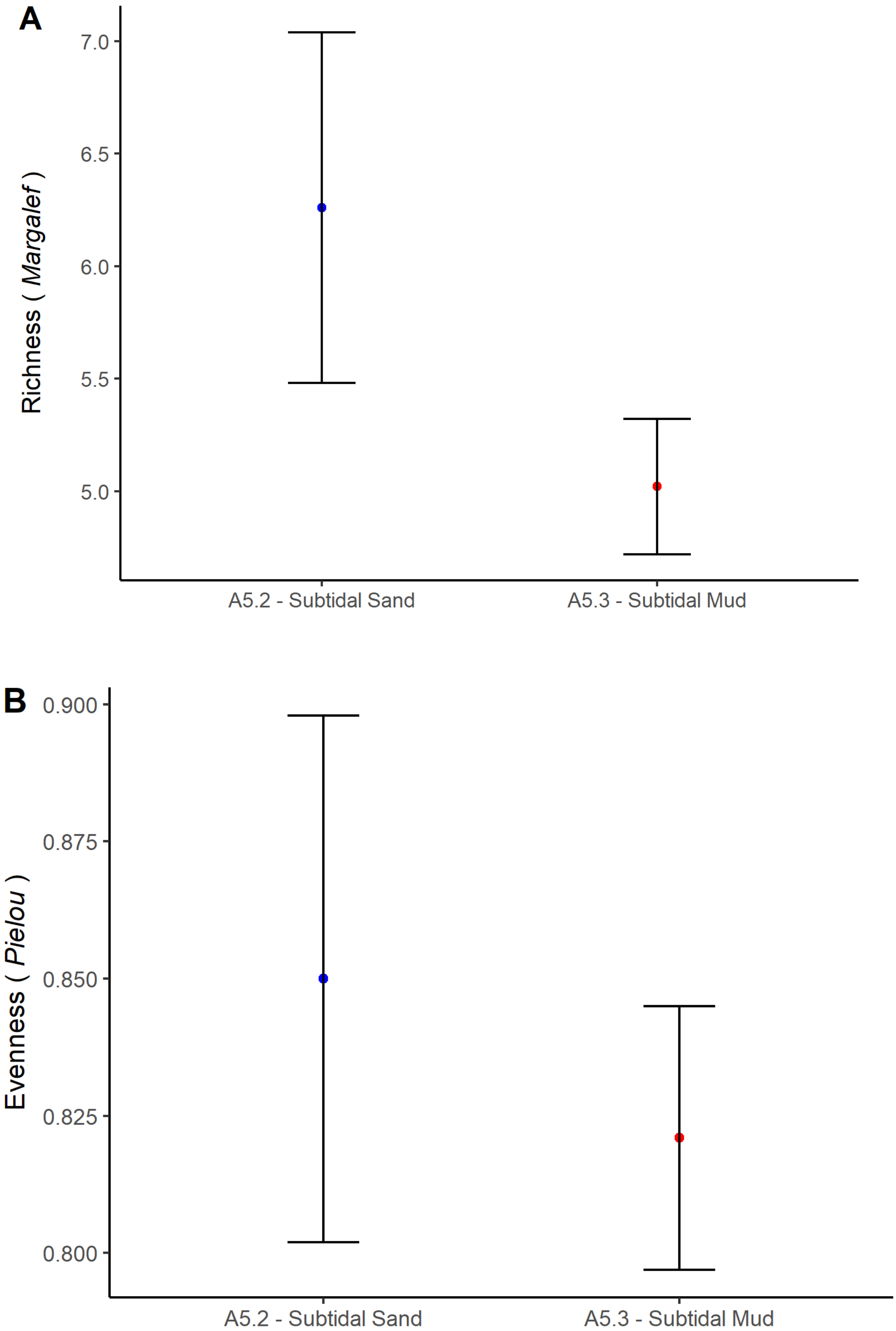
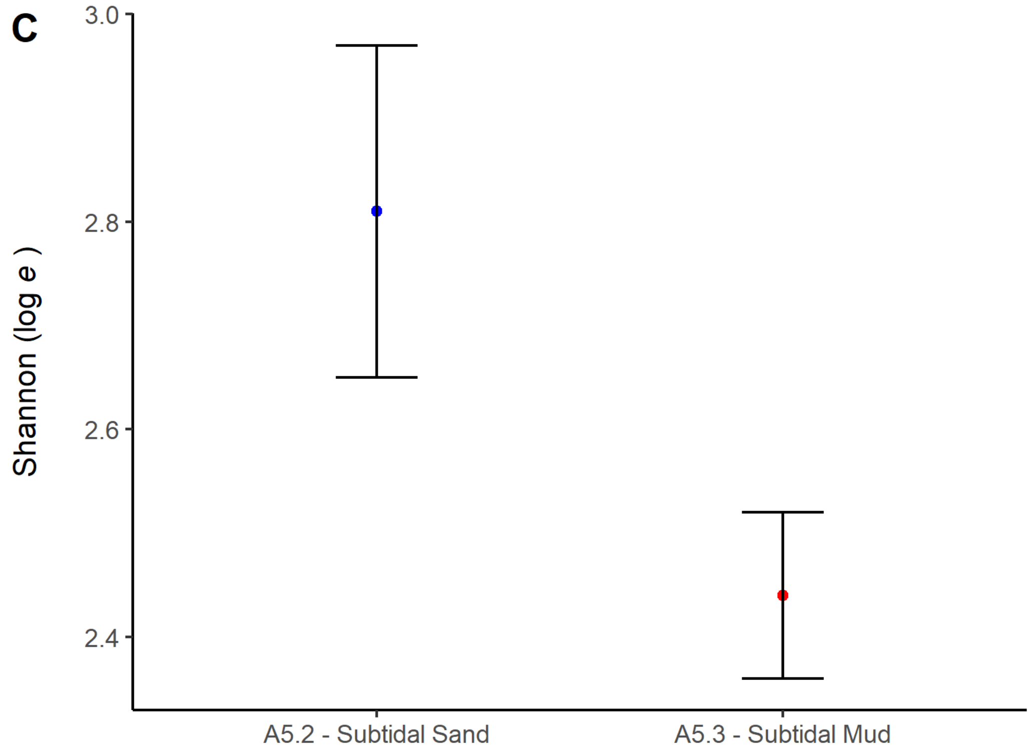


Figure 14. Mean values and 95 % confidence intervals for the univariate metrics of infaunal diversity between BSHs at West of Walney MCZ in 2018 (Blue circles = A5.2 Subtidal sand and red circles = A5.3 Subtidal mud) (© Natural England and Cefas 2022).



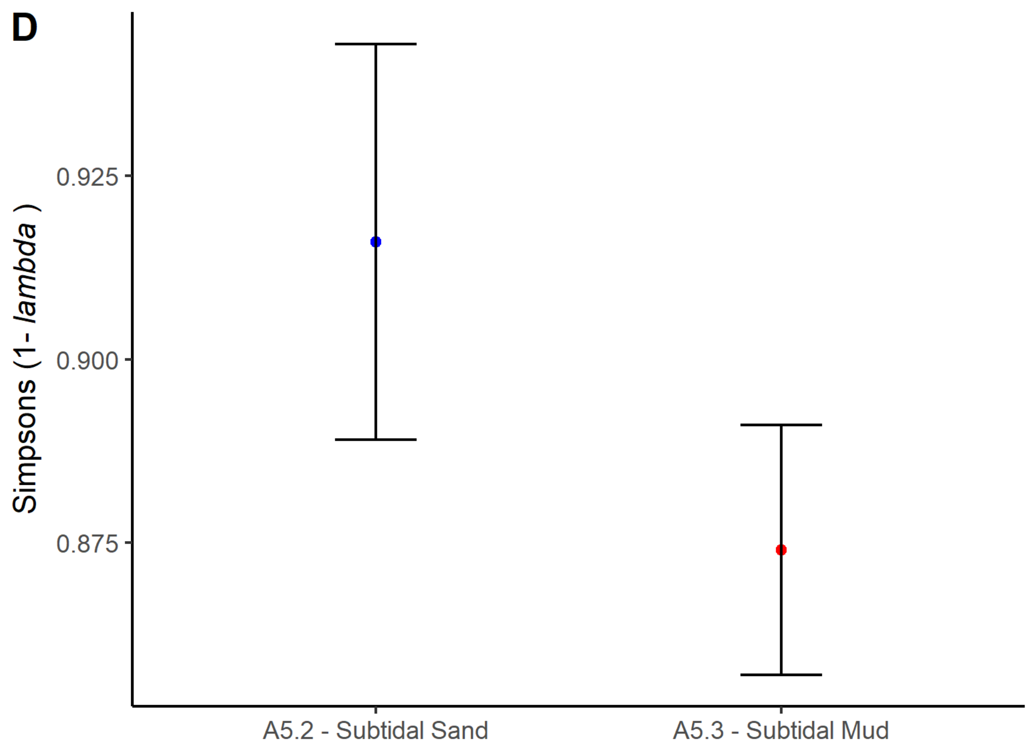


Figure 14 (continued). Mean values and 95 % confidence intervals for the univariate metrics of infaunal diversity between BSHs at West of Walney MCZ in 2018 (Blue circles = A5.2 Subtidal sand and red circles = A5.3 Subtidal mud) (© Natural England and Cefas 2022).

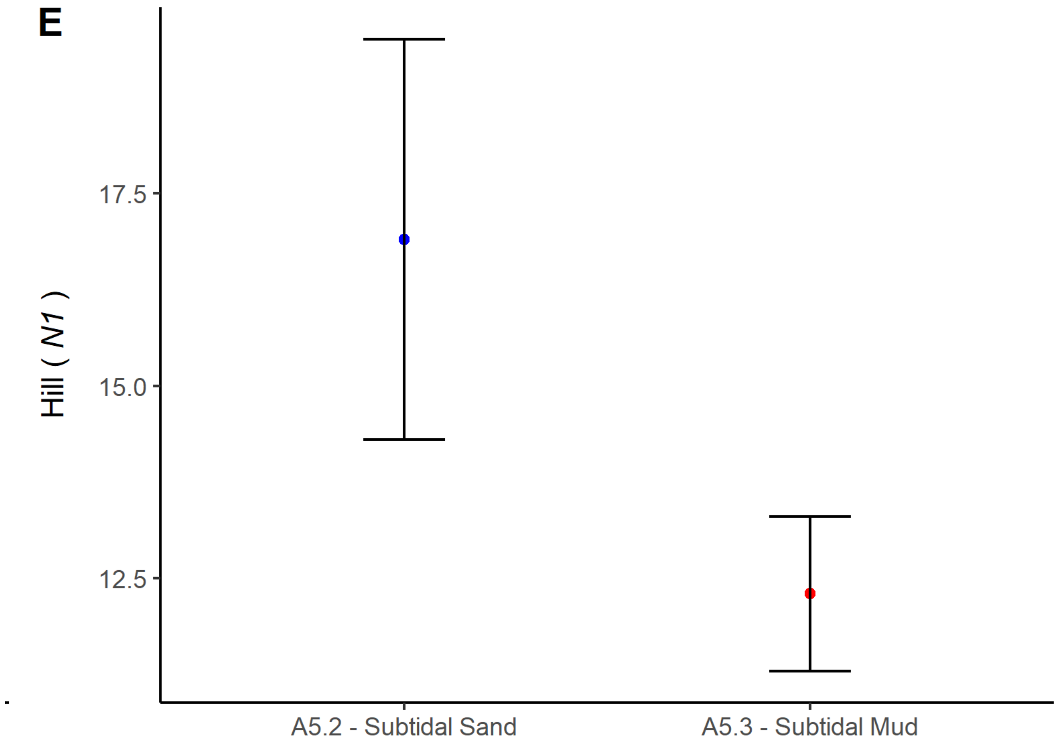


Figure 14 (continued). Mean values and 95 % confidence intervals for the univariate metrics of infaunal diversity between BSHs at West of Walney MCZ in 2018 (Blue circles = A5.2 Subtidal sand and red circles = A5.3 Subtidal mud) (© Natural England and Cefas 2022).

Table 6. Comparison between BSHs at West of Walney MCZ in 2018, showing the significantly different ($p < 0.05$) univariate metrics (*in bold*) (© Natural England and Cefas 2022).

Comparison	Metric	T statistic (df)	P value
'Subtidal sand' and 'Subtidal mud'	Margalef's richness (*d*)	3.2 (13.9)	***0.006***
	Pielou's evenness (*J'*)	1.2 (16.6)	0.247
	Shannon (*H'*)	4.6 (17.4)	***0.0002***
	Simpsons (1-λ)	2.9 (22.7)	***0.009***
	Hill (*N1*)	3.7 (13.8)	***0.003***
	Number of taxa (*S*)	2.3 (13.3)	***0.042***
	Abundance (*N*)	0.27 (13.3)	0.79
	Biomass (*g*)	-2.4 (88.1)	***0.015***

There was a small, yet significant, difference between 'Subtidal sand' and 'Subtidal mud' infaunal assemblages (R = 0.17, p = 0.02) and the average dissimilarity between the BSHs was relatively high: 73 %. Five taxa were only found in a single sample of ‘Subtidal sand’ and were absent from sediment samples classified as ‘Subtidal mud’. Sixty-three taxa were present only in samples classified as ‘Subtidal mud’. None of these taxa were hugely dominant and differences in the abundances of infrequent taxa appear to be driving the dissimilarity between BSHs (Figure 15). Note, this may not be a reliable assessment of the assemblages due to the limited number of ‘Subtidal sand’ samples available and the average within group similarity was low (36 and 31 % for ‘Subtidal sand’ and ‘Subtidal mud’ respectively).

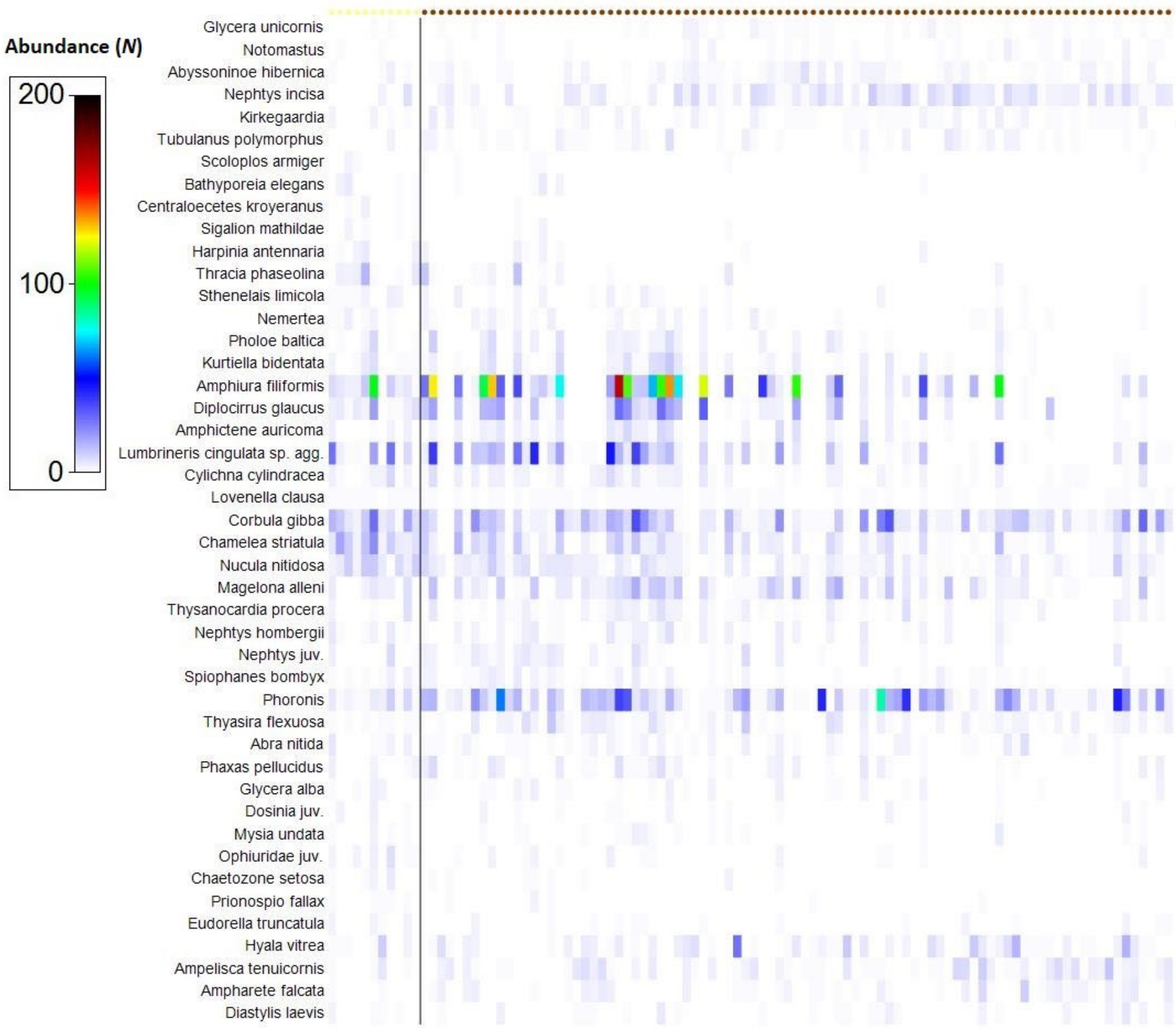


Figure 15. The distribution of infaunal abundance of those taxa which contribute 80 % of the cumulative difference between 'Subtidal sand' (yellow) and 'Subtidal mud' (brown) samples at West of Walney MCZ in 2018 (© Natural England and Cefas 2022). Stations are ordered by increasing mud content and taxa are ordered by Bray-Curtis similarity scores.

The infaunal assemblage, from the samples collected during the 2018 survey, clusters into 20 statistically different groups when using the non-hierarchical 'k-R Clustering' method (R = 0.9, Min:2- Max:20). Samples belonging to each group appear relatively close to each other geographically (Figure 16), however, there are too few samples in each group to allow for robust analysis of any characterising species e.g. using the SIMPER routine. The characterising taxa of groups comprising more than five samples are described below and the abundance of the characterising taxa of all groups is shown in Annex 10.

Table 7. Number of samples allocated to each *k*-R cluster group at West of Walney MCZ in 2018 (© Natural England and Cefas 2022).

Number of samples	29	10	8	7	6	4	3	2	1
***k*-R cluster group**	S	G	E	T	P	F, K	C, H, I, M, N, O, Q	B, D, J, L, R	A

A large proportion (89 %) of the total abundance of the brittle star *Amphiura filiformis* occurred in group S samples and 85 and 82 % of the total abundance of the polychaetes *Amphictene auricoma* and *Diplocirrus glaucus* respectively occurred in nearly all of the group S samples (*A. auricoma* was absent from one sample in group S). Two of the samples in group S were classified as 'Subtidal sand'; the remaining 27 samples were classified as 'Subtidal mud'.

Almost half (49 %) of the total abundance of the gastropod mollusc *Turitella communis* was present in four of the ten samples allocated to group G. Another gastropod mollusc *Hyala vitrea* was present in all group G samples, accounting for 44 % of their total abundance in the dataset. The polychaete *Nephtys incisa* was present in all samples in group G (16 % of total). A single station was classified as 'Subtidal sand'.

The eight samples in group E comprised 22 % of the total abundance of the Annelid *Abyssoninoe hibernica* which occurred in all samples belonging to the group. The polychaete *Nephtys incisa* was also present in all samples in group E (11 % of total). All samples in group E were classified as 'Subtidal mud'.

Group T was dominated by the amphipod *Ampelisca tenuicornis* (31 % total abundance) and the polychaete *Nephtys incisa* (13 % total abundance) which occurred in all group T samples (which were classified as 'Subtidal mud').

Group P was dominated by the brittle star *Amphiura filiformis* (72 individuals present across all samples in group P) and bivalve molluscs. Notably, 59 % of the total abundance of *Thracia phaseolina* (*n* = 40) was present across all the samples in group P and large proportions of the total abundance of *Chamelea striatula* (19 %) and *Nucula nitidosa* (18 %) were also present across all group P samples. Almost half the total abundance of the amphipod crustacean *Harpinia anntenaria* (45 %, *n* = 10) was present in group P samples. Four samples in group P were classified as 'Subtidal sand'.

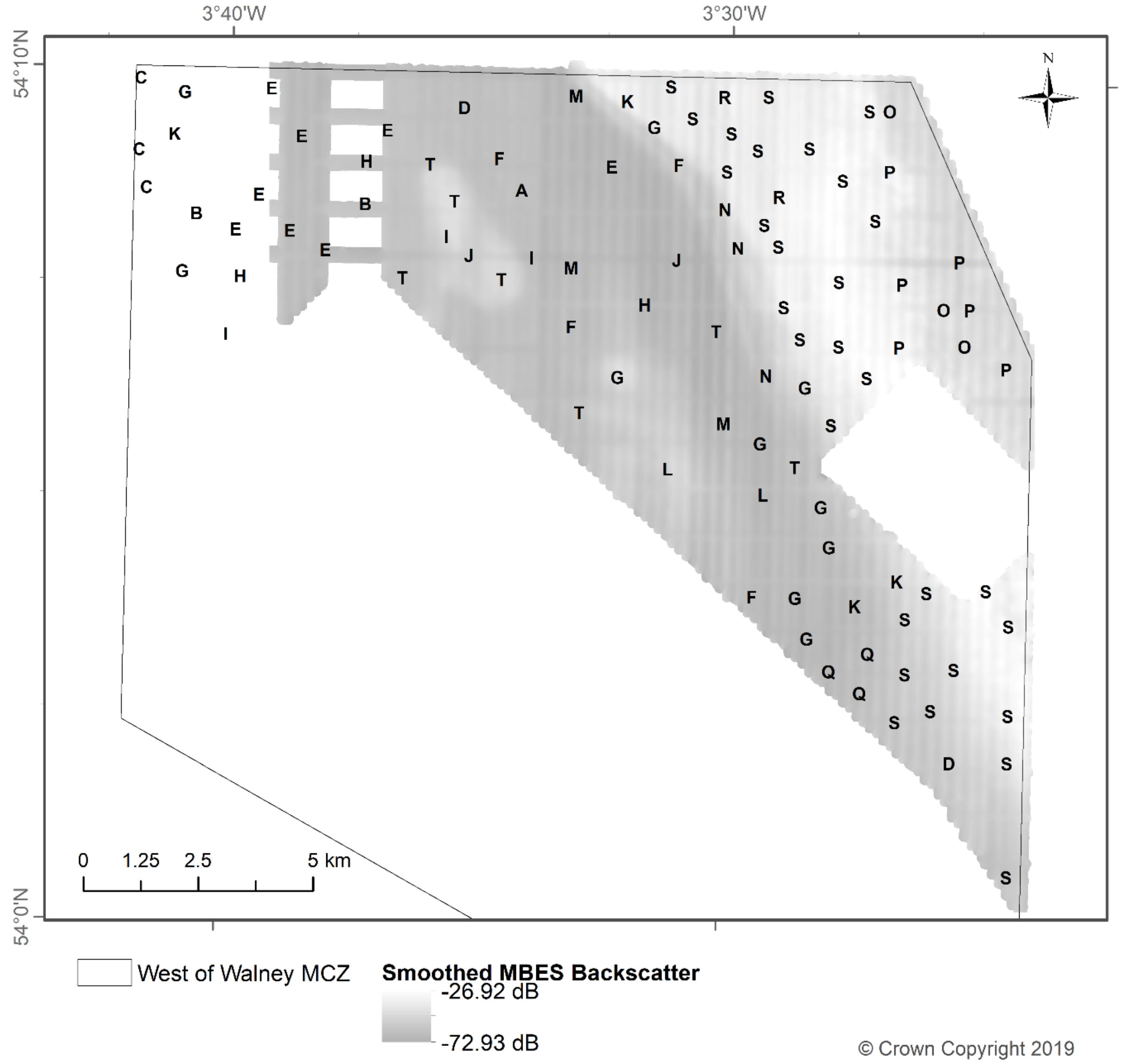


Figure 16. Infauna group allocation plotted on the smoothed MBES backscatter (dB) values, showing the numerous *k*-R cluster groups (Table 7) and their geographical association at West of Walney MCZ in 2018.

Aggregating the infaunal abundance data to a lower classification (family level) results in a large number (13) of *k*-R cluster groups ($R = 0.88$; $p = 0.05$), which are also geographically associated, but does not provide a more comprehensive understanding of the assemblage at the West of Walney MCZ.

An alternative presentation of infaunal communities is given in Figure 17. Biotopes for infaunal communities were determined by the external contractors undertaking analysis of the infaunal samples. Samples were matched to the most appropriate biotope from the JNCC Marine Habitat Classification system; 04.06, and equivalent EUNIS 2007-2011 codes. Level 5 biotopes were assigned wherever possible. Notes were recorded where samples could not be assigned to a single biotope code and were deemed to constitute species indicative of two or more different communities which could be interpreted as representing a transitional zone between two or more dominating communities / biotopes.

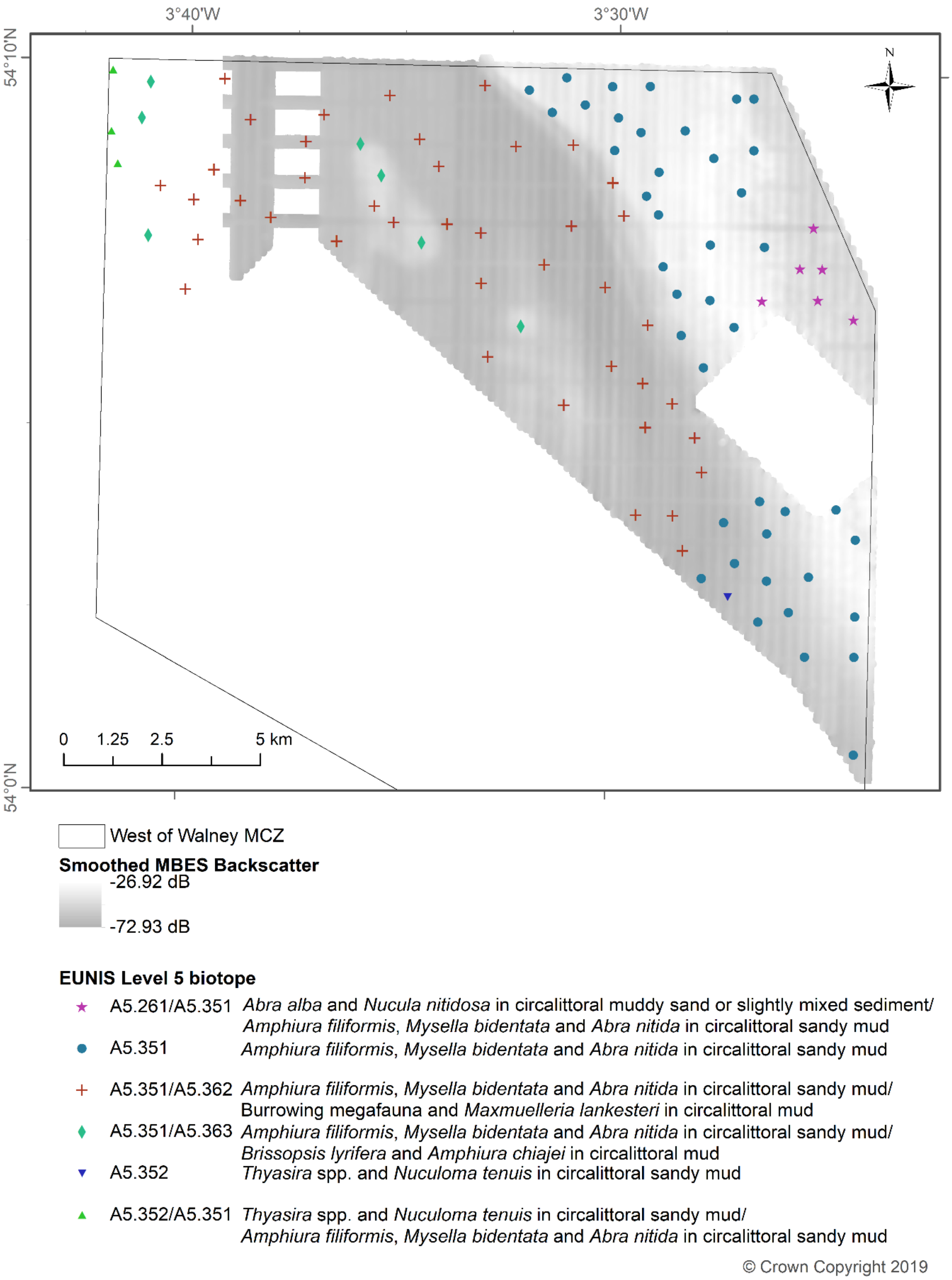


Figure 17. 2018 grab samples classified based on EUNIS level 5 biotopes plotted on the smoothed MBES backscatter (dB) values.

3.5 2016 Gear comparison

In total, 171 taxa were collected during the 2016 survey, of which 137 were present in samples acquired using the Box corer (*n* = 57, 19 stations; three replicates from each) and 131 were collected using the Day grab (*n* = 99, 20 stations; five replicates at 19, four replicates at one). In total, 7860 individuals were counted from the Box corer sampler compared to 4520 in the Day grab. Similarly, the Box corer collected more biomass (1819.8 *g* vrs 240.2 *g*). The Box corer penetrated deeper into the sediment than the Day grab and variability in penetration depth was low for both gears (Box_{Depth} = 48 cm ± 7.6 s.d.; Day_{Depth} = 15 cm ± 1.1 s.d.).

The Box corer consistently collected more species, individuals and biomass per sample than the Day grab and Box corer samples were observed to have a significantly more diverse assemblage (Figure 18). With particular respect to taxa considered representative of the habitat FOCI, the abundances of these taxa collected by each gear type is shown in Annex 11. When including gear type as variable in the model, the number of taxa collected by the Day grab was significantly less than that collected by the Box core (Figure 19). The inclusion of penetration depth did not aid in the model (AIC increased from 126.89 to 130.71). However, as penetration depth varied so little within gears, it is impossible to separate the effect of gear from that of penetration depth as they indicate essentially the same thing.

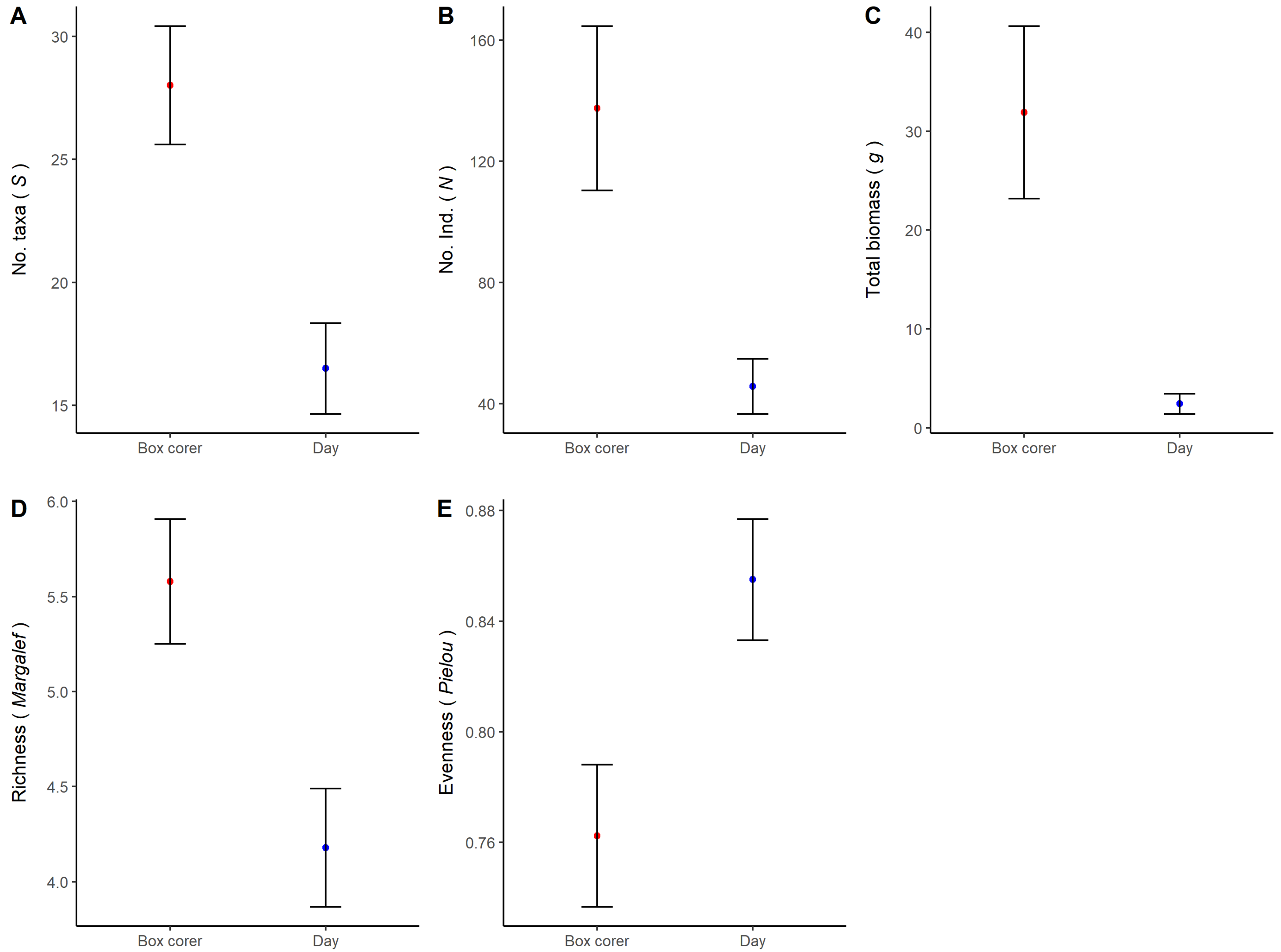


Figure 18. Mean and 95 % confidence interval plots showing the differences in metrics tested between gears at West of Walney MCZ in 2016 (© Natural England and Cefas 2022).

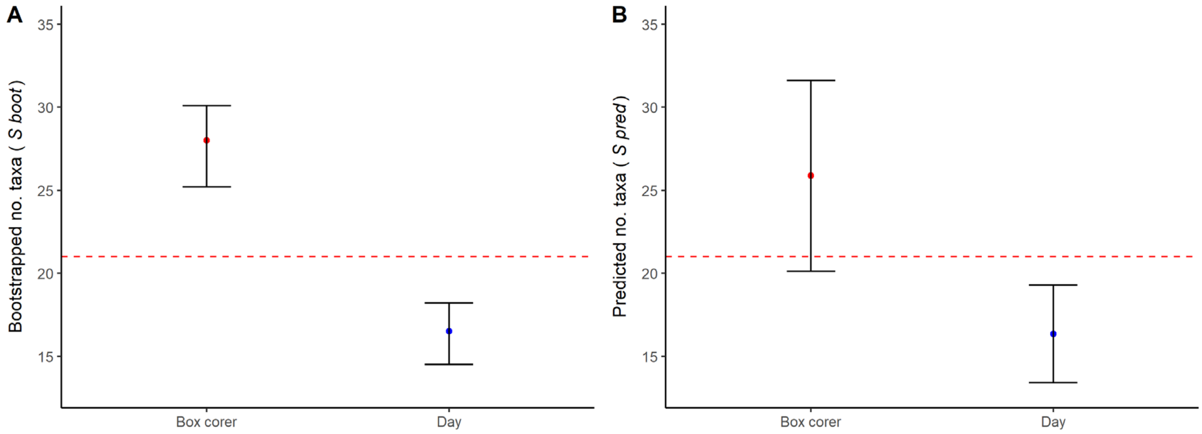


Figure 19. Plots showing the A) bootstrapped mean number of taxa (S boot) and B) mean number of taxa accounting for gear type and penetration depth (S pred) with associated 95 % confidence intervals (© Natural England and Cefas 2022). The red dashed lines show the mean number of taxa across both samplers. Data from West of Walney MCZ in 2016.

Table 8 shows, for each gear, the total abundance (summed and percentage contribution to the total value) and the percentage occurrence in the samples for the most numerically dominant taxa. The burrowing spoon worm (*Maxmuelleria lankesteri*) was not collected by the Day grab and yet was frequently collected by the Box corer (60 individuals at 80 % of the stations). The majority (98 %) of the total abundance of the bivalve *Saxicavella jeffreysi* was present in the Box corer samples. The majority (90 %) of the burrowing mud shrimp *Callianassa subterranea* individuals were found in samples collected by the Box corer. All samples collected by the Box corer had *C. subterranea* individuals present, while the Day grab collected *C. subterranea* in 35 % of samples.

A table summarising the abundance and biomass values for the most dominant taxa is presented in Annex 7.

Table 8. Total abundance (summed across replicates for each gear) for the most numerically dominant taxa showing the contribution of each and the number of samples in which they are found (© Natural England and Cefas 2022).

Taxon	**Summed total abundance**		**Percentage of total abundance (%)**		**Percentage occurrence in samples (%)**		**Dominance in sample (summed abundance in numerical order)**	
	Box core	**Day grab**	**Box core**	**Day grab**	**Box core**	**Day grab**	**Box core**	**Day grab**
Phoronis spp.	2572	1235	68	32	100	100	1	1
Corbula gibba	613	195	76	24	100	100	2	4
Saxicavella jeffreysi	421	9	98	2	79	25	3	61
Nephtys incisa	382	228	63	37	100	100	4	3
Hyala vitrea	381	348	52	48	100	100	5	2
Abra nitida	363	118	75	25	100	85	6	6
Mediomastus fragilis	338	92	79	21	68	65	7	10
Callianassa subterranea	177	19	90	10	100	35	8	41
Prionospio multibranchiata	168	149	53	47	100	100	9	5
Ampelisca tenuicornis	161	88	65	35	100	90	10	12
Phaxas pellucidus	111	117	49	51	89	70	12	7
Jaxea nocturna	84	96	47	53	100	95	14	8
Lumbrineris cingulata sp. agg.	68	91	43	57	32	30	19	11
Magelona minuta	43	94	31	69	74	80	23	9

Notably, the ten most numerically dominant taxa collected by the Box corer were also numerically dominant in the Day grab dataset, although not nessecarily in the same order. While 35 taxa were present in the Box corer samples and absent from the Day grab, 29 taxa were present only in the Day grab. However, the majority of these taxa occurred in low numbers in the benthic assemblage (Annex 11).

There was a small yet significant difference in the overall assemblage collected by the different gears ($R = 0.17$, $p < 0.0001$), which appears to be a result of differences in the abundances of taxa encountered by both gears (Figure 19). The average similarity among stations sampled using the Day grab and Box corer was relatively low at 29.4 and 45.7 % respectively and the average dissimilarity between stations collected by each of the gears was 73.8 %.

Figure 20.20 Abundances of those taxa responsible for 80 % of the cumulative dissimilarity between Day grab and Box corer samples from West of Walney MCZ. Samples ordered by station code (A – S Box core; A – T Day grab) (© Natural England and Cefas 2022).

3.6 Habitat FOCI

Sea-Pen and Burrowing Megafauna Communities

All stations classified as ‘Subtidal mud’ were observed to contain species indicative of the Habitat FOCI ‘Sea-Pen and Burrowing Megafauna Communities’ (Figure 21; Annex 4). In total, 11 stations were classified as ‘Subtidal sand’ (all from the Day grab survey undertaken in 2018; see section 3.3.1). These stations were dominated by ‘sandy’ sediments with a mean percentage contribution of ‘mud’ sized grains of only 9 % (which ranged between 0 – 17 %; Figure 12), and were not determined to be representative of the habitat FOCI ‘Sea-Pen and Burrowing Megafauna Communities’.

Sea-pens, and most other taxa most characteristic of the habitat FOCI ‘Sea-Pen and Burrowing Megafauna Communities’ were not recorded in the sediment samples from any station in 2018. Taxa considered as characterising of the habitat FOCI are listed in Annex 4 and were based on 1) inclusion in the list of species linked through Table 4 of the JNCC definition development paper[7] and 2) expert judgement investigation of the taxa sampled.

The abundances of these taxa present in samples from both 2016 and 2018 surveys and from both gear types are shown as the shade plots in Annex 4.

[7] http://jncc.defra.gov.uk/pdf/Advice_Document_MudHabitats_FOCIdefinitions_v1.0.pdf [Accessed 04/06/20]

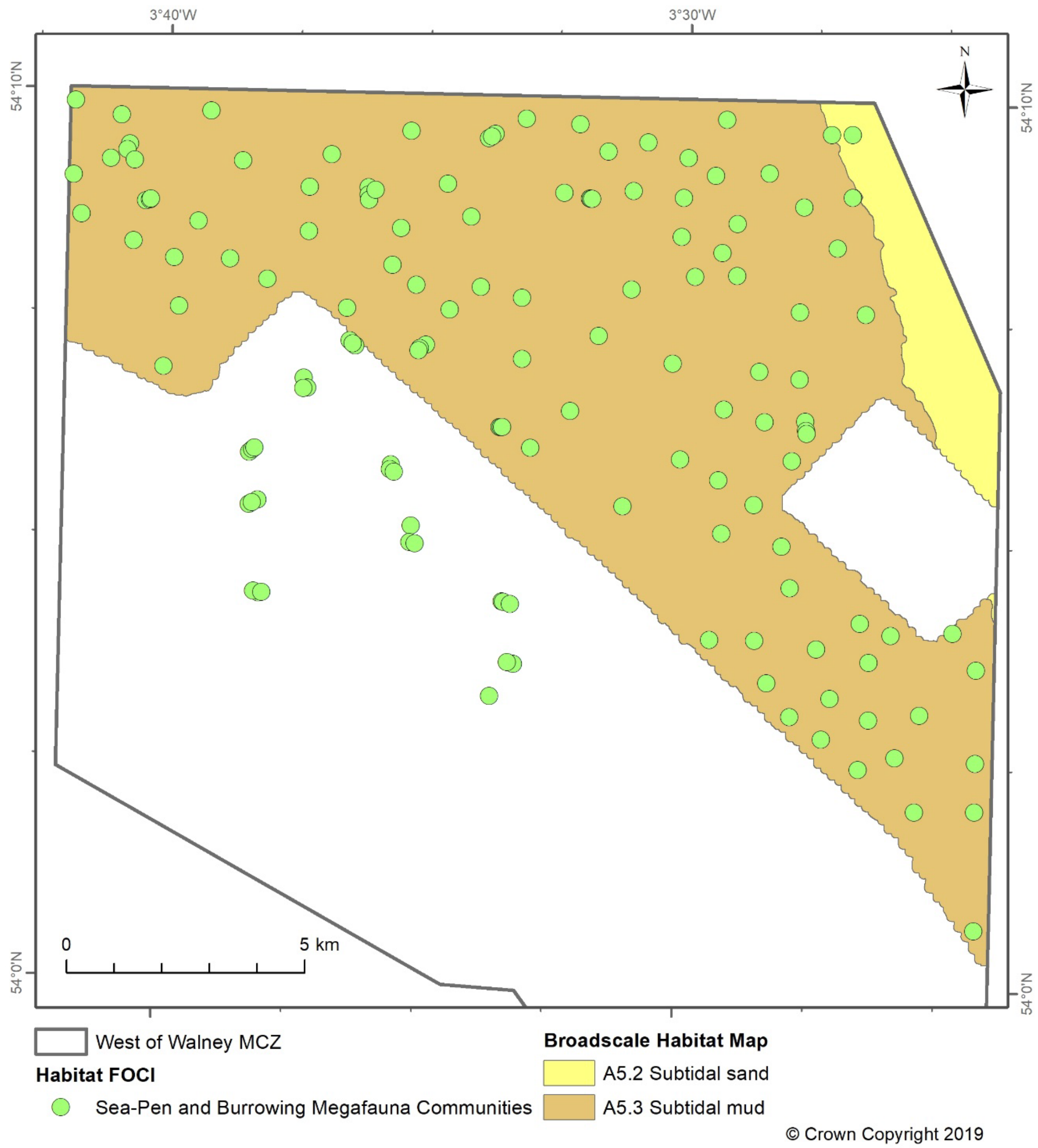


Figure 21. Locations where species indicative of 'Sea-Pen and Burrowing Megafauna Communities' were observed in grab samples collected during the 2016 and 2018 surveys of the West of Walney MCZ.

Megafauna measurements

In total, additional measurements were collected from seven taxa during laboratory processing of the 2018 Day grab samples. These taxa were considered characteristic of burrowing megafauna and were selected due to the ability to easily record measurements per individual (Table 9).

Table 9. Additional measurements recorded from a sub-set of megafauna collected in Day grabs during the 2018 survey (© Natural England and Cefas 2022).

Taxon	No. of individuals	Total biomass (g)	Measurements acquired (per individual)
Jaxea nocturna	14	1.2045	Biomass (g) Length of carapace (mm) Total length (mm)
Callianassa subterranea juv.	3	0.0036	
Upogebia deltaura juv.	2	0.0221	
Callianassa subterranea	1	0.0012	
Cerianthus lloydii	1	0.3718	Biomass (g) Total length (mm)
Echinocardium cordatum	5	19.3327	
Corystes cassivelaunus	2	0.6097	Biomass (g) Width of carapace (mm) Total length (mm)
Goneplax rhomboides	16	18.9777	

No taxa were collected in enough numbers to carry out an assessment of their size distribution across the MCZ. Plots showing the frequency histograms of the measurements recorded for taxa with a total of more than ten individuals (*Jaxea nocturna* and *Goneplax rhomboides*) are provided in Annex 12.

3.7 Species FOCI

No species FOCI were observed in the 2016 and 2018 surveys. The surveys reported here were not designed to specifically monitor (or identify the presence of) species FOCI. As such, this should not be interpreted as an absence of these species FOCI from the site.

3.8 Non-indigenous species

There were no instances of NIS collected in sediment samples from either of the surveys reported (2016 and 2018).

3.9 Marine litter

Litter found in grab samples were categorised based using the MSFD litter codes. A: Plastic or B: Metal litter (greater than 1 mm) were found in 62 sediment samples collected during the 2018 Day grab survey (62 % of samples) (Figure 22) and litter were found in all stations surveyed during 2016. Replicate samples (n = 3) collected using the Box corer yielded litter at all stations visited, while there was no evidence of litter in replicate samples (n = 5) collected using the Day grab from nine of these same stations where litter was sampled by the Box core (Figure 23).

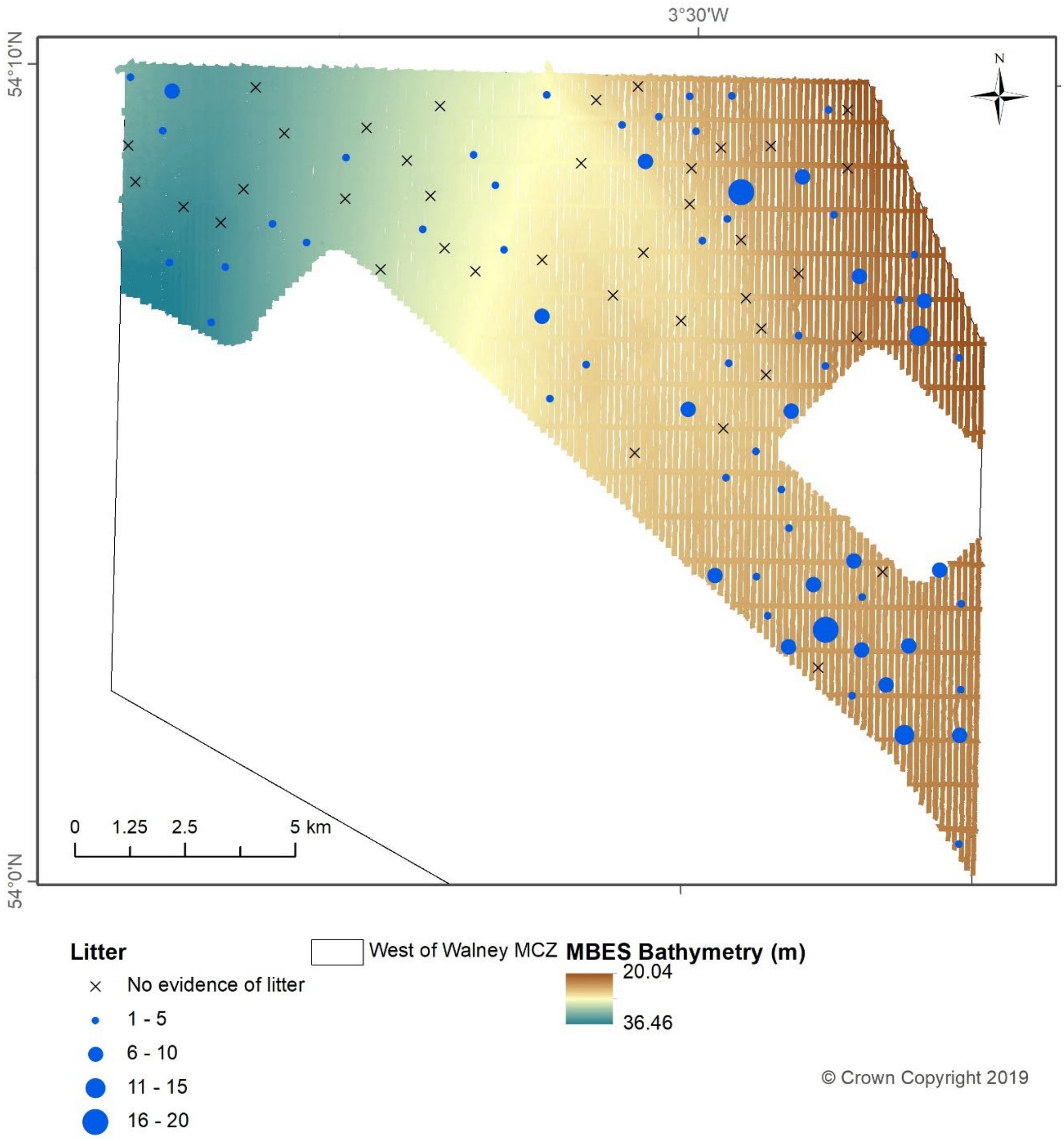


Figure 22. The abundance of litter greater than 1 mm in the 2018 Day grab samples from West of Walney MCZ.

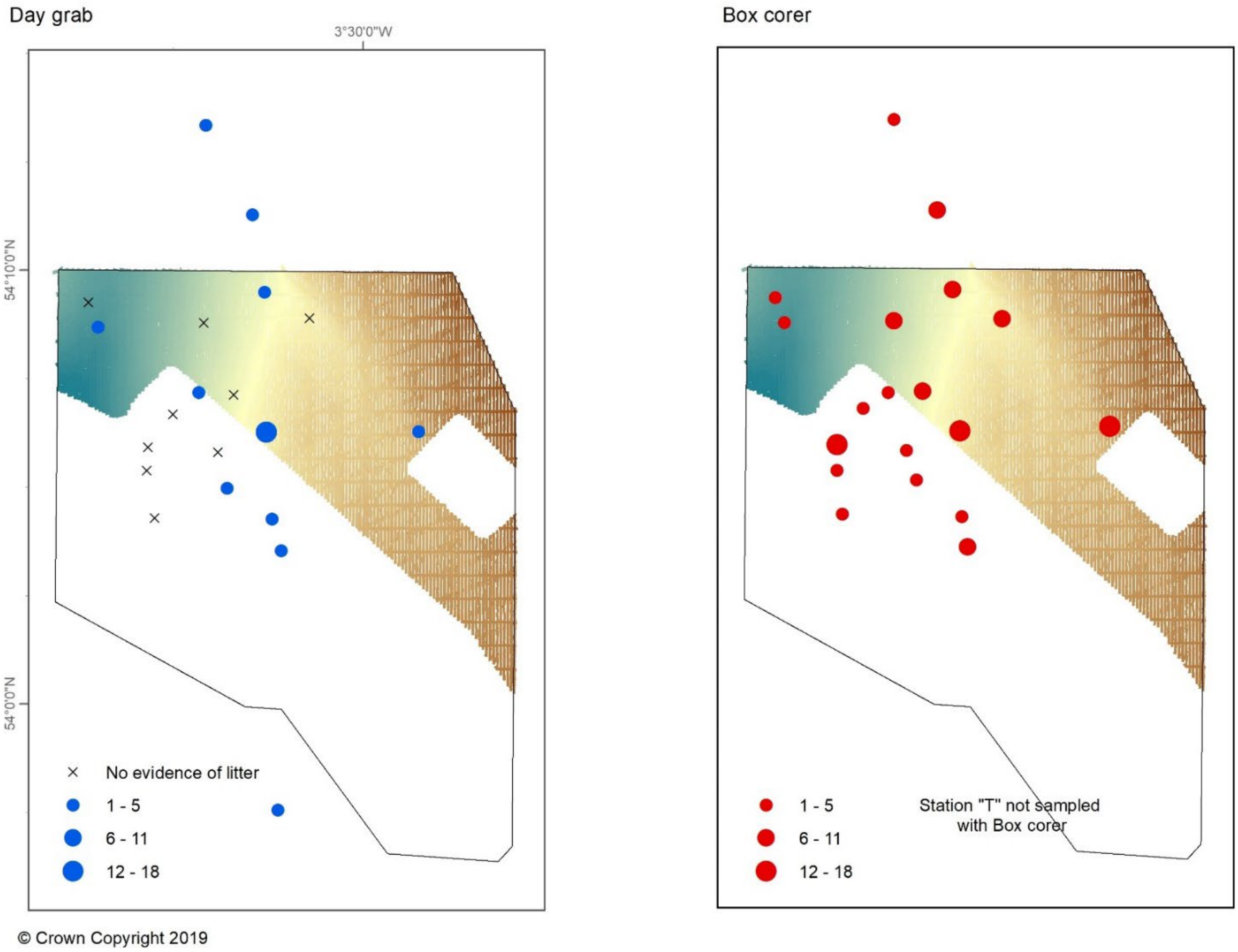


Figure 23. The abundance of litter in the Day grab (blue circles) and Box corer (red circles) samples (summed replicates) collected in 2016 at West of Walney MCZ.

3.10 Observed anthropogenic activities and pressures

An objective of this report was to document any anthropogenic activities or pressures encountered during the monitoring surveys for this site (objective 5; see section 1.3.3). The presence of OWF developments within the West of Walney MCZ are well documented. However, in the northern section of the MCZ, which is the focus of this report, there were no anthropogenic activities or pressures reported in the 2016 survey (Monnington, 2017) and 2018 survey report (Pritchard, 2019).

3.11 Environmental data

All stations sampled for additional environmental data were classified as ‘Subtidal mud’. See Annex 2 for a full suite of the contaminants assessed and the resulting values. The percentage contribution (by weight) for each half ϕ size class, of sediment samples collected from each station shows the varying degree of fine silts and clays present in each sample (Figure 24).

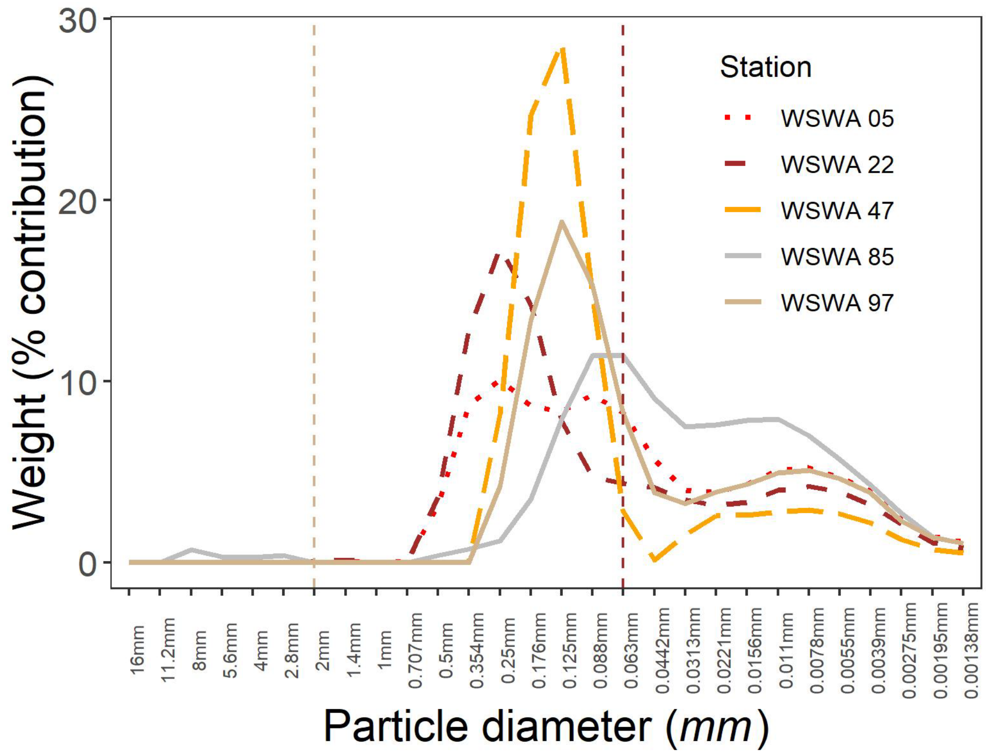


Figure 24. Percentage contribution (weight) of each half ϕ particle size band showing the distribution for each station sampled for contaminants at West of Walney MCZ in 2018 (© Natural England and Cefas 2022). Dashed vertical lines indicate the boundary between gravel and sands (left), and sands (left), and sands and muds (right).

Each analyte was measured against OSPAR thresholds, after performing the relevant normalisation (5 % aluminium for metals and 2.5 % carbon for chlorobiphenyls), using the assessment criteria for contaminants in sediment[8].

Most metals were below or close to their respective Background Assessment Criteria (BAC) levels. However, a single station (WSWA075 in the north-east of the MCZ) had elevated levels of Nickel (83 mg kg^{-1} dry weight); almost four times the effects range (Effects Range Low) developed by the United States Environmental Protection Agency for assessing the ecological significance of sediment concentrations.

All chlorobiphenyls and organo-bromines measured were either below detectable limits or below or close to their respective BAC levels.

Assessment criteria were not available for manganese, lithium and iron (metals); tributyl tin (organo-metals); hexachlorobutadiene and hexachlorobenzene (pesticides).

[8] http://dome.ices.dk/osparmime2018/main.html [Accessed 04/06/20]).

4 Discussion

This monitoring report has achieved the broad objective 1 (see section 1.3.3 and 1.3.4) through the creation of a BSH map and by describing the distribution and structural attributes of designated features within the north of the West of Walney MCZ. Aside from considering the tidal and wave models, no further data have been presented or discussed in order to inform on the supporting processes of the designated features (report objective 2; see section 1.3.3). No targeted sampling was undertaken in 2016 or 2018 to inform on wider supporting processes and none is expected to be planned in the future, therefore, data to support this objective must be sought from wider, existing monitoring programmes in the future. Objective 3 has been addressed through noting the species indicative of potential habitat FOCI within the site (no species FOCI were observed within the site). As no NIS were observed within the 2016 and 2018 surveys this satisfies the requirements of objective 4. Objective 5, which relates to anthropogenic activities and pressures, have been addressed through observations of marine litter and sampling for sediment contamination. The following sections discuss the evidence pertaining to these objectives and provide monitoring recommendations for the designated features in order to meet broad objective 6. Objective 7 has been addressed through a comparison of sediment sampling gear to identify the most suitable gear for future sampling.

4.1 Benthic and environmental overview

Based on the available acoustic data and ground truth samples collected in 2016 and 2018, an updated BSH map has been generated for the northern section of the West of Walney MCZ. The presence of the BSHs ‘Subtidal sand’ and ‘Subtidal mud’ were confirmed based on samples collected in 2016 and 2018. The spatial extents of these features were not investigated for the rest of the MCZ by these surveys or the mapping undertaken for this report.

The evidence indicates that the West of Walney MCZ is not subject to changes in the distribution of BSHs through the natural hydrodynamic forces which are more commonly observed in shallower sites. No mobile seabed morphologies were observed in the bathymetry data and modelled hydrodynamic data (Figure 6) suggests that the seabed is a low energy environment. Considering the high mud content of the seabed across this site, predicting natural change is complex as the sediment would display cohesive behaviour in response to wave and current disturbance (Aldridge *et al.,* 2015).

4.2 Subtidal sediment BSH

Extent and distribution

The site is predominantly made up of fine-grained muds classified as 'Subtidal mud' with a gradual coarsening of grain size towards the east. Figure 11 shows the percentage of sand in each sample generally increased towards the east, which based on the Folk 16 sediment classes (Folk, 1954) was reflected as a transition from Mud, to Sandy Mud, to Muddy Sand. Based on the BSH classification, however, which only separates the sediment into four classes as opposed to 15, these were all classified as 'Subtidal mud'. In the east of the study area there is the presence of the BSH 'Subtidal sand', which was predicted to cover less than 7 % of the mapped extent (Figure 7 and 5). While the underlying acoustic data were not available at 100 % coverage, the gradual change in sediment type across the site meant sediment types were interpolated across the gaps in data to product a full coverage habitat map. This lack of full coverage acoustic data resulted in two levels of MESH confidence scores assigned to the map (Figure 8), but it is unlikely that any variation would have been observed in the areas of no data, considering how homogenous the rest of the site was.

Despite the more complex variation in MBES backscatter intensity, backscatter did not correlate with variations in BSHs (Figure 5). As such the mapped extent of these BSHs was determined based on the depth alone which may be a proxy for wave exposure (Figure 6). The current mapped boundary should be considered indicative only, as the relationship between BSH and depth was derived from a limited number of samples and is likely to reflect a correlation with other more relevant variables for which data are not available. Based on the 100 sediment samples from 2018 and a further eight samples that overlapped the MBES data from 2016, the updated BSH map agrees with 100 of these samples (or 93 %). While this estimation of accuracy was based on the samples used to derive the model, and therefore is not as robust as accuracy measures derived from independent data, it is promising. As this map is the first map generated for this section of the West of Walney MCZ derived from high resolution data specifically collected across the AOI within the site, it provides the best map currently available.

Biological communities

There are small differences between metrics and assemblages associated with each BSH. However, these are not particularly distinct nor is any one aspect characteristic of one BSH. Furthermore, too few samples were classified as 'Subtidal sand' to allow a more detailed analysis and reliable interpretation of results. Grouping metrics by BSH may have made observing any spatial differences more difficult. Most of the variance occurred within the BSH 'Subtidal mud', which if further subdivided into the Folk 16 classifications may better reflect differences in infauna communities. There appears to be some differences in infauna communities along the backscatter gradient (Annex 9), which also appear to coincide with different Folk 16 sediment classes (Figure 11). However, as the West of Walney MCZ is to be managed at a BSH level this was not investigated further.

4.3 Gear comparison

The use of the Box core resulted in higher mean values for each of the metrics assessed than the Day grab. Furthermore, the Box core successfully collected more burrowing animals such as the mud shrimp *Callianassa subterranea*. However, the overall benthic assemblage sampled using either of gears appears similar.

4.4 Undesignated BSH

No undesignated BSHs were observed within the West of Walney MCZ.

4.5 Habitat FOCI

Taxa considered indicative of the habitat FOCI 'Sea-Pen and Burrowing Megafauna Communities' (listed in Annex 4) were observed in the samples from both the 2016 and 2018 surveys (Figure 20). The JNCC advice on identifying 'Sea-pen and Burrowing Megafauna Communities' (JNCC, 2014) indicates between one and nine burrows should be recorded per 10 m^2, in conjunction with the collection of infaunal samples confirming the presence of relevant taxa, and PSD data confirming a fine mud habitat. Therefore, with only PSD and infauna data available it was not possible to confirm the full extent of this habitat FOCI. The absence of larger burrowing animals from Day grab samples, in particular, should not be considered to preclude the habitat FOCI 'Sea-Pen and Burrowing Megafauna Communities' from occurring there. For that reason, the presence of the habitat FOCI 'Sea-Pen and Burrowing Megafauna Communities' was presented as point locations with no inference made regarding the spatial extent of these communities. In the absence of a specifically targeted sampling regime to assess extent, based on the data available the extent of 'Subtidal mud' could potentially be used as a proxy for extent of the HOCI within this MCZ.

4.6 Species FOCI

No species FOCI were observed during the 2016 and 2018 surveys of the West of Walney MCZ. The surveys reported here were not designed to specifically monitor (or identify the presence of) all species FOCI. As such, this should not be interpreted as an absence of these species from the site.

4.7 Non-indigenous species

No evidence of NIS is presented in this report. The surveys reported here were not designed to specifically monitor (or identify the presence of) the full list of marine (planktonic, benthic, epibenthic) NIS. As such, this should not be interpreted as an absence of these species from the site. Furthermore, five OWF are co-located with the MCZ. Impacts associated with the presence of these OWF should be monitored closely in relation to the presence and distribution of NIS.

4.8 Marine litter

Litter was reported from the sediment samples as records of “plastic” greater than 1 mm in the infaunal species abundance dataset. These samples were collected primarily to assess the benthic assemblage rather than an assessment of litter and as such litter content was not analysed further. Consideration of the requirement to determine the presence of litter (microplastics) in the surficial sediments of an MCZ at the survey design stage would facilitate a more accurate assessment to be made by adopting relevant best practices during sample collection and sample processing.

4.9 Observed anthropogenic activities and pressures

Although no anthropogenic activities or pressures were reported during the survey conducted in 2018, the Ormonde, Walney 1, Walney 2, Walney Extension and West of Duddon Sands OWF are co-located with the MCZ. Furthermore, the area is subject to pressures associated with the *Nephrops norvegicus* fishery which currently operates within the West of Walney MCZ (Monnington, 2017).

Based on the five sediment samples collected within the BSH ‘Subtidal mud’, one sample was observed to have elevated levels of Nickel that was higher than the Effects Range Low level. It would be inadvisable to draw conclusions from a single observation; however, this elevated level of contamination may be worthy of further investigation.

5 Recommendations for future monitoring

There appears to be some level of sorting of sediment grain size throughout the MCZ. Towards the east of the MCZ the sediment is generally coarser (Figure 11), which coincides with higher backscatter reflectivity. However, any relationship between the sediment grain fractions and backscatter reflectivity is not reflected at the level of BSHs. The variation is predominantly within the range of gravel, sand and mud fractions classified as ‘A5.3 Subtidal mud’. As this BSH fraction groups sediments with between 100 % mud and 20 % mud into the same BSH this sorting across the site is not evident in a BSH classification. More quantitative approaches to model sediment fractions independently from classification scheme are available (Misiuk *et al.,* 2019; Mitchell *et al.,* 2019). Similarly, applying a more detailed classification scheme that subdivides the ‘A5.3 Subtidal mud’ class (e.g. Folk 16; Misiuk *et al.,* 2019) may also produce classes that can be accurately mapped from the backscatter. These were not considered in this report as the feature designation is based at a BSH level. However, understanding the distribution of biological communities and monitoring change over time may require the consideration of more subtle changes in grain size that do not relate to changes in BSH.

5.1 Operational and survey strategy

- Subtidal sand is present in a small portion of the mapped area (less than 7 %) and was not sampled sufficiently to allow for robust investigation of the community present or comparison of measures of diversity between sand and mud communities. In order to provide a robust exploration of the benthic community in this BSH and to enable, for example, trend analysis in the future, a greater sampling effort will be required to establish a robust T0 baseline specifically within this BSH. Given the small area of the site mapped as this BSH however it would need to be carefully considered if this expenditure of survey effort was considered appropriate for such a comparatively small area.
- The diversity metrics derived from the Box core and Day grabs differ, particularly with regards to burrowing species. This suggests that time series data should be based on one gear type. The Box core outperformed the Day grab, returning more species, individuals and biomass per sample. However, other considerations may influence the choice of gear used during future monitoring surveys. Restrictions on the safe deployment and successful operation of the larger Box core may require large and stable survey platforms, while the Day grab is a relatively light sampler which can be deployed from smaller vessels and has been shown to sample a very similar community to that sampled using the Box core.
- If the collection of various megafauna taxa is required for any future assessment e.g., metrics derived from individual measurements used as

indicators of environmental quality, the sampling strategy to acquire these taxa must result in an accurate estimate of the population present and may be best carried out using combination of different gears. As demonstrated in 2016 (Monnington, 2017), the Box core is more favourable than the Day grab for sampling larger and burrowing infauna species, while a scientific beam trawl in combination with seabed imagery, e.g., images from a towed camera sledge, would best sample epifaunal communities and allow for investigation of spatial extent of communities.

- If there is to be a future requirement for accurate determination of litter from sediment samples, the collection method and processing procedure, must be specified clearly as this currently sits outside of the nationally accepted standards (NMBAQC invertebrate scheme component). Collecting and storing sediment samples specifically collected for the analysis of microplastics, and other anthropogenic material, as occurs on the Clean Seas Environmental Monitoring Programme, would permit more reliable assessment as laboratory techniques advance.
- NIS monitoring should take into consideration the OWFs in terms of the provision of colonising structure and any licence conditions placed upon the developer(s). Similarly, any benthic monitoring of the wider region, that is conducted as part of the OWF operators deemed marine license conditions, should be conducted in a manner that is consistent with the monitoring strategy designed for the West of Walney MCZ to allow robust analysis and efficient data collection.

5.2 Analysis and interpretation

- While several metrics are presented in this report, those to be monitored going forward have not yet been set. Any temporal assessment of these data, as and when metrics become available, must therefore incorporate and reanalyse these data in an appropriate manner to address change over time.
- Benthic assemblage groupings must be reanalysed when additional monitoring data become available. Cooper and Barry (2017) provide a potentially useful method for determining grouping of infauna samples in the context of the UK infauna prior to any more detailed analysis which assigns newly acquired data to one of several established faunal groups.
- Numerous datasets are available for inclusion in any assessment of the benthic assemblage from this site, particularly from the OWF industry. The inclusion of these datasets may allow better assessment of any temporal change in the benthic assemblage at the West of Walney MCZ.
- Individual measurements of conspicuous megafauna may provide insights into the size classes present within the site and therefore increase our understanding of recruitment, mortality etc in response to the pressures at the

site. However, adequate assessment requires a more comprehensive understanding of the empirical relationships between organism size and any chosen measure to be investigated.

- Sediment sampling for litter needs to be bespoke, fit for purpose and follow specific methodology, classification and lab processes to enable a more robust dataset for spatial and temporal comparison.

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Annex 1. Non-indigenous species lists

Table A. Taxa listed as NIS (present and horizon) which have been selected for assessment of Good Environmental Status in GB waters under MSFD Descriptor 2 (Stebbing *et al*., 2014). Some cells left blank intentionally.

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		

Table B. Additional taxa listed as NIS 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. Some cells left blank intentionally (species name has not been updated).

Species name (1997)	Updated name (2017)
Thalassiosira punctigera	
Thalassiosira tealata	
Coscinodiscus wailesii	
Odontella sinensis	
Pleurosigma simonsenii	
Grateloupia doryphore	
Grateloupia filicina var. luxurians	Grateloupia subpectinata
Pikea californica	
Agardhiella subulate	
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 torquate	
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	

Species name (1997)	Updated name (2017)
Petricola pholadiformis	
Mya arenaria	

Annex 2. Sediment contaminants and water quality

Table C. Complete list of concentration of contaminants from the five analysed samples collected in 2018 (© Natural England and Cefas 2022).

Class (unit)	Analyte	WSW A097	WSW A005	WSW A022	WSWA 085	WSWA 047
Metals (mg kg $^{-1}$ dw)	Mercury	0.0208	0.0261	0.0161	0.0227	0.0188
	Copper	15.6	17.9	17	16.2	15.2
	Zinc	109	123	105	109	101
	Cadmium	0.123	0.138	0.119	0.145	0.147
	Aluminium	49400	53500	49300	48500	47400
	Lead	39.8	44.9	36.2	39.6	35.7
	Arsenic	12.8	14.9	13.2	12.9	13.2
	Nickel,	31.6	32.8	82	29.2	30.8
	Chromium	95.2	102	90.5	122	120
	Manganese	739	743	598	794	965
	Lithium	53.1	56.2	57.3	49.6	44.1
	Iron	26400	29300	25200	26800	26400
Polycyclic aromatic hydrocarbons (µg kg $^{-1}$ dw)	Benzo(a)Anthracene	11.5	26	38.6	33.9	38.4
	Benzo(g,h,i)Perylene	17	27.8	55.7	48.4	50.8
	Fluoranthene	20.2	52.9	66.5	57.6	66.9
	Phenanthrene	16.6	44	57.7	43.7	52.9
	Indeno(1,2,3-cd)pyrene	17.6	29.1	57.7	50.8	52.1
	Chrysene + Triphenylene	14.4	29	47.5	41.7	46.3
	Benzo(a)Pyrene	15.3	29.3	50.2	44.7	49.5
	Pyrene	19.7	44.7	67.7	57.7	64.5
	Anthracene	2.58	13.6	9.69	8.68	9.79
	Naphthalene	6.83	10.2	23.9	19.2	21

Class (unit)	Analyte	WSW A097	WSW A005	WSW A022	WSWA 085	WSWA 047
Organo-bromines (µg kg $^{-1}$ dw)	2,4,4-TriBromoDiphenylEther {PBDE 28}	0.02	0.02	0.02	0.02	0.02
	2,2,4,4,5,5-Hexabromodiphenyl ether {PBDE 153}	0.02	0.02	0.02	0.02	0.02
	2,2,4,4,5,6-Hexabromodiphenyl ether {PBDE 154}	0.02	0.02	0.022	0.033	0.027
	2,2,4,4,5-Pentabromodiphenyl ether {PBDE 99}	0.05	0.05	0.05	0.05	0.05
	2,2,4,4,6-Pentabromodiphenyl ether {PBDE 100}	0.02	0.02	0.02	0.02	0.02
	2,2,4,4-Tetrabromodiphenyl ether {PBDE 47}	0.07	0.07	0.07	0.07	0.07
Chlorobiphenyls (µg kg $^{-1}$ dw)	PCB - 028	0.1	0.1	0.171	0.224	0.179
	PCB - 052	0.1	0.1	0.1	0.1	0.1
	PCB - 101	0.1	0.1	0.1	0.109	0.102
	PCB - 118	0.1	0.1	0.112	0.124	0.12
	PCB - 138	0.1	0.1	0.101	0.127	0.125
	PCB - 153	0.1	0.1	0.13	0.17	0.149
	PCB - 180	0.1	0.1	0.1	0.1	0.1
Pesticides (µg kg $^{-1}$ dw)	Hexachlorobutadiene	0.1	0.102	0.1	1.25	0.17
	Hexachlorobenzene	0.1	0.109	0.18	0.242	0.245
Organo-metals (cation)	Tributyl Tin (TBSN+)	1	1	2	2	2
Carbon (% dw)	Total Organic Carbon	0.226	0.346	0.645	0.673	0.668
Nitrogen (mg kg $^{-1}$ dw)	Total Organic Nitrogen	450	436	1050	879	889
Latitude	WGS84	54.06686	54.14372	54.14711	54.1382	54.14107

Class (unit)	Analyte	WSW A097	WSW A005	WSW A022	WSWA 085	WSWA 047
Longitude	WGS84	-3.43216	-3.48389	-3.46266	-3.48854	-3.50177
Sample depth	cm	23.05	24.11	28.88	21.44	23.39
Salinity *in situ*	Parts per thousand	32.15	32.32	32.52	32.41	32
Moisture content (%)	Air dried at 30 °C	74.7	68.9	59.5	58.5	60.1

Annex 3. Infauna data truncation

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 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 have to 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 West of Walney 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 (with the exception of 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 infaunal data collected at West of Walney MCZ: where a species level identification was labelled 'juvenile', the record was combined with the associated species level identification, when present.
- Records of meiofauna (i.e., nematodes) were removed.

Table D. List of truncated taxa which showing which have been removed from the analyses and which have been combined with other records for the 2018 abundance and biomass data matrices (© Natural England and Cefas 2022). Some cells have been left blank intentionally.

Taxon	Qualifier	Truncation action
Animalia		Remove
Nemertea	Parts	Combine
Sigalion mathildae	Juvenile	Combine
Eumida bahusiensis	Parts	Combine
Lycera unicornis	Parts	Combine
Ancistrosyllis groenlandica	Parts	Combine
Glyphohesione klatti	Parts	Combine
Nereididae	Parts	Remove
Nephtys	Parts	Combine
Lumbrineris cingulata	Parts	Combine
Orbiniidae	Parts	Remove
Scoloplos armiger	Parts	Combine
Scolelepis korsuni	Parts	Combine
Chaetozone setosa	Parts	Combine
Notomastus	Parts	Combine
Leiochone tricirrata	Parts	Combine
Owenia	Parts	Combine
Pectinariidae	Parts	Remove
Ampelisca brevicornis	Parts	Combine
Ampelisca tenuicornis	Parts	Combine
Callianassa subterranea	Juvenile	Combine
Lucinoma borealis	Juvenile	Combine
Spisula subtruncata	Juvenile	Combine
Chamelea striatula	Juvenile	Combine
Mysia undata	Juvenile	Combine
Thracia phaseolina	Juvenile	Combine
Phoronis	Parts	Combine
Ophiura	Parts	Remove
Echinocardium	Parts	Combine
Leptosynapta	Parts	Combine
Actinopterygii	eggs	Remove
Plocamium cartilagineum		Remove
Cladophora		Remove

Annex 4. Characterising taxa of biotopes that may be a component part of ‘Sea-Pen and Burrowing Megafauna Communities’

Table E. List of taxa considered as characterising biotopes that may contribute the Habitat FOCI ‘Sea-Pen and Burrowing Megafauna Communities’ (© Natural England and Cefas 2022). Some cells have been left blank intentionally.

Circalittoral fine mud (SS.SMu.CFiMu) and Burrowing megafauna and Maxmuelleria lankesteri in circalittoral mud (SS.SMu.CFiMu.MegMax)	Circalittoral sandy mud (SS.SMu.CSaMu)	
Virgularia mirabilis	Kirchenpaueria pinnata	Pecten maximus
Cerianthus lloydii	Nemertesia ramosa	Mysella bidentata
Maxmuelleria lankesteri	Virgularia mirabilis	Phaxas pellucidus
Ophiodromus flexuosus	Cerianthus lloydii	Abra alba
Nephtys hystricis	Nemertea spp.	Abra nitida
Chaetozone setosa	Thysanocardia procera	Phoronis spp.
Nephrops norvegicus	Pholoe inornata	Asterias rubens
Calocaris macandreae	Nephtys incisa	Ophiuroidea
Jaxea nocturna	Lumbrineris gracilis	Amphiura filiformis
Callianassa subterranea	Chaetopterus variopedatus	Amphiura filiformis
Pagurus bernhardus	Cirratulidae	Ophiura albida
Liocarcinus depurator	Diplocirrus glaucus	Ophiura ophiura
Carcinus maenas	Scalibregma inflatum	Echinus esculentus
Buccinum undatum	Owenia fusiformis	
Mysella bidentata	Lagis koreni	
Abra alba	Melinna palmata	
Corbula gibba	Terebellidae	
Asterias rubens	Lanice conchilega	
Amphiura chiajei	Pomatoceros triqueter	

Circalittoral fine mud (SS.SMu.CFiMu) and Burrowing megafauna and Maxmuelleria lankesteri in circalittoral mud (SS.SMu.CFiMu.MegMax)	Circalittoral sandy mud (SS.SMu.CSaMu)	
Amphiura chiajei	Pariambus typicus	
Lesueurigobius friesii	Pagurus bernhardus	
Pomatoschistus minutus	Pagurus prideaux	
	Liocarcinus depurator	
	Turritella communis	
	Aporrhais pespelecani	
	Nuculoma tenuis	

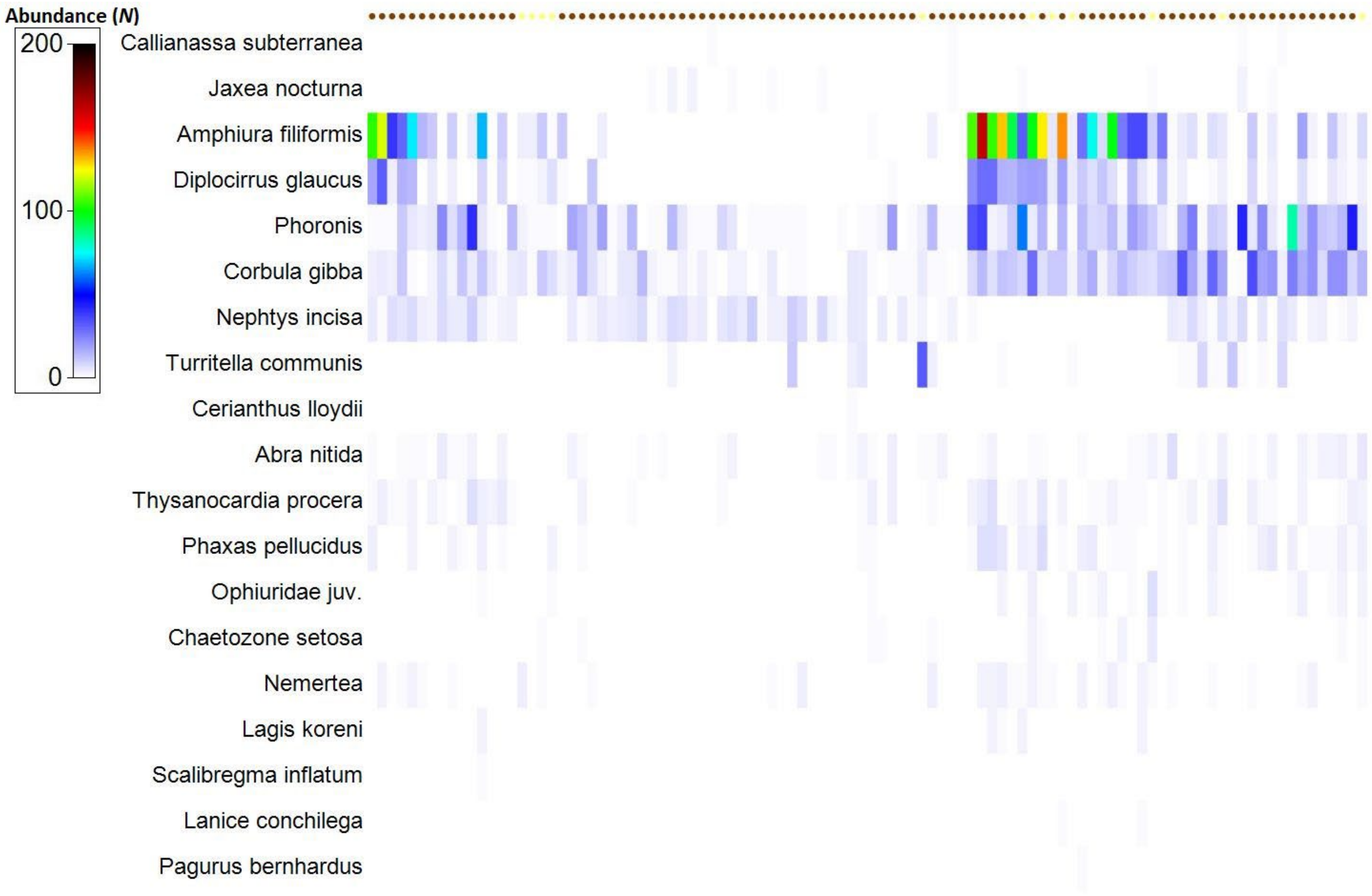

Figure A. Abundances of species characteristic of biotopes that may be a component part of 'Sea-Pen and Burrowing Megafauna Communities' that are present in the 2018 samples at West of Walney MCZ ('Subtidal sand' = yellow circles, 'Subtidal mud' = brown circles) (© Natural England and Cefas 2022).

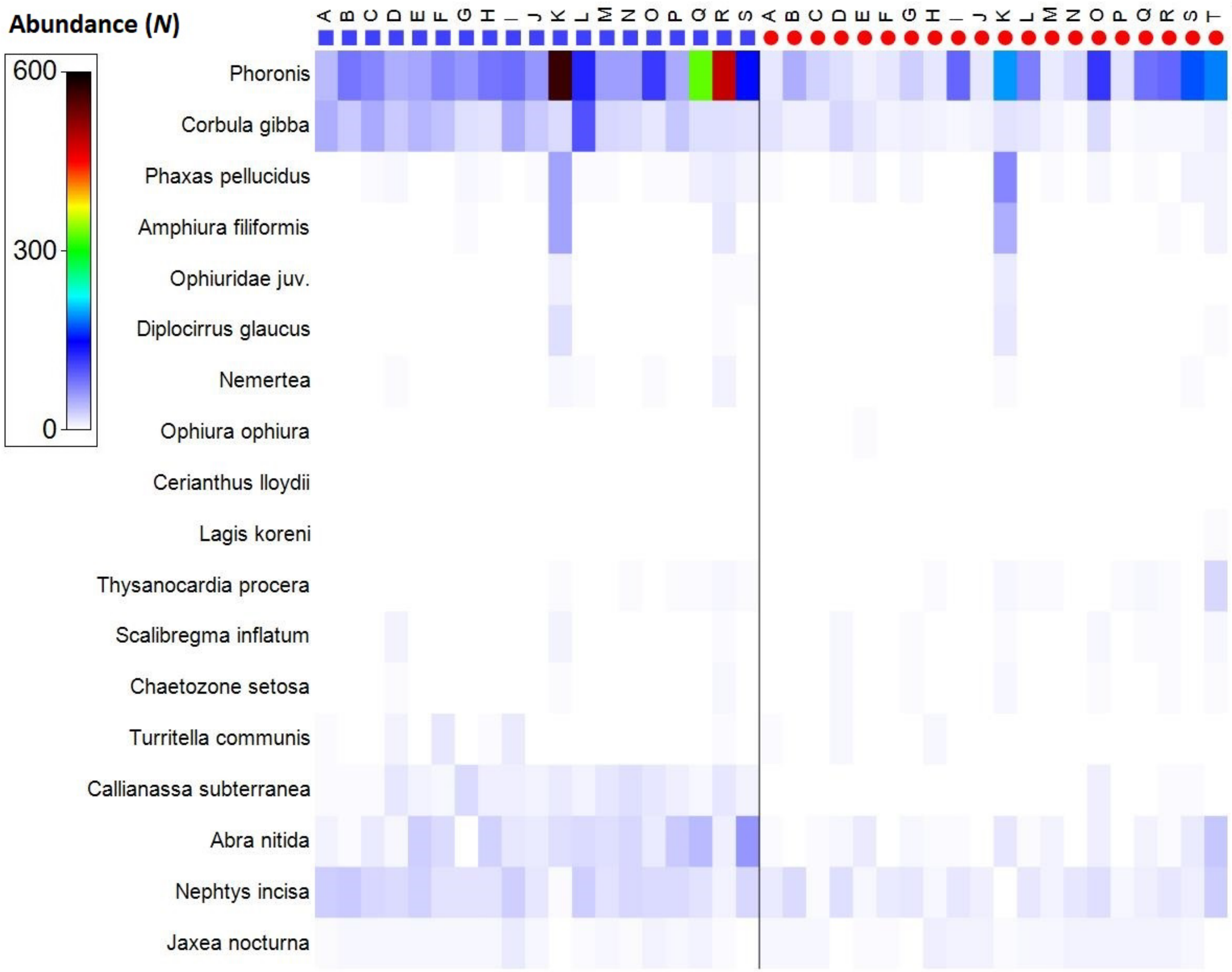

Figure B. Abundances of species characteristic of biotopes that may be a component part of 'Sea-Pen and Burrowing Megafauna Communities' that are present in the 2016 Box corer (blue squares) and Day grab (red circles) samples at West of Walney MCZ (© Natural England and Cefas 2022).

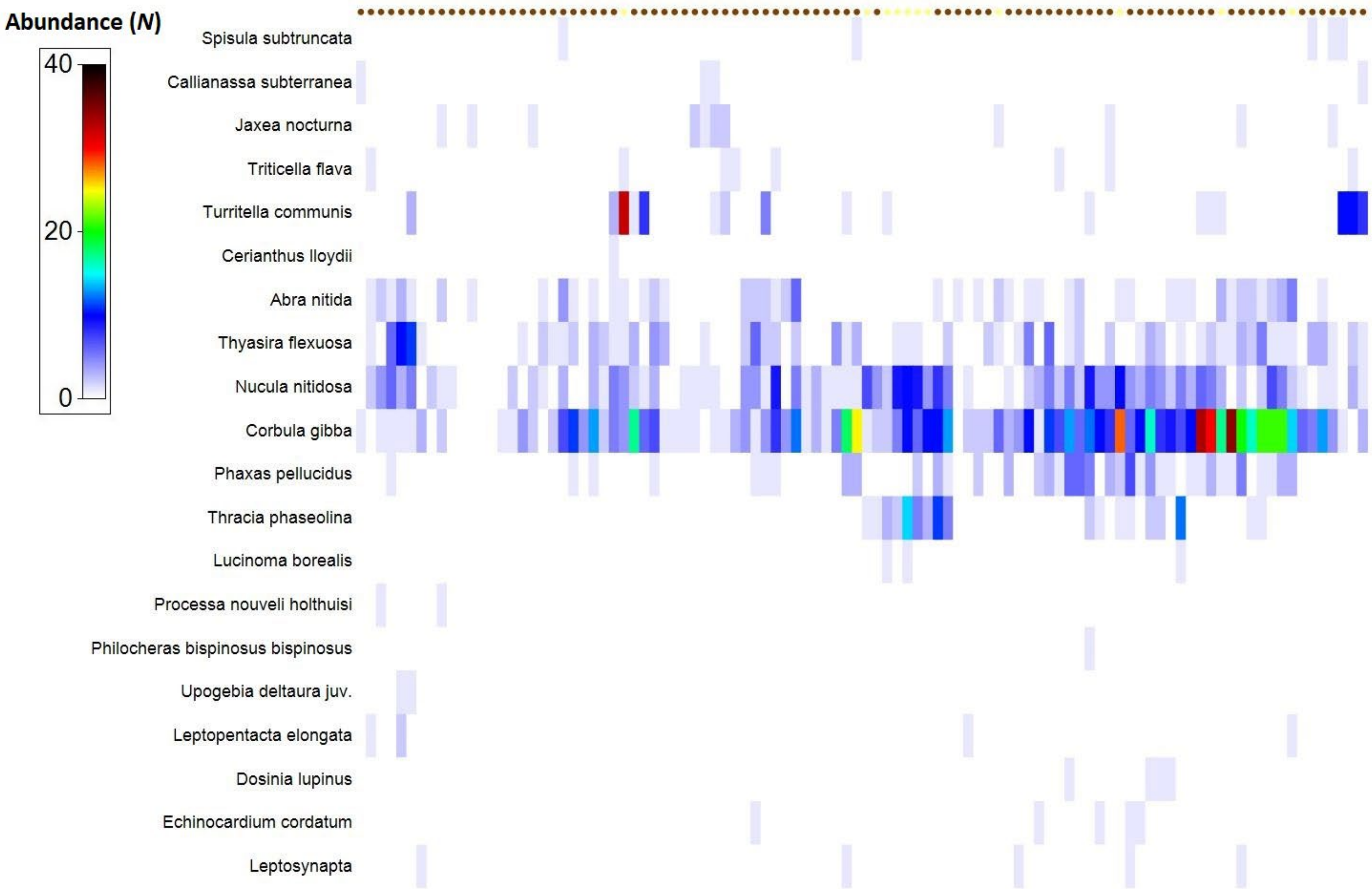

Figure C. Abundances of species that may reasonably be considered representative of or associated with 'Sea-Pens and Burrowing Megafauna Communities' that are present in the 2018 samples ('Subtidal sand' = yellow circles, 'Subtidal mud' = brown circles) at West of Walney MCZ (© Natural England and Cefas 2022).

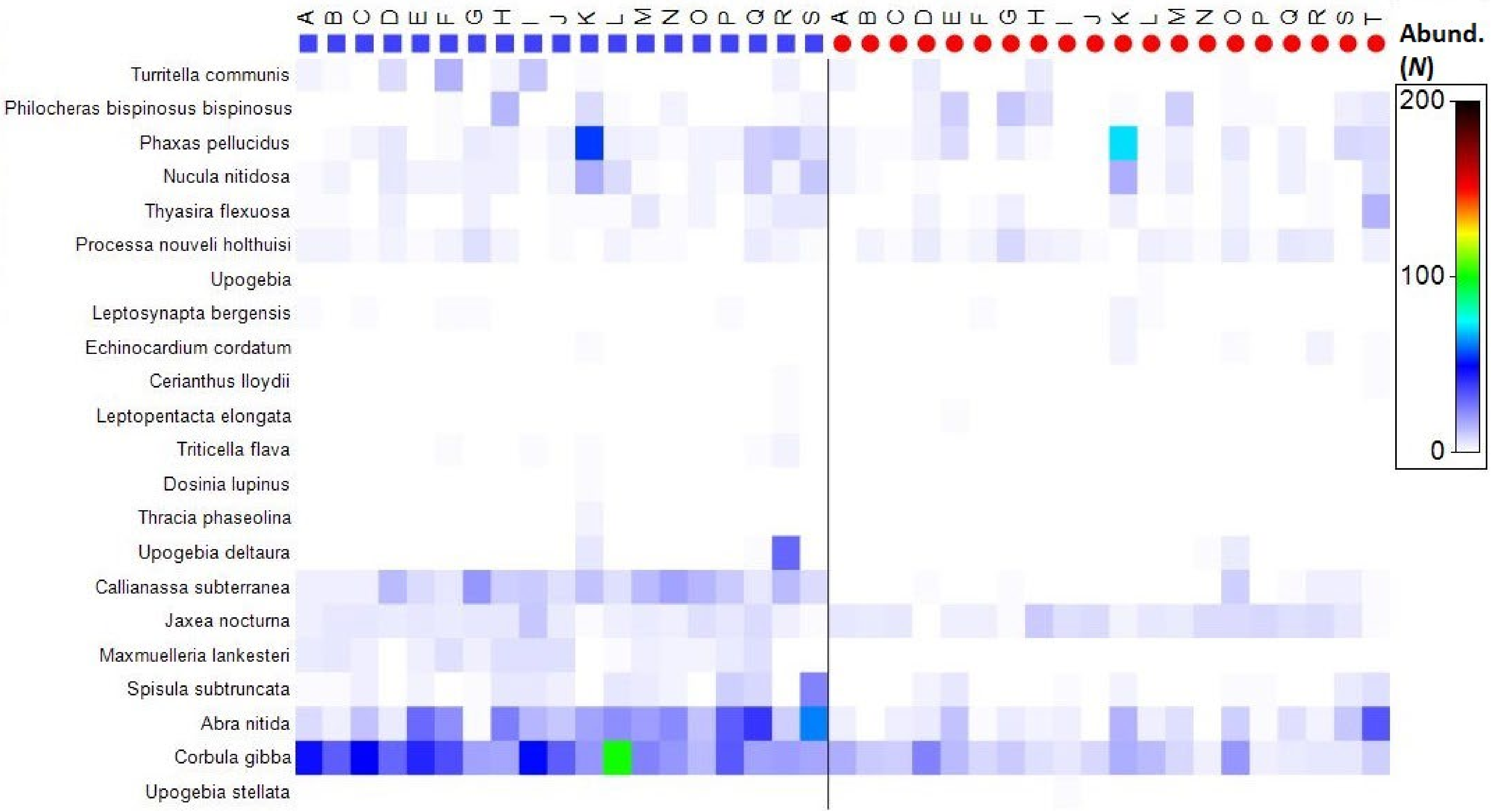

Figure D. Abundances of species that may reasonably be considered representative of or associated with 'Sea-Pens and Burrowing Megafauna Communities' that are present in the 2016 Box corer (blue squares) and Day grab (red circles) samples at West of Walney MCZ (© Natural England and Cefas 2022).

Annex 5. Marine litter categories

Categories and sub-categories of litter items for seafloor from the OSPAR/ICES/IBTS for North-East Atlantic and Baltic. Guidance on Monitoring of Marine Litter in European Seas, a guidance document within the Common Implementation Strategy for the Marine Strategy Framework Directive, MSFD Technical Subgroup on Marine Litter, 2013.

A: Plastic	B: Metals	C: Rubber	D: Glass/ Ceramics	E: Natural products/ Clothes	F: Miscellaneous
A1. Bottle	B1. Cans (food)	C1. Boots	D1. Jar	E1. Clothing/ rags	F1. Wood (processed)
A2. Sheet	B2. Cans (beverage)	C2. Balloons	D2. Bottle	E2. Shoes	F2. Rope
A3. Bag	B3. Fishing related	C3. Bobbins (fishing)	D3. Piece	E3. Other	F3. Paper/ cardboard
A4. Caps/ lids	B4. Drums	C4. Tyre	D4. Other		F4. Pallets
A5. Fishing line (monofilament)	B5. Appliances	C5. Other			F5. Other
A6. Fishing line (entangled)	B6. Car parts				
A7. Synthetic rope	B7. Cables				
A8. Fishing net	B8. Other				
A9. Cable ties					
A10. Strapping band					
A11. Crates and containers					
A12. Plastic diapers					
A13. Sanitary towels/ tampons					
A14. Other					

Related size categories
A: ≤ 5*5 cm = 25 cm^2
B: ≤ 10*10 cm = 100 cm^2
C: ≤ 20*20 cm = 400 cm^2
D: ≤ 50*50 cm = 2500 cm^2
E: ≤ 100*100 cm = 10000 cm^2
F: ≥ 100*100 cm = 10000 cm^2

Annex 6. Particle size distribution

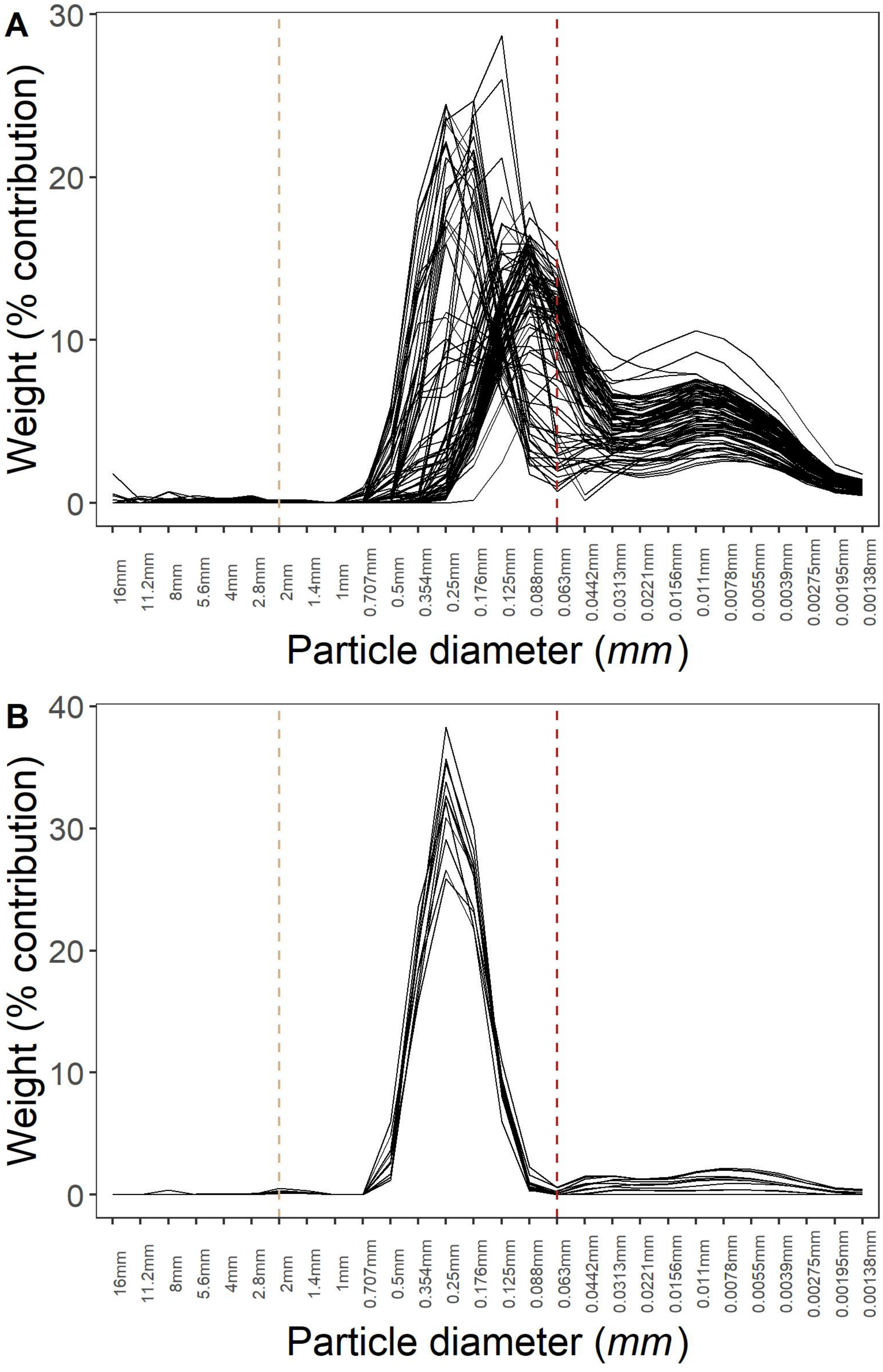

Figure E. 2018 sediment samples at West of Walney MCZ. A) Subtidal mud. B) Subtidal sand. Dashed vertical lines indicate the boundary between gravel and sand grains (left), and sand and mud grains (right) (© Natural England and Cefas 2022).

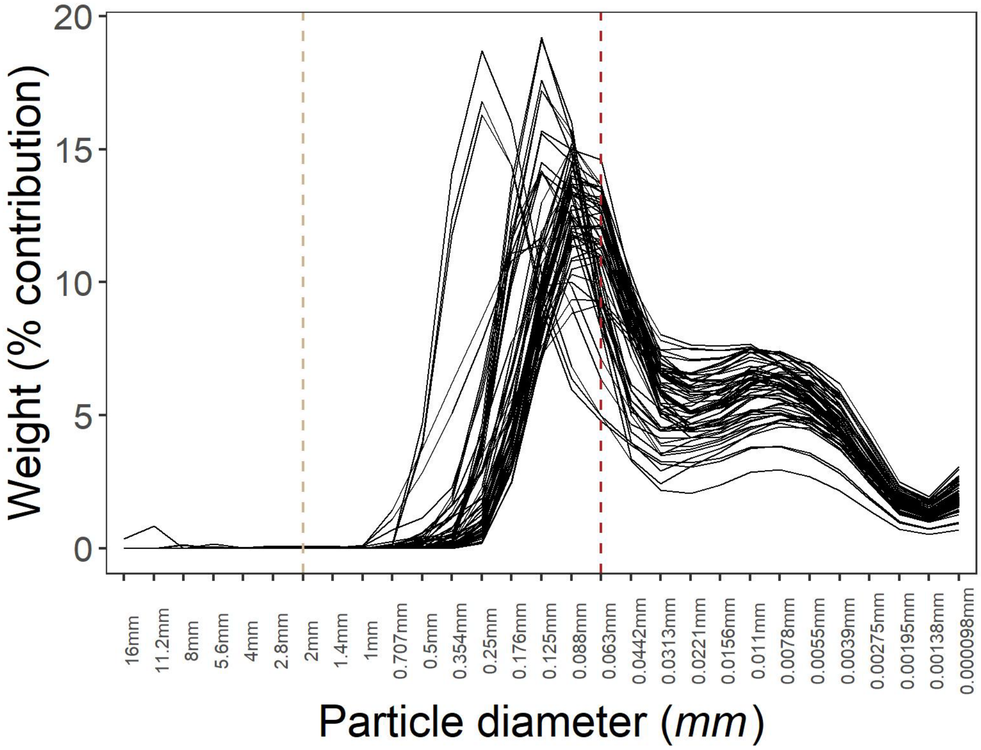

Figure F. 2016 sediment samples, composing of three Day grabs per station at West of Walney MCZ. Dashed vertical lines indicate the boundary between gravel and sands (left), and sands and muds (right) (© Natural England and Cefas 2022).

Annex 7. Abundant infauna

Table F. Summary of abundance and biomass of the most dominant infauna taxa from the 2018 samples at West of Walney MCZ (© Natural England and Cefas 2022).

Taxon	Summed abundance	Occurrence	Percentage contribution to total abundance	Mean abundance per sample	Summed biomass (g)
Phoronis	894	91	10.0	8.9	6.7
Corbula gibba	700	89	7.9	7.0	1.3
Nucula nitidosa	257	75	2.9	2.6	3.4
Chamelea	340	69	3.8	3.4	27.7
Nephtys incisa	255	66	2.9	2.6	9.0
Magelona alleni	322	64	3.6	3.2	1.4
Lovenella clausa	60	60	0.7	0.6	0.0
Thyasira	136	57	1.5	1.6	0.2
Ampelisca	162	57	1.8	1.4	0.2
Hyala vitrea	200	56	2.2	2.0	0.4
Lumbrineris	586	54	6.6	5.86	1.7
Amphiura	1920	52	21.6	19.2	22.6
Abyssoninoe	82	51	0.9	0.8	0.5
Turritella	89	17	1.0	0.9	61.7
Acanthocardia	2	2	0.0	0.0	54.3
Echinocardium	5	5	0.1	0.1	19.3
Goneplax	16	14	0.2	0.2	18.9
Leptopentacta	5	4	0.1	0.1	10.2
Glycera unicornis	38	34	0.4	0.4	2.9

Table G. Summary of abundance and biomass of the most dominant infauna taxa from the 2016 Day grab samples at West of Walney MCZ (© Natural England and Cefas 2022).

Taxon	Summed abundance	Occurrence	Percentage contribution to total abundance	Mean abundance per sample	Summed biomass (g)
Abra nitida	115	48	1.1	1.2	0.2
Abyssoninoe	87	51	1.1	0.9	0.6
Callianassa	19	12	0.3	0.2	6.1
Corbula gibba	183	67	1.5	1.9	0.6
Echinocardium	6	5	0.1	0.1	68.5
Glycera	58	40	0.9	0.6	8.2
Goneplax	62	38	0.8	0.6	50.9
Hyala vitrea	348	82	1.8	3.6	0.7
Jaxea	95	58	1.3	1.0	4.7
Nephrops	5	3	0.1	0.1	26.0
Nephtys incisa	221	80	1.7	2.3	5.7
Notomastus	57	45	1.0	0.6	8.3
Phaxas	115	29	0.6	1.2	1.6
Phoronis spp.	1230	97	2.1	12.6	8.1
Prionospio	143	55	1.2	1.5	0.1
Turritella	10	6	0.1	0.1	7.4
Upogebia	1	1	0.0	0.0	8.0

Table H. Summary of abundance and biomass of the most dominant infauna taxa from the 2016 Box corer samples (© Natural England and Cefas 2022).

Taxon	Summed abundance	Occurrence	Percentage contribution to total abundance	Mean abundance per sample	Summed biomass (g)
Phoronis spp.	2572	100	1.3	45.1	9.7
Corbula gibba	613	98	1.3	10.8	1.3
Nephtys incisa	382	95	1.2	6.7	5.3
Callianassa	177	89	1.1	3.1	157.5
Abra nitida	363	88	1.1	6.4	0.7
Ampelisca	161	86	1.1	2.8	0.1
Hyala vitrea	381	84	1.1	6.7	0.7
Abyssoninoe	137	82	1.1	2.4	0.5
Prionospio	168	75	1.0	2.9	0.1
Saxicavella	421	67	0.9	7.4	0.6
Chamelea	109	60	0.8	1.9	5.0
Phaxas	111	58	0.7	1.9	1.1
Maxmuelleria	60	56	0.7	1.1	1176.1
Mediomastus	338	37	0.5	5.9	0.5
Turritella	42	26	0.3	0.7	109.2
Upogebia	36	11	0.1	0.6	205.0

Annex 8. Univariate metrics

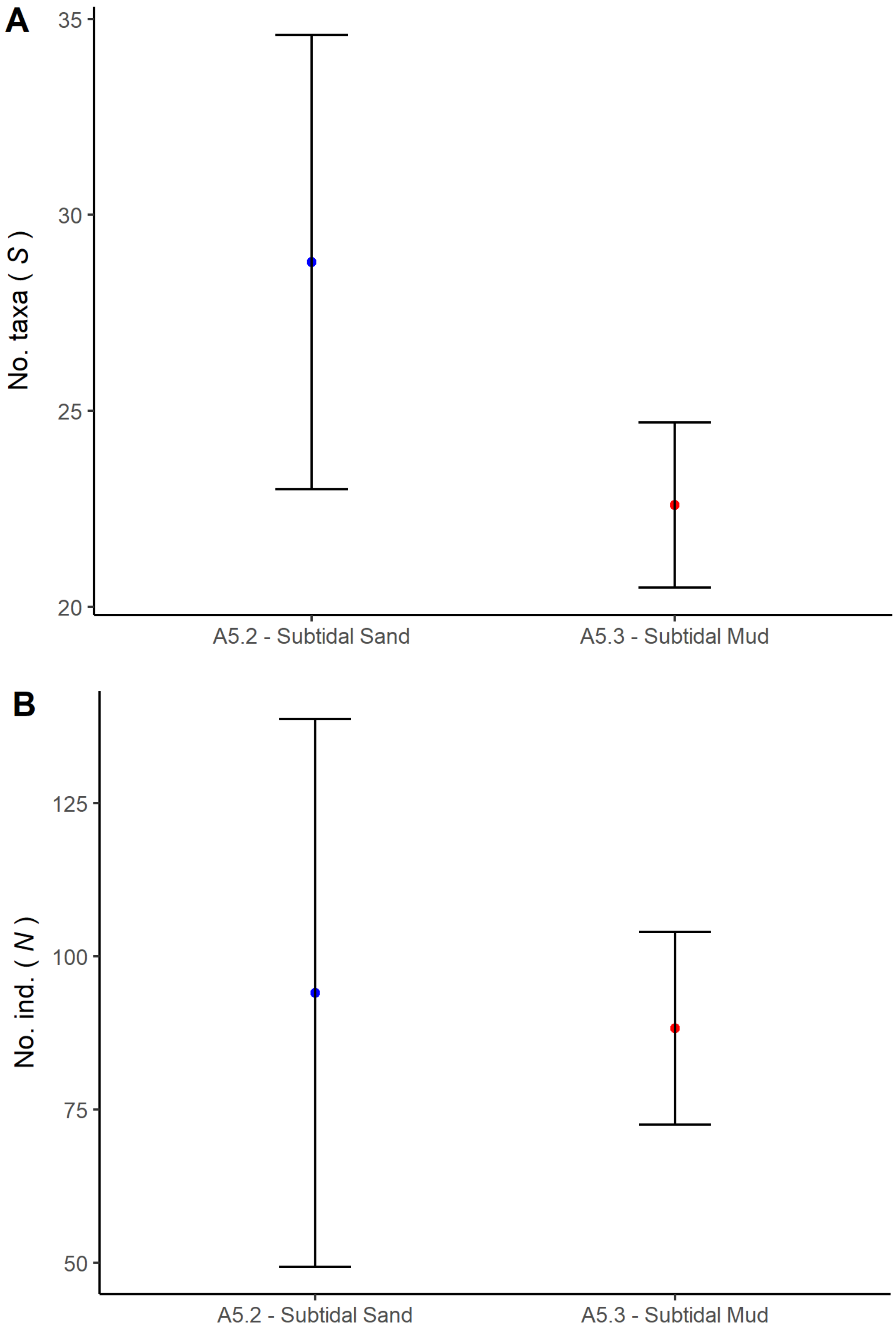

Figure G. Mean number of taxa (A) and abundance (B) with associated 95 % confidence intervals of the Day grab samples collected at West of Walney MCZ in 2018 and classified as 'Subtidal sand' and 'Subtidal mud' (© Natural England and Cefas 2022).

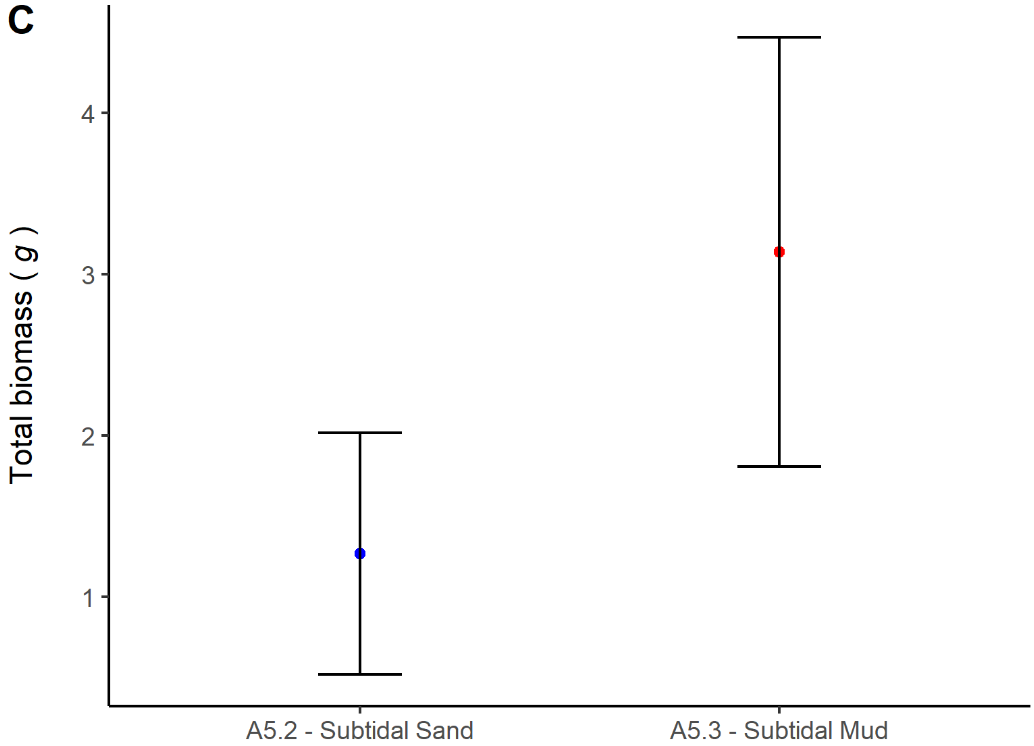

Figure G (continued). Mean total biomass (C) with associated 95 % confidence intervals of the Day grab samples collected at West of Walney MCZ in 2018 and classified as 'Subtidal sand' and 'Subtidal mud' (© Natural England and Cefas 2022).

Annex 9. Notable differences in the distribution of richness and diversity measures

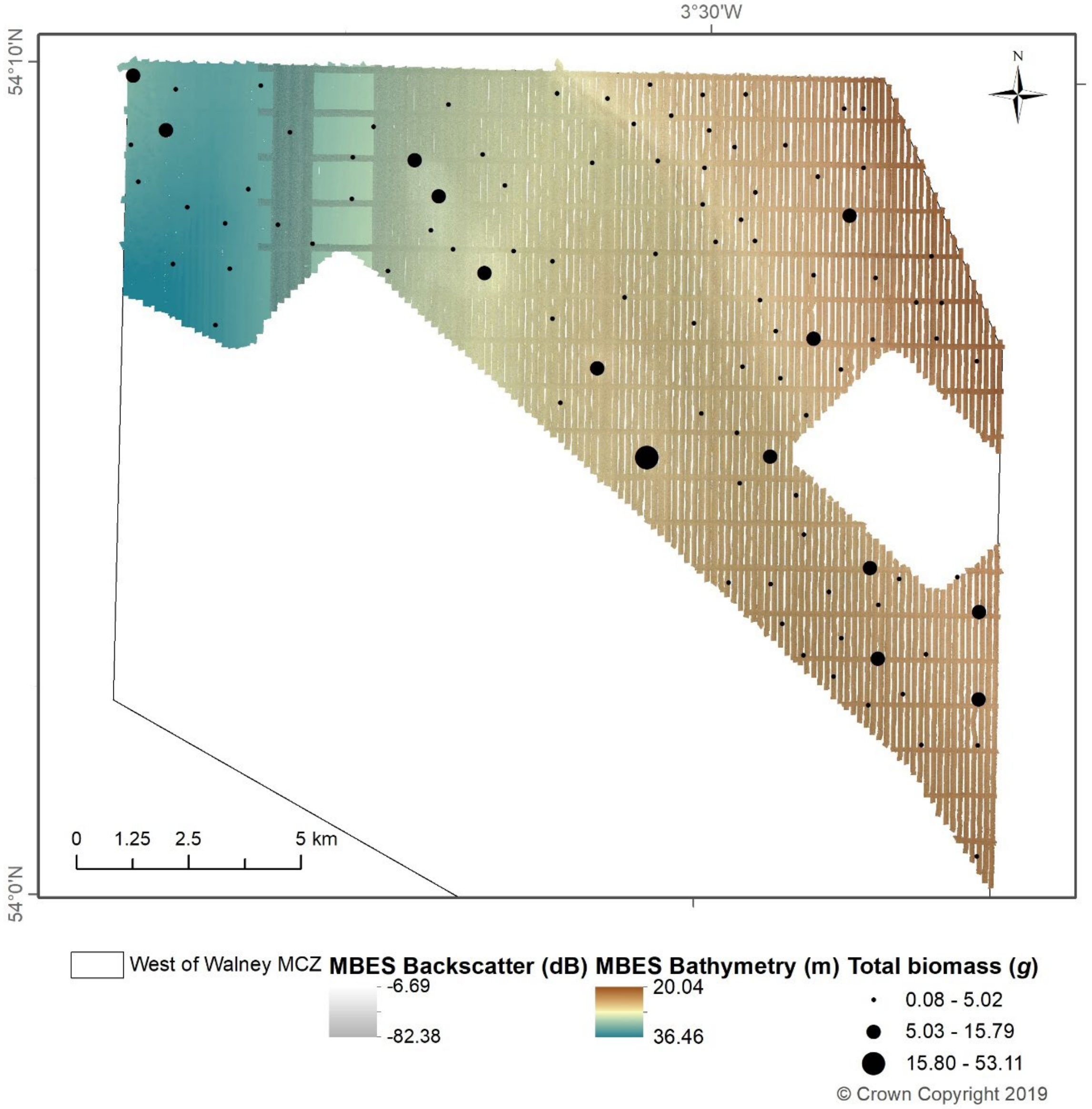

Figure H. Spatial distribution of total biomass (wet weight in grams *g*) of infaunal taxa collected at West of Walney MCZ in 2018 using the Day grab.

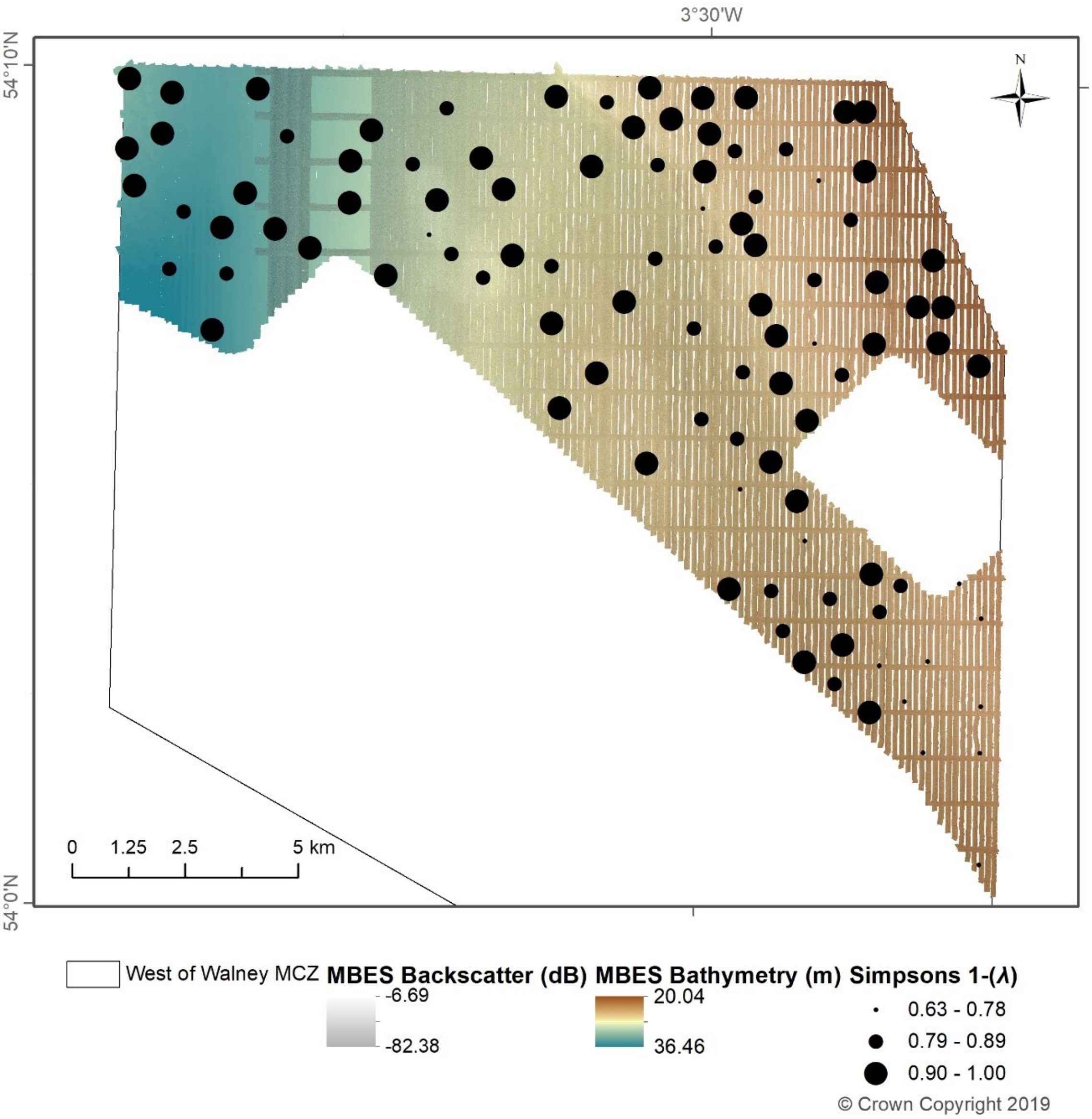

Figure I. Spatial distribution of Simpsons diversity index (1-λ) of infaunal taxa collected using the Day grab at West of Walney MCZ in 2018.

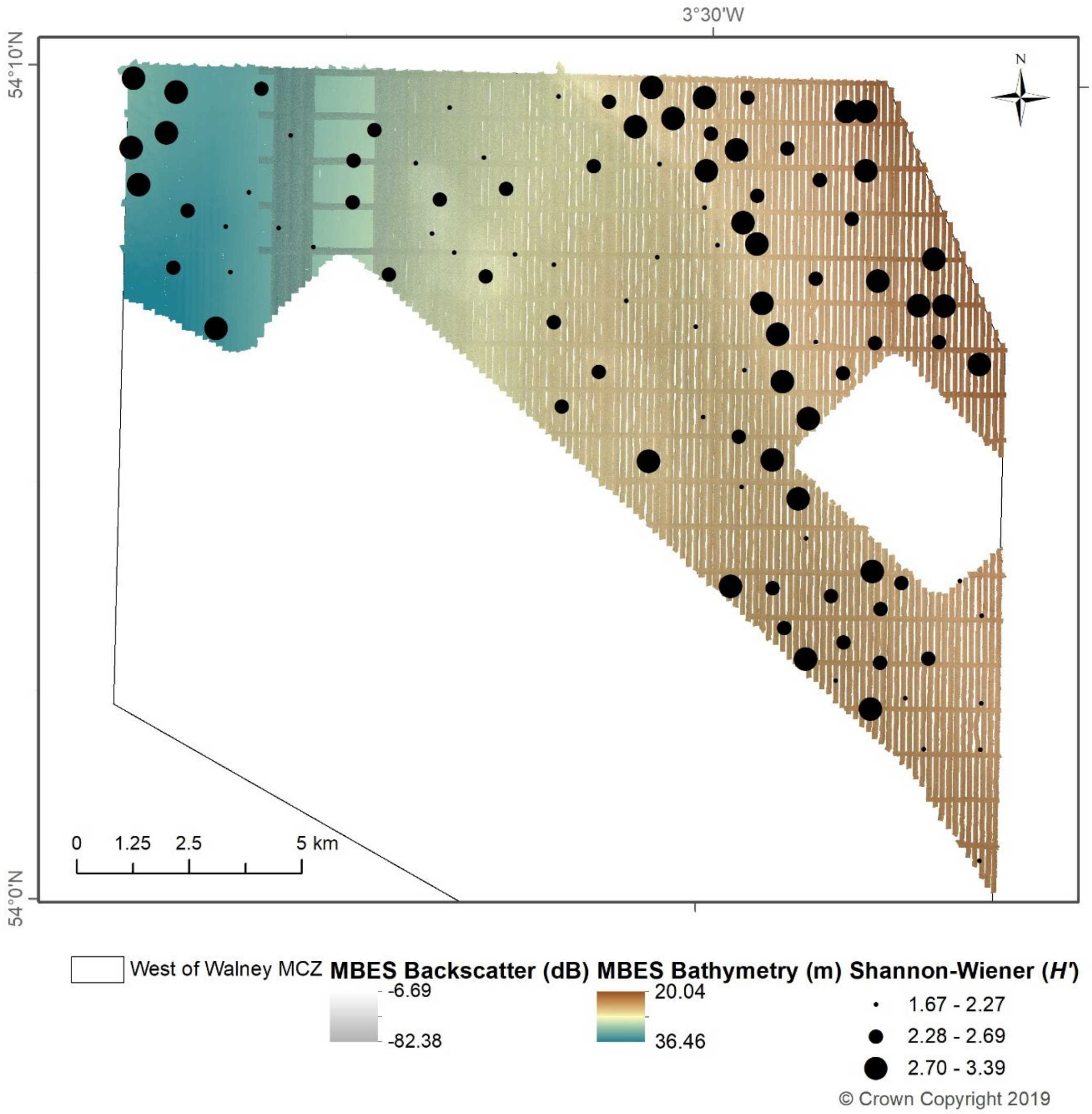

Figure J. Spatial distribution of Shannon-Wiener diversity index (*H'*) of infaunal taxa collected at West of Walney MCZ using the Day grab in 2018.

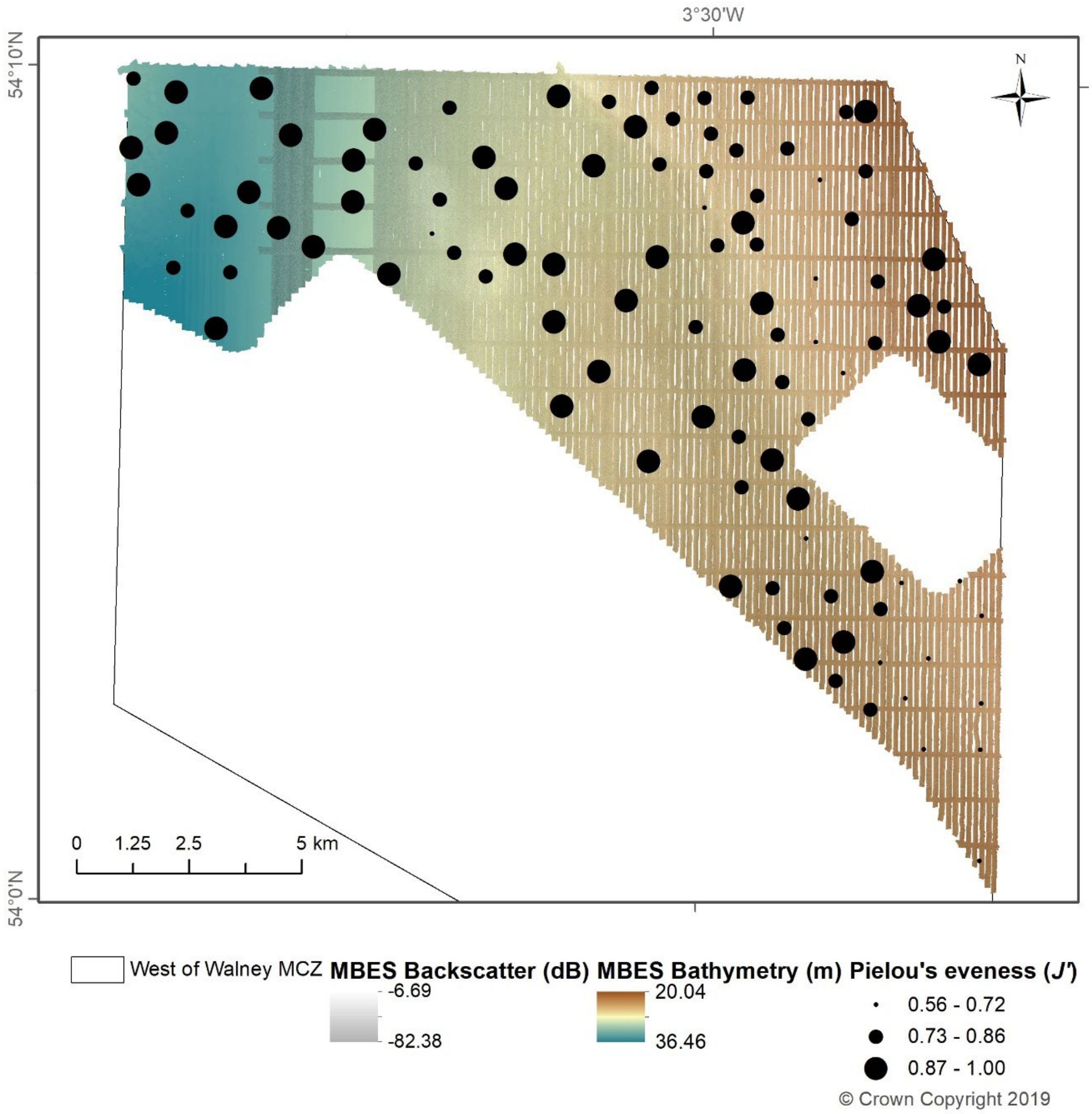

Figure K. Spatial distribution of Pielou's evenness measure (*J'*) of infaunal taxa collected using the Day grab at West of Walney MCZ in 2018.

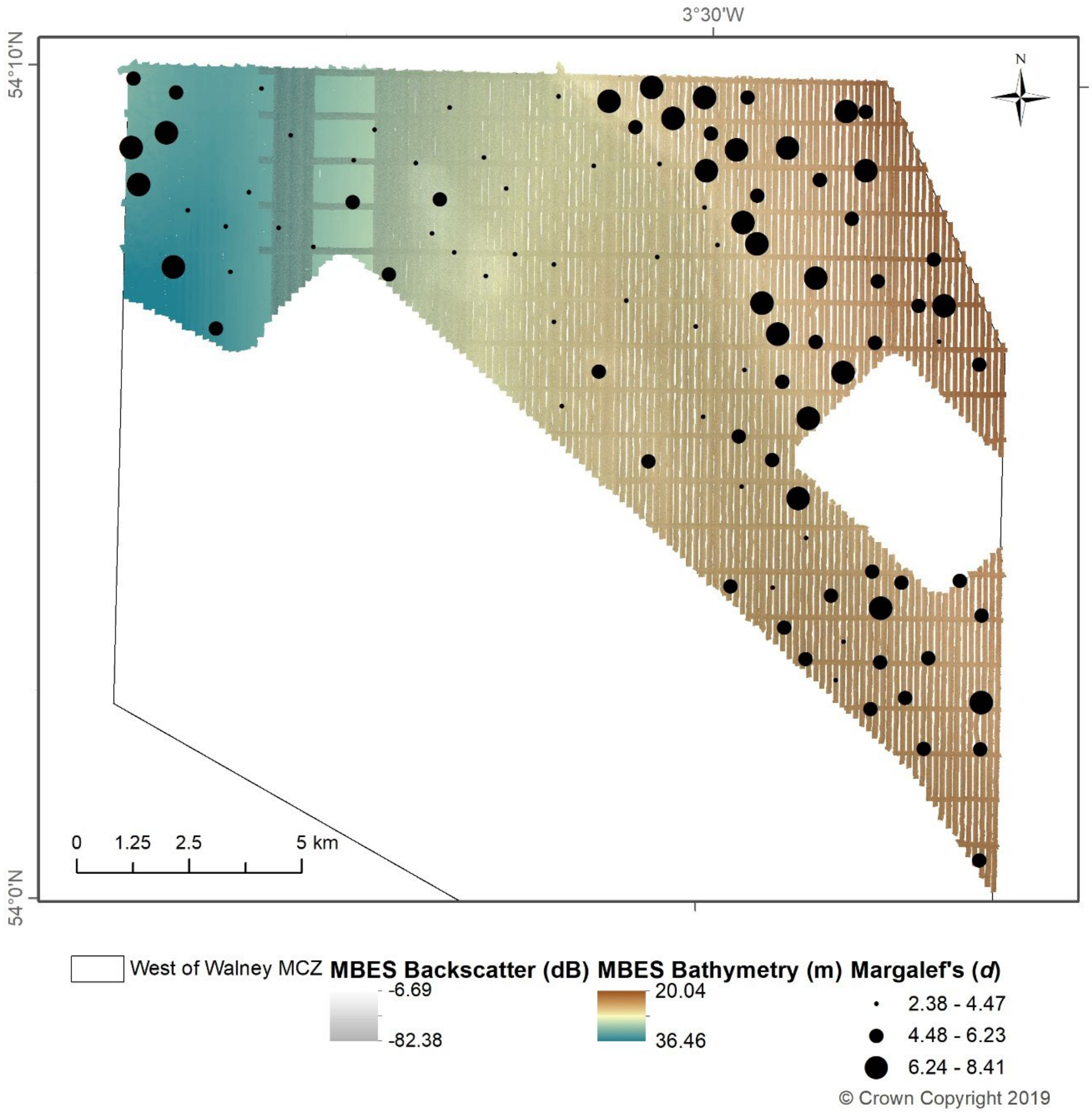

Figure L. Spatial distribution of Margalef's diversity index (*d*) of infaunal taxa collected using the Day grab at West of Walney MCZ in 2018.

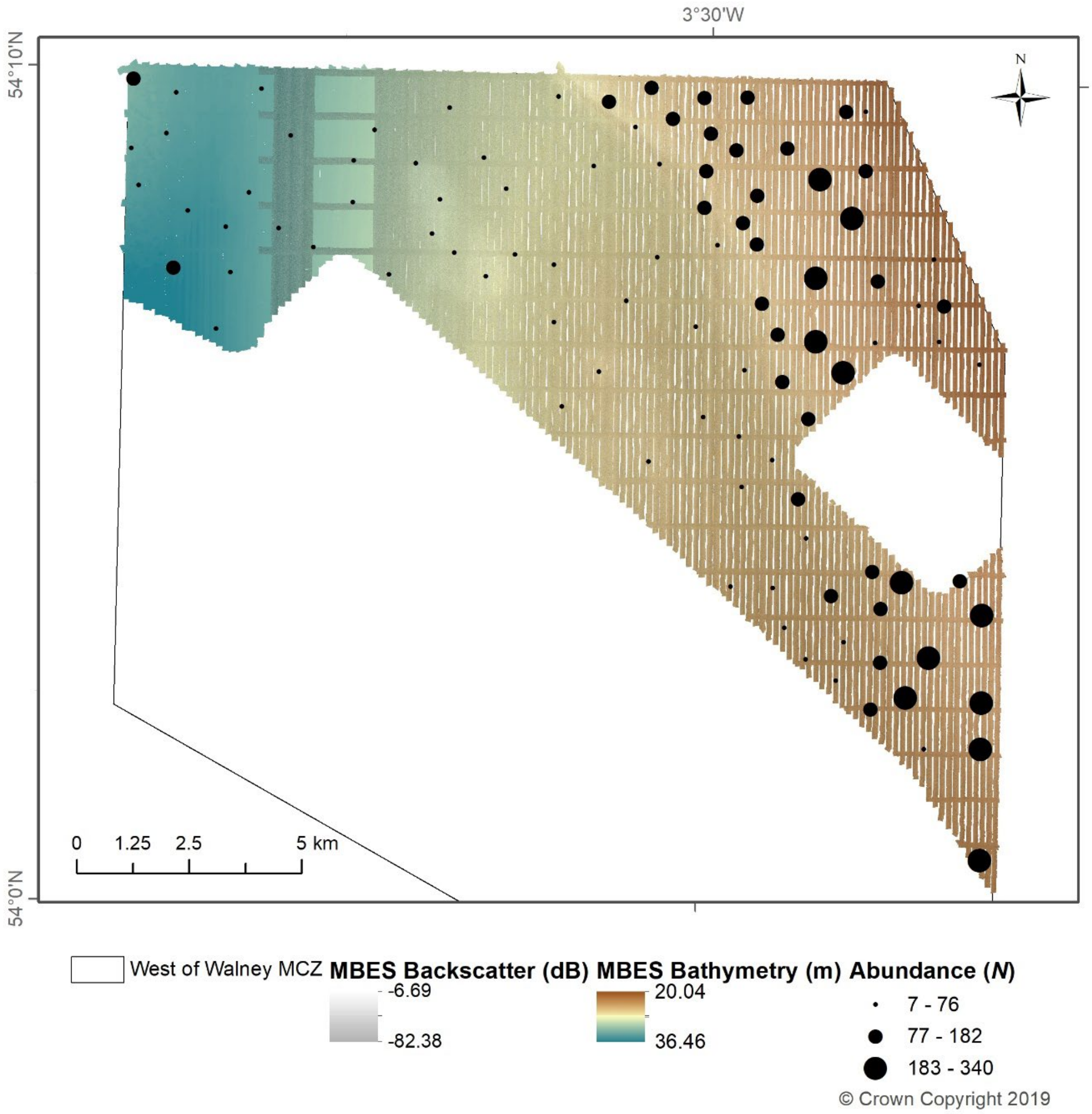

Figure M. Spatial distribution of the total abundance (*N*) of infaunal taxa collected using the Day grab at West of Walney MCZ in 2018.

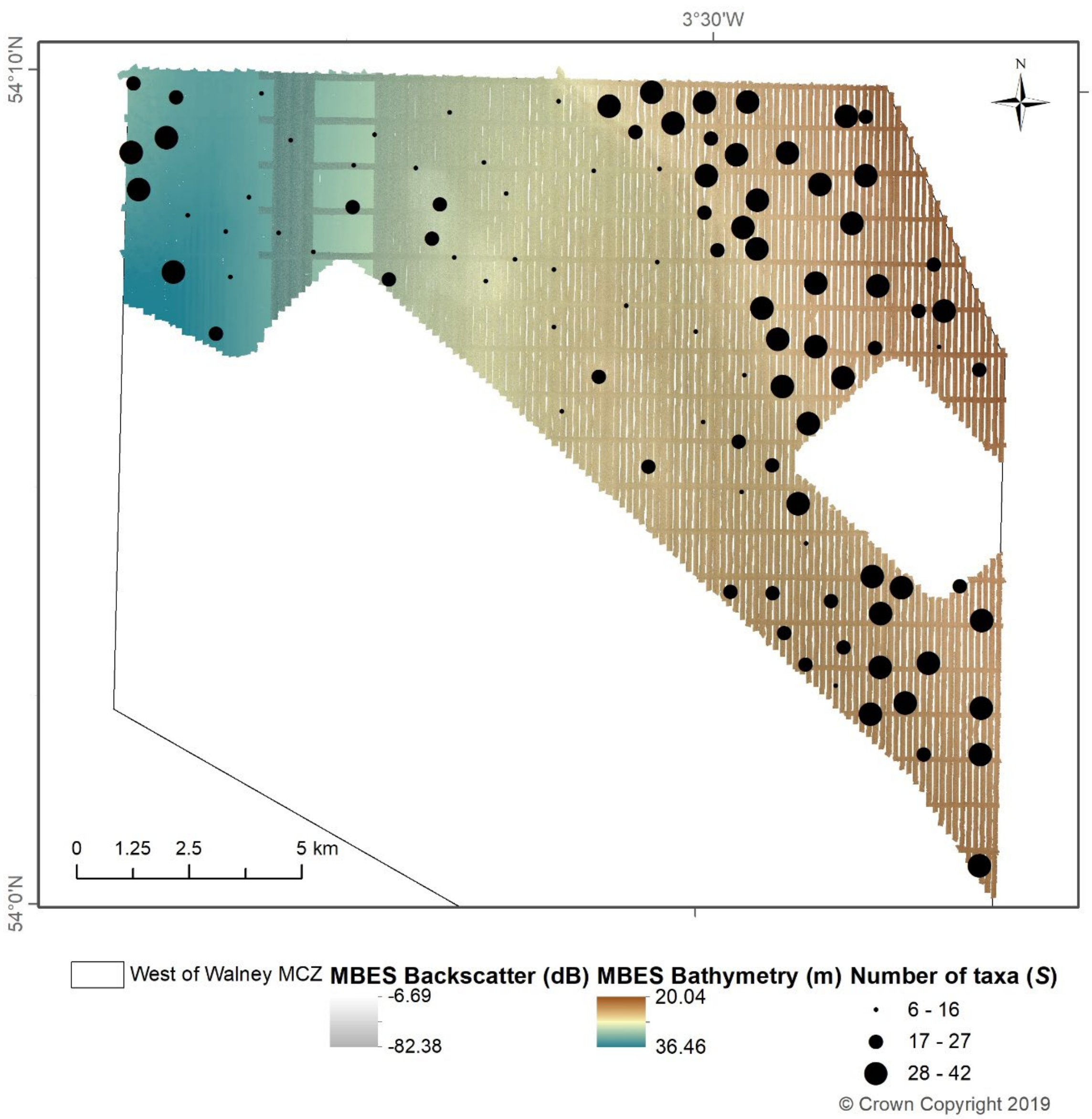

Figure N. Spatial distribution of species richness (number of taxa *S*) of infaunal taxa collected using the Day grab at West of Walney MCZ in 2018.

Annex 10. Characterising infauna

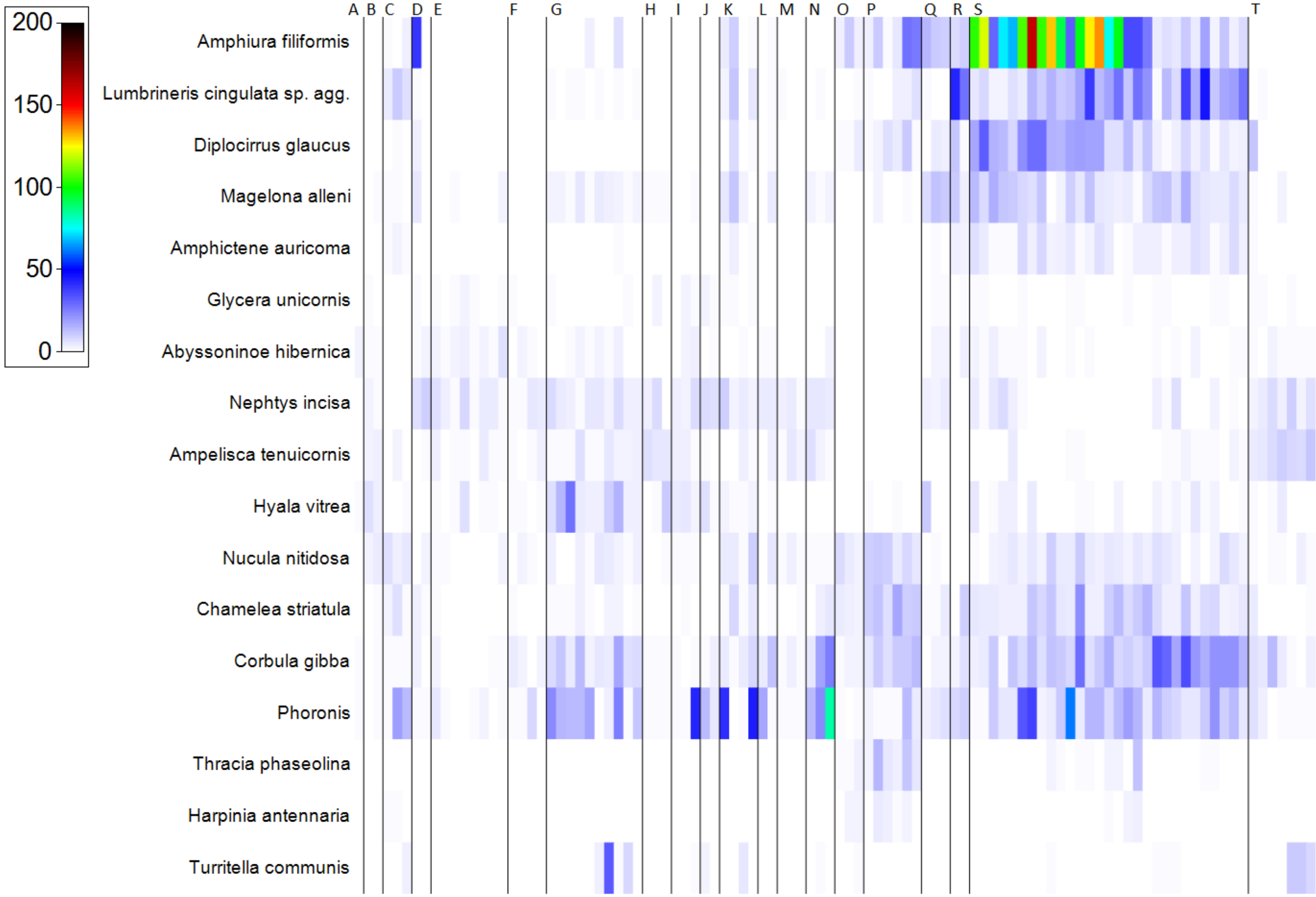

Figure O. Abundance of the characterising taxa in *k*-R clustering groups (A – T) from the 2018 Day grab samples at West of Walney MCZ (© Natural England and Cefas 2022).

Annex 11. Infauna exclusively collected by one gear

Table I. Comparison of the taxa exclusively collected by one gear type during the 2016 survey at West of Walney MCZ that has been used for sample gear comparison (© Natural England and Cefas 2022).

Taxon	Total abundance		Phylum
	Box core	Day grab	
Aphelochaeta A	1	0	Annelida
Aphroditidae juv.	1	0	
Atherospio disticha	41	0	
Goniada maculata	2	0	
Harmothoe clavigera	0	2	
Malmgrenia darbouxi	0	2	
Maxmuelleria lankesteri	60	0	
Nephtys kersivalensis	2	0	
Nephtys sp. indet.	0	3	
Paranaitis kosteriensis	0	1	
Pectinariidae	0	3	
Phyllodoce groenlandica	2	0	
Phyllodoce longipes	1	0	
Terebellides	0	1	
Tubificoides amplivasatus	0	1	
Acanthomysis longicornis	1	0	Arthropoda
Alpheus glaber	0	2	
Bodotria scorpioides	1	0	
Crangon sp.	1	0	
Crangon allmanni	0	3	
Diastylis bradyi	0	1	
Eusirus longipes	0	2	
Gyge branchialis	5	0	
Harpinia antennaria	1	0	
Harpinia pectinata	1	0	
Hemimysis lamornae	0	1	
Heteromysis (Heteromysis) norvegica	4	0	
Hippolytidae sp. indet.	3	0	
Inachus juv.	0	1	
Ione thoracica	9	0	
Janira maculosa	0	2	
Jassa	1	0	
Liocarcinus depurator	0	1	
Mysidae sp. indet.	0	1	
Photis longicaudata	1	0	
Pisidia longicornis	0	9	

Taxon	Total abundance		Phylum
	Box core	Day grab	
Sacculina gonoplaxae	0	1	
Upogebia sp.	0	1	
Upogebia stellata	0	1	
Amathia	1	0	Bryozoa
Bugulina flabellata	1	0	
Bugulina fulva	1	0	
Triticella flava	6	0	
Chaetognatha	1	0	Chaetognatha
Didemnidae	0	1	Chordata
Gonothyraea loveni	0	1	Cnidaria
Tubulariidae	1	0	
Amphiuridae juv.	0	11	Echinodermata
Astropecten irregularis	1	0	
Ophiothrix fragilis juv.	2	0	
Enteropneusta	1	0	Hemichordata
Abra alba	1	0	Mollusca
Bivalvia	1	0	
Cheirocratus female	0	5	
Devonia perrieri	1	0	
Dosinia lupinus	1	0	
Euspira nitida	0	1	
Lepton squamosum	5	0	
Mactra stultorum	0	1	
Mytilus edulis juv.	0	1	
Thracia convexa	0	1	
Thracia phaseolina	2	0	
Veneridae	1	0	
Golfingia (Golfingia) vulgaris vulgaris	0	3	Sipuncula

Annex 12. Megafauna measurements

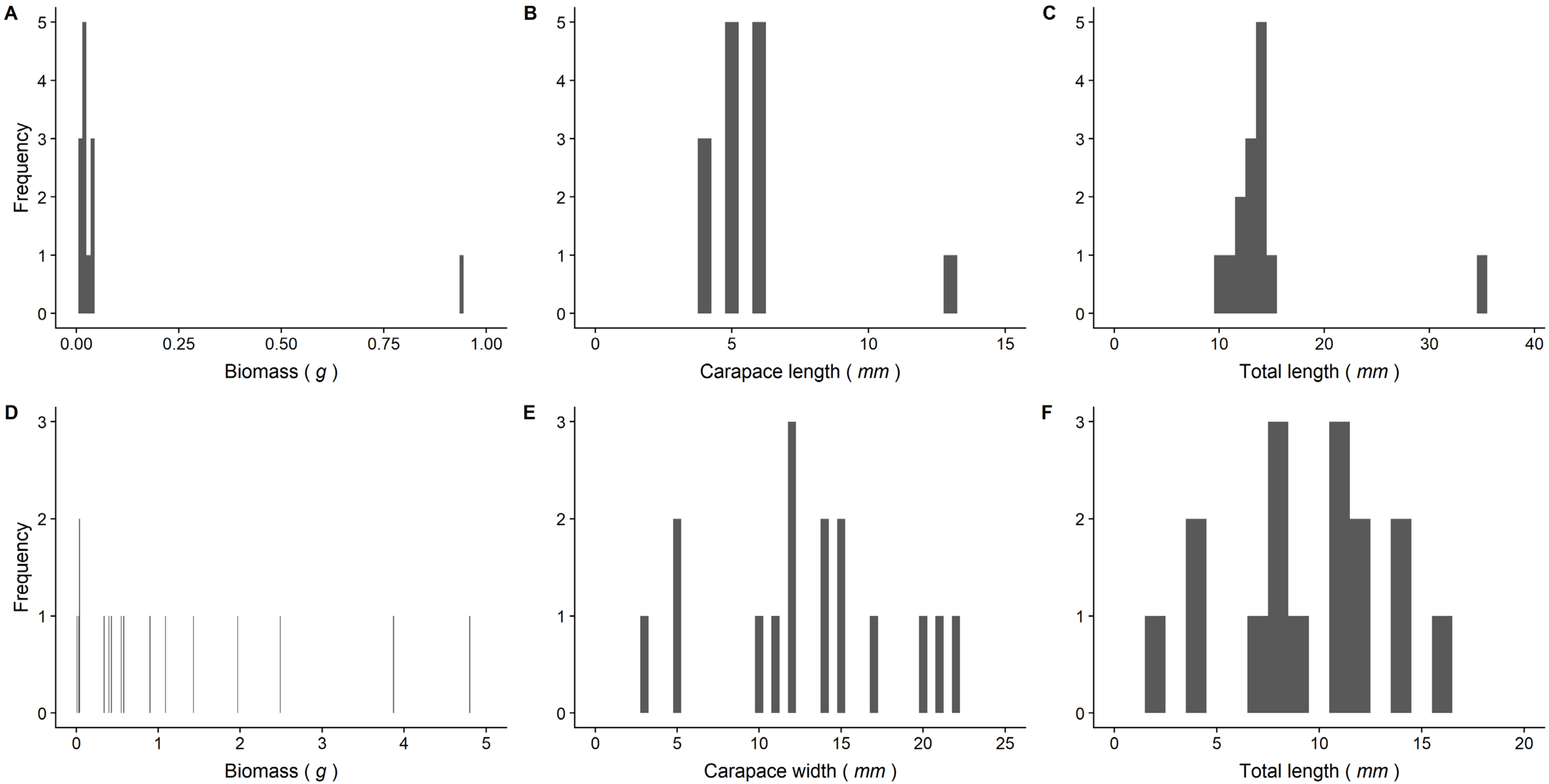

Figure P. Frequency histograms showing the individual measurements recorded for *Jaxea nocturna* (A-C) and *Goneplax rhomboides* (D-F) at West of Walney MCZ (© Natural England and Cefas 2022).

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