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East of Start Point Marine Conservation Zone (MCZ) Monitoring Report 2021

Bolam, S.G., McIlwaine, P., Lake, I., Archer-Rand, S., Hawes, J., Hatton, J. & Savage, J.

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East of Start Point Marine Conservation Zone (MCZ)

Monitoring Report 2021

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Executive Summary

This report includes recommendations to inform ongoing improvement and development of sample acquisition, analysis and data interpretation for future surveys 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.

The East of Start Point Marine Conservation Zone (MCZ) is an offshore Marine Protected Area (MPA) located off the south coast of Devon, within the Eastern English Channel Charting Progress 2 (CP2) sea area. This report provides evidence on the Subtidal sand Broadscale Habitat (BSH) feature for which the MCZ has been designated, based primarily on data from the first dedicated monitoring survey conducted in 2021. The 2021 data will form the first point in a time series, against which change can be monitored through time (Type 1 monitoring; see Kröger & Johnston 2016).

The new habitat map created for the East of Start Point MCZ (Objective 1) provides a full coverage interpretation of the different sediment types (Subtidal sand and Subtidal mud) distributed across the site. Interpretation of the underlying acoustic data (collected in 2012 and 2014) in relation to the ground-truthing data (collected in 2021) and a site-specific tidal model showed that the boundary between the two sediment types may have changed in the intervening years. Caution is therefore recommended when interpreting any future changes in mapped extent, particularly regarding the use of extent in assessments of feature condition.

The particle size analysis of the 2021 samples (Objective 1) highlighted a coarsening of sediments associated with the gradual north to south increasing depth gradient from approximately 25 m to 50 m. This is different to the situation observed within most MCZs across the coast of England and Wales where softer, muddier sediments are correlated within depth increases. A relatively linear gradient in environmental conditions was observed between sediment type, depth and tidal flows, a gradient that is reflected in its associated macrofaunal assemblages. Indeed, the description of the faunal assemblages (Objective 1) almost directly corresponds to changes in key environmental conditions, with the six infaunal k-R clusters grading from one to the next along a north to south gradient.

Importantly, the infaunal community data also provides evidence of two potential biotopes not currently present in the Marine Habitat Classification of Britain and Ireland (MHCBI), one present on muddy sand and one on sand.

Analysis of the sediment and infaunal variability within replicated stations (Objective 2) showed that at the station scale, seabed sediments were rather homogeneous and variance in univariate metrics of community structure was lower in the station replicates, compared to the those based on single samples in the wider survey area.

No habitat or species features of conservation interest (FOCI) were observed in the 2021 samples (Objective 3) whilst one record of a non-indigenous taxon was found (Objective 4); one individual of the polychaete worm *Goniadella gracilis*. Evidence of human activities was observed at the site (Objective 5), including a number of wrecks (all chartered) and trawl scars.

Seven practical recommendations for future monitoring at the East of Start Point MCZ were identified:

1) undertake epifaunal data collection (imagery or scientific beam trawl) analysis to complement the existing information on the ecological characteristics of the site,

- provide some contextual information on the temporal variability of infaunal assemblages using data from the Clean Seas Environment Monitoring Programme (CSEMP),
- 3) a combination of two sampling approaches (replication at a subset of stations and an array of single samples) is likely to be the most effective design for future monitoring,
- 4) future surveys should be carried out at the same time of year as the 2021 survey and should follow the same truncation process for the infaunal data,
- 5) future monitoring of the site should gather evidence on the continued presence of the rarely encountered gastropod *Eulima glabra*,
- 6) investigation of fish spawning and nursery locations would facilitate the ecologically coherent management of the site, and
- 7) it would be advisable to conduct further acoustic surveys of the site.

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Abbreviations

ANOSIM	Analysis of Similarity
BGS	British Geological Survey
BPI	Bathymetric Position Index
BSH	Broadscale Habitat
Cefas	Centre for Environment, Fisheries and Aquaculture Science
CHP	Civil Hydrography Programme
CSEMP	Clean Seas Environmental Monitoring Programme
Defra	Department for Environment, Food & Rural Affairs
DEM	Digital Elevation Model
EUNIS	European Nature Information System
FOCI	Feature of Conservation Interest
GES	Good Environmental Status
JNCC	Joint Nature Conservation Committee
MBES	Multibeam echosounder
MCZ	Marine Conservation Zone
MESH	Mapping European Seabed Habitats project
МНСВІ	Marine Habitat Classification for Britain and Ireland
MPA	Marine Protected Area
MSFD	Marine Strategy Framework Directive
NIS	Non-Indigenous Species
NMBAQC	North East Atlantic Marine Biological Analytical Quality Control Scheme
nMDS	Non-metric Multidimensional Scaling
OSPAR	The Oslo-Paris Convention for the Protection of the Marine Environment of the North East Atlantic
PSA	Particle Size Analysis
PSD	Particle Size Distribution
SACO	Supplementary Advice on Conservation Objectives
SIMPER	Similarity Percentages analysis
SIMPROF	Similarity Profile analysis
SNCB	Statutory Nature Conservation Body
SOCI	Species of Conservation Interest
UKHO	United Kingdom Hydrographic Office

1 Introduction

The East of Start Point ('EOSP' hereafter) Marine Conservation Zone (MCZ) is part of a network of sites designated under the Marine and Conservation Access Act (2009), which provides the legal mechanism to assist in the conservation and recovery of the protected wildlife and habitats within them. These sites will also contribute to an ecologically coherent network of Marine Protected Areas (MPAs) across the North East Atlantic agreed under the Oslo-Paris Convention (OSPAR), and other international commitments to which the UK is a signatory.

Under the UK 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. Statutory Nature Conservation Bodies (SNCBs) carry out a programme of MPA monitoring to fulfil this objective. The SNCB responsible for nature conservation offshore (between 12 nm and 200 nm from the coast) is the Joint Nature Conservation Committee (JNCC) and within inshore waters (between 0 nm and 12 nm from the coast) the responsible SNCB is Natural England (NE). 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 the EOSP MCZ in 2021. This dataset will form the initial point in a monitoring time series, against which feature condition can be assessed in the future. The specific aims and objectives of the report are discussed in more detail in Section 1.3.

1.1 Site overview

The EOSP MCZ is an offshore site in the English Channel, located approximately 20 km east of Torquay and 19 km southwest of Lyme Bay (Figure 1). The site, which was designated in May 2019 and falls within the wider 'Charting Progress 2' (CP2) area 'Eastern English Channel', covers 116 km² and ranges in depth from 25 m to 50 m below Chart Datum (Table 1). The site is predominantly in offshore waters, beyond the 12 nm boundary, with the northwest corner extending inside the 12 nm inshore/offshore boundary into inshore waters. Advice for this MPA is therefore jointly delivered by JNCC and NE.

The EOSP MCZ was designated to protect the Broadscale Habitat (BSH) feature 'Subtidal sand'. The seabed within the site supports a range of organisms found on the surface of the sand and buried within it, such as worms, bivalve molluscs (such as razor clams and mussels) and flat fish. The site is a spawning and nursery ground for a number of fish species such as lemon sole (*Microstomus kitt*), sand eels (*Ammodytes tobianus*), mackerel (*Scombre scombrus*), thornback ray (*Raja clavata*) and spotted ray (*Raja montagui*).

Charting Progress 2 Region	Eastern English Channel
Spatial Area (km ²)	116
Water Depth Range (m)	25 to 50
Designated Feature	Subtidal sand (Broadscale Habitat)

 Table 1. East of Start Point MCZ overview.



Figure 1. Location of East of Start Point MCZ in relation to other MCZ boundaries.

1.2 Existing data and habitat maps

1.2.1 Sediment samples

Empirical data describing the sediment particle size for the EOSP MCZ was previously limited.

British Geological Survey (BGS) activity from 1974 and 1978 coincides with the area that was subsequently designated as EOSP MCZ. This survey activity collected particle size data using three different methodologies: borehole (one sample), sediment gravity corer (one sample) and Shipek grab (five samples).

Additionally, as part of the Clean Seas Environmental Monitoring Programme (CSEMP), a government program initiated in the 1980s, sediment samples were collected from a single station within the site boundary. Infauna samples have been collected from this station (CSEMP536) over a number of consecutive years, between 1998 and 2008, using a Day grab (five replicates during each survey).

No seabed imagery data or beam trawl samples were previously acquired from within the EOSP MCZ.

1.2.2 Habitat maps and acoustic data

The designation of EOSP MCZ was based on a combination of two habitat maps which indicated that the entire MPA was comprised of the BSH Subtidal sand.

A modelled habitat map was created using data from a Cefas central channel survey in 2006 (survey codes CEND1406 and CEND1206) which partially overlapped with the eastern side of the MPA. The data were acquired using a combination of multibeam echosounder (MBES) and side scan sonar (SSS) for three lines within the area, with the remainder of the site being modelled (Coggan, Diesing & Vanstaen 2006).

A map covering much of the site was created by The Devon Wildlife Trust for the Lyme Bay Marine Spatial Mapping Project (Marine Planning Consultants Ltd, 2014). This map was created using data collated from the General Bathymetric Chart of the Oceans (GEBCO) and from OLEX data collected from across the site. OLEX systems utilise Fisheries and low resolution MBES sounders to chart seabed features. Data are primarily used to identify features and hazards for fishing but can provide useful contextual information for habitat mapping. At the time of production, the map received a MESH score of 53, which is considered low.

The Civil Hydrography Programme (CHP) has collected multibeam data that covers the entire extent of the site via two separate surveys in 2012 and 2014. These data were acquired from the CHP for the purpose of creating a new habitat map for the MCZ. The raw bathymetry data and the processed backscatter data are displayed in Figure 2.



Figure 2. Bathymetry (left) and backscatter (right) data for the East of Start Point MCZ. Data collected by the Civil Hydrography programme (CHP) during 2012 and 2014.

1.3 Aims and objectives

1.3.1 High-level conservation objectives

High-level 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'.

The conservation objective for the site is that 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.

1.3.2 Definition of favourable condition

Favourable condition, with respect to the BSH feature (Subtidal sand), means that:

- a) The extent and distribution are stable or increasing.
- b) The **structures and functions**, including their quality, and the composition of their characteristic biological communities, are such as to ensure that the habitats remain in a condition which is healthy and not deteriorating.
- c) The 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 associated biological features, such as key and influential species present and characterising assemblages. Physical structure refers to topography, sediment composition and distribution and 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).

1.3.3 Report aims and objectives

The primary aim of this monitoring report is to explore and describe the designated Subtidal sand feature within the EOSP MCZ, to enable future assessment and monitoring of feature condition. The results presented will be used to develop recommendations for future monitoring.

The specific objectives of this monitoring report are to:

- 1) Present evidence on the **extent**, **distribution**, **structural** and **functional** feature attributes of broadscale habitats (Subtidal sand), based on the 2021 data.
- 2) Analyse the sediment and infaunal variability within replicated stations, relative to that between single-sampled stations.
- 3) Note observations of any habitat or species Features of Conservation Importance (FOCI).
- 4) Present evidence on the abundance and distribution of non-indigenous species.
- 5) Note any observations of anthropogenic activities or pressures.
- 6) Provide practical recommendations for appropriate future monitoring approaches for both the designated feature and its natural supporting processes (e.g. metric

selection, sampling design, data collection approaches) with a discussion of their requirements.

1.3.4 Feature attributes and supporting processes

A list of selected feature attributes and supporting processes considered in this report is presented in Table 2, alongside the methods used to address each attribute. At the time of reporting, site specific Supplementary Advice on Conservation Objectives (SACO) was not available for EOSP MCZ, therefore proxy advice from a site with similar features, Swallow Sand MCZ (JNCC, 2018), was used.

Table 2. Feature a	attributes and supporti	ing processes add	dressed to achieve	report Objective
1.		-		-

Feature	Feature attribute / supporting process	Methods
Extent and distribution	Extent and distribution	Review the extent and distribution of Broadscale Habitats (BSH) based on PSA analysis and compare with EUSeaMap 2021. Produce two new substrate maps based on BSH and Folk7 classes, using the new sediment samples and the CHP multibeam echosounder data from 2012 and 2014.
	Sediment composition	Folk trigon plot showing stations Map showing BSH per station Map showing the PSA composition per station Describe and map sediment distribution across the site
Structure and function	Characteristic biological communities Key and influential species	 Identify patterns in infaunal assemblages using multivariate analysis: k\R clustering in PRIMER Describe spatial patterns in infaunal assemblages Describe infaunal assemblage variation in terms of environmental gradients SIMPER analysis to identify characterising taxa Allocate Marine Habitat Classification of Britain and Ireland (MHCBI) habitat classes to each station Identify any key and influential species
Supporting processes	Hydrodynamic regime	Present and describe a tidal model for the site

The report **does not** aim to assess the condition of the designated feature. SNCBs use evidence from MPA monitoring reports in conjunction with other available evidence (e.g. activities, pressures, historical data, survey data collected from other organisations or collected to address different drivers) to make assessments on the condition of designated features.

2 Methods

2.1 2021 survey design

The 2021 survey at EOSP MCZ was conducted aboard the RV *Cefas Endeavour* (CEND0121) between 22 and 23 January 2021 (Stones *et al.* 2023). The survey was designed to acquire the data for the first point in a Type 1 monitoring time series to enable long-term monitoring (as part of Objective 1).

After assessment of available data and following guidance given in Noble-James *et al.* (2016) a random sampling design was selected, with stations placed across the entire MCZ.

Thirty-eight grab stations were randomly generated using ArcGIS, with a 1 km minimum distance between points to reduce the probability of spatial autocorrelation (Olea 1984) (Figure 3). A 500 m buffer from the edge of EOSP was used to ensure stations were not close to its boundary. The number of grab stations was decided based on time available to attain sufficient samples for good geographical coverage of the site.

Replicated grab samples (n = 3) were collected from a subset of stations (EOSP36, EOSP37, EOSP38) (Figure 3) to investigate within-station variability of sediments and communities (Objective 2). These data were acquired to improve our understanding of whether single replicate sampling offers a reliable approach for future sampling within this MCZ.



Figure 3. Station locations for 0.1 m^2 Hamon Grab (HG) sampling in East of Start Point MCZ created using random point generation with a minimum distance between points of 1 km (0.54 nm). Three stations were selected for replicate grab sampling (n = 3).

2.2 Sample acquisition and processing

This section provides an overview of sample acquisition and processing methodologies, whilst a detailed account is available in the CEND0121 survey report (Stones *et al.* 2023).

2.2.1 Seabed sediments

Seabed sediment samples for particle size analysis (PSA) and benthic infauna were obtained from each station using a 0.1 m^2 Hamon grab (also known as a 'mini' Hamon grab). A 500 ml sub-sample for PSA was taken and immediately stored at -20°C. PSA analysis was conducted in accordance with the recommended methodology of the North-East Atlantic Marine Biological Analytical Quality Control (NMBAQC) scheme (Mason, 2016). 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. The sediment distribution data were used to classify samples using several approaches (see Section 2.3.2.2).

The remaining sediment, the infaunal fraction, was processed onboard. The sediment was sieved using a 1 mm mesh, photographed then fixed and preserved in buffered 4% formaldehyde. The infaunal samples were later processed to extract all infauna present, each individual being identified to the lowest taxonomic level possible. Specimens of each taxa were enumerated and weighed (blotted wet weight) to the nearest 0.0001 g following the recommendations of the NMBAQC scheme (Worsfold *et al.* 2010). An external audit of the processing was conducted, and the infaunal data were corrected following this process.

2.3 Data preparation and analysis

2.3.1 Tidal model

Maximum (peak ebb and peak flood) tidal current velocities (m s⁻¹) at the seabed were predicted using a tidal model built for the English Channel, using an unstructured triangular mesh, using the software Telemac2D (v7p1). The mesh had a resolution of approximately 3 km along the open boundary. In the area of the EOSP MCZ, the resolution was refined to approximately 25 m. Bathymetry for the model was sourced from the Defra Digital Elevation Model (Astrium 2011). The resolution of the dataset was 1 arc second (~30 m). After a spin up period of 5 days, the model was run for 30 days to cover a full spring-neap cycle.

2.3.2 Habitat map production

2.3.2.1 Acoustic data acquisition

MBES bathymetry data for the site were collected on two separate third party surveys conducted in 2012 and 2014. The data for the southern part of the site was collected in 2012 by Netsurveys Ltd. on behalf of the Maritime and Coastguard Agency (MCA). Data were downloaded as a pre-processed raster from the UK Hydrographic Office (UKHO) Seabed Mapping data portal at 4 m x 4 m resolution. Data for the northern quarter of the site were collected in 2014 by MMT (UK) Ltd. on behalf of the MCA. Data were downloaded as a pre-processed raster from the UKHO Seabed Mapping data portal at 1 m x 1 m resolution. Data were resampled to a 4 m x 4 m resolution.

Backscatter data from the MBES data were only available from the 2012 dataset and were downloaded directly from the British Geological Survey GeoIndex (offshore) data portal as a pre-processed backscatter geotiff.

2.3.2.2 PSA data

Based on the percentages of sand, gravel and mud, PSA samples were classified both into BSH and into Folk7 classes (Folk 1954). The two classification schemes differ slightly in the way they delineate sediments with low (< 5%) proportions of gravel. The BSH scheme uses an 8:2 ratio (sand:mud) to separate sediments into 'Subtidal sand' and 'Subtidal mud'. The Folk7 classification, however, divides the low gravel sediments into four categories based on three ratio boundaries at 1:9, 1:1 and 9:1 (sand:mud) (Figure 4).



Figure 4. Classification triangles for Broadscale Habitats (left) and Folk7 (right) with the percentages and ratios of mud, sand and gravel.

2.3.3 Acoustic data processing

The acoustic data were processed in a stepwise manner (see Appendix 2 for further details), initiated by merging the bathymetry datasets to a new raster. Based on this new raster layer, it was evident that the data for the southern three quarters of the site had a number of acquisition artefacts, so a low pass filter with a bounding box of 3 x 3 pixels. As part of the habitat classification process, several derivative layers from the bathymetric data were created to aid the identification of geomorphological features which underpin a number of the different habitats. Segmentation was then conducted in the eCognition software to divide the image into objects, based on their spectral and spatial characteristics. The goal of the segmentation is to create meaningful objects that represent areas of homogeneous values in the map image. The PSA data were used to classify the coincidental objects based on a hybrid classification using both the BSH and Folk7 classifications (See Sections 2.3.2.2 and 2.3.4).

As backscatter data were not available for the northern section (approximately one-fifth of the MCZ), this area was mapped separately to the rest of the site. For each classified object the mean and standard deviation of the derivative layers were calculated, exported from eCognition and imported in Python for investigation. A delineation between the classified objects classed as 'Sand (10–20% mud)' and 'Muddy sand (20–50% mud)' was statistically identified using boxplots, based on the standard deviation of aspect. The threshold was set so that objects with a standard deviation of aspect of less than 88 were classified as 'Muddy sand (20–50% mud)'. The remaining habitats were classified as the hybrid habitat 'Sand (10–20% mud)'.

2.3.4 Particle size analysis (PSA)

Sediment particle size distribution data (half phi classes) were grouped by the percentage contribution of gravel, sand and mud derived from the BGS-modified version (Long 2006) of the classification proposed by Folk (1954). Each sample was assigned to:

- i) a sediment BSH, and
- ii) a Folk7 sediment class.

In addition, the full-resolution PSD data (at 0.5 ϕ intervals) were grouped using Entropy, a non-hierarchical clustering method that groups large matrices of PSD datasets into a finite number of groups (Stewart *et al.* 2009). The notable difference between categorising sediments using this approach as opposed to the Folk (1954) approach is that it uses data regarding all size distribution classes, as opposed to the composition of gravel, sand and mud. The optimum number of sediment clusters was achieved when the Calinski–Harabasz (C–H) statistic is at its maximum (Orpin & Kostylev 2006).

2.3.5 Infaunal data

The infaunal dataset was reviewed to ensure consistent nomenclature using the WORMS 'Match Taxa' tool. The species abundance-by-taxon matrix was then truncated according to the truncation steps presented in Appendix 3. The 'indicators' and 'sum' functions in PRIMER v7 were then used to allow grouping of the data for analyses.

Bray-Curtis similarity matrices were produced from the fourth root-transformed infaunal data within PRIMER v7 (Clarke & Gorley 2015) following a review of the resulting shade plots of other, less suitable, abundance data transformations. A single replicate (the 'A' replicate) from each of the three replicate sampled stations was selected and included in the assessment of wider patterns of infaunal assemblage structure.

Infaunal assemblage groups were derived using a non-hierarchical 'k-R Clustering' method. This method determines the optimum number of groups using the Analysis of Similarity (ANOSIM) R statistic and the Similarity Profile (SIMPROF) algorithm to test whether a suitable number of groups has been reached (with a minimum of two and a maximum of 20 groups). This non-hierarchical clustering approach enables samples to be reallocated at later points in the clustering process, without becoming isolated as similarity measures are developed during algorithm computation (Clarke *et al.* 2016). Non-metric multidimensional scaling ordinations (nMDS) were produced to illustrate differences in assemblage structure within and between group classifications. The ANOSIM routine was used to determine any significant differences in infaunal assemblage composition between groups and the Similarity Percentage (SIMPER) routine was used determine within- and among-group similarity (Clarke & Warwick 1994).

Several univariate metrics of community structure were generated using the DIVERSE routine in Primer v7 for each sample (together with total biomass to reflect assemblage function):

- species richness (*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,
- Pielou's evenness $(J') \frac{H'}{\ln (S)}$: where H' is the Shannon Wiener diversity. Quantifying how evenly the individuals in a sample are distributed, J' ranges from zero (uneven

distribution or dominance of a taxon) to one (even distribution of individuals across taxa, less dominance by a taxon,

total biomass (g): the summed mass of all enumerable taxa; blotted wet weight. Taxa were removed from the dataset before the calculation of total biomass if they (a) contributed to approximately ≥ 10 % of the total biomass recorded at the site; and/or (b) possessed an average biomass per individual of greater than four grams. These arbitrary delineations were imposed to reduce the resulting biomass composition being dominated by large individuals whose population density and/or biomass are insufficiently estimated using single grab samples. Five taxa were removed according to this approach (see Section 3.4).

To quantify the relative magnitude of spatial variability within each of the three stations for which replicate grab samples were acquired (Objective 2), the larger-scale variability in the vicinity of each station was quantified by selecting data from a suite of 'near field' stations (i.e. those less than 3 km from each of the three intensively sampled (triplicate) stations (Figure 5)). Six, eleven and nine 'near field' stations were used to quantify larger spatial variation for stations EOSP036, EOSP037 and EOSP038 respectively (Figure 5). Relative spatial variability was assessed by reviewing the within- and among-group similarity and dissimilarity values (Bray-Curtis values; synonymous with a beta diversity proxy in this respect) and the nMDS ordination of the samples included in the analysis. The suite of univariate metrics of community structure adopted for Objective 1 was also compared between the two spatial scales. The total number of taxa sampled at EOSP036, EOSP037 and EOSP038 were compared based on one, two and three replicates to help evaluate whether diversity estimates based on single sampling approaches may under-represent actual diversity.

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Figure 5. 'Near field' stations for each of the three replicate sampled stations from which the data are used to quantify larger scale spatial variability within the East of Start Point MCZ.

2.3.6 Biotopes

Statistical analysis was undertaken for each sample to categorise it according to the Marine Habitat Classification of Britain and Ireland (MHCBI; v22.04) based on its physical (depth, sediment particle size composition) and biological features. To make this approach consistent between the single sample station and the three stations where replicates were sampled, replicates were treated separately as opposed to combining replicate data.

Species data were first compared to the WORMS database using the 'worrrms' package (v 0.4.3) in R (v 4.2.3) to ensure consistent nomenclature. The species list was reviewed to ensure that only infaunal species were used in further analysis.

K-means clustering was then used to create community groups and identify indicator values for component species using the 'labdsv' package (v 2.1.0). These indicative species were then compared to the characterising species lists for existing MHCBI biotopes on relevant substrates to create a shortlist of closest biotopes. Core biotopes records from Marine Recorder of the closest matches where then compared to the new community clusters using a Bray-Curtis dissimilarity matrix and nMDS plots to determine if there were any matches.

2.3.7 Non-indigenous species (NIS)

To address Objective 4, the raw infaunal data were cross-referenced against a list of 49 nonindigenous species which have been selected for assessment of GES in UK waters under MSFD Descriptor 2 (Stebbing *et al.* 2014; Appendix 4). The list includes two categories: species which are already known to be present within the assessment area (present) and species which are not yet thought to be present but have a perceived risk of introduction and impact (horizon). An additional list of taxa, which were identified as invasive in the 'Nonnative marine species in British waters: a review and directory' (Eno *et al.* in 1997) was also used to cross reference against the observed taxa (Appendix 4).

3 Results

3.1 Hydrodynamic regime (Objective 1)

Modelled peak ebb and flood tidal current magnitudes within the EOSP MCZ are generally considered low, varying between 0.134 and 0.217 m s⁻¹ (Figure 6). The highest magnitudes are observed within the south-west section of the site at peak flood tide. Tidal current directions at peak ebb and peak flood vary according to the state of the tide, with a north-east flow direction during flood and a south-west flood during peak ebb tide (as expected for the English Channel). The model predicts the presence of an amphidromic point slightly to the north-north-west of the site. Modelled points within the Telemac mesh had a wide spatial distribution and low density for the site, however, given the generally low velocities and limited gradient, this limitation of resolution is considered acceptable.



Figure 6. Tidal direction and maximum velocity at peak ebb tide (A) and peak flood tide (B) for the East of Start Point MCZ.

3.2 Sediment Composition (Objective 1)

Sand was the dominant component of the sediments (ranging from 75% to 95%) throughout the 44 PSA samples across the EOSP MCZ (35 single sample stations, three triplicate sample stations). Mud, the next most dominant sediment fraction ranged from 4% to 24% of the total sediment composition, whilst gravel (where present) comprised a maximum of just 3%). The sediments, when assigned to the BGS-modified Folk broad sediment classes (Long 2006; Folk 1954), are classified as Subtidal sand (33 samples) and Subtidal mud (11 samples) (Figure 7).



Figure 7. Classification of particle size distribution (half phi) information for each sampling point at East of Start Point MCZ (2021) into one of the sediment BSHs (coloured areas) plotted on a true scale subdivision of the BGS-modified Folk triangle (Long 2006; Folk 1954). Plot on the right shows the enlarged section shown.

There is an evident spatial pattern in the distribution of the main sediment group proportions across the site, with the slight increase in mud content being restricted to the stations towards the north of the site, while those containing the minor gravel component are located only in the south (Figure 8). It is in this northern, muddier region where the sediments classed as Subtidal mud were observed.



Figure 8. Distribution of sediment fractions at the 38 grab sample stations from the 2021 survey at the East of Start Point MCZ overlying the new BSH habitat map (described in Section 3.3).

The PSA data from the 44 sediment samples were classified into three main Entropy groups, two of which were further sub-divided into two groups (Table 3). The ten samples in Group 3, which comprised the coarsest sediments, were all classified as BSH Subtidal sand. Samples from all the other Entropy groups comprised both BSH Subtidal sand and Subtidal mud, particularly group 2b (mean mud content of 21.1%, Table 3) where the majority of samples were classified as this BSH. The distribution of the Entropy group samples spatially reflected the north to south gradient of BSH (Figure 9). Group 2b was associated with BSH Subtidal mud in the north, with Group 2a samples being located south of this. The boundary between Subtidal mud and Subtidal sand comprised Groups 1a and 1b. All the samples representing Group 3 were located within the southern-most region of the MCZ, within the areas mapped as Subtidal sand (Figure 9).

Entropy group	n	% gravel	% sand	% mud	BSH (number of samples)
1a	9	0.2	85.1	14.7	Subtidal sand (8); Subtidal mud (1)
1b	7	0.6	88.5	10.7	Subtidal sand (6); Subtidal mud (1)
2a	7	0.2	83.4	16.4	Subtidal sand (6); Subtidal mud (1)
2b	11	0.2	78.8	21.1	Subtidal sand (3); Subtidal mud (8)
3	10	1.7	92.0	6.4	Subtidal sand (10)

Table 3. Summary statistics for the Entropy groups based on 2021 PSA data of the 44 samples (across the 38 stations) across the East of Start Point MCZ.



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Figure 9. Distribution of Entropy groups of the 2021 PSA samples across the 38 stations at East of Start Point MCZ.

3.3 Extent and distribution (Objective 1)

Habitats were mapped across the whole site using two different classification schemes; BSH and Folk7, following the methods set out in Section 2.3.2. Two different habitats were identified across the site for both these two classifications (Table 4).

Table 4. Broadscale Habitats and Folk7 sediment classifications identified in East of Start Point MCZ along with total areas calculated for each classification, based on the new BSH map.

Classification	Habitat	Area [km ²]
BSH	Subtidal sand	78.0
	Subtidal mud	37.4
Folk7	S	43.1
	mS	72.4

Based on the BSH classification, Subtidal sand was predominant in the southern area of the site (Figure 10). This included several areas of sand waves which were visible from the acoustic data. The sand wave features were identified in the south-central part of the site, with the crests running north-west to south-east, indicating that the predominant current at the centre of the site runs south-west to north-east (or vice versa). Moving north, the sediment changes to a mosaic of Subtidal sand and Subtidal mud, until the northern-most area of the site, which is dominated by Subtidal mud. From the acoustic data, this area was evidently composed of a finer grain material (mud) with a distinctly different acoustic signature and evidence of trawl scars.

Based on the Folk7 classification, the northern two-thirds of the MCZ is classified as muddy sand (mS; Figure 11), with sand comprising the lower third. The northern muddy sand habitat (72.4 km²) based on the Folk7 classification approach is, therefore, more spatially extensive than the Subtidal mud (37.4 km²) in the northern part of the MCZ, as classified using the BSH approach (Table 4).

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Figure 10. Revised broadscale habitat map for the East of Start Point MCZ.



Figure 11. 2023 Folk7 habitat map for the East of Start Point MCZ.

The confidence in the resulting habitat map, based on the MESH confidence Assessment Tool, ranged from 81 to 83 (Figure 12). The lower score in the north reflects the lack of backscatter data available for mapping. Both scores can be considered good, however, some caveats should be considered (see Section 4.1 Broad findings).



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Figure 12. MESH overall quality scores for the new East of Start Point MCZ habitat maps.

3.4 Characteristic biological communities (Objective 1)

3.4.1 Univariate metrics and biomass of infaunal assemblages

A total of 224 taxa remained following truncation of the infaunal abundance data from the 38 stations sampled in 2021. This included 102 annelid taxa, 46 molluscan taxa, 32 arthropod taxa, 15 echinoderm taxa, 12 cnidarian taxa and 5 bryozoan taxa. Other phyla (n = 9) accounted for the remaining 5% of the total number of taxa. A table summarising the abundance and biomass values for the most dominant taxa is presented in Table 5. The bivalve mollusc, *Varicorbula gibba*, was numerically dominant within the MCZ, with 283 individuals identified from the samples collected (Table 5). This taxon was found to be the most common taxon present, occurring in 93% of samples and contributing to almost 10% of the total abundance at the site. Another bivalve mollusc, *Nucula nitidosa*, was also commonly found across the site, occurring in 75% of the samples collected, with a mean abundance of three individuals per sample collected (±3).

The most diverse stations, EOSP008, EOSP019 and EOSP035, were in the southern area of the MCZ and contained 45, 44 and 56 taxa per grab, respectively (Figure 13). Station EOSP007 was the least speciose of the stations sampled, with comparatively fewer taxa (18 per grab) and was located just to the north of the three most diverse stations in a relatively low diversity region (Figure 13 and Figure 14). Stations in this central region, where BSHs transitioned from Subtidal mud in the north to Subtidal sand in the south, were consistently less diverse and typically had fewer individuals present. However, six of these stations contained a large proportion (35%) of the biomass at EOSP MCZ (Figure 15). Stations where relatively larger biomass values were recorded were typically located in the southern half of the MCZ, although sample location was not consistently predictive of high biomass as five stations in this region contained lower total biomass values.

Taxon	Summed abundance	Occurrence in samples (%)	Contribution to total abundance (%)	Mean abundance per sample	Summed biomass (g)
Varicorbula					
gibba	283.0	93.2	9.2	6.4	66.6
Eulima glabra	207.0	68.2	6.7	4.7	2.2
Echinocyamus	100.0	50.0	5.0	0.7	
pusilius	162.0	56.8	5.3	3.7	0.8
Nucula nitidosa	127.0	75.0	4.2	2.9	5.7
Nuculidae	111.0	70.5	3.7	2.5	0.2
Amphiura filiformis	103.0	29.5	33	23	15
	100.0	20.0	0.0	2.0	1.0
Lumbrineris	02.0	70.5	3.0	2.1	0.8
ciligulata ayy.	92.0	70.5	5.0	Z. 1	0.0
Nemertea	71.0	63.6	2.3	1.6	4.1
Poecilochaetus serpens	66.0	63.6	2.1	1.5	0.2

Table 5. Numerical and biomass dominant taxa sampled across the East of Start Point MCZ in 2021.
Taxon	Summed abundance	Occurrence in samples (%)	Contribution to total abundance (%)	Mean abundance per sample	Summed biomass (g)
Nephtys	43.0	47.7	1.4	1.0	4.5
Phaxas pellucidus	40.0	40.9	1.3	0.9	3.8
Chamelea striatula	31.0	52.3	1.7	0.7	21.7
Lovenella clausa	28.0	63.6	1.6	0.6	0.0
Thyone fusus*	25.0	9.1	0.8	0.6	111.8
Dosinia	22.0	25.0	0.7	0.5	27.8
Echinocardium cordatum*	14.0	25.0	0.5	0.3	143.1
Turritellinella tricarinata	10.0	11.4	0.3	0.2	6.3
Gari fervensis	7.0	13.6	0.2	0.2	4.1
Corystes cassivelaunus*	6.0	13.6	0.2	0.1	20.6
Echinocardium pennatifidum*	3.0	6.8	0.1	0.1	41.9
Glycera oxycephala	1.0	2.3	0.0	0.0	4.9
Echinocardium*	1.0	2.3	0.0	0.0	0.1

* Taxa removed from overall assessment of total biomass in subsequent analyses.



Figure 13. Map showing the number of infaunal taxa in 2021 grab samples (0.1 m² Hamon) at East of Start Point MCZ. Data are overlain on the new Broadscale Habitat map for the MCZ.



Figure 14. Map showing the number of infaunal individuals in 2021 grab samples (0.1 m² Hamon) at East of Start Point MCZ. Data are overlain on the new Broadscale Habitat map for the MCZ.



Figure 15. Map showing the total wet biomass of infaunal taxa in 2021 grab samples (0.1 m^2 Hamon) at East of Start Point MCZ. Data are overlain on the new Broadscale Habitat map for the MCZ.

3.4.2 Infaunal cluster groups

A total of six statistically different infaunal groups were identified using k-R clustering of the fourth root transformed abundance dataset (R = 0.82). The most widespread infaunal cluster (Group 'B') comprised 16 samples, while the smallest (Group 'D') comprised only two samples (Table 6). The average Bray-Curtis dissimilarity measure was generally high for comparisons between infaunal cluster groups, ranging from 70% (comparing 'Group B' and 'Group C') to 93% (comparing 'Group A' and 'Group F'). Bray-Curtis dissimilarity was highest when comparing geographically distant groups and lowest when comparing bordering groups. Groups 'E' and 'F', for example, to the south of the MCZ, had relatively similar benthic assemblages and an average dissimilarity of 74% (Table 6). The geographical distribution of the six infaunal groups shows a transition of Groups 'A' to 'F' from the north to the south of the MCZ (Figure 16). There is an evident relationship between the spatial distribution of the assemblage clusters and the subtle changes in sediment type. For example, Group 'A' in the north of the site is largely comprised of stations with the highest mud fraction (being classified as the BSH Subtidal mud), whilst Groups 'E' and 'F' in the south-west of the MCZ are typically associated with the less muddy and slightly gravelly sediments. The sediments of all stations belonging to Groups 'E' and 'F' were classed as Subtidal sand. There was a statistically significant association between faunal group allocation and mud content (categorised into < 5%, $\ge 5\%$ to < 10%, $\ge 10\%$ to < 20% and \geq 20% bins), (Fisher's Exact test, p< 0.001) (Figure 17).

Whilst harbouring assemblages of different taxonomic composition, a number of the k-R cluster groups also vary in their univariate metrics of community structure and total biomass (Figure 18). For example, Group 'E', to the south of the MCZ, is particularly species-rich and contains the greatest infaunal density. Some cluster groups have similar mean univariate values yet harbour contrasting species. For example, Groups 'A' and 'F' contain a similar level of diversity (31 taxa \pm 4 and 32 taxa \pm 1, respectively) and average number of individuals present (n = 63 \pm 11 and n = 63 \pm 7, respectively; Figure 18), yet they represent the most dissimilar assemblages with respect to taxonomic composition (93% dissimilarity; Table 6).

Faunal group	n	Average within group similarity (%)	Average dissimilarity (%) B	Average dissimilarity (%) C	Average dissimilarity (%) D	Average dissimilarity (%) E	Average dissimilarity (%) F
А	8	39	74	82	85	89	93
В	16	39	-	70	81	83	88
С	4	38	-	-	77	82	86
D	2	33	-	-	-	82	84
E	5	37	-	-	-	-	73
F	3	36	-	-	-	-	-

Table 6. SIMPER analysis results for 2021 infaunal data from East of Start Point MCZ.



Figure 16. K-R cluster groups (as defined using the ANOSIM R statistic) based on 2021 infaunal abundance data from East of Start Point MCZ. Data are overlain on the new Broadscale Habitat map for the MCZ.



Figure 17. Mean (± 95% CI) sediment mud content for infaunal cluster Groups A to F, based on the 2021 data collected from East of Start Point MCZ.



Figure 18. Mean (± 95% CI) univariate metrics of community structure and total wet biomass of the samples belonging to each of the six infaunal k-R cluster groups, based on 2021 data collected from East of Start Point MCZ.

3.5 Within-station variability (Objective 2)

3.5.1 Sediment PSA

The stations sampled in triplicate displayed an evident lack of small-scale spatial variability in sediment particle size distribution, with respect to percentage contribution of major sediment fractions and Entropy group allocation (Table 7). The mud content of the sediments sampled at EOSP036, for example, had a range of only 5.6% to 7.6%, with a gravel content range of 2.0% to 3.0%. Comparable small ranges of these sediment fractions were witnessed across the replicates of both EOSP037 and EOSP038. The consistent Entropy allocations across replicates was not replicated by BSH classes. Although replicates from one station (EOSP036) were exclusively classified as Subtidal sand, those from both EOSP037 and EOSP038 also represented Subtidal mud.

Station	Replicate	% Gravel	% Sand	% Mud	BSH	Entropy group
EOSP036	А	2.3	90.5	7.2	Subtidal sand	3a
	В	3.0	89.4	7.6	Subtidal sand	3a
	С	2.0	92.4	5.6	Subtidal sand	3a
EOSP037	А	0.0	77.4	22.6	Subtidal mud	2b
	В	0.0	81.1	18.9	Subtidal sand	2b
	С	0.0	78.1	21.9	Subtidal mud	2b
EOSP038	А	0.2	78.6	21.2	Subtidal mud	1a
	В	0.1	84.1	15.8	Subtidal sand	1a
	С	0.2	84.6	15.2	Subtidal sand	1a

Table 7. Sediment PSA data for each of the three replicate samples from the three replicate stations sampled in 2021 at the East of Start Point MCZ.

3.5.2 Infauna

The infaunal data obtained from the three replicate-sampled stations and those of their 'near field' stations (each single replicates; see Figure 5 in Section 2.3.5 Infaunal data) were compared using both multivariate and univariate approaches. Small (among replicates) and large-scale (among 'near field' sites) variability of selected univariate measures is presented in Figure 19. Wilcoxon's rank sum tests comparing the univariate metrics of replicates of a station and the associated near field stations were not significant (p > 0.05). Replicate samples from EOSP037 also had similar variability in species diversity (number of taxa *S*) and number of individuals (abundance *N*) to that of their associated 'near field' stations. Variability in species diversity and number of individuals was lower among replicate samples of EOSP036 and EOSP038 than among their 'near field' stations. The number of taxa in the sediments sampled at EOSP036, for example, had a range of only 29 to 33 while for the 'near field' stations this ranged from 31 to 56 (Figure 19).

Due to the level of replication (n = 3) a robust assessment of how the number of taxa sampled varies with increased sampling effort was not deemed possible. However, there was a small trend (positive linear) in the relationship between number of taxa from one replicate and number of taxa in three replicates (R = 0.46) and Smax (R = 0.40), where Smax is the extrapolated number of species that would be observed as the number of samples tends to infinity. The nature of these relationships indicates that single samples significantly under-sample the true number of taxa present at a particular station. The total number of taxa from one sample and across three samples increased from: 32 to 59; 33 to 53; and 28 to 49 for EOSP036, EOSP037 and EOSP038, respectively.

The low two-dimensional stress value in the non-parametric nMDS ordination (0.15) indicated a good 2D representation of Bray-Curtis similarity scores as distances between samples. Small-scale variability in benthic assemblage structure, as demonstrated by the distance between replicates of the same station (filled shapes), is generally lower than large-scale variability (Figure 20). At stations EOSP036 and EOSP038, for example, replicate samples appear close together on the nMDS and the 'near field' stations are spread over a larger space. However, station EOSP037 displays similar levels of small- and large-scale variability, with a similar distance between replicates and between 'near field' stations. Within-group similarity was relatively high for the replicate stations (ranging from 43% to

55%) compared to that of the 'near field' stations, which only ranged from 31% to 34% (Table 8).

The percentage dissimilarity values of comparisons between replicate stations and their associated 'near field' stations were consistently lower than that of the values for other group comparisons (Table 8). For example, the infaunal assemblage of station EOSP036 from its three replicates was only 69% dissimilar to that of its 'near field' samples, whilst 61% and 57% dissimilarity was calculated for EOSP037 and EOSP038, respectively.

Infaunal groups were assigned to 38 samples for the analyses to address Objective 1 (Section 3.4.2 Infaunal cluster groups), thus two samples from each of the replicate stations did not have an assigned faunal group. Each of the 'near field' groups are generally dominated by samples belonging to a single faunal group. The 'near field' stations for comparison with EOSP038, for example, comprise mostly Group 'B' (with only single representatives of Groups 'C' and 'D'). Also, the 'near field' EOSP037 stations were mostly represented by the entirety of Group 'A' (plus two stations categorised as Group 'B' on the geographic boundary between the two; Figure 16). Although the 'near field' EOSP036 stations (to the south of the MCZ) were made up of two groups ('E' and 'F'), the faunal assemblages of these groups are relatively similar (Section 3.4.2 Infaunal cluster groups).



Figure 19. Scatter plots showing the range in infaunal univariate metric values at replicate sampling stations compared to their associated 'near field' stations for 2021 samples from East of Start Point MCZ.



Figure 20. Non-parametric Multi-Dimensional Scaling ordination of the 2021 fourth root transformed infaunal abundance data, showing the relative differences in variability within and among replicate stations and 'near field' stations at East of Start Point MCZ.

Table 8. SIMPER results (Bray-Curtis dissimilarity values) showing relative differences in variability among replicates (percentage similarity, in bold) and pairwise comparison between replicate stations and their 'near field' stations (percentage dissimilarity, in italics) based on 2021 infaunal data sampled at the East of Start Point MCZ.

Replicate	n	Average within group similarity (%)	Average dissimilarity (%) EOSP037	Average dissimilarity (%) EOSP038	Average dissimilarity (%) Near EOSP036	Average dissimilarity (%) Near EOSP037	Average dissimilarity (%) Near EOSP038
EOSP036	3	48	94	89	69	93	90
EOSP037	3	43	-	62	92	61	72
EOSP038	3	55	-	-	83	70	57
Near EOSP036	6	31	-	-	-	90	84
Near EOSP037	9	34	-	-	-	-	75
Near EOSP038	11	34	-	-	-	-	-

3.6 MHCBI habitat classification

Biotopes were explored primarily using species data, with secondary consideration given to environmental variables (depth and sediment particle size composition), as biotopes can be found on a greater range of sediment types than where they are in the MHCBI hierarchy.

The most abundant taxa across the site were the bivalve *Varicorbula gibba* (n = 283), the brittlestar *Amphiura filiformis* (103), the nut clam *Nucula nitidosa* (127), records noted as Nuculidae that were likely juveniles of *N. nitidosa* (111), the pea urchin *Echinocyamus pusillus* (162) and the gastropod *Eulima glabra* (see Section 3.7 below for a discussion on this species' presence and distribution within the EOSP MCZ). Species data were then reviewed to ensure only infaunal species were included as the presence of epiphytic species growing on shell fragments may skew results away from existing infaunal biotopes (Appendix 4). The resulting truncated species list was then analysed to identify community clusters.

K-means clustering using the Calinski-Harabasz Index determined that the species data form five cluster groups (C1–C5), of these C5 was discounted as analysis of the clustering revealed this cluster to be unstable (for further details see Appendix 4). Although stable clusters, C1 and C3 only comprised single stations and thus insufficient data to accurately determine biotope: these were also discounted from further biotope determination.

The charactering species of the remaining two clusters (C2 and C4) were then compared to the species lists of existing MHCBI biotopes for a range of sand and mud habitats. This process identified seven closely matching biotopes:

- SS.SMu.CSaMu.AfilEten
- SS.SMu.CSaMu.AfilKurAnit
- SS.SSa.IMuSa.EcorEns
- SS.SSa.IMuSa.FfabMag
- SS.SMu.ISaMu.NhomMac
- SS.SMu.ISaMu.MelMagThy
- SS.SSa.CMuSa.AalbNuc

The core biotope records for these existing biotopes were then compared to the two clusters to determine similarity. However, nMDS plots revealed clear separation between the existing records and the clusters, and a Bray-Curtis dissimilarity matrix resulted in > 89 % dissimilarity to the closest biotope (SS.SSa.CMuSa.AalbNuc). Therefore, these two clusters are proposed as new biotopes to be included in further analysis as part of updates to the MHCBI (Figure 21). Summary biotope descriptions for these clusters have been suggested below.

C2: Potential muddy sand biotope

"Predominantly muddy sand, but occasionally sand sediments, characterised by the bivalves Nuculidae and the bivalve mollusc *Chamelea striatula*. Other important taxa include the polychaetes *Pholoe baltica* and *Spiophanes bombyx*. Taxa which are common but not characterising the biotope include *Varicorbula gibba* and *Eulima glabra*. This biotope has been identified through grab surveys recorded in the East of Start Point Marine Conservation Zone."

C4: Potential sand biotope

"Sand characterised by *Aglaophamus agilis*, *Dosinia* and Nuculidae. Other important taxa include *Chamelea striatula* and *Pholoe baltica*. Taxa which are common but not characterising for the biotope include *Echinocyamus pusillus*, *Diplodonta rotundata*, *Hydroides norgegica*, *Nucula nitidosa* and *Varicorbula gibba*. This biotope has been recorded in the East of Start Point Marine Conservation Zone."

The five stations not included in C2 or C4 are instead identified as Level 4 in the MHCBI classification using sediment data (Figure 21).



Figure 21. Distribution of Marine Habitat Classification for Britain and Ireland (MHCBI) habitats (Level 4) for each sample collected in 2021. Data are overlain on the new Broadscale Habitat map for the MCZ.

3.7 Key and influential species (Objective 1)

Key and influential species are those that have a core role in determining the structure and function of habitats. For example, for subtidal sedimentary habitats bioturbating species (animals that forage and burrow tunnels, holes and pits in the seabed) help recycle nutrients and oxygen between the seawater and the seabed, supporting the organisms that live within and on the sediment. The few taxa sampled across EOSP which might qualify as key/influential in this respect were the larger (high biomass) organisms which were found to be low in abundance. These were the sea cucumber *Thyone fusus*, several *Echinocardium* species and the crustacean *Corystes cassivelaunus*. Large species generally occur in relatively lower densities relative to smaller, often shallower sediment dwelling and their population densities are generally not suitably estimated by single grab sampling.

The rarely encountered gastropod mollusc, *Eulima glabra* (da Costa 1778) (Figure 22), was recorded from the MCZ in relatively high abundances from the 2021 samples. In total, 207 individuals were collected from across the 38 stations (44 samples). The maximum number of individuals collected from any one sample was 31 (EOSP032 in the west of the site) and most stations sampled (26 of the 38 stations) contained at least one specimen of *E. glabra*. On average, seven individuals (\pm sd 7) were collected from each sample. However, *E. glabra* was absent from 12 stations, generally located to the south-east of the area sampled (Figure 23).

E. glabra is a small (approximately 20 mm) and slender (approximately 5 mm) gastropod mollusc characterised by a robust and highly glossy, almost transparent shell, with red banding on each whorl. It is presumed to be an ectoparasite of echinoderms and lives in the sublittoral zone, down to approximately 200 m water depth. Although the exact hosts are not known (and E. glabra is known to be free living), it has been suggested that brittlestars (ophiuroids) are the likely hosts (Wigham 2017). While this species might not, sensu stricto, represent a 'key/influential species', its presence across the EOSP is noteworthy, as it has a sparse UK distribution based on records from the National Biodiversity Network Atlas (National Biodiversity Network 2017), OneBenthic (OneBenthic database 2020) and Marine Recorder (Marine Recorder 2022), (Figure 24). Wigham (2017) does not record E. glabra from the southern North Sea and the sparse records identified imply a west of the UK distribution (southwest coast of England, Irish sea). The nearest records of E. glabra to the MCZ are south of Plymouth and north of Brixham (Figure 24). The records from the 2021 survey provide more information on its known distribution and the relatively high abundances imply that the EOSP MCZ may potentially be an important area for it. Further monitoring surveys may provide insight into its recurring distribution within the site.



Figure 22. Image of typical *Eulima glabra* specimen, collected at East of Start Point MCZ in 2021. Image taken by APEM Ltd.

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Figure 23. Distribution and densities of *Eulima glabra* in 0.1 m² Hamon grab samples from East of Start Point MCZ. Data are overlain on the new Broadscale Habitat map for the MCZ. Data are overlain on the new habitat map (based on a BSH classification) for the MCZ.



Documented presence of Eulima glabra prior to 2021 survey

Figure 24. Records of *Eulima glabra* from the National Biological Network Atlas, OneBenthic and Marine Recorder showing its distribution around the UK.

3.8 Habitat and species FOCI (Objective 3)

No habitat or species features of conservation interest (FOCI) were observed in the 2021 grab survey of the EOSP MCZ.

3.9 Non-indigenous species (NIS) (Objective 4)

One individual (0.0003 g wet weight) of the polychaete worm *Goniadella gracilis*, listed as invasive in the 'Non-native marine species in British waters: a review and directory' (Eno *et al.* 1997), was identified from the grab sample collected from station EOSP013 (north of the centre of the MCZ – see Figure 3 or Figure 21 to view the location of station EOSP013). No other records of non-indigenous taxa were identified.

3.10 Observed anthropogenic activities and pressures

Across the site a number of wrecks were observed from the acoustic data, all corresponding with charted wreck locations (Figure 25). In the north-west of the site in the areas classified as having a muddier substrate, evidence of trawl scars was seen from the backscatter data. Although not mapped here it is likely that these continue into the muddier sediments further north.



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Figure 25. Evidence of human activities and pressures across (and in the vicinity of) the East of Start Point MCZ.

4 Discussion and recommendations for future monitoring

4.1 Broad findings

The new habitat maps created here for the EOSP MCZ provide, for the first time, a full coverage assessment of the different sediments distributed across the whole site. The maps scored well with regards to the MESH guality assessment, however, there are several factors which may influence the accuracy of these maps. The underlying acoustic data used to create the new habitat maps date from 2012 and 2014, whilst the groundtruthing data used to map against these datasets were collected during the 2021 survey. The nine-year period between the two datasets may therefore have some bearing on the accuracy of the new maps. In stable, deep-sea environments or in areas with little current or wave action, this temporal mismatch between acoustic and ground-truthing data is unlikely to affect accuracy of the mapping process. For the EOSP MCZ, although peak ebb and flood tidal current magnitudes are generally considered low (Section 3.1), it appears that the sediments are regularly swept by a current with sufficient energy to mobilise sediments and form features such as sand waves (Figure 26). This current, therefore, may affect the distributions of the different sediments across the site and temporally affect the boundaries between the different classifications (although total distributions of sediments may remain consistent). Indeed, some evidence of temporal change was highlighted during the mapping process, where an area which appeared to be of a different sediment to the surrounding area on the 2012 backscatter acoustic data had very similar PSA results from the 2021 samples. This indicates that the sediment boundary may have moved in the intervening years. Caution should therefore be applied in interpreting any future changes in mapped extent, particularly regarding the use of extent in assessments of condition.

The PSA samples across the site were classified using two different classification approaches (BSH and Folk7). However, the actual differences in the sediment type were found to be extremely small, with the proportions of sand:mud only changing slightly across the site. Therefore, the boundaries drawn on the new habitat maps are likely to be more suggestive of a gradual sediment change than the location of a hard boundary with a noticeable change in sediment type. This is supported by the PSA data, which reveal a slow transition of sediments from the south-east corner of the site to the north-west. In the south-east section of the MCZ sediments have a higher proportion of sand and an increased, albeit very minor, proportion of gravel. Towards the north-east of the MCZ, the proportion of mud shows a consistent increase across the site to a maximum of 24.2% at EOSP010 (the most northerly station sampled; Figure 8).

One noteworthy feature of the EOSP MCZ is that the observed coarsening of sediments is associated with the gradual north to south increased depth gradient (Figure 2), from *approximately* 25 m to 50 m. The muddier sediments in the shallower northern section grade to less muddy and increasingly gravelly sediments towards the deeper, more southerly regions of the site. It is in this southern region where the highest (although still comparably low) peak tidal flows are witnessed (Figure 6). This is different to the situation observed within most MCZs across the coast of England and Wales where softer, muddier sediments are correlated within depth increases. Further investigation of the EOSP MCZ could include an assessment of whether the hydrodynamic conditions and associated sediment characteristics of the site are representative of the wider western English Channel region or represents a unique example of more localised coastal processes.



Figure 26. Sand wave features across the East of Start Point MCZ indicating a currentswept seabed. Underlying data is the bathymetric derivative BPI5.

The relatively linear gradient in environmental conditions evidenced here for the EOSP MCZ (sediment type, depth and tidal flows) have been demonstrated to manifest in a rather similar gradient in its associated macrofaunal assemblages. The site, one may argue, represents a good example whereupon infaunal assemblages more-or-less directly reflect changes in key environmental conditions. The six infaunal k-R clusters grade from one to the next along a north to south gradient (Figure 16). Indeed, some of the key taxa discriminating the clusters reflect the observed changes in sediment composition. For example, the assemblages of Groups 'E' and 'F, located in the deeper, coarser sediments, were not only more speciose and densely populated than the other assemblages but they represented those where several attached taxa such as sponges were observed. The macrofaunal gradient is aligned with the habitats delineated for the site based on both BSH and the Folk7 classification. Given the similar nature of these two habitat maps, this is not an unexpected observation. Thus, in terms of which map should be applied going forward, one might consider the commonly used BSH-based map to be most appropriate.

Analysis of the infaunal data, together with the observed minor variation in sediment composition, identified the potential of two new biotopes which will undergo further review for addition to the MHCBI classification system. The first newly suggested biotope was associated with predominately muddy sands and characterised by the bivalves Nuculidae and the bivalve mollusc *Chamelea striatula*. The second biotope was associated with sand and characterised by the taxa *Aglaophamus agilis*, *Dosinia* and Nuculidae. There is currently a lack of data from the Eastern English Channel for use in habitat classification system. The discovery and categorisation of new biotopes is an important aspect of monitoring work as it allows for a more accurate picture of the seabed to be created and increases our knowledge of species distribution and biological communities. Further sampling will be

required to provide the confidence that these are novel biotopes, and to more accurately define the species which make up this community.

Objective 2 aimed to ascertain the relative spatial variability in sediments and infauna at the station scale (i.e. between replicates within a 50 m sampling bullring) with the wider environs, to allow inferences regarding the utility of sampling/monitoring based on single samples across the site, compared to a reduced number of stations with replication. The data revealed that at the station scale, seabed sediments at all three stations were rather homogenous, showing small ranges in the proportions of mud, sand and gravel and no variation in sediment Entropy groups between replicates (although the replicates straddled the two BSH classes at two of the stations). Perhaps not unexpectedly, variations in univariate metrics of community structure were lower in the station replicates compared to the those based on single samples in the wider environs (i.e. 'near field') (Figure 19) and that was also mirrored in multivariate community structure as evidenced through 2-D nMDS plots (Figure 20). However, the three samples of one of the three stations EOSP037 did show a comparable variability in multi-dimensional space as those of the nine samples across the wider spatial scale. Finally, it was evident that estimates based on single samples significantly under-represent total number of taxa at each station. Fundamentally, however, one would consider that the outcomes of the data comparisons in Objective 2 are in accordance with expectation. That is, the contrasting approaches of:

- i) replication at the station level; and
- ii) ii) single samples across a wider region, each provide different information about the seabed.

The decision regarding which offers a more suitable approach for monitoring change at a site is a more involved one, that must consider wider issues such as habitat distribution complexity (e.g. a mosaic of habitats compared to a linear gradient as is the case for the EOSP MCZ) and whether monitoring aims to quantify the effectiveness of a measure to reduce a pressure to all or part of a site.

4.2 Recommendations for future monitoring

The data acquired through the 2021 grab survey at the EOSP MCZ have improved our understanding of the types and spatial variability of seabed sediments and infaunal assemblages at the site. These data form an important baseline for a monitoring time-series, eventually allowing assessment of changes in condition. There are, of course, some evident gaps in our current knowledge of the MCZ and some important details to highlight here for any future survey efforts and/or management of the site. These are presented below.

- 1. The EOSP MCZ has not previously been targeted for sampling to assess its ecological characteristics, and the 2021 data provide a real advancement for the site in this respect. However, with the present lack of imagery data within the site, there remains no information on the epifaunal communities present. It would be prudent to undertake targeted video and/or still approaches (preferably using a camera sledge) and/or 2 m beam trawl approaches to capture such missing information. These data would fill the distinct gap in our understanding of the ecological characteristics of the MCZ beyond that which is captured by grab sampling alone.
- 2. To provide some context regarding the temporal variations in infaunal assemblages for the EOSP MCZ, one relatively cost-effective, albeit spatially limited, approach would be to review the temporal data obtained within the site under the CSEMP program. One CSEMP station (CSEMP536) has been subject to temporal sampling and an appraisal of the magnitude of temporal variability observed may offer a good insight regarding the temporal stability of the site's infauna. The sediment

contaminants data collected as part of the CSEMP program may also provide a useful context regarding the concentrations of a suite of chemicals and their temporal signatures.

- 3. Both the sediment granulometric properties and infaunal assemblages displayed a reduced spatial variability (based on three stations) at small scales (within 50 m station bullring) relative to that of the wider environs. This implies that a greater statistical power to detect a change in any associated metric will result from replicate station sampling as opposed to single sampling over a wider spatial extent. These two sampling principles fundamentally provide differing information about the seabed and, although it is recognised that any design should be tailored specifically to meet the specific objectives of the survey, we would advocate that a combination of both approaches (replication at a subset of stations and an array of single samples) is likely to be the most effective design for subsequent monitoring. Stations subject to replication should ideally be positioned away from likely boundaries between habitats or benthic assemblage types. The outcomes of the 2021 survey may be used to enable such station positioning.
- 4. Temporal assessments using data from a number of surveys is a fundamental tenet of any monitoring program. To facilitate this and ensure that conclusions based on the outcomes are as robust as possible, surveys should be aligned as much as possible with respect to sampling season. The 2021 survey, conducted in January, therefore, should set a precedent for the timing of subsequent sampling events if changes in the health and status of benthic assemblages at the EOSP are to be robustly assessed. Similarly, the truncation process followed for the infaunal data presented here should be mirrored for any data which are to be used as part of such assessments.
- 5. The 2021 infaunal data has evidenced that EOSP MCZ, and possibly the wider environs within which it is located, is potentially important for the rarely encountered gastropod *Eulima glabra*. Any future monitoring of the site should gather evidence on its continued presence.
- 6. The seabed within the EOSP MCZ site supports a range of animals found on the sediment surface (see Section 1.1 Site overview)). While there is a need for increased data regarding the numbers and species of such epifaunal species (see point 1 above) which includes demersal fish (e.g. flatfish), there is also the need to understand better the nature of the relationships of these species, particularly the fish species, with the seabed within the EOSP MCZ. The site is a spawning and nursery ground for a number of fish species such as lemon sole (*Microstomus kitt*), sand eels (*Ammodytes tobianus*), mackerel (*Scombre scombrus*), thornback ray (*Raja clavata*) and spotted ray (*Raja montagui*) and it would be prudent to better understand where such spawning and nursery areas within the site are located. This may facilitate better management of the site by managing activities that have the potential to affect the specific characteristics for which these fish species rely.
- 7. Given the temporal mismatch of the acquisition of the acoustic data for EOSP (2012 and 2014) and that of the groundtruthing data (2021), and the potential for the observed sediment habitat boundaries to have changed over time, it would be advisable to conduct further acoustic surveys for either all of, or targeted parts of, the site.

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Appendix 1. Glossary

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

Term	Definition
Activity	A human action which may have an effect on the marine environment; (e.g. fishing, energy production (Robinson <i>et al.</i> 2008)).*
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).*
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 Condition	When the ecological condition of a species or habitat is in line with the conservation objectives for that feature. The term 'favourable' encompasses a range of ecological conditions depending on the objectives for individual features.*
Feature	A species, habitat, geological or geomorphological entity for which an MPA is identified and managed.*
Feature Attributes	Ecological characteristics defined for each feature within site-specific Supplementary Advice on Conservation Objectives (SACO). Feature Attributes are monitored to determine whether condition is favourable.
Features of Conservation Importance (FOCI)	Habitats and species that are rare, threatened or declining in Secretary of State waters.*
Impact	The consequence of pressures (e.g. habitat degradation) where a change occurs that is different to that expected under natural conditions (Robinson <i>et al.</i> 2008).*

Term	Definition
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 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).*
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.
Natural England	The statutory advisor to Government on conservation on land and in UK territorial waters. Its specific remit in the marine environment ranges from 0–12 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 <i>et al.</i> 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 <i>et al.</i> 2008).*
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.

Appendix 2. Acoustic data processing

Acoustic data pre-processing

The bathymetry datasets were merged to a new raster and resampled to a common 4 m x 4 m grid and then clipped to an area slightly larger than the EOSP MCZ boundary. Upon visual inspection of the data, it was evident that the data for the southern three quarters of the site had a number of acquisition artefacts. A regular heave motion was discernible throughout the majority of the data. To help remedy this, a low pass filter with a bounding box of 3×3 pixels was applied to the bathymetry data.

As part of the habitat classification process, several derivative layers from the bathymetric data (Table 9) were created to aid the identification of geomorphological features which underpin a number of the different habitats.

Layer Name	Detail
Bathymetric Position Index	Bathymetric position index (Lundblad <i>et al.</i> 2006); radii of five and ten cells.
Aspect	Identifies the direction of maximum rate of change in depth from each cell.
Roughness	Calculated as the difference between the maximum and minimum value of each cell and its 8 neighbours.
Northness	Calculated as the cosine of the aspect in degrees. Values range from -1 to 1 with a value of 1 indicating a north facing slope
Slope	The slope in degrees using the maximum change in elevation of each cell and its 8 neighbours.

Table 9. Bathymetric derivatives calculated from combined 2012 and 2014 data.

Segmentation

Segmentation divides the image into objects, based on their spectral and spatial characteristics. The resulting objects can be characterised by their various features such as layer values (mean, standard deviation, skewness, etc.), geometry (extent, shape, etc.) and texture. The input layers used were the primary acoustic data layers (bathymetry and backscatter strength) and their derivatives (Table 9). The initial segmentation was conducted using the multi-resolution segmentation algorithm in eCognition. This is an optimisation procedure that starts with an individual pixel and consecutively merges it with neighbouring pixels to form an object. The process continues until a threshold value (determined by the operator) for a scale parameter determining the variability allowed in the objects is reached. The goal of the segmentation is to create meaningful objects that represent areas of homogeneous values in the map image. The size of the objects is influenced by the scale parameter and the heterogeneity of the image. For a fixed value of the scale parameter, a homogeneous area of seabed will have larger objects than a heterogeneous area. Likewise, for a fixed seabed heterogeneity, larger values of the scale parameter produce larger objects than smaller values. For the EOSP bathymetric data, a scale parameter of 100 was set, shape was set to 0.5 and compactness was set to 0.5.

Classification

Following segmentation, the PSA data were used to classify the coincidental objects. A hybrid classification using both the BSH and Folk7 classifications (See Sections 2.3.2.2 and 2.3.4 Particle size analysis (PSA)) was applied to the samples based on proportions of sand, gravel and mud (Table 10), the PSA samples being categorised into three different classifications.

Hybrid Class	BSH	Folk7	Sand: mud ratio limits
Sand	Subtidal Sand	Sand	> 9:1
Sand (10–20% mud)	Subtidal Sand	Muddy Sand	9:1 > x > 8:2
Muddy sand (20–50% mud)	Subtidal Mud	Muddy Sand	8:2 > x > 1:1

Table 10. Hybrid classification and how they relate to BSH and Folk7.

As backscatter data were not available for the northern section (approximately one-fifth of the MCZ), this area was mapped separately to the rest of the site.

For each classified object the mean and standard deviation of the derivative layers were calculated, exported from eCognition and imported in Python for investigation. Statistical differences between the three different hybrid class habitats (Table 10) were identified using boxplots.

A rather clear distinction between the samples classified as 'Sand' and the other two categories (i.e. 'Sand (10–20% mud)', 'Muddy sand (20–50% mud)', was identified based on the mean backscatter values (Figure 27)). A threshold value of 92 was applied to the unclassified objects and those with a mean backscatter greater than 92 were classified as 'Sand'.



Sediment Class

Figure 27. Boxplot showing the sample values of the derivative layers (y axis) for object mean backscatter based on the acoustic data for East of Start Point MCZ.

A delineation between the classified objects classed as 'Sand (10–20% mud)' and 'Muddy sand (20–50% mud)' was statistically identified based on the standard deviation of aspect (Figure 28). The threshold was set so that objects with a standard deviation of aspect of less than 88 being classified as 'Muddy sand (20–50% mud)'. The remaining habitats were classified as the hybrid habitat 'Sand (10–20% mud)'.





Figure 28. Boxplots showing the sample object mean and standard deviation (of the derivative layers (y axis)) of aspect based on the acoustic data for East of Start Point MCZ.

Appendix 3. Infauna data truncation protocol

Raw taxon abundance and biomass matrices can often contain entries that include the same taxa recorded differently, erroneously or differentiated according to unorthodox, subjective criteria. Therefore, ahead of analysis, data should be checked and truncated to ensure that each row represents a legitimate taxon and 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 East of Start Point MCZ ahead of the analyses reported here are provided below:

- 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 East of Start Point MCZ: where a species level identification was labelled 'juvenile', the record was combined with the associated species level identification, when present, or the 'juvenile' label removed where no adults of the same species had been recorded.
- Records of meiofauna were removed.
- Records of eggs and fragments of individuals were removed.
- Records of algae, fish and litter were removed.
- Unique records at a Kingdom, Phylum or Order taxonomic level were removed (Animalia, Bivalvia, Sessilia).
- 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.

Appendix 4. Biotope determination methodology

Biotopes were explored primarily using species data, with secondary consideration given to environmental variables (depth and sediment particle size composition), as biotopes can be found on a greater range of sediment types than where they are located in the MHCBI hierarchy. Species data were first reviewed to ensure only infaunal species were included as the presence of epiphytic species growing on shell fragments may skew results away from existing infaunal biotopes. The resulting truncated species list was then analysed to identify community clusters.

The Calinski-Harabasz Index (CH Index) and the Total within Sum of Squares (WSS) were calculated to determine a suitable K value when clustering (Figure 29). To determine the K value using the WSS, the number at which the decrease in value begins to flat out is identified (K = 5). Sometimes this value is not obvious and can be highly subjective but was identified to be either K = 5 or K = 7. Using the CH Index, the value at which the K number spikes is used to determine the cluster number. With some data (such as the present data), there is an initial peak at two clusters, however this is not considered ideal and instead the next peak should be used (K = 5). Combining these two methods, the suitable K value of K = 5 was concluded.



Figure 29. Calinski-Harabasz Index (left) and the Total within Sum of Squares (WSS) (right).

Using the calculated K value, the samples were then clustered using the UPGMA clustering method (Figure 30).



Sample - UPGMA

Figure 30. The clusters created from UPGMA clustering. The labels are sample IDs. The red boxes identify how the samples should be clustered when K = 5: From left to right, clusters C1, C2, C3, C4, and C5.
After the clusters were identified, the Bootstrap method was used to evaluate the stability of the clusters (Table 11). When clustering, clustering algorithms may produce several clusters that represent actual grouping of data, and one or two 'other' clusters that contain data with little relationship with one another, but very distinct from the other clusters.

Table 11. Bootstrap method results. 'clust' is the name of the cluster identified through UPGMA clustering; 'bootmean' is a measure of consistency or stability. A higher bootmean value indicates a more consistent cluster; 'bootdissolved' quantifies how frequently clusters undergo dissolution or exhibit variability in the context of repeated bootstrapping. A higher bootdissolved indicates increased instability.

clust	bootmean	bootdissolved
C1	0.8834387	1
C2	0.6400000	36
C3	0.6526643	25
C4	0.5200000	48
C5	0.4473333	53

As C5 has a low bootmean (< 0.6) and a high bootdissolved (> 45) (Table 11), this cluster was considered to be unstable, and therefore was removed from further analysis. While C1 and C3 qualify as stable clusters, they included too few samples to accurately place them into a biotope (n = 1), therefore, they were also removed from further analysis.

The package "labdsv" (v 2.1.0) was used to calculate the indicator value (IndVaI) of species in each cluster, an alternative to SIMPER for identifying species indicative of each cluster (Table 12 and Table 13).

Species	Abu	Frq	IndVal	pval
Nuculidae	0.7943445	0.9000000	0.7149100	0.001
Chamelea striatula	0.2532468	0.6333333	0.1603896	0.021
Lumbrineris cingulata	0.1296625	0.8666667	0.1123742	0.035
Pholoe baltica	0.1304348	0.3666667	0.0478261	0.010
Kurtiella bidentata	0.0148662	0.4333333	0.0064420	0.017
Turritellinella tricarinata	0.0604027	0.1000000	0.0060403	0.041
Amphiura filiformis	0.0085113	0.2000000	0.0017023	0.022
Chaetozone zetlandica	0.0209790	0.0666667	0.0013986	0.028
Dosinia	0.0076336	0.0666667	0.0005089	0.047

Table 12. Species from cluster C2 that had a significant (p < 0.05) Indicator Value (IndVal).

Species	Abu	Frq	IndVal	pval
Aglaophamus agilis	1.0000000	0.8888889	0.8888889	0.013
Dosinia	0.1526718	0.7777778	0.1187447	0.047
Nuculidae	0.2056555	0.444444	0.0914025	0.001
Chamelea striatula	0.0649351	0.2222222	0.0144300	0.021
Pholoe baltica	0.0457666	0.2222222	0.0101704	0.010
Amphiura filiformis	0.0149321	0.5555556	0.0082956	0.022
Lumbrineris cingulata	0.0177620	0.3333333	0.0059207	0.035
Chaetozone zetlandica	0.0349650	0.1111111	0.0038850	0.028
Turritellinella tricarinata	0.0335570	0.1111111	0.0037286	0.041
Kurtiella bidentata	0.0039643	0.1111111	0.0004405	0.017

Table 13.	Species from	cluster C4	that had a	significant (p	< 0.05) Indicator	Value ((IndVal)
						/		(/

The characterising species of the following biotopes were compared against the species in each cluster with a significant IndVal:

##	[1]	"SS	.SSa.	CFiSa	.Epus(DborAp	ri"	"SS	.SSa	a.CF	iSa./	Apri	BatP	o"
##	[3]	"SS	.SSa.	CFiSa	.SiphN	lephVe	n"	"SS	.SS	a.CN	/luSa	a.Ăa	albNu	с"
##	[5]	"SS	.SSa.	CMuS	a.Abra	Airr"	"(SS.S	Sa.0	CMu	Sa.C)opł	ר"	
##	[7]	"SS	.SMu	.CSalv	lu.AfilE	ten"	"(SS.SI	Mu.(CSal	Mu.T	⁻ hyE	Eten"	
##	[9]	"SS	.SMu	.CSalv	lu.Afilk	(urAnit"		SS.S	Mu.	CSa	Mu.	VirC	DphPi	max"
##	[11]	"SS	S.SMu	ı.CSal	/u.VirC	OphPm	ax.l	HAs"	"SS	.SM	u.CS	SaM	lu.Lko	orPpel"
##	[13]	"SS	S.SSa	.OSa.	OfusAf	il"	"SS	S.SS	a.OS	Sa.N	lalEo	def"		-
##	[15]	"SS	S.SSa	.IMuS	a.Ssub	Nhom"		"SS	.SS	a.IM	uSa.	.Are	ISa"	
##	[17]	"SS	S.SSa	.IMuS	a.Ffabl	Mag"	'	SS.S	SSa.	IMu ^s	Sa.E	cor	Ens"	
##	[19]	"SS	S.SSa	.IFiSa	IMoSa	ı" [–]	"SS	S.SS	a.IFi	Sa.I	NcirE	Bat"		
##	[21]	"SS	S.SSa	.IFiSa	TbAm	Po"	"(SS.S	Mu.	SaN	lu.C	ap"		
##	[23]	"SS	S.SMu	ı.ISaM	u.Amp	Plon"		"SS.	SΜι	ı.ISa	Mu.	Cun	dAas	p"
##	[25]	"SS	S.SMu	ı.ISaM	u.KurA	\br"	"(SS.S	Mu.I	Sa№	lu.N	hom	וMac'	
##	[27]	"SS	S.SMu	ı.ISaM	u.MelN	/lagThy	/"							

The following biotopes were found to be the most closely matching:

##	[1] "SS.SMu.CSaMu.AfilEten"	"SS.SMu.CSaMu.AfilKurAnit"
##	[3] "SS.SSa.IMuSa.EcorEns"	"SS.SSa.IMuSa.FfabMag"
##	5] "SS.SMu.ISaMu.NhomMac"	"SS.SMu.ISaMu.MelMagThy"
##	[7] "SS.SSa.CMuSa.AalbNuc"	

Current biotopes in MHCBI were classified using data stored in Marine Recorder. During this classification, groups of data with similar biological and environmental characteristics were clustered together to form biotopes, these were then used the basis for the biotope descriptions and identified as core biotope records on which the biotope is based (Connor *et al.* 2004). These core biotope records were obtained from Marine Recorder for each of closely matching biotopes to compare to C2 and C4. The core data were quality checked as described in previously. An nMDS plot was then created to visualise the similarity between the clusters and the core data of the biotopes (Figure 31).

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C2 & C4 comp. Core records



Figure 31. nMDS plot including core data from all the most closely matching Marine Habitat Classification biotopes, and the identified clusters C2 and C4. (Stress: 0.223).

A Bray-Curtis dissimilarity matrix was also calculated to inform our decision on which biotope was most closely matched.

Biotope	C2	C4	Afil Eten	AfilKur Anit	Ecor Ens	Ffab Mag	Nhom Mac	MelMag Thy	Aalb Nuc
C2	-	-	-	-	-	-	-	-	-
C4	0.804	-	-	-	-	-	-	-	-
AfilEten	0.9139	0.974	-	-	-	-	-	-	-
AfilKurAnit	0.9214	0.9784	0.7503	-	-	-	-	-	-
EcorEns	0.9143	0.9593	0.926	0.9612	-	-	-	-	-
FfabMag	0.9068	0.9681	0.9105	0.9443	0.7299	-	-	-	-
NhomMac	0.961	0.9939	0.949	0.9954	0.9673	0.9469	-	-	-
MelMagThy	0.907	0.9635	0.8706	0.9549	0.8956	0.8598	0.9343	-	-
AalbNuc	0.8873	0.9434	0.91	0.9159	0.878	0.8204	0.9529	0.8884	-

Table 14. Bray-Curtis Dissimilarity between C2, C4, and core data for Marine Habitat Classification biotopes.

C2 and C4 had the lowest dissimilarity between one another (80% dissimilarity). AalbNuc was the least dissimilar biotope to C2 and C4 (89 % and 94 % dissimilarity respectively).

The nMDS plot shows that SS.SMu.ISaMu.MelMagThy and SS.SSa.CMuSa.AalbNucis were the most similar biotopes to C2 and C4, so another nMDS plots was created with just C2, C4, and SS.SMu.ISaMu.MelMagThy and SS.SSa.CMuSa.AalbNuc core data (Figure 32).



Figure 32. nMDS plot including core data from the Marine Habitat Classification biotopes SS.SMu.ISaMu.MelMagThy and SS.SSa.CMuSa.AalbNucis, and the identified clusters C2 and C4. (Stress: 0.222).

As the Bray-Curtis dissimilarity resulted in a percentage of at least > 89%, and the nMDS plot displayed a clear separation between the clusters and the biotopes, C2 and C4 were not considered to be within any currently existing Marine Habitat Classification biotopes. However, the nMDS plot also reports a high stress value (> 0.2) which is usually indicative of plots which should be treated with a great deal scepticism as they do not represent a good portrayal of the data. These two clusters are therefore proposed as new biotopes to be included in further analysis as part of updates to the MHCBI.

Appendix 5. Non-indigenous species (NIS)

Table 15. Taxa listed as non-indigenous species (present and horizon) which have been selected for assessment of Good Environmental Status in GB waters under MSFD Descriptor 2 (Stebbing *et al.* 2014).

Species name	List	Species name	List
Acartia (Acanthacartia) tonsa	Present	Alexandrium catenella	Horizon
Amphibalanus amphitrite	Present	Amphibalanus reticulatus	Horizon
Asterocarpa humilis	Present	Asterias amurensis	Horizon
Bonnemaisonia hamifera	Present	Caulerpa racemosa	Horizon
Caprella mutica	Present	Caulerpa taxifolia	Horizon
Crassostrea angulate	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 16. Additional taxa listed as non-indigenous species in the JNCC 'Non-native marine species in British waters: a review and directory' report by Eno *et al.* (1997) which have not been selected for assessment of Good Environmental Status in GB waters under MSFD Descriptor 2.

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

Species name (1997)	Updated name (2017)
Rhithropanopeus harrissii	-
Potamopyrgus antipodarum	-
Tiostrea lutaria	Tiostrea chilensis
Mercenaria mercenaria	-
Petricola pholadiformis	-
Mya arenaria	-







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