JNCC/Cefas Partnership Report Series

Report 44

Offshore Overfalls Marine Conservation Zone (MCZ) Monitoring Report 2019

Jon Hawes, Riccardo Arosio, Stefan Bolam and Chris McCabe

October 2024

ISSN 2051-6711

Offshore Overfalls Marine Conservation Zone (MCZ)

Monitoring Report 2019

Jon Hawes, Riccardo Arosio, Stefan Bolam and Chris McCabe

October 2024

ISSN 2051-6711

For further information please contact:
JNCC, Quay House, 2 East Station Road, Fletton Quays, Peterborough, PE2 8YY

https://jncc.gov.uk/

Marine Monitoring Team (marinemonitoring@jncc.gov.uk)

This report should be cited as:
Hawes, J. Arosio, R., Bolam, S. & McCabe, C. (2024). Offshore Overfalls Marine Conservation Zone (MCZ) Monitoring Report 2019. JNCC/Cefas Partnership Report 44. JNCC, Peterborough, ISSN 2051-6711, Crown Copyright 2024.

JNCC EQA Statement:
This report is compliant with the JNCC **Evidence Quality Assurance Policy** https://jncc.gov.uk/about-jncc/corporate-information/evidence-quality-assurance/.

Funded by:
Department for Environment, Food and Rural Affairs (Defra)
Marine and Fisheries
Nobel House
17 Smith Square
London
SW1P 3JR

Please Note:
This work was delivered by Cefas and JNCC on behalf of the Marine Protected Areas Survey Coordination & Evidence Delivery Group (MPAG) and sponsored by Defra. MPAG was established in November 2012 and continued until March 2020. MPAG was originally established to deliver evidence for Marine Conservation Zones (MCZs) recommended for designation. In 2016, the programme of work was refocused towards delivering the evolving requirements for Marine Protected Area (MPA) data and evidence gathering to inform the assessment of the condition of designated sites and features by Statutory Nature Conservation Bodies (SNCBs), to inform Secretary of State reporting to Parliament. MPAG was primarily comprised of members from Defra and its delivery bodies which have MPA evidence and monitoring budgets and/or survey capability. Members included representatives from Defra, JNCC, Natural England, Cefas, the Environment Agency, the Inshore Fisheries Conservation Authorities (IFCAs) and the Marine Management Organisation (MMO).

Since 2010, offshore MPA surveys and associated reporting have been delivered by JNCC and Cefas through a JNCC/Cefas Partnership Agreement (which remained the vehicle for delivering the offshore survey work funded by MPAG between 2012 and 2020).

Executive Summary

Offshore Overfalls MCZ is an offshore Marine Conservation Zone (MCZ) located off the southern coast of Sussex, within the ‘Eastern Channel’ Charting Progress 2 (CP2) sea area. Several features are designated for protection within Offshore Overfalls MCZ, including the Broadscale Habitats (BSH) *‘Subtidal coarse sediment’, ‘Subtidal sand’ and ‘Subtidal mixed sediments’*), and a geomorphological feature (‘*English Channel outburst flood features: Quaternary fluvio-glacial erosion features’*).

This report explores environmental and ecological sample data, primarily acquired from the CEND0119 survey of Offshore Overfalls MCZ in 2019, intended to serve as the first point in a monitoring time series.

In this report we use the 2019 imagery and grab sample data to describe the epifaunal assemblages and (within the context of data limitations) the infaunal assemblages which characterise the designated features of the site. An improved habitat map has also been produced, combining 2019 acoustic data and derived epifaunal assemblage groups to provide greater resolution and confidence in the extent and distribution of the designated features across the site.

Five of the faunal assemblages identified were used to derive the new habitat map: four epifaunal assemblages derived from seabed imagery and a single infaunal assemblage from grab sample data. Three of the four epifaunal assemblages were associated with the BSH ‘Subtidal coarse sediment’, and one with ‘Moderate energy circalittoral rock’. The infaunal assemblage was associated with the BSH ‘Subtidal sand’. The approach to multivariate analysis of epifaunal data used in this report has allowed for a greater understanding of the complex assemblages associated with the ‘Subtidal coarse sediment’ BSH. Furthermore, additional work was undertaken to assess the efficacy of short (50 m) camera transects within a 100 m ‘bullring’ for characterising the epifaunal assemblage of stations. This was tested using an increased replication approach at a subset of stations, finding that assemblage did not vary spatially within the bullring, but did change significantly with increased seabed coverage. This indicates a required minimum surveyed area for effective characterisation of epifaunal assemblages at Offshore Overfalls MCZ.

In addition to improved insights on biological community composition and habitat extent within the MCZ, this report also provides significant insight into the fine scale topography of the geomorphological feature, through the exploration of the first multibeam echosounder (MBES) derived bathymetric dataset from the palaeovalley geomorphological feature.

In the discussion and recommendations sections this report addresses the challenges of establishing a monitoring regime when seabed imagery (from drop down camera platforms) is the primary means of data acquisition, offering operational and analytical guidance for future monitoring surveys at the site.

Contents

Tables

Figures

Abbreviations

ANOSIM	Analysis of Similarity
ANOVA	Analysis of Variance
AUC	Area Under Curve
BSH	Broadscale Habitats
CATAMI	Collaborative and Automated Tools for Analysis of Marine Imagery
Cefas	Centre for Environment, Fisheries and Aquaculture Science
C-H	Caliński-Harabasz criterion
CHP	Civil Hydrography Programme
CP2	Charting Progress 2
Defra	Department for Environment, Food and Rural Affairs
FOCI	Feature of Conservation Importance
FoV	Field of View
GES	Good Environmental Status
HD	High Definition
ICES	International Council for the Exploration of the Seas
IndVal	Indicator Value Metric
JNCC	Joint Nature Conservation Committee
MBES	Multibeam echosounder
MCA	Maritime and Coastguard Agency
MCC	Matthews Correlation Coefficient
MCZ	Marine Conservation Zone
MESH	Mapping European Seabed Habitats project
MHCBI	Marine Habitat Classification for Britain and Ireland
MPA	Marine Protected Area
MPAG	Marine Protected Areas Survey Coordination and Evidence Group

MSFD	Marine Strategy Framework Directive
NE	Natural England
NIS	Non-Indigenous Species
NMBAQC	North-East Atlantic Marine Biological Analytical Quality Control Scheme
nMDS	Non-metric Multidimensional Scaling
OBIA	Object-based Image Analysis
OOVR	Offshore Overfalls
OSPAR	The Convention for the Protection of the Marine Environment of the North-East Atlantic
PCA	Principal Components Analysis
PSA	Particle Size Analysis
PSD	Particle Size Distribution
RDA	Redundancy Analysis
RV	Research Vessel
SACFOR	Superabundant-Abundant-Common-Frequent-Occasional-Rare scale
SACO	Supplementary Advice on Conservation Objectives
SAD	Site Assessment Document
SAPA	Surface Area to Planar Area
SIMPER	Similarity Percentages analysis
SIMPROF	Similarity Percentages analysis
SNCB	Statutory Nature Conservation Body
SpAC	Species Accumulation Curve
SSS	Sidescan sonar
TESS	Total Error Sum of Squares
UKHO	UK Hydrographic Office
WoRMS	World Atlas of Marine Species
XGBC	Extreme Gradient-boosted Classifier

1 Introduction

This monitoring report explores data acquired from the first dedicated monitoring survey of Offshore Overfalls Marine Conservation Zone (MCZ), (hereafter referred to as ‘Offshore Overfalls’ or OOVR), survey CEND0119, which will form the initial point in a monitoring time series against which feature condition can be assessed in the future. The specific aims of the report are discussed in more detail in Section 1.2.

This report **does not** aim to assess the condition of the designated features. Statutory Nature Conservation Bodies (SNCBs) use evidence from Marine Protected Area (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 within an MPA.

1.1 Site overview

Offshore Overfalls MCZ is located in the Eastern English Channel, approximately 18 km south-east of the Isle of Wight and just north of Offshore Brighton MCZ (Figure 1). This site is jointly managed by the JNCC and Natural England, as the site straddles the 12 nm limit of inshore and offshore waters. The site is in the ‘Eastern Channel’ Charting Progress 2 biogeographic region.

The MCZ protects 593 km^2 of seabed with a depth range of 18.3 to 74.1 m below sea level (chart datum). The protected features of the site include the English Channel outburst flood geomorphological features, examples of Quaternary fluvial-glacial erosion features, as well as the Broadscale Habitats (BSH) ‘Subtidal coarse sediment’, ‘Subtidal mixed sediments’, and ‘Subtidal sand’. A 2012 site verification survey (Defra 2015), conducted by JNCC and Cefas, confirmed the presence of ‘Subtidal coarse sediment’, ‘Subtidal mixed sediments’, and ‘Subtidal sand’ at the site, leading to their designation as protected features of the site. These designated habitat features are listed in Table 1.

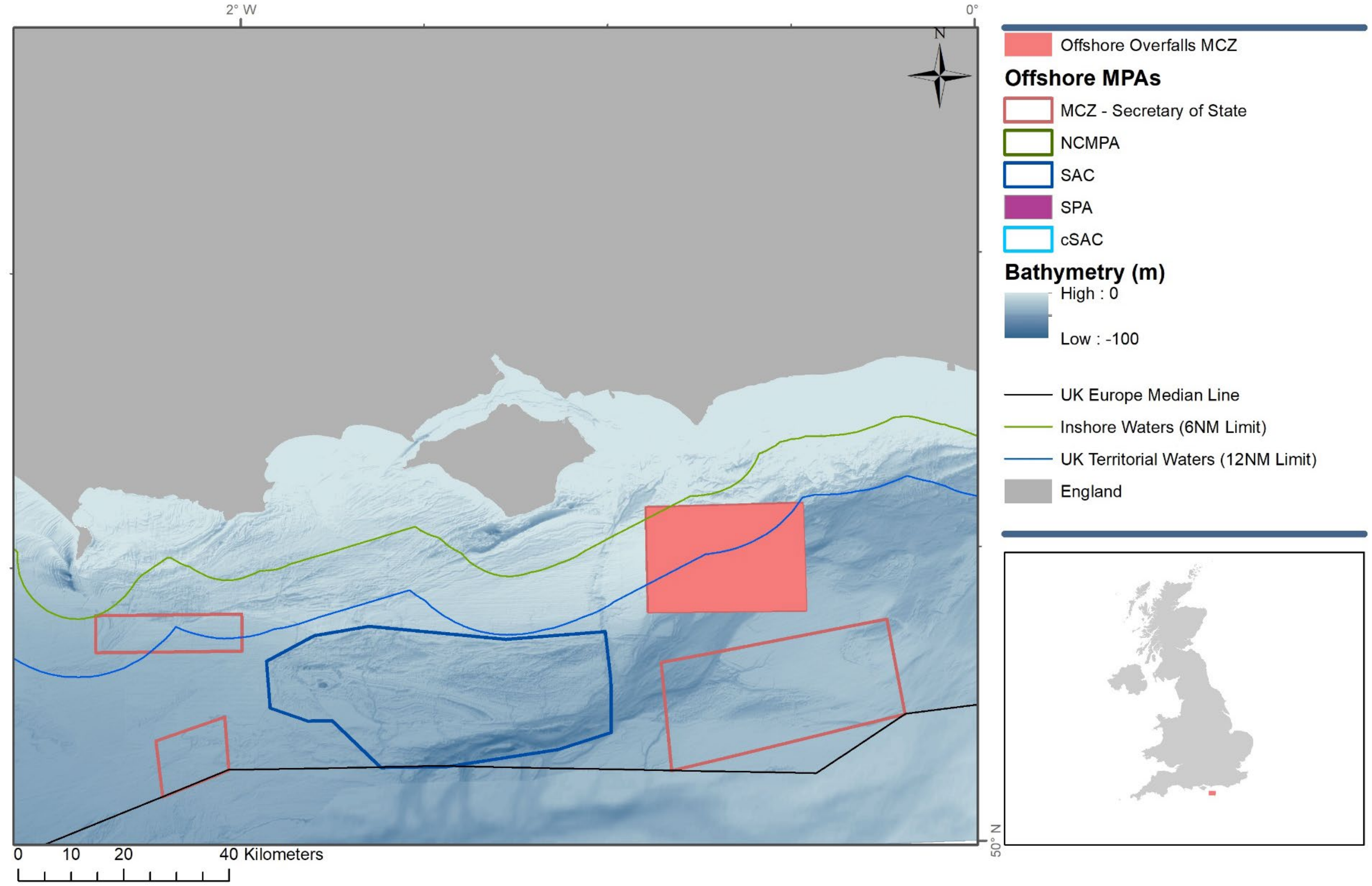

Figure 1. Location of the Offshore Overfalls MCZ. Bathymetry is taken from the Astrium (1 arc second) dataset (Astrium 2015)

Table 1. Designated features of Offshore Overfalls MCZ.

Designated Features Present within MCZ	Corresponding Marine Habitat Classification for Britain and Ireland (MHCBI) class	Designated	Addressed in this report?
Broadscale Habitat (BSH)			
Subtidal coarse sediment	Subtidal coarse sediment ((unstable cobbles and pebbles, gravels, and coarse sands) (SS.SCS)	✓	✓
Subtidal mixed sediments	Subtidal mixed sediments (SS.SMx)	✓	✓
Subtidal sand	Subtidal sands and muddy sands (SS.SSa)	✓	✓
Geomorphological Feature			
English Channel outburst flood features (Quaternary fluvio-glacial erosion features)	N/A	✓	✓

1.2 Existing data and habitat maps

A site verification survey was undertaken of the Offshore Overfalls (as was then) recommended MCZ (rMCZ) in 2012, under the MB0120 Defra data collection programme (Vanstaen *et al.* 2016). Ground truth samples were acquired from the RV *Cefas Endeavour* in 2012 (CEND0812c). Multibeam echosounder (MBES) and side scan sonar (SSS) data were collected along prospecting lines across the site, with additional areas targeted for potential features of conservation interest. Sediment samples were collected using a 0.1 m^2 mini-Hamon grab and imagery data using an underwater camera sledge. In total 59 grab sample attempts were made, alongside 21 video tows and 289 still images collected across the site. However, only 22 of the 59 grab samples met the minimum sediment volume threshold of 5L recommended by Ware and Kenny (2011), indicating the coarse nature of the substrate at the site; this contributed to the consequent rationale for future survey efforts to focus on seabed imagery. A full account of the survey methods and results of the verification survey can be found in Mellett and Green (2015).

MBES bathymetry data for Offshore Overfalls were collected during the CEND0812c survey and as part of the MCA (Maritime and Coastguard Agency) and UKHO (United Kingdom Hydrographic Office) Civil Hydrography Programme (CHP). During CEND0812c, transit MBES data was collected and two MBES and SSS lines were run across the site in an east-west orientation, covering less than 8% of the site. Low resolution (30 Arc Second) seabed bathymetric data are also available for the whole site in the form of the Defra Digital

Elevation Model (Astrium 2015). Extensive CHP bathymetry data were acquired in 2017 (under codes HI1498 and HI1499), however these did not include a large section (259.9 km^2) in the south and east of the site (Figure 2). A predictive habitat map was created from these MBES and ground truth data, herein referred to as the '2015 habitat map' and shown in Figure 3.

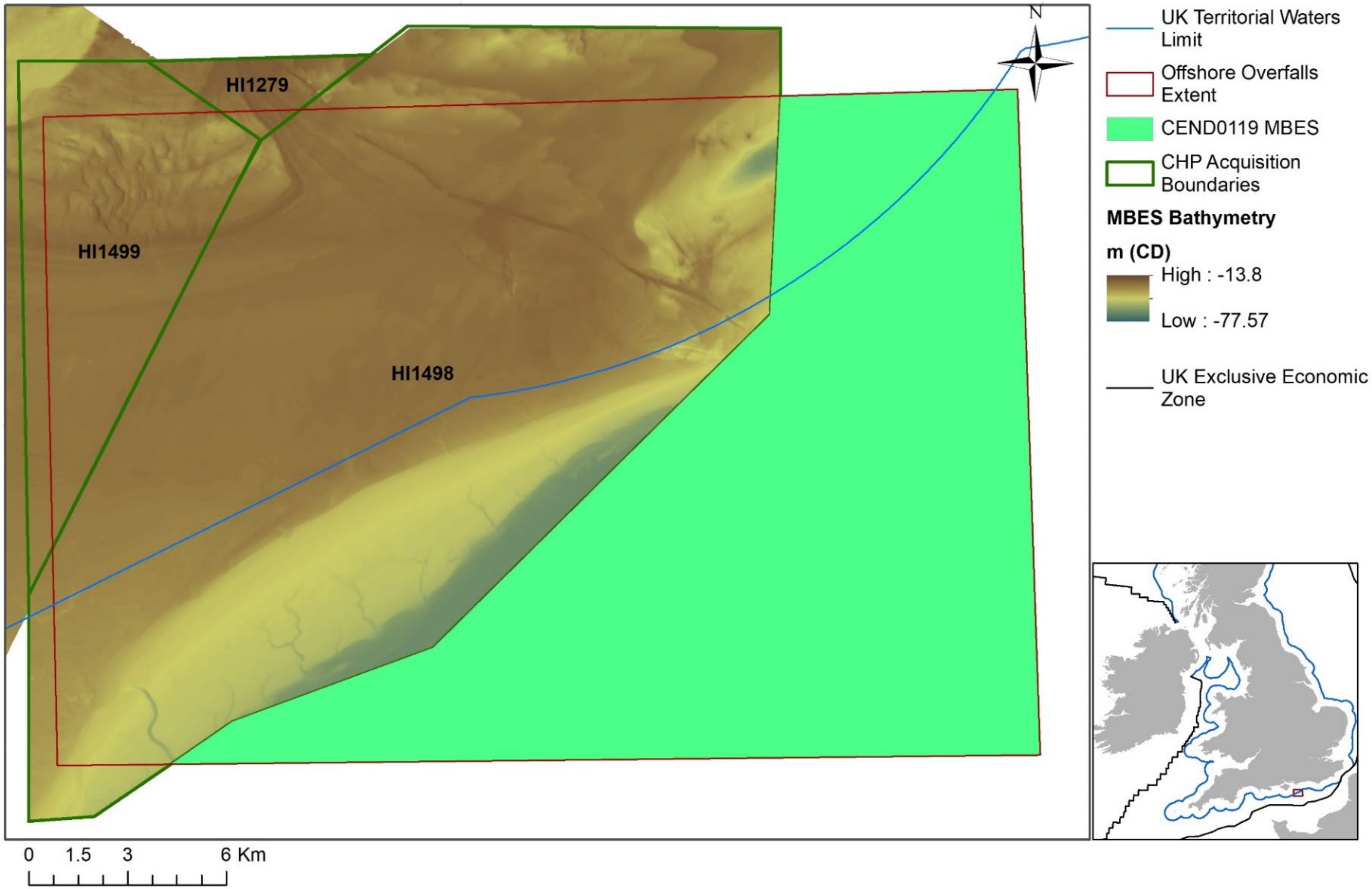

Figure 2. The area of pre-existing MBES-derived bathymetric data available for Offshore Overfalls MCZ. Maritime and Coastguard Agency data is available from three survey areas at Offshore Overfalls, which are annotated with their 'HI' number.

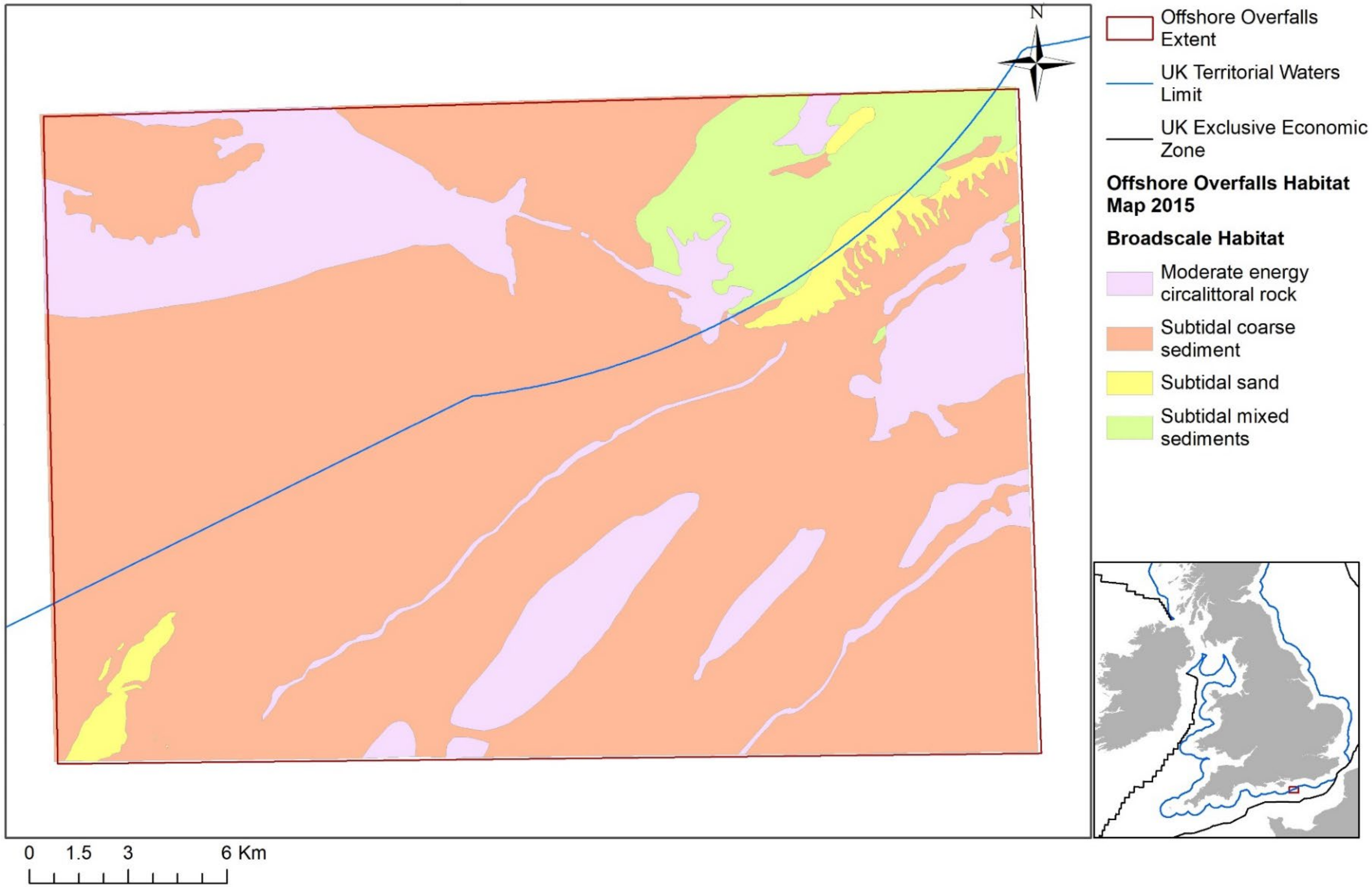

Figure 3. 2015 habitat map for Offshore Overfalls MCZ, showing predicted extent of observed Broadscale Habitat types.

1.2.1 Tidal dynamics

Maximum (peak ebb and peak flood) tidal current velocities (m s^{-1}) at the seabed were predicted using a tidal model built for Offshore Overfalls MCZ. The depth-averaged model for Offshore Overfalls MCZ is nested within a larger English Channel model (which extends into the Bristol Channel) and has been built using an unstructured triangular mesh, using the software Telemac2D (v7p1).

Modelled peak flood and peak ebb tidal current magnitudes within Offshore Overfalls MCZ varied between 0.02 m s^{-1} (Figure 4) and 0.65 m s^{-1}, respectively (Figure 5). The highest magnitudes are observed within the south-east section of the site (along the palaeovalley feature) at peak ebb tide, and within the north-west section at peak flood tide. Tidal current directions at peak ebb and peak flood vary significantly across the south-east section of the site, with a north-east flow during flood and a south-west flow 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, resulting in the unidirectional south-west flow seen at both states of tide in the north-west of the site. Of note is the low predicted tidal magnitude within the palaeovalley channel at peak flood, in comparison to the strong tidal flow (south-west) at peak ebb, indicating a highly dynamic seabed environment.

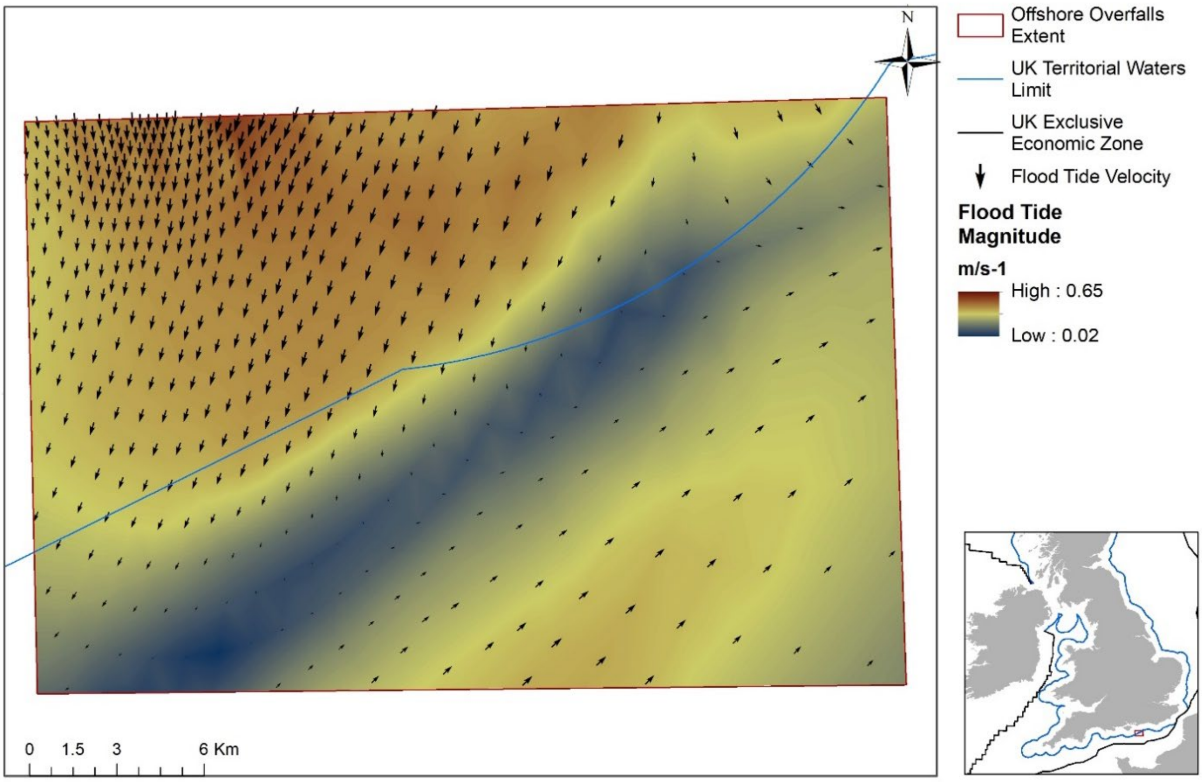

Figure 4. Direction and magnitude (black arrows) of tidal flow at peak flood tide at Offshore Overfalls MCZ.

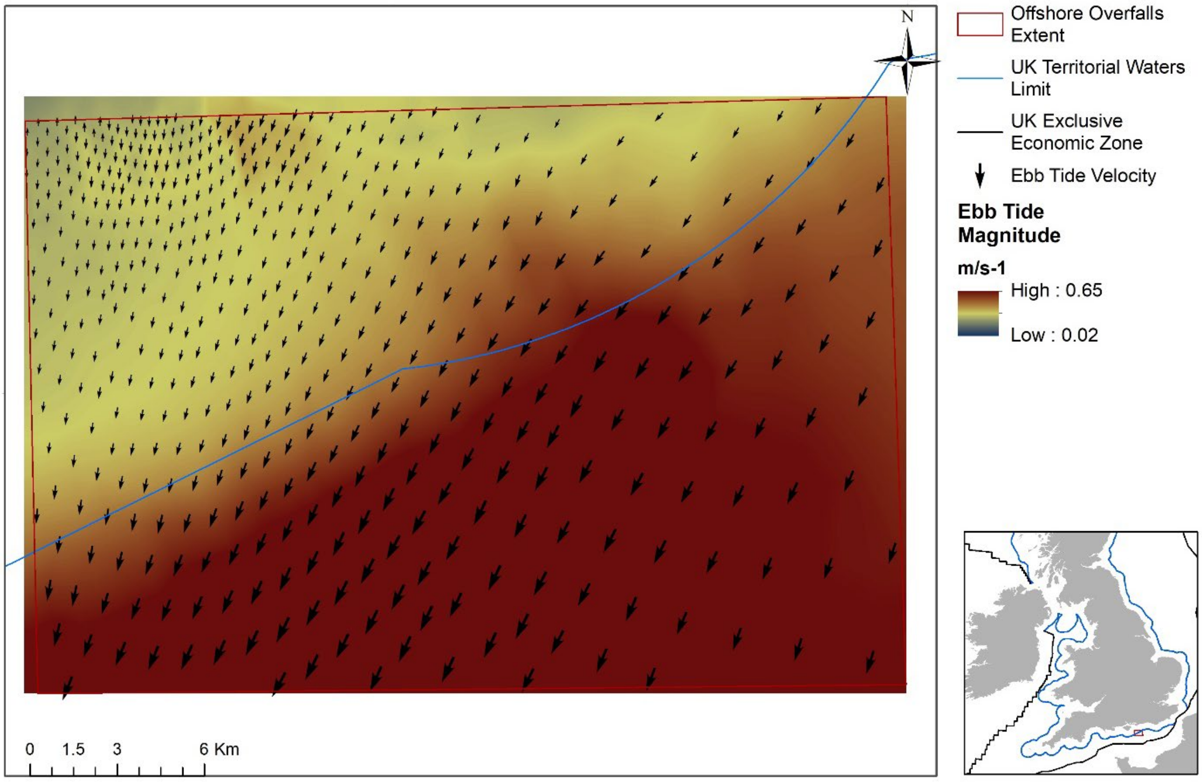

Figure 5. Direction and magnitude (black arrows) of peak tidal flows at peak ebb at Offshore Overfalls MCZ.

1.3 Aims and objectives

1.3.1 Conservation objectives

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

As detailed in the conservation objectives for the site, the designated features must:

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 Report aims and objectives

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

The objectives of this monitoring report, and the associated outputs are provided in Table 2.

To achieve report objective 1, selected Feature Attributes and supporting processes of the designated features are described as defined in JNCC Supplementary Advice on

Conservation Objectives (SACOs) for Offshore Brighton MCZ from March 2018 (JNCC 2018). Offshore Brighton is geographically adjacent and designated for the same features (see Table 2) and has been used as Offshore Overfalls did not have a site-specific SACO when this report was completed.

Table 2. Offshore Overfalls MCZ report objectives and outputs.

Objective		Feature Attribute*	Broadscale Habitat (BSH) Features / Entire MCZ	Output
Objective 1	Provide a description of the **extent distribution and structural** attributes of the designated features within the site.	**Biological structure**: Characteristic communities Key and influential species	Subtidal coarse sediments Subtidal mixed sediments Subtidal sand	a) Conduct multivariate analysis of epifaunal data to: I. Identify patterns in biological assemblages using *k*-mean clustering. II. Relate the derived assemblages to each of the three BSH features. III. Identify key and influential species associated with the derived assemblages. IV. Identify any potential indicator taxa (and evaluate them according to the criteria in Table 22). V. Assess relationship between environmental variables and assemblages using Redundancy Analysis (RDA).
		Extent and distribution	Subtidal coarse sediments Subtidal mixed sediments Subtidal sand	b) Generate an updated habitat map to determine the predicted extent of BSH features within the MCZ. I. Assign and map biotopes (where possible)

Objective		Feature Attribute*	Broadscale Habitat (BSH) Features / Entire MCZ	Output
				c) Plot the point distribution of BSH within the MCZ.
		Physical structure: finer scale topography	Subtidal coarse sediments Subtidal mixed sediments Subtidal sand English Channel outburst flood feature (Quaternary fluvio-glacial erosion features)	d) Generate a geomorphological map of seabed features within the site, with reference to features associated with the English Channel outburst flood. I. Evaluate biotopes in the context of the geomorphological classes.
		Physical structure: sediment composition	Subtidal coarse sediments Subtidal mixed sediments Subtidal sand	e) Describe and display the composition and distribution of sediments across the MCZ, with reference to the designated features and habitat map.
Objective 2	Assess within-station variability using assemblage data acquired from high-replication imagery stations.	n/a	Entire MCZ	a) Explore and discuss the efficacy of the sampling strategy for future monitoring survey. I. Comparison of Species Accumulation Curves and univariate metrics to assess optimum seabed sample area.

Objective		Feature Attribute*	Broadscale Habitat (BSH) Features / Entire MCZ	Output
				II. Comparison of epifaunal assemblages by four treatments of epifaunal assemblage – multivariate analysis of variance.
Objective 3	Note observations of any FOCI or SOCI not covered by Designation Order as features of the site.	n/a	Entire MCZ	a) Plot locations of undesignated FOCI or SOCI.
Objective 4	Present any evidence of non-indigenous species and marine litter within the site	n/a	Entire MCZ	a) Plot locations and densities of non-indigenous species and marine litter.
Objective 5	Provide practical recommendations for appropriate future monitoring approaches for the designated features (e.g. metric selection, survey design, data collection approaches) with a discussion of their requirements	n/a	Entire MCZ	

* As defined in Supplementary Advice on Conservation Objectives (SACO) for the Offshore Overfalls MCZ.

2 Methods

2.1 Survey design

In January 2019 a dedicated monitoring survey was conducted at the Offshore Overfalls MCZ, onboard the RV *Cefas Endeavour* (CEND0119). Further information regarding this survey can be found in the corresponding survey report (Wood *et al.* 2020).

2.1.1 Acoustic acquisition

Acoustic acquisition was planned to fill in the large gaps to the east of the site (as seen in Figure 2). Acoustic data were collected using a Kongsberg EM2040 multibeam echosounder (MBES) at 300 kHz.

2.1.2 Benthic sampling overview

Seabed sampling was split by equipment type into grab samples and seabed imagery (camera) samples. Further division of stations according to analytical purpose was then undertaken, with ‘grid’ stations (spatially distributed over a regular grid) representing the primary monitoring points. ‘Increased replication’ stations were a subset of the grid stations which were to have replicate samples taken (imagery and grab) to investigate within-station variation. ‘Ground truth’ stations were imagery-only stations, with longer transects planned (15 minutes) to assess features observed from the acoustic data. Table 3 presents the benthic sampling strategy in full.

Table 3. Overview of station types and samples in the 2019 survey of Offshore Overfalls MCZ.

	Sample type	***Stations planned***	***Stations attempted***	***Replicates***	***Ad-hoc stations***	***Ad-hoc stations sampled***	***Total samples***
Camera	Grid stations	60	60	1 x 5 min transect	—	—	60
	Increased replication stations	8	8	4 x 5 min transects	—	—	32
	Ground truth stations	7*	7	1 x 15 min transect	7	7	14
Mini Hamon	Grid stations	~20	12	1	—	—	7
	Increased replication stations	—	—	4	7	—	17
	Ground truth stations	—	—	3	14	14	31

2.1.3 Infaunal survey strategy

As a review of 2012 sediment sampling success had shown a limited potential for acquisition of suitable sediment samples (i.e. greater than 5 L of sediment retained), it was determined appropriate to reduce the minimum sample volume threshold to 4 L. As such, a limited programme of sediment sampling was undertaken with the following aims:

- A single replicate grab sample was to be collected from a subset of the grid and ground truth camera stations, where the substrate type was deemed suitable for sediment sampling.
- A subset of the stations where grab sampling was possible was to be used as increased replication stations (five replicates per station).
- An additional aim was included whilst at sea to sample the ‘Subtidal sand’ feature as provisionally mapped whilst on survey (‘Ad-hoc’ sampling).

Drop down video operations were undertaken across all stations before sediment sampling (as described in Section 2.1.2). This allowed for onboard review of the likely success of acquiring a valid sediment sample from each station. After review, sediment sampling was deemed appropriate at nine stations, with five chosen for increased replication. Fourteen further (‘Ad-hoc’) sediment sampling stations were positioned during the survey, in an area of mobile megaripples in the north-east of the site and the area of predicted ‘Subtidal sand’ in the south-west (seven in each area). This sediment sampling strategy is presented in Figure 6.

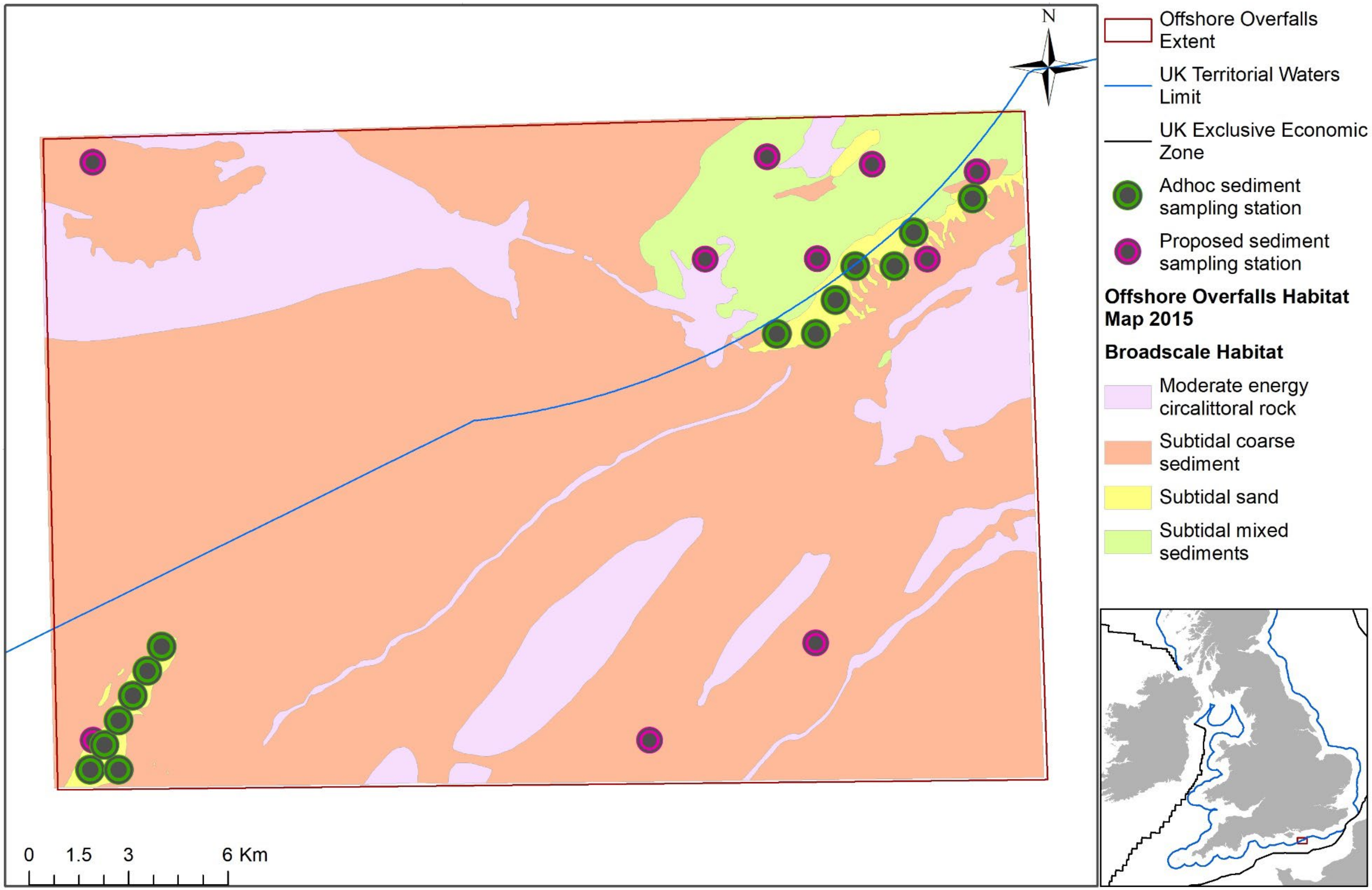

Figure 6. Location of planned sediment sampling stations at Offshore Overfalls MCZ in 2019, overlaying the 2015 habitat map.

2.1.4 Epifaunal survey strategy

Given the known nature and extent of the coarse gravel substrates at the site (from review of grab success data from the 2012 characterisation survey), a drop camera system was selected for seabed imagery acquisition, as the primary source of monitoring data.

An equidistant triangular grid (3.5 km spacing) of 60 stations (the ‘grid stations’) was planned as the primary means of addressing Objective 1, providing a Time 0 (T0) ‘Sentinel Monitoring’ dataset (see Figure 7). The total number of grid stations was chosen based on available time and the complexity of the existing habitat map (Figure 3), as opposed to being based on power analysis (due to insufficient data availability). Bullrings with 100 m diameters were superimposed on the navigation software for each sampling station and a single 50 m drop camera transect was run, crossing the centre of the bullring, with still images taken every 10 seconds. Shorter transects were selected (relative to previous MPA surveys) based on the rationale that greater transect lengths at this MCZ would result in likely higher habitat variability covered on a single line, and thus that longer transects increase ‘within-station’ variability. The drop camera system utilised obliquely mounted (downward facing) High Definition (HD) video and stills cameras, whilst the altitude of the camera frame was maintained and recorded, resulting in standardised and quantitative fields of view (FoVs) for each still image, thereby allowing density values to be calculated for each taxon.

A subset of eight grid stations was selected for acquisition of replicate samples (the ‘increased replication stations’), designed to assess within-station variability and address Objective 2. A total of five transects (50 m / 5 minutes) were located within the bullrings of these stations, orientated in parallel to the central transect, spaced 10 m apart. The increased replication stations were chosen to include representation from all predicted BSH types. A study by Lim *et al*. (2018) has indicated that still images from replicate parallel transects taken across small areas (e.g. squares of 50 m x 50 m) yield highly resolved epifaunal community datasets which can account for within-station variability and provide a basis for monitoring of these communities. Data were acquired from these increased replication stations to assess the benefits of this approach, versus a single 50 m transect.

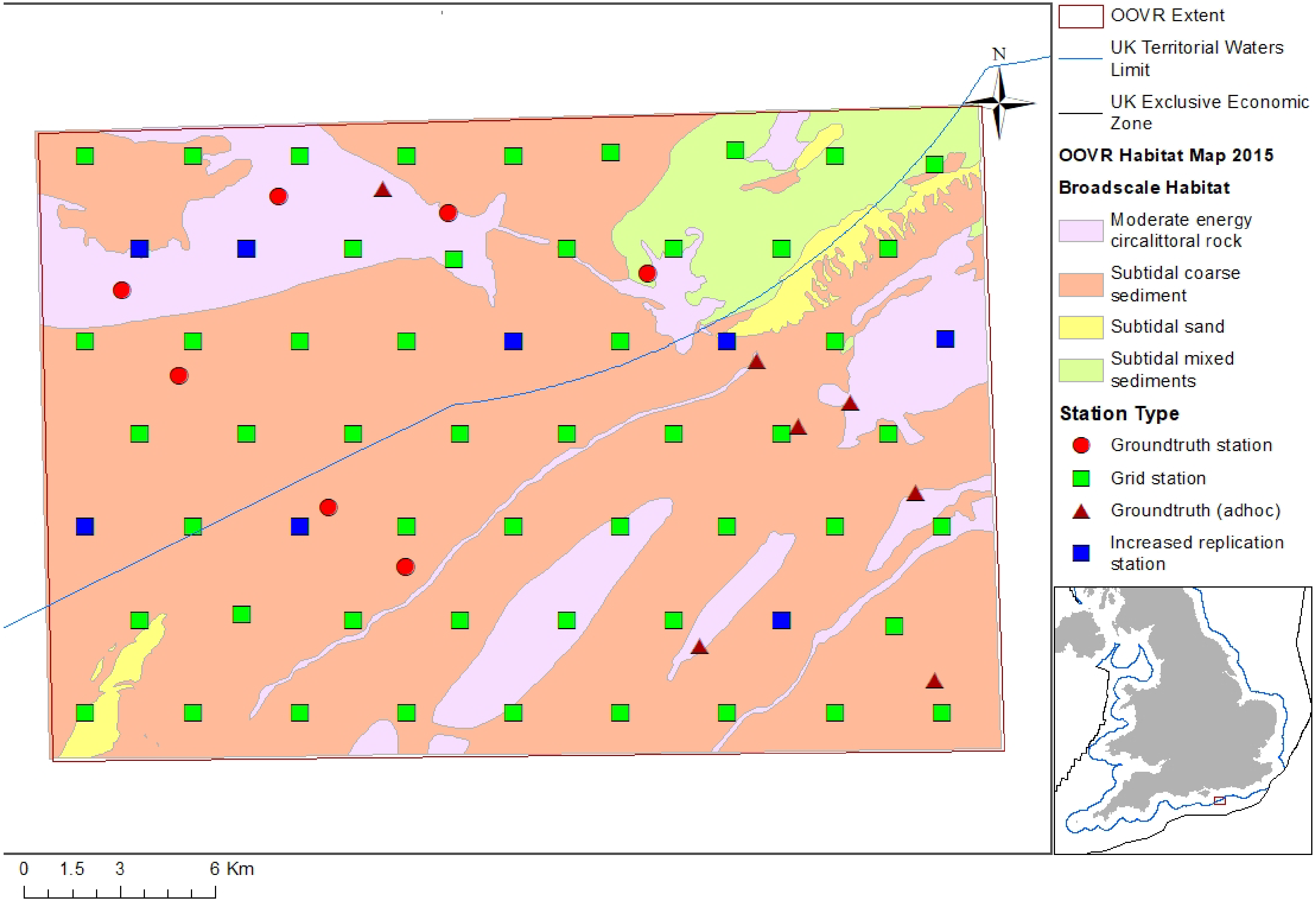

Figure 7. Locations of planned imagery stations at Offshore Overfalls (OOVR) MCZ in 2019, overlaying the 2015 habitat map**.**

Additional 'ground truth' stations were located in the areas of previously (CHP HI1498) and newly (CEND0119) acquired MBES data at Offshore Overfalls. These 15 minute (300 m) transects were located to ground truth bathymetric, predicted habitat and geomorphological features not covered by the grid stations. Seven ground truth stations were chosen prior to the survey, having been manually identified using the HI1498b data. Identification of these stations was achieved through Object-Based Image Analysis (OBIA), with an initial segmentation allowing for classification of areas of homogenous depth, backscatter intensity and topography. During the CEND0119 survey seven additional ground truth stations were chosen based upon OBIA of the newly acquired MBES data.

2.2 Data acquisition

2.2.1 Sediment sample acquisition and processing

Sediment samples for particle size distribution (PSD) and infaunal analyses were collected using a 0.1 m^2 mini Hamon grab (see Annex 3.2 for more detailed information). Owing to the findings of the previous survey (namely the inability to acquire 5 L minimum sediment sample volume from the site) the decision was taken to reduce the threshold to 4 L.

2.2.2 Seabed imagery acquisition

Seabed imagery data (video footage and stills) were collected using an STR SeaSpyder 'Telemetry' drop down camera system, following an approach modified from the Mapping European Seabed Habitats (MESH) recommended operating guidelines (Coggan *et al.* 2007; see Annex 3.3 for more detailed information).

2.3 Data preparation and analysis

2.3.1 Infaunal data preparation

The infaunal data were checked against the World Register of Marine Species (WoRMS) to ensure consistent nomenclature. Discrepancies were resolved using expert judgement following the truncation steps presented in Annex 4.

2.3.2 Seabed imagery selection and preparation

With quantification being a key aim of these analyses, the decision was taken to analyse a subset of the still images from each of the acquired transects. The images were filtered by field of view (given the acquired altimetry data, laser image scaling, and the oblique angle of orientation). The altitude range used for image filtering was between 0.7 and 1.2 m from the seabed. As a still image was taken every 10 seconds, the resulting number of raw images was too large for cost-effective analysis. As such, a randomly selected subset of a constant number of images per station and station type was selected for annotation. It was considered that 15 images over a 50 m transect was likely to retain sufficient information for the purpose of broad assemblage characterisation. The number of images per station type is detailed in Table 4.

Table 4. Seabed imagery acquired sample numbers.

Station Type	Number of Stations	No. of Replicates	Transect Length (m)	No. of Utilised Stills	Objective
Grid	60 (imagery) 9 (sediment)	1	50	15	1.a. i-v & 1.b.i
Increased replication	8	5	50	15 (per replicate)	3.a. i-ii
Ground truth	15	1	250	15 (per segment)	1.b.i

To further improve repeatability and accuracy of image analysis, the BIIGLE online image annotation platform was used (Langenkämper *et al.* 2017). This platform allows for instant collaboration and review of annotations between analysts, alongside controllable label hierarchies and accurate point placement. Furthermore, polygon annotation tools allow for much greater accuracy in ground cover annotations.

The ability to control the label hierarchy enabled each entry (taxonomic or morphological) to be nested within a morpho-taxonomic tree, as presented in Jones *et al.* (2020), which in turn is derived from the CATAMI structure (Hill *et al.* 2014). The resulting matrices (ground cover and point count) were then exported from BIIGLE, with the ground cover values converted into percentage cover values (a ratio of annotation pixel area to image pixel area). Both matrices were then truncated as described in Annex 3, with the resulting taxa checked using the WoRMS ‘Match Taxa’ tool.

As Table 4 indicates, not all station types were used to address all objectives. Specifically, the increased replication stations were not included in the primary selection (addressing Objectives 1a and 1b). These were instead used only to address Objective 2. Ground truth stations were used in the assemblage analysis for mapping purposes only, and this was achieved by creating samples from a random selection of 15 still images from each of the distinct video segments (areas of continuous BSH type) which comprised the transect.

The resulting matrices were then normalised and combined (see Annex 3.4) to produce a single ‘relative abundance matrix’, which was in turn further transformed into a ‘relative density’ matrix. An overview of the data preparation and analysis process is provided in Figure 8.

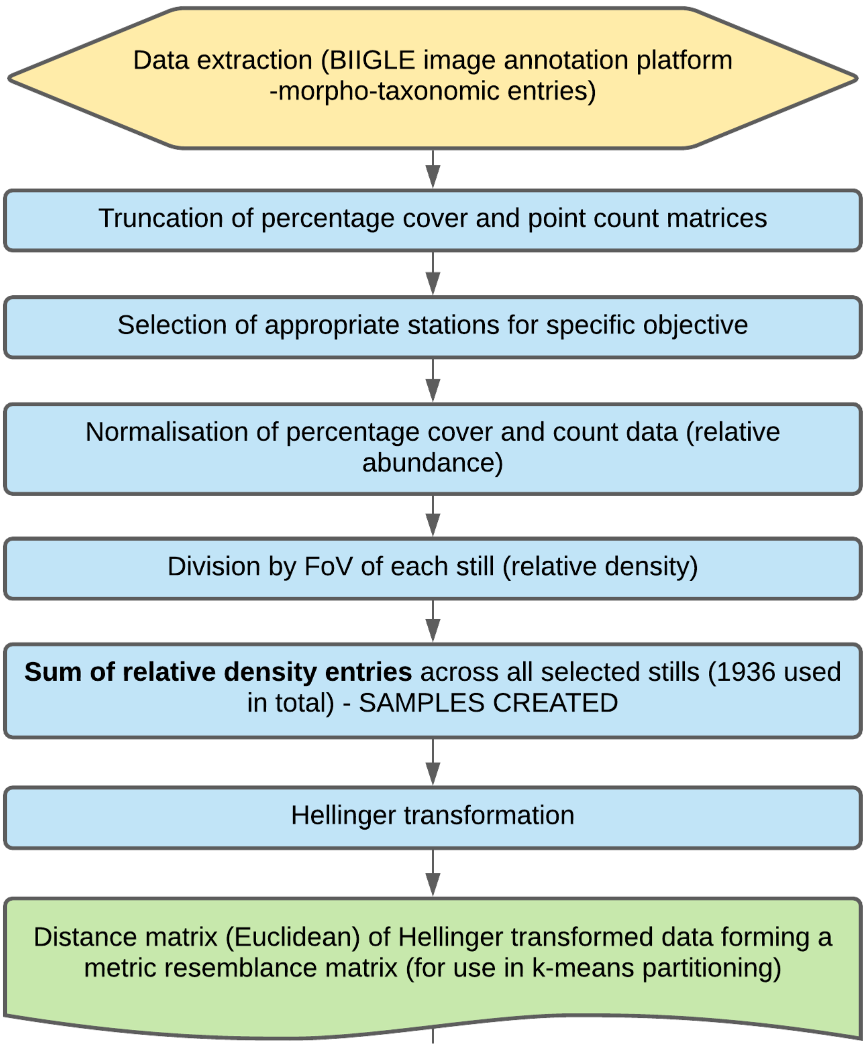

Figure 8. Preparation and processing schematic for seabed imagery data.

2.4 Statistical analyses

2.4.1 Infaunal assemblage analysis

A very limited number of successful (greater than 4 L) sediment samples were acquired from the site, with many successful samples being located in the targeted areas of the 'Subtidal sand' BSH type.

Highly variable taxon counts were down-weighted in the infaunal matrices using a dispersion weighting (Clarke *et al.* 2006) within Primer v7 (Clarke & Gorley 2015) and Bray-Curtis similarity matrices were produced from the square root transformed data for both samples and variables. Non-metric multidimensional scaling ordinations (nMDS) were produced for the infaunal data to illustrate differences in assemblage structure within and between samples, as depicted by their sediment BSH class.

Infaunal assemblages were derived using the hierarchical agglomerative clustering routine in Primer v7, whereby the Similarity Profile (SIMPROF) algorithm was used to test whether a suitable number of cluster groups had been reached at the 5% significance level. The similarity percentage (SIMPER) routine was used to highlight the taxa contributing to within-group similarity (Clarke & Warwick 1994).

2.4.2 Epifaunal assemblage analysis

Several statistical analyses, both univariate and multivariate, were used to address Objectives 1 and 2 (some outputs were used to address multiple objectives or sub-objectives). These methods are described in detail in Annex 3.4. An overview of the methods alongside the objectives and sub-objectives they were used to address is presented in Figure 9.

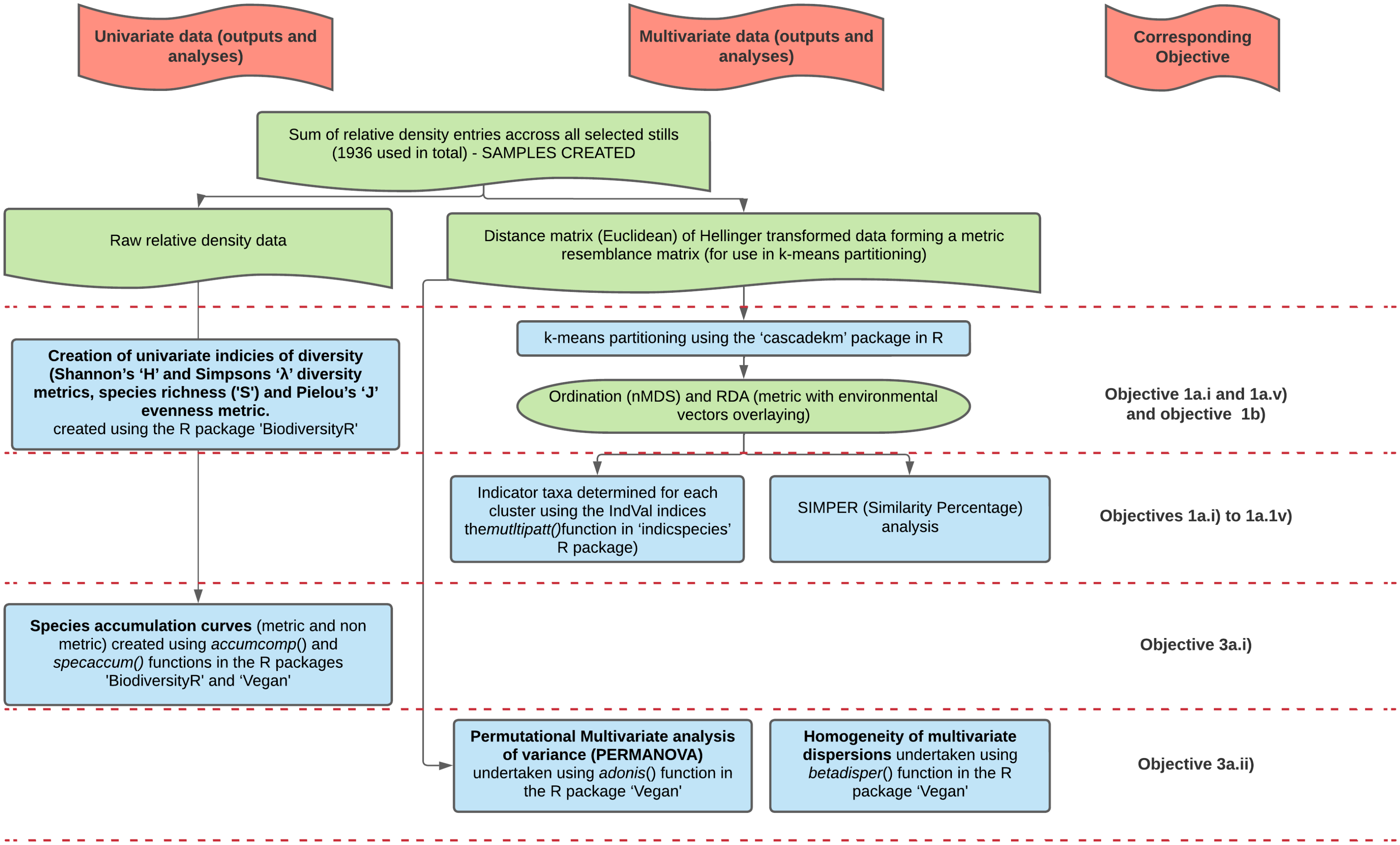

Figure 9. Univariate and multivariate analyses schematic for seabed imagery data.

2.5 Habitat mapping

A new habitat map was created to predict the extent of BSH features within the MCZ. Owing to the difficulty in assigning BSH type to imagery data (through subjectivity of grain size estimation in gravel and cobble dominated environments) and the robust quantitative nature of the assemblage and RDA analyses, the habitat mapping process was driven by assemblage, and geomorphological data.

Bathymetric, acoustic reflectance, and tidal magnitude data (the ‘environmental data layers’) were first segmented in eCognition 9. A morphological map was then created using a threshold-based classification of the resulting objects. A machine learning algorithm was then trained using the five identified assemblage classes, and the model was used to predict the class of each object based on that object’s environmental data values. Based on the strong relationship between assemblage class and observed (subjective particle size, recorded depth) and remotely sensed (backscatter, bathymetry, and derivatives) environmental parameters, each assemblage class could be back-correlated with an equivalent BSH class. The full methodology is provided in Annexes 3.5 and 3.6.

This back-correlation was then cross-validated for accuracy, with the predicted (back-correlated) BSH compared against the observed (subjective) BSH. Figure 10. presents the habitat mapping workflow.

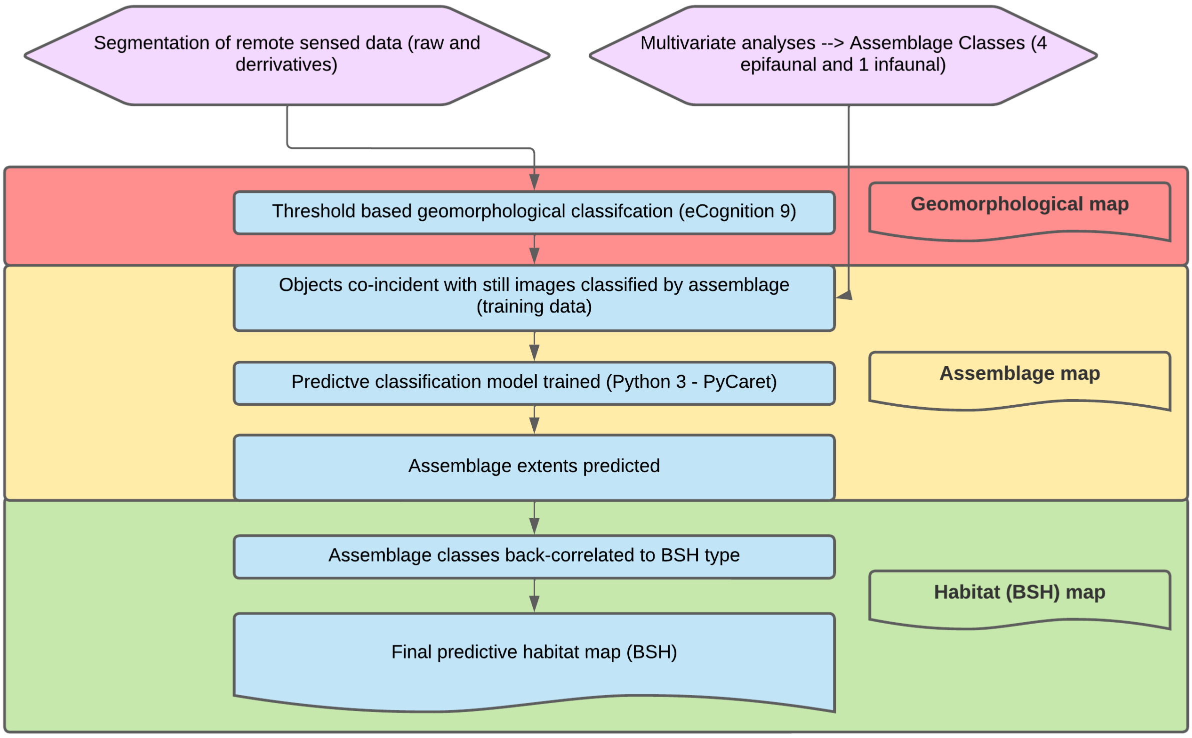

Figure 10. Schematic outlining predictive habitat methodology at Offshore Overfalls MCZ.

3 Results

Objective 1.b of this report was to create an updated BSH map. This updated map was informed by epifaunal community analysis and predictive assemblage mapping, the results of which are presented in Section 3.5 and Section 3.6. The updated BSH map, and extent and distribution results, are presented here first for clarity.

3.1 Broadscale Habitats

Broadscale Habitats were assigned to stations primarily from analysis of video segments, as a single still image cannot provide a sufficient seabed area to accurately (and consistently) determine BSH type. This ‘initial’ analysis of BSH type (video segment, still images and sediment samples) found ‘Subtidal coarse sediment’ to be the dominant habitat, with 83% of all video segments classified as this BSH.

Of primary significance in a comparison of the 2015 and 2019 BSH classifications and extents is the discrepancy between the assigned energy levels of the circalittoral rock BSH types. In 2015 the initial analysis of BSH by video segments indicated that all circalittoral rock observed was ‘Moderate energy’. In 2019, analysts concluded that almost all observations (17 out of 18 segments) were ‘High energy’. Table 5 presents the results of the 2015 to 2019 comparison.

Table 5. Number of samples collected in each Broadscale Habitat. ‘In’ represents those samples which match the underlying 2015 prediction, and ‘Out’ represents those which do not match the 2015 predictions.

Broadscale Habitat (BSH)	Grab – PSA and Infauna (44)		Video Segments (129)		Stills (2116)
	In	Out	In	Out	In
High energy circalittoral rock	n/a	n/a	0	17	127
Moderate energy circalittoral rock	n/a	n/a	1	0	40
Subtidal coarse sediment	27	5	71	31	1857
Subtidal sand	6	3	0	1	42
Subtidal mixed sediments	11	23	2	4	36
Subtidal biogenic reef	n/a	n/a	0	2	14

3.1.1 2019 updated BSH habitat map

The creation of the assemblage map (see Annex 3.5) allowed for a process of back-correlation to derive a BSH habitat map with an accuracy of 65% (moderate to high) in post-hoc validation, as compared against the initial BSH assignments of 2019 video segments. The final predicted BSH extents are presented in Figure 11. Table 6 shows the habitat classes and associated BSH types, alongside the area covered by each BSH. The final habitat map based on the 2019 acquired data is shown in Figure 11 and includes the distribution of BSH types observed from both seabed imagery and sediment sampling.

Table 6. Broadscale Habitat extents predicted by the 2019 map at Offshore Overfalls MCZ.

Broadscale Habitat (BSH)	Equivalent Assemblage Class	Area 2019 (km^2)
High energy circalittoral rock	1	100.34
Moderate energy circalittoral rock	1	0
Subtidal coarse sediment	3 & 4	419.12
Subtidal sand	5	7.96
Subtidal mixed sediments	2	68.45
Subtidal biogenic reef	1	n/a

Direct comparison of the 2019 acquired BSH samples (assigned through initial analysis of the video data and used to produce the 2019 habitat map) with those used to produce the 2015 habitat map shows a substantially smaller number of 2019 samples classed as ‘Moderate energy circalittoral rock’ than previously predicted. This is due to the issue discussed above, wherein analysts assigned all circalittoral rock identified in the 2014 survey as ‘moderate energy’, whereas separate analysis of the 2019 data determined the energy level to be, for the most part, ‘high’. The decision was taken to re-classify these 2019 samples as the BSH ‘Moderate energy circalittoral rock’ after review of the epifaunal structure, characterising species, tidal magnitudes, and most relatable biotopes.

The 2015 and 2019 habitat maps are shown to be highly comparable, with very small percentage reductions in the extent of three of the features and a moderate increase in the predicted extent of ‘Subtidal mixed sediments’. This is not an actual change, simply a more accurate reflection of the real distribution of these habitats. The 2015 map was based on the limited data which was available at the time.

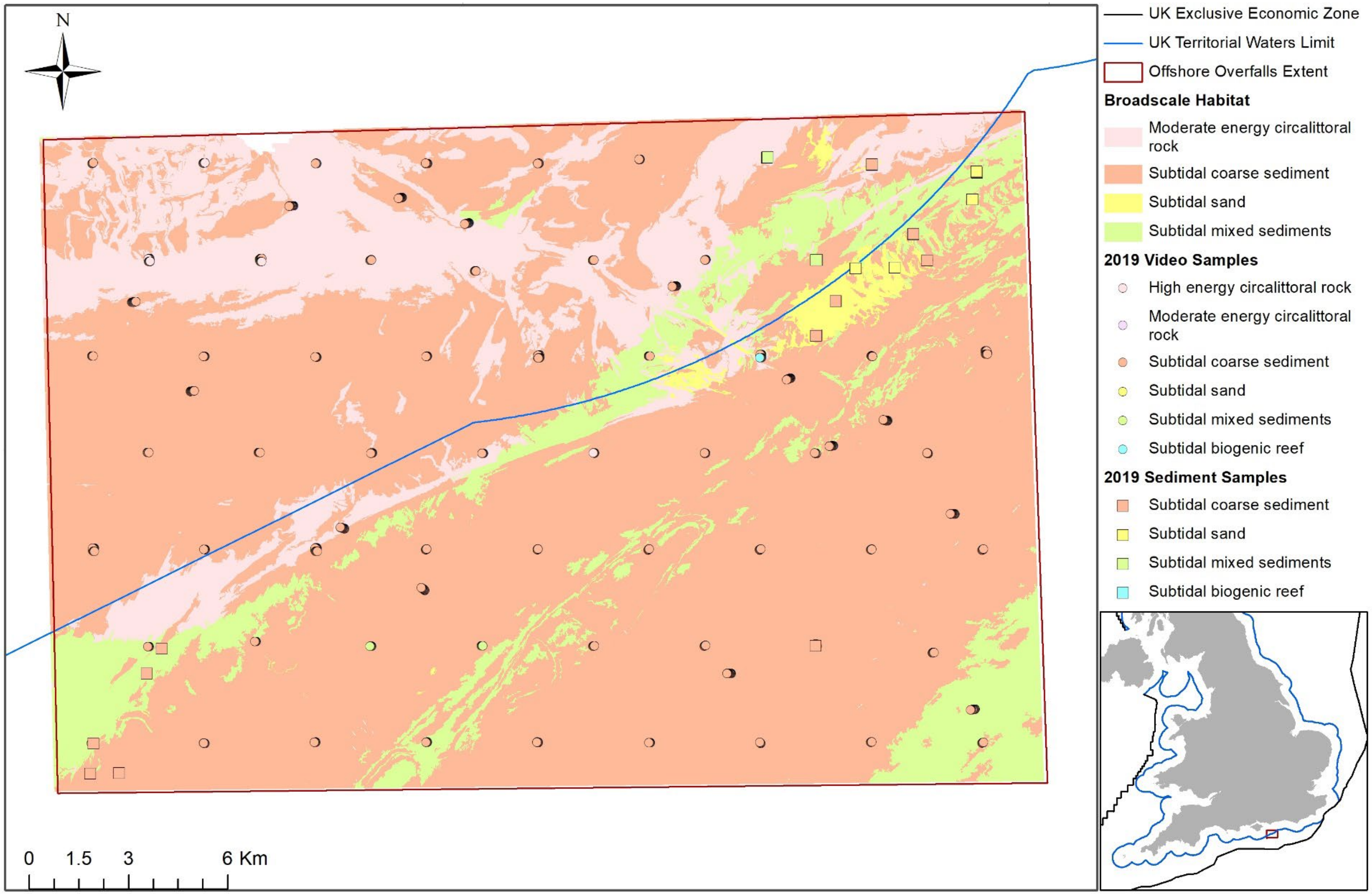

Figure 11. 2019 habitat map showing Broadscale Habitat (BSH) extent and distribution at Offshore Overfalls MCZ. Circles represent still images with video segments assigned BSH. Squares indicate BSH of sediment samples.

3.2 Benthic and environmental overview

3.2.1 Overview of sampling success

All planned seabed imagery stations were sampled successfully, as displayed in Figure 11. Sediment sampling success was limited, with the initial plan of 20 stations revised whilst on survey, after review of the seabed imagery. Following the data review, nine grid stations were selected for sediment sampling, yielding successful samples at six stations. Increased sediment sampling replication was planned for five stations and successful only at three; however, at two stations only three and four replicates were achieved, resulting in an imbalanced dataset. Table 7 presents an overview of the planned and acquired sediment samples, whilst sediment sampling success is presented spatially in Figure 12.

Table 7. Mini Hamon grab sampling for PSA and macrofauna at Offshore Overfalls MCZ. Note that invalid samples and failed attempts are not included. *It was estimated that 20 stations would provide valid samples; however, in practice only 12 showed suitable substrate.

Survey objective	Stations planned	Stations successful	Planned replicates	Total successful samples
Grab stations for monitoring and ground truthing (grid stations)	20*	6	1	6
Grab stations for monitoring (Increased replication stations)	—	4 (subset of above)	5	21 (including A1 reps from 6 grid stations)
Grab stations for monitoring the ‘Subtidal sand’ BSH	—	12	3	23

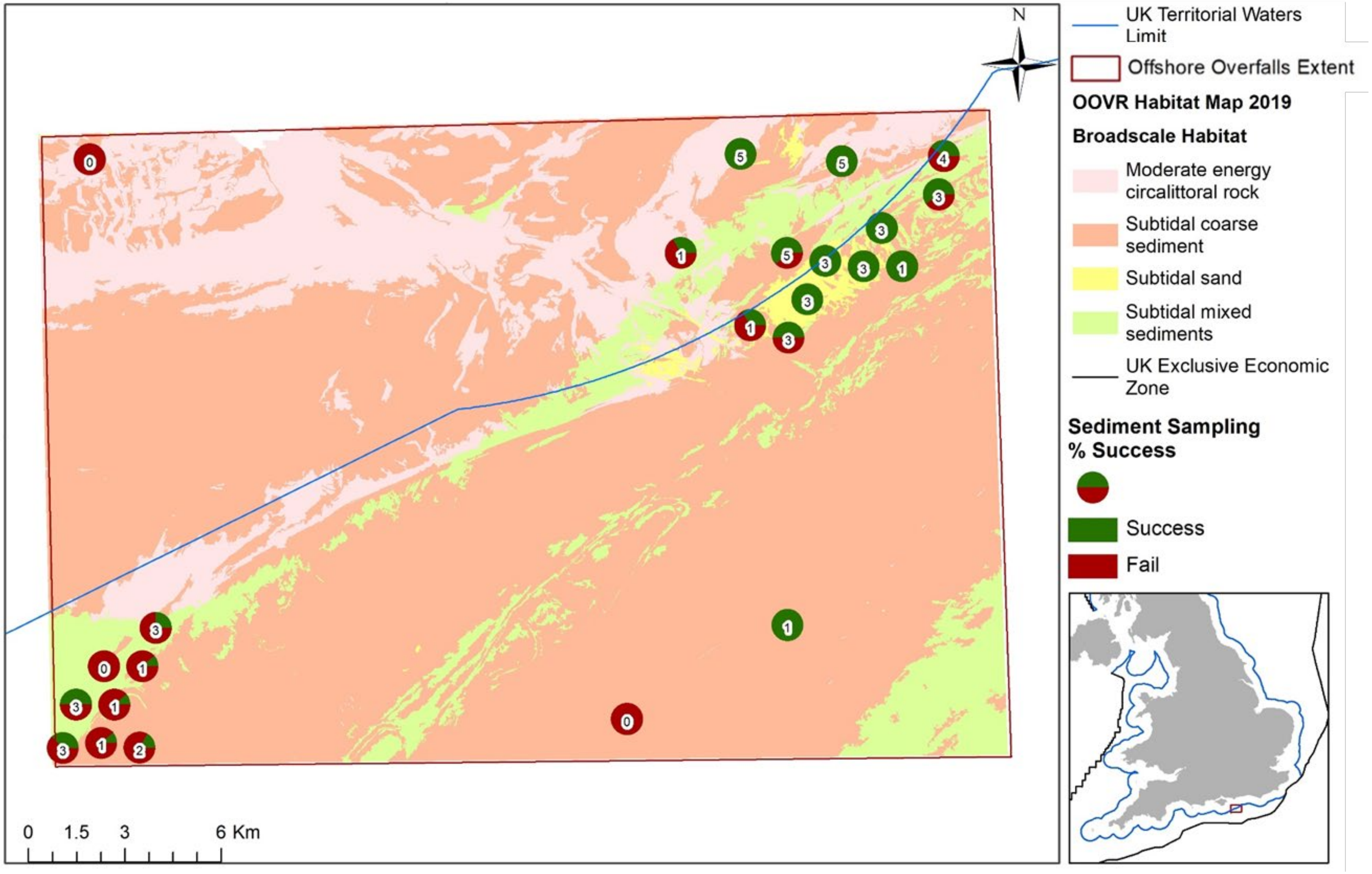

Figure 12. Spatial distribution of successful sediment sampling at Offshore Overfalls (OOVR) MCZ in 2019, overlying the 2019 habitat map. Pie charts represent the proportion of successful to failed attempts, whilst the number in the centre indicates successful replicates acquired.

3.3 Physical structure

3.3.1 Particle size analysis

Particle size distribution (PSD) analysis was undertaken for 44 replicates from 17 stations. Figure 13 presents the PSD analysis in the form of a trigon plot, indicating that many stations comprised sand-dominated ‘Subtidal coarse sediment’. Sand was the dominant sediment component at all stations in the north-east of the site, and gravel fractions dominated the stations in the south-west (Figure 14).

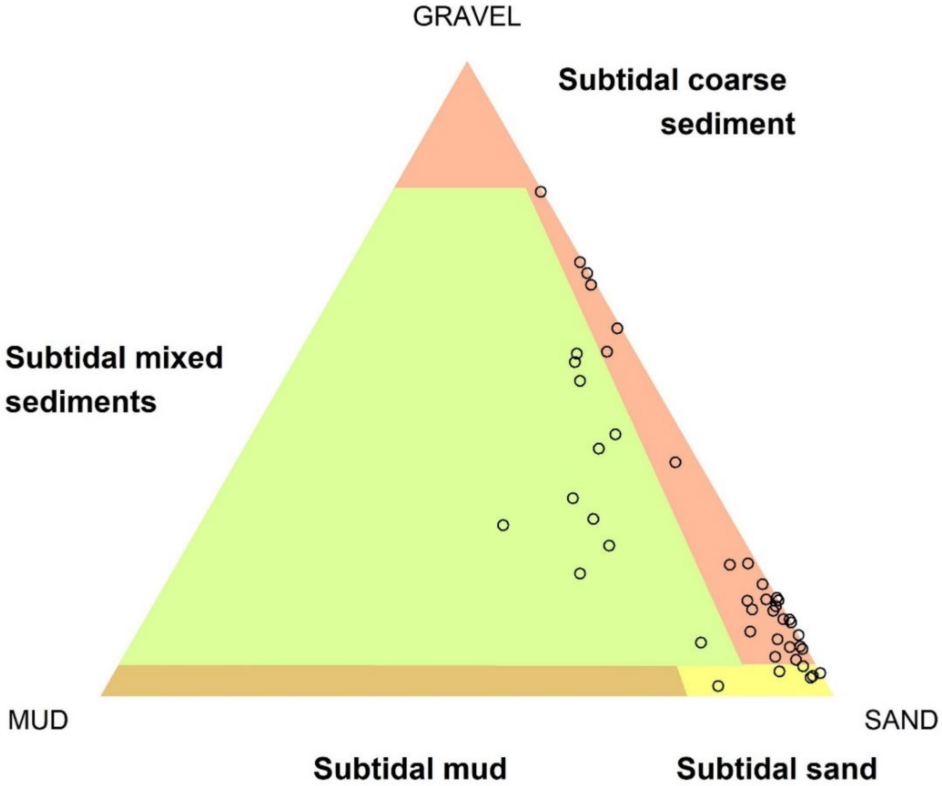

Figure 13. Classification of particle size distribution (half phi) information for each sampling point at Offshore Overfalls MCZ (2019) into one of the sediment BSHs (coloured areas) plotted on a true scale subdivision of the BGS-modified Folk triangle (Folk 1954; Long 2006).

Spatial distribution of dominant sediment fractions varied substantially between the two predicted soft substrate zones (the small patch of ‘Subtidal sand’ in the south-west and the larger area of megarippled ‘Subtidal sand’ in the north-east of the site). The sediment samples from the south-west patch showed a dominance of gravel within each sample, in comparison with the larger proportion of sand observed in the samples acquired from the north-east megaripple field. Outside of these two zones (which were targeted for monitoring by sediment sampling), ad-hoc sediment sampling in the area predicted to be ‘Subtidal mixed sediments’ showed a greater proportion of fine sediments (mud fraction) and gravel.

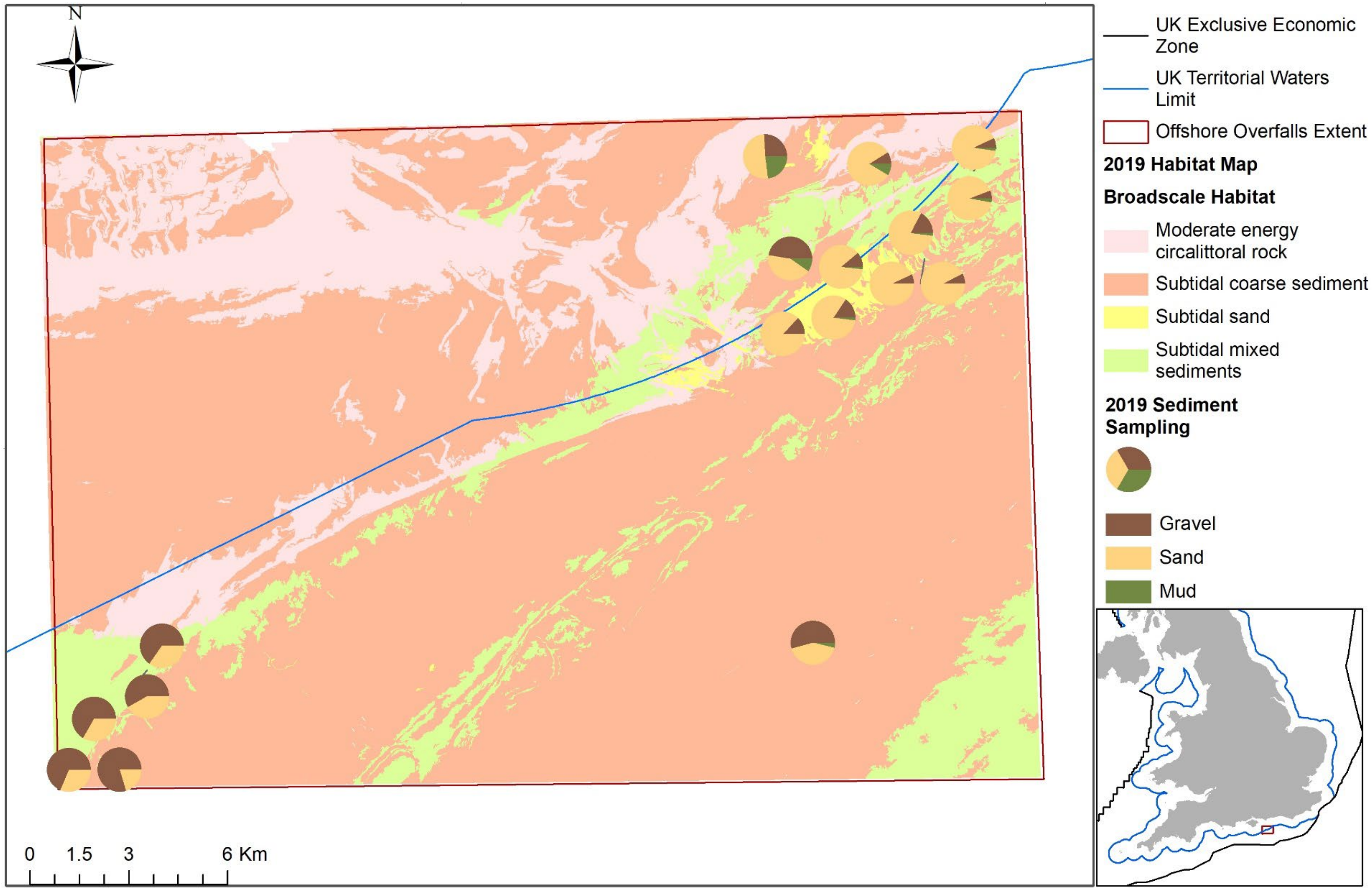

Figure 14. Average sediment fractions (% gravel, sand, and mud) of sediment samples acquired from Offshore Overfalls MCZ in 2019.

3.3.2 Finer scale topography – morphological mapping

High resolution MBES bathymetry data collected as part of the UKHO CHP in 2014, covering nearly 50% of the MCZ, was combined with the MBES data acquired during CEND0119. This allowed for a more complete (100% coverage) assessment of the large-scale topography (morphology) of the both the Northern Palaeovalley feature and the wider site. Morphological mapping of the combined Offshore Overfalls MBES dataset was undertaken at 1 m resolution (as described in Annex 3). The resulting seven classes highlight the variety in seabed morphology associated with the site. These classes, shown in Figure 15, highlight the main Northern Palaeovalley floor and escarpment (the ‘platform’ class) well, alongside the sinuous palaeo-tributary valleys located on the northern escarpment. The five lemniscate (tear-drop shaped) mounds in the centre of the main palaeo-valley are well described by the classification, as are the depression and ridge features in the northern, constricted section of the channel floor. In addition to the palaeovalley feature, the ridge class highlights the location of the hard Palaeogene bedding (‘ridge’ feature running north-west to south-east across the top of the site) and the lower Cretaceous (‘Wealdon group’) formations which form the sub-cropping rock feature in the north-west of the site. A full description of the morphology of the site can be found in published work from the report authors (Arosio *et al.* 2021).

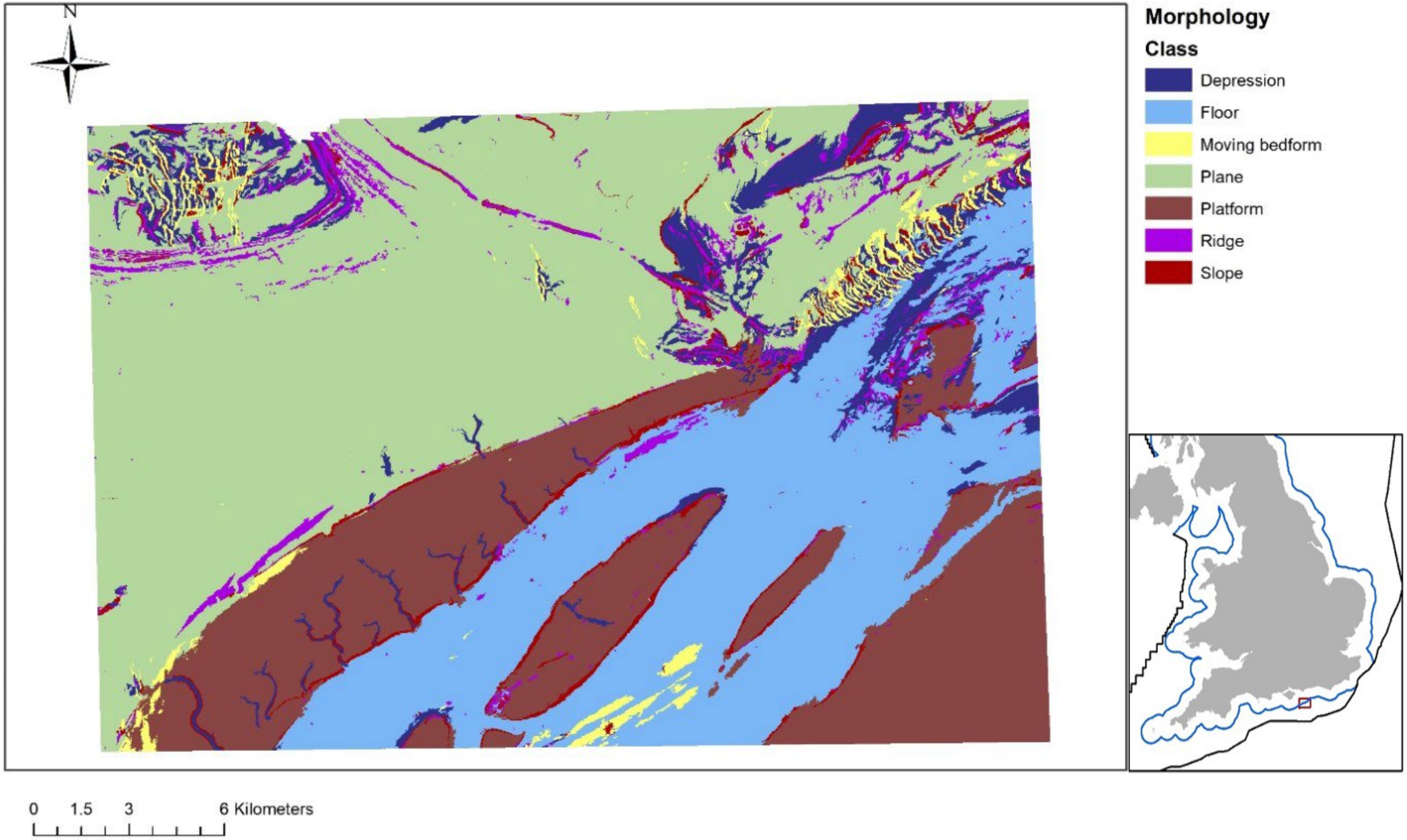

Figure 15. Morphological map of Offshore Overfalls MCZ, using high resolution MBES data acquired through the Civil Hydrography Programme and the CEND0119 survey.

3.4 Infaunal assemblage analysis

As discussed in Section 3.2.1, the limited success of sediment sampling at this site (owing to the coarse nature of the dominant gravel substrates) resulted in limited utility of the infaunal data. A cluster analysis was undertaken to detect meaningful infaunal assemblages present, and to define the taxa which best characterise them. This was undertaken primarily in order to ascertain the biological composition associated with the 'Subtidal sand' BSH.

The relationships between macrofaunal assemblages of the 44 grab samples (which included varying numbers of replicate samples from 18 stations) are presented in a dendrogram, following averaging of raw abundance data per station and hierarchical agglomerative clustering (Figure 16). An nMDS ordination was created to visualise the relationships between samples in 2D space and is presented in Figure 17.

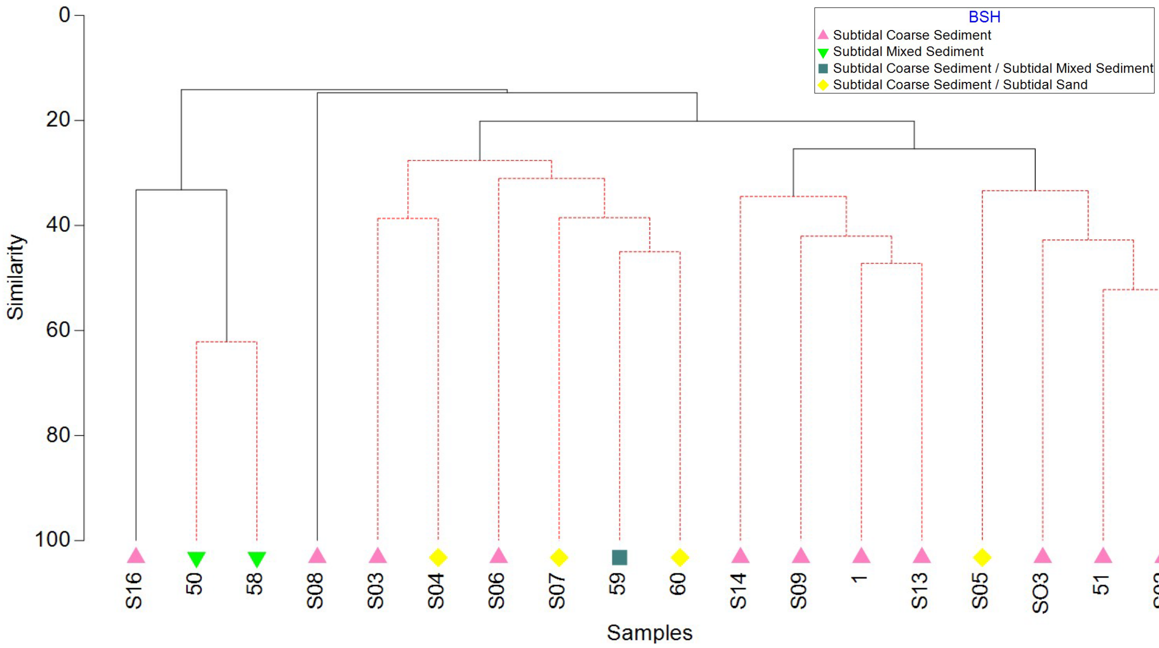

Figure 16. Dendrogram following hierarchical agglomerative clustering (group average linking) on a Bray-Curtis similarity matrix, based on square root transformed abundance data. Labels reflect sample codes and symbols indicate the BSH as defined by the sample's sediment PSA data. Cluster groups were defined by SIMPROF (at 5% similarity).

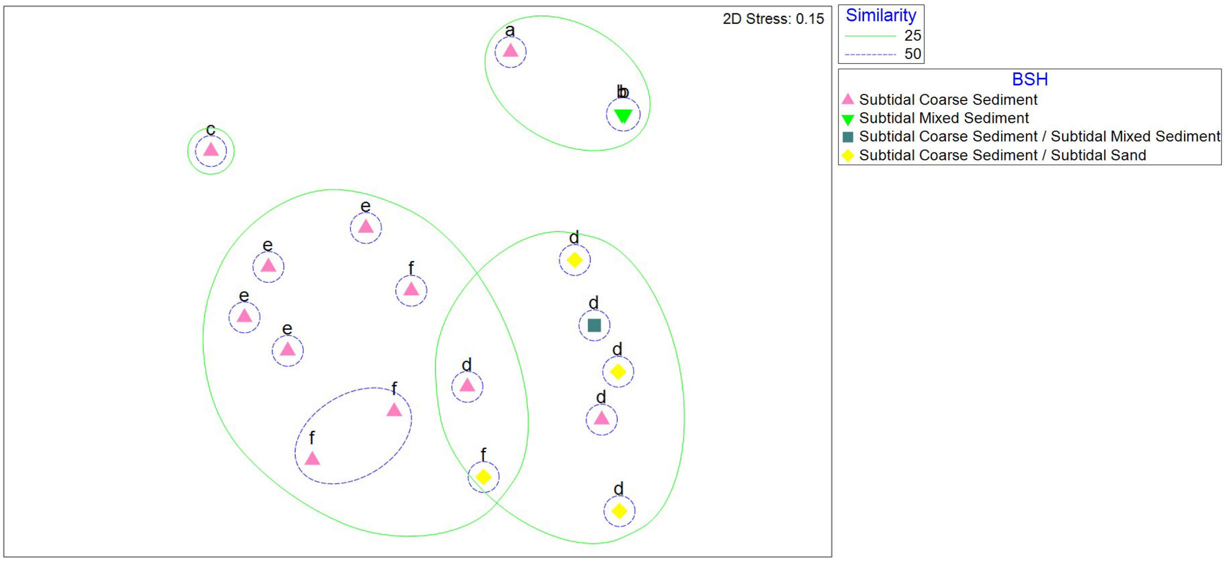

Figure 17. nMDS showing the distribution of the infaunal communities in non-metric space, with Broadscale Habitat (BSH) overlain and labelled according to SIMPROF clusters.

The 2D stress of the plot (0.15) was considered low-moderate and is considered an indicative portrayal of the samples in multivariate space. Eight of the 11 stations within the BSH 'Subtidal coarse sediment' show a relatively similar macrofaunal assemblage structure (forming clusters e and f and d - plus two outliers), whilst those of the remaining stations, which represent 'Subtidal sand' (4 stations) and 'Subtidal mixed sediment' (2 stations), showed greater variability. SIMPROF analysis (at 5% significance) revealed four discernible assemblage clusters (plus two outliers). The top five species which characterised the assemblages (as derived from SIMPER analysis) are presented in Table 8.

Cluster 'f' is dominated by *Glycera lapidum* and cluster 'd' by *Schizomavella sp.* respectively; both are characteristic of assemblages associated with coarse sand and can be spatially linked with the areas of BSH 'Subtidal sand' as predicted by the2019 map (Figure 18). Furthermore, a gradation to slightly gravelly sand can be ascertained by the shift in assemblage to cluster 'e' in the south western area of coarser substrate. Cluster 'e' being differentiated from cluster 'f'; *by* the encrusting bryozoan *Escharella immersa* and by the errant nereid polychaete *Rullierinereis ancornunezi* – which favour slightly coarser sand and has only been observed in two locations in the UK, including the areas south of the Isle of Wight (Wasson & Núñez 2013). A marked north / south divide is noted between the two sets of assemblages, with cluster 'e' being more strongly associated with the southern section of predicted coarser sand, and clusters d' and 'f' being associated with the large patch of sand in the north of the site. Co-located PSA samples (matched geographically and not tested statistically) showed that clusters 'd' and 'f' are associated with a greater proportion of sand, with samples assigned to the BSH 'Subtidal sand'. Cluster 'e' appears to be associated with coarser substrates, with a higher percentage of gravel included. Clusters 'd' and 'f' have therefore been selected as the representative assemblage for the BSH 'Subtidal sand' and taken forward as assemblage group '5' in the predictive mapping exercise undertaken in Section 3.7.

Table 8. Top five discriminating infaunal taxa (SIMPER routine) which describe the six assemblage cluster groups defined by SIMPROF (5%).

Group	N	Taxon	Ave. Abundance	Contrib. %	Group	N	Taxon	Ave. Abundance	Contrib. %
a	1	N/A			d	6	*Schizomavella* sp.	0.76	8.5
							Lumbrineris cingulata	0.82	7.51
							Echinocyamus pusillus	1.34	6.84
							Nephtys cirrosa	0.81	6.62
							Glycera oxycephala	0.64	6.1
b	2	*Lumbrineris cingulata*	4.59	5.23	e	4	*Glycera lapidum*	1.21	4.85
		Echinocyamus pusillus	4.62	4.6			Nemertea	1.00	4.60
		Sabellaria spinulosa	5.85	4.45			*Escharella immersa*	1.00	4.60
		Nemertea	3.25	3.5			*Rullierinereis ancornunezi*	1.29	3.97
		Notomastus	3.33	2.75			*Syllis pontxioi*	0.96	2.76
c	1	N/A			f	4	*Glycera lapidum*	1.22	14.41
							Folliculinidae	0.95	13.36
							Nemertea	1.04	11.72
							Pseudonotomastus southerni	0.93	11.43
							Puellina bifida	0.85	10.62

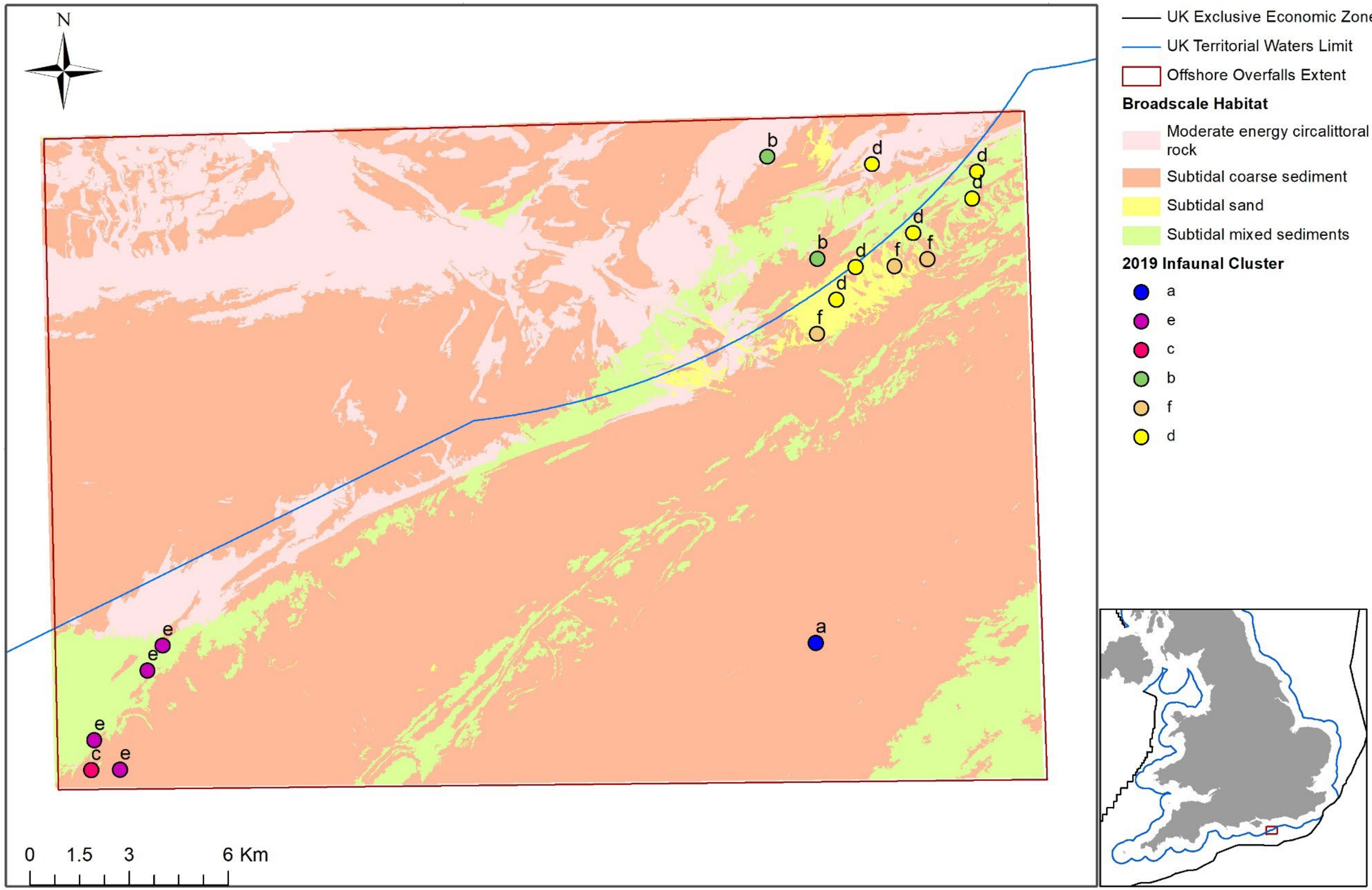

Figure 18. Spatial distribution of SIMPROF derived infaunal assemblages across Offshore Overfalls MCZ in 2019.

3.5 Epifaunal assemblage analysis

3.5.1 Structure

Following truncation and segmentation of still image data, the analysed relative density matrix resulted in 110 distinct morpho-taxonomic entries, including indeterminate taxa described at the morphological level (morpho-taxa) and consistently identified taxa at the species and genus levels. Amongst the most prominent phyla observed in the taxonomic list were: 13 bryozoan entries (including six encrusting taxa distinguished by colour alone), 27 poriferan entries (of which 12 were encrusting taxa distinguished by colour alone), 16 molluscan entries, four hydroid entries, eight Actiniaria, two colonial actinians, and three colonial ascidians. Initial *k*-means clustering of the benthic morpho-taxa relative density data identified four clusters as the optimal number of partitions, as indicated by the highest Caliński-Harabasz (C-H) criterion value of 0.40 (Caliński and Harabasz, 1974). Figure 19 shows the partition diagram for the *k*-means clustering.

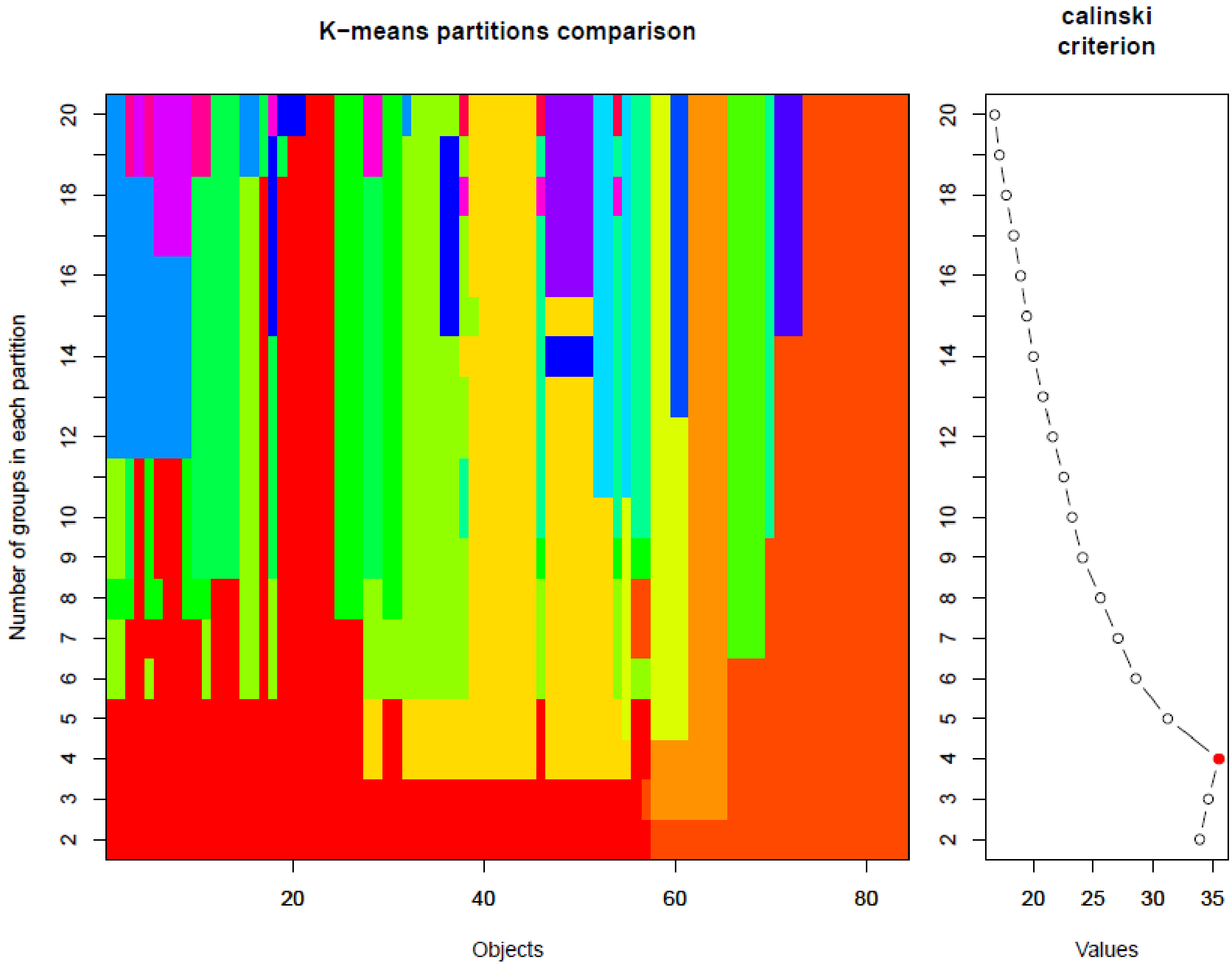

Figure 19. Partition diagram resulting from *k*-means clustering of Offshore Overfalls epifaunal samples with Caliński-Harabasz criterion values presented by partition in the right hand figure**.**

Figure 19 shows the distribution of samples (‘objects’) by partition, for between 2 and 20 groups. The right-hand figure presents a plot of the C-H criterion values, showing the highest value (35) to be associated with the partition containing four groups.

Figure 20 shows an nMDS plot of *k*-means clustered samples with an evident division into the three primary cluster groups and a left-right separation.

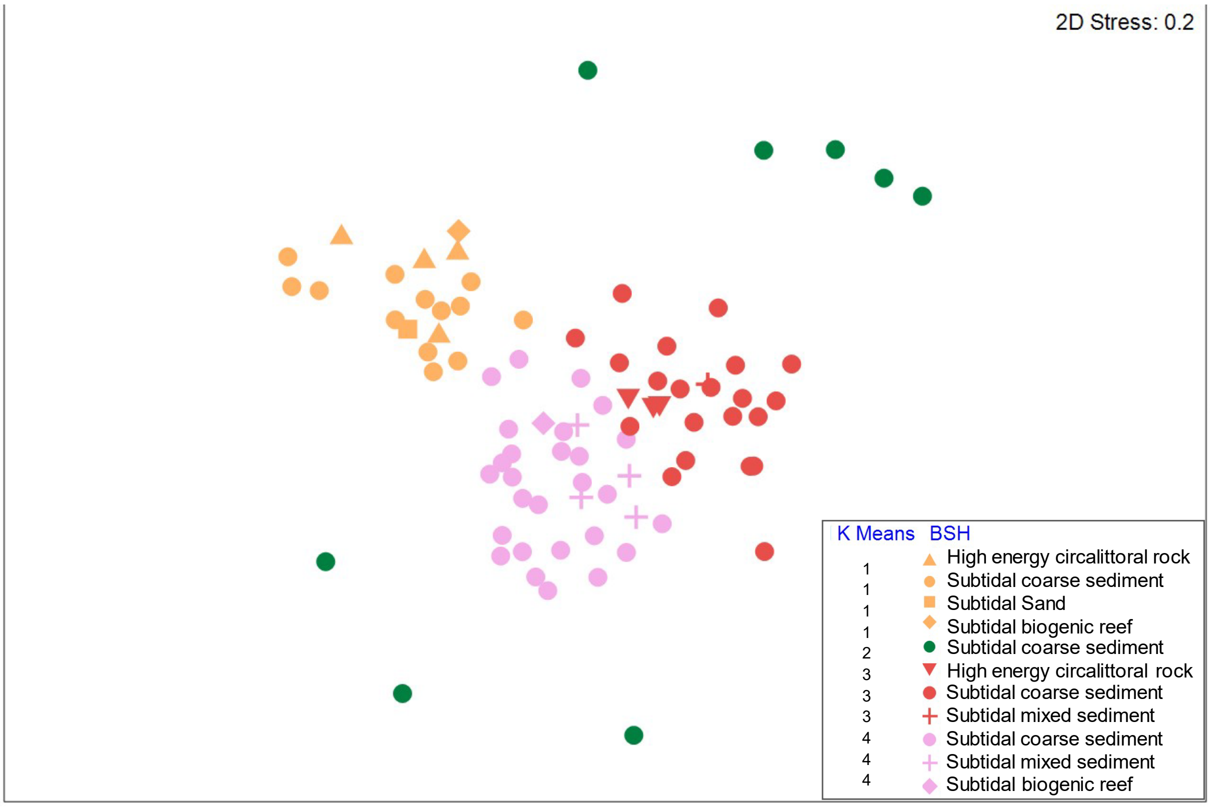

Figure 20. Non-metric multidimensional ordination of the epibiotic percentage cover data, based on the still images collected at Offshore Overfalls MCZ in 2019. SIMPROF cluster memberships are overlain, and stations are coloured by the Broadscale Habitat (BSH) type they represent (the most common BSH from each video segment)**.**

Most right-side orientated samples were classed as assemblage groups 3 and 4, which each clustered tightly together with some apparent top-bottom separation. Assemblage 1 forms the left side division, with some assemblage 4 overlap. Assemblage 2 is ill-defined in the nMDS, presenting as outliers to the top and bottom of the main three assemblages.

With respect to the relation of BSH classes (whole transect or transect as defined from video analysis) to the *k*-means cluster and the left-right separation, the results are more difficult to interpret. Both right hand and left-hand groupings are dominated by 'Subtidal coarse sediment', which was by far the most common BSH observed across the site. The BSH types which make up the remaining samples within each grouping, are 'Atlantic and Mediterranean high energy circalittoral rock' (hereafter referred to as 'High energy circalittoral rock') in the left-hand cluster and 'Subtidal mixed sediments' in the right.

The associated stress value of 0.20 indicates that the 2-dimensional representation gives a moderately useful picture of the relationships between the stations in multivariate space, therefore too much reliance should not be accorded to interpretation of the plot.

Pairwise Analysis of Similarities (ANOSIM) testing with BSH as an *a-priori* factor (Table 9) shows no significant differences in community structure between any of the samples, as classified by BSH type.

Table 9. Results of ANOSIM tests for differences in epibiotic assemblage composition between the Broadscale Habitat types sampled at Offshore Overfalls in 2019.

Broadscale Habitat comparison		R statistic	Significance (*p*-value)	Average % dissimilarity (SIMPER)
Subtidal coarse sediment	Subtidal mixed sediments	-0.197	95.3	70.93
Subtidal coarse sediment	High energy circalittoral rock	0.153	16.6	81.87
Subtidal coarse sediment	Subtidal sand	0.069	24.3	79.23
Subtidal coarse sediment	Subtidal biogenic reef	-0.013	42.0	80.32
Subtidal coarse sediment	Moderate energy circalittoral rock	-0.171	84.0	72.86
Subtidal mixed sediments	High energy circalittoral rock	0.981	0.8	81.90
Subtidal mixed sediments	Subtidal sand	1	16.7	82.28
Subtidal mixed sediments	Subtidal biogenic reef	0.527	14.3	78.11
Subtidal mixed sediments	Moderate energy circalittoral rock	-0.005	41.1	57.37
High energy circalittoral rock	Subtidal sand	0.417	20.0	83.10
High energy circalittoral rock	Subtidal biogenic reef	0.286	26.7	64.06
High energy circalittoral rock	Moderate energy circalittoral rock	1	2.9	80.75
Subtidal sand	Subtidal biogenic reef	0	66.7	86.76
Subtidal sand	Moderate energy circalittoral rock	1	25.0	79.22
Subtidal biogenic reef	Moderate energy circalittoral rock	0.833	10.0	75.51

ANOSIM testing shows limited identifiable structure when BSH is used as the *a-priori* factor (Table 9), whilst the nMDS (Figure 20) indicated separation (with a degree of overlap) between three of the *k*-means identified clusters. Redundancy Analysis (RDA) was used to investigate the relationship between the environmental data and the biological assemblages. The RDA tri-plot (Figure 21) shows the stations in metric space, with eigenvectors for the principal environmental factors overlain. The plot shows that assemblage 1 appears to be associated with bedrock and coarse sand. Assemblage 2 has no apparent single environmental driver, and many stations are associated with the mud vector. Assemblage 3 is strongly associated with depth, mud, and fine sand. Assemblage 4 appears to be driven by coarser substrates containing pebbles, and particularly gravels.

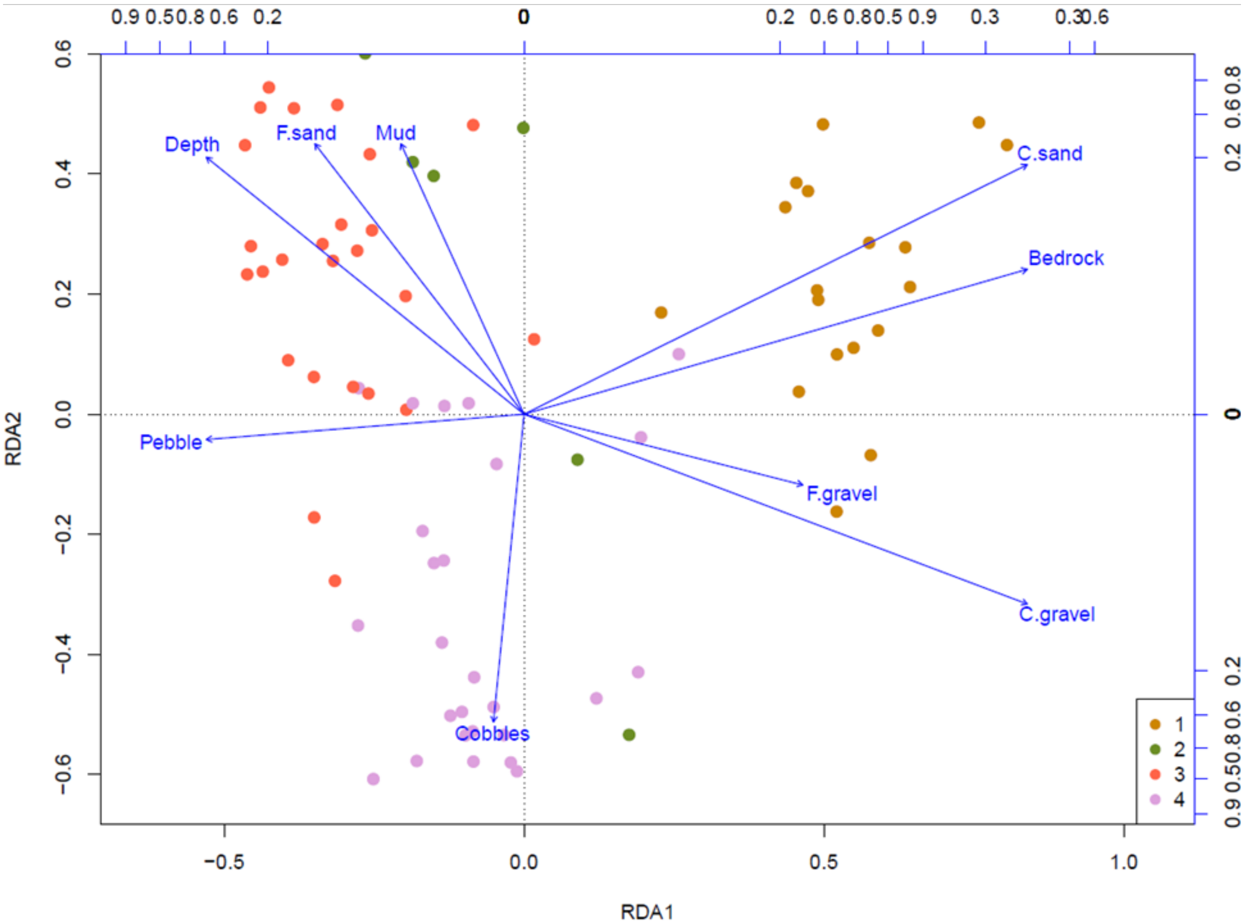

Figure 21. Redundancy Analysis (RDA) tri-plot showing the *k*-means cluster groups of Offshore Overfalls epifaunal grid and ground truth stations in metric space, with eigenvectors for the principal environmental parameters overlain. Abbreviations; C = coarse and F = fine.

3.5.2 Key and influential taxa

Table 10 presents the results of the Multi-level Pattern analysis (see Annex 3.4) on the four primary cluster groups, as defined by *k*-means clustering. Only those taxa entries that were identified as significant ($p > 0.10$) with an IndVal statistic of > 0.50 are presented within each cluster. The majority of these were significant at $p < 0.05$. Within-group similarity analysis (SIMPER) was also undertaken, with comparatively low values observed across the groups, ranging from 19.96% to 49.50%. Encrusting sponges were common across two assemblage groups (1 and 3).

Assemblage 1 showed a comparatively high SIMPER within-group similarity (46.38%) and can be characterised very effectively by the presence of Ross worm aggregations (*Sabellaria spinulosa)* (IndVal = 0.97, $p < 0.05$*)* and to a lesser extent by yellow encrusting sponges (IndVal = 0.68, $p < 0.05$*)* and by orange encrusting sponges (IndVal = 0.67 $p < 0.05$*)*. Visual assessment of still images indicates the prevalence of gravels with a mobile coarse sand veneer and frequent sub-cropping rock associated with this assemblage, in line with the RDA output. Assemblage 2 was only significant to the 90% confidence level (p <

0.1); it is included for information, but assemblage 2 characterisation must be considered indicative only and interpreted with caution.

Table 10. Results from the Multi-level Pattern analysis of epifaunal assemblages (*k*-means clustering) showing indicator epifaunal taxa by assemblage across Offshore Overfalls MCZ in 2019. A and B are components of the IndVal statistic. Taxa have been included if the IndVal statistic is > 0.5 and *p* <0.1.

Cluster (Assem-blage)	Percent similarity (SIMPER)	Main taxa	A	B	IndVal Statistic	*p* - Value
1	46.38	*Sabellaria spinulosa*	0.95	1.00	0.97	<0.01
		Encrusting Yellow Sponge	0.67	0.68	0.68	0.04
		Encrusting Orange Sponge	0.65	0.68	0.67	0.05
		Solitary Unstalked Ascidian	0.61	0.63	0.62	0.04
		Flustra foliacea	0.43	0.74	0.57	0.08
2	19.96	*Ophiopthrix fragilis* / *Ophiocomina nigra* bed	0.97	0.50	0.70	0.10
3	49.5	Branching Hydroids (Indet)	0.90	1.00	0.95	<0.01
		Aequipecten opercularis	0.78	0.96	0.87	0.01
		Encrusting Grey Sponge	0.83	0.52	0.66	0.01
		Encrusting Green Sponge	0.86	0.44	0.61	<0.01
		Chitons (Indet)	0.76	0.48	0.60	0.01
		Nemertesia sp.	0.60	0.56	0.58	0.02
		Ebalia sp.	0.52	0.64	0.58	0.02
4	46.26	Serpulidae	0.58	1.00	0.77	<0.01
		Gastropods (Indet)	0.42	1.00	0.65	0.05
		True Anemones (Indet)	0.41	1.00	0.64	0.05

Assemblage 2 had the lowest within-group similarities, with ophiuroid beds (mixed *Ophiocomina nigra* and *Ophiothrix fragilis)* having a high specificity (IndVal component 'A' score of 0.97) and a moderate fidelity (IndVal component 'B' score of 0.75). This indicates that although assemblage 2 has a low within-group similarity, ophiuroid beds can be used to characterise this cluster with a moderate probability of accuracy (IndVal = 0.70, $p < 0.1$). These samples were associated with gravel and coarse/mixed sediments. Notably more fines were observed at the sediment sampling station in this location, however detection of fines from imagery is not reliable.

Indeterminate branching hydroids were particularly characteristic of assemblage 3 (IndVal = 0.95, p <0.01). This was alongside the Queen Scallop (*Aequipecten opercularis* (IndVal = 0.87, p <0.01)) and encrusting grey and green sponges (IndVal = 0.66 & 0.61 respectively, p <0.01). Branching hydroids (indet.) had an 'IndVal B' component score of 1.00, and an 'IndVal B' score of 0.96, indicating complete fidelity to assemblage 3. Greater depths and increased proportions of fine sand and mud were associated with this assemblage.

Assemblage 4 was characterised by serpulid worms (IndVal = 0.77, $p < 0.01$) and indeterminate gastropods (IndVal = 0.65, $p < 0.05$). True anemones were also found to be prominent characterising taxa (IndVal = 0.64, $p = 0.05$). All three entries had low 'IndVal A' component scores (0.53, 0.42 and 0.41, respectively) and perfect fidelity to the cluster group, meaning that these taxa will always be observed within samples belonging to assemblage 4, although they are not uniquely associated with it. Environmental parameters associated with this group include cobbles, and pebbles to a lesser degree than seen in assemblage 3.

Figure 22 presents a selection of representative still images from each of the four identified assemblage groups.

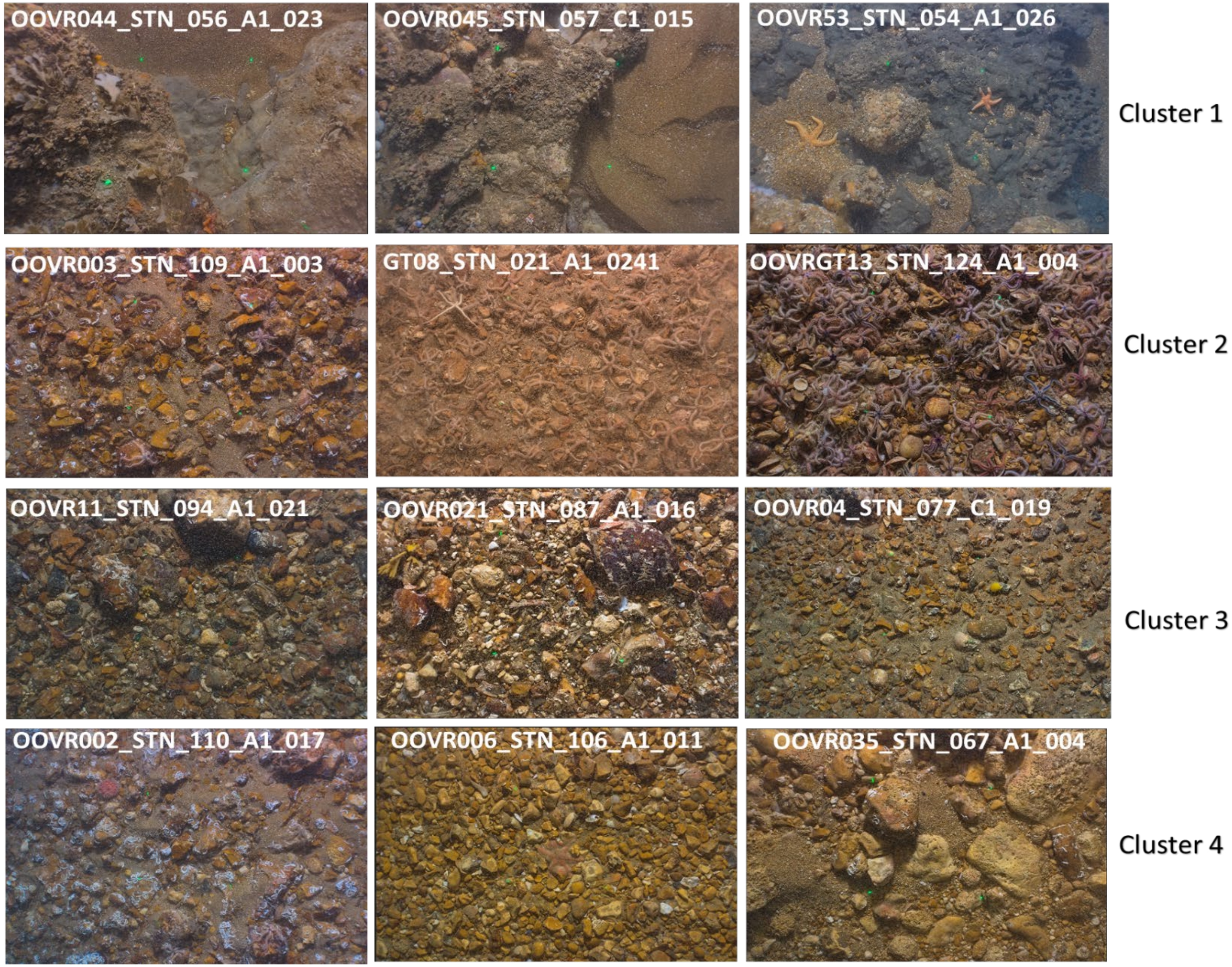

Figure 22. Representative still images from each of the four *k*-means identified epifaunal assemblages (as defined by *k*-means cluster analysis).

3.5.3 Spatial distribution of epifaunal assemblages

The spatial distribution of the four epifaunal assemblages (Figure 23) shows evident north-west / south-east separation between assemblages. Assemblage 1 is associated with the 2015 predicted extents of the features ‘Moderate energy circalittoral rock’ and ‘Subtidal mixed sediments’ (Mellett & Green 2015). Assemblage 2 was observed in areas of ‘Subtidal coarse sediment’ predicted to be adjacent to the ‘Subtidal sands’ feature (north-east and south-west locations). Assemblages 3 and 4 were the most frequently observed, distributed with a distinct north-west / south-east separation, with assemblage 3 possibly associated with the palaeovalley feature in the south-west, and assemblage 4 with the ‘plain’ feature to the north-east.

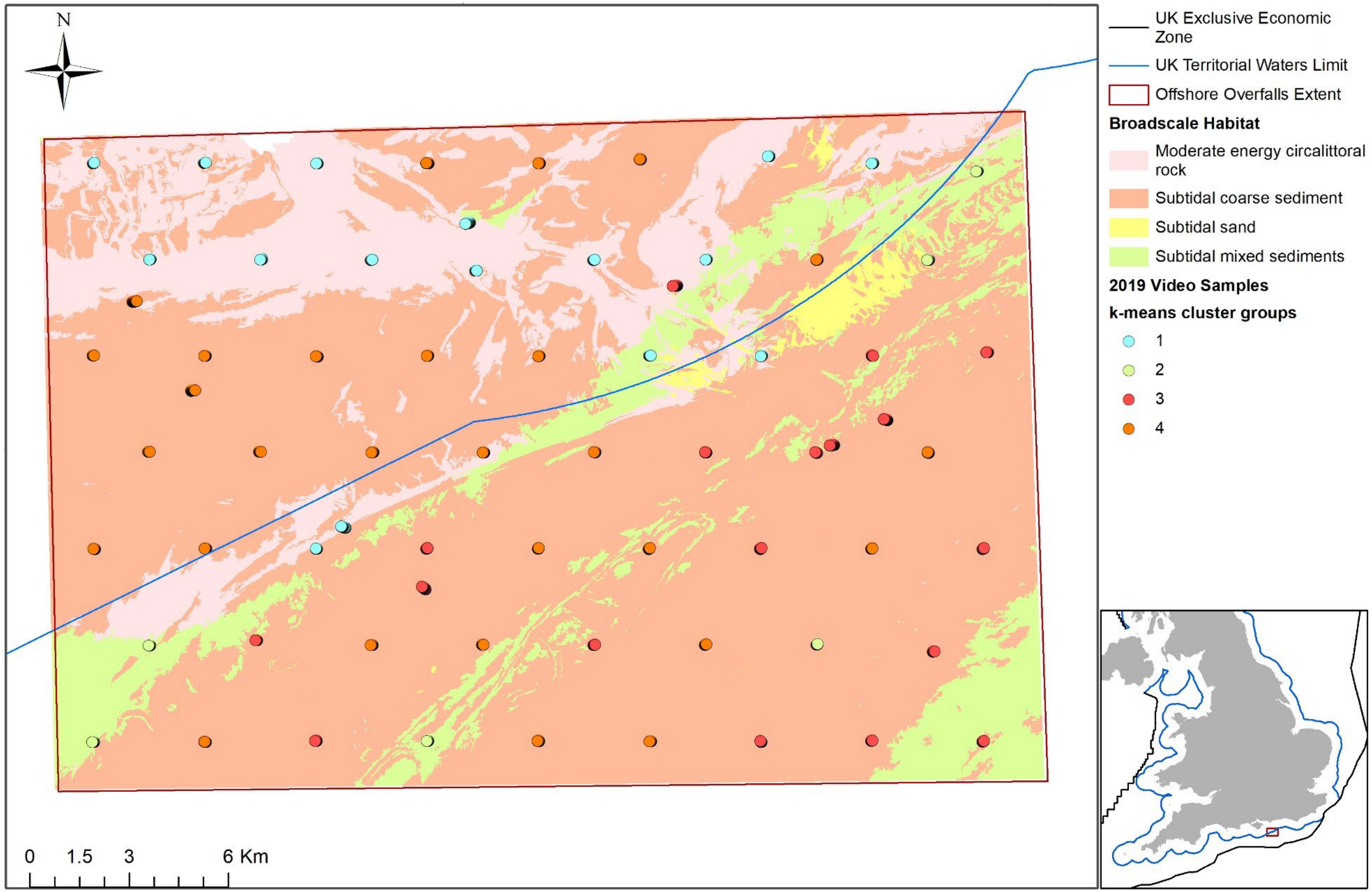

Figure 23. Spatial distribution of *k*-means derived epifaunal assemblages in 2019 across the 2019 habitat map of Offshore Overfalls MCZ.

3.6 Predictive habitat mapping

3.6.1 Segmentation

Object-based image analysis (OBIA) was undertaken using the methodology presented in Annex 3. Initially, a segmentation which utilised bathymetric, backscatter reflectance and geomorphic layers was undertaken and comprised 12,213 objects. These objects were then classified following the approach outlined in Annex 3 and presented in Figure 10.

3.6.2 Training sample selection

Initially, four epifaunal assemblage classes were defined from the *k*-means-derived epifaunal assemblages. The samples were broken down into their component still images, such that each image which constituted a sample was assigned the class of the sample as a whole. The still image locations were then overlain onto the segmentation, and objects corresponding to the location of each still were classified accordingly. The predictive mapping process involved training a machine learning algorithm on the physical parameters associated with the segments coincident with derived assemblage classes. The result is a prediction of assemblage extent, given the physical parameters at any given location, i.e. a habitat map.

Seabed imagery data were not acquired from the two areas predicted as the BSH 'Subtidal sand', as these were characterised using sediment samples. As such, a fifth habitat class (class 5) was assigned based on the locations of sediment samples with a dominant sand fraction (as indicated by particle size analysis) and the infaunal assemblage analysis (cluster K). Table 11 presents the descriptions of each habitat class, and the number of training samples (classified objects) for each.

Table 11. Assemblage mapping class description and training sample numbers.

Habitat Class	Class Description	Training Sample No.
1	Epifaunal assemblage 1: Ross worm dominated sub-cropping rock with heavy (mobile) sand veneer	33
2	Epifaunal assemblage 2: Mixed assemblages dominated by brittlestars	25
3	Epifaunal assemblage 3: Coarse sediment characterised by branching hydroids, green and grey encrusting sponges, and queen scallops	37
4	Epifaunal assemblage 4: Exposed / impoverished coarse / cobble dominated sediment characterised by serpulid worms, gastropods, and anemones	50
5	Sand and sand-influenced coarse sediment (as defined by sediment sampling and infaunal cluster K)	20

3.6.3 Model generation and feature selection

The predictive model was trained and selected as described in Annex 3.5, using a custom-written Python 3 script. The model was evaluated in this script using several functions available in the PyCaret package. The evaluation metrics used were Accuracy, Area Under Curve (AUC), Recall, Precision, F1, Kappa and Matthews Correlation Coefficient (MCC). The results of the testing indicated that the most accurate model was an 'Extreme Gradient-boosted Classifier' (XGBC). Feature (i.e. data layer) importance for the final predictive model was presented (*post hoc*) using the 'Feature Importance Plot' algorithm, available in PyCaret. This algorithm uses a combination of several supervised feature selection techniques to select the subset of features that are most important for modelling. Figure 24 presents the results of this selection, showing that mean backscatter intensity ('Mean_BS') was found to be the most informative, followed closely by the topographical derivative Surface Area to Planar Area (SAPA) and the predicted maximum ebb tide magnitude. Explanations of these data layers can be found in Annex 3.5.

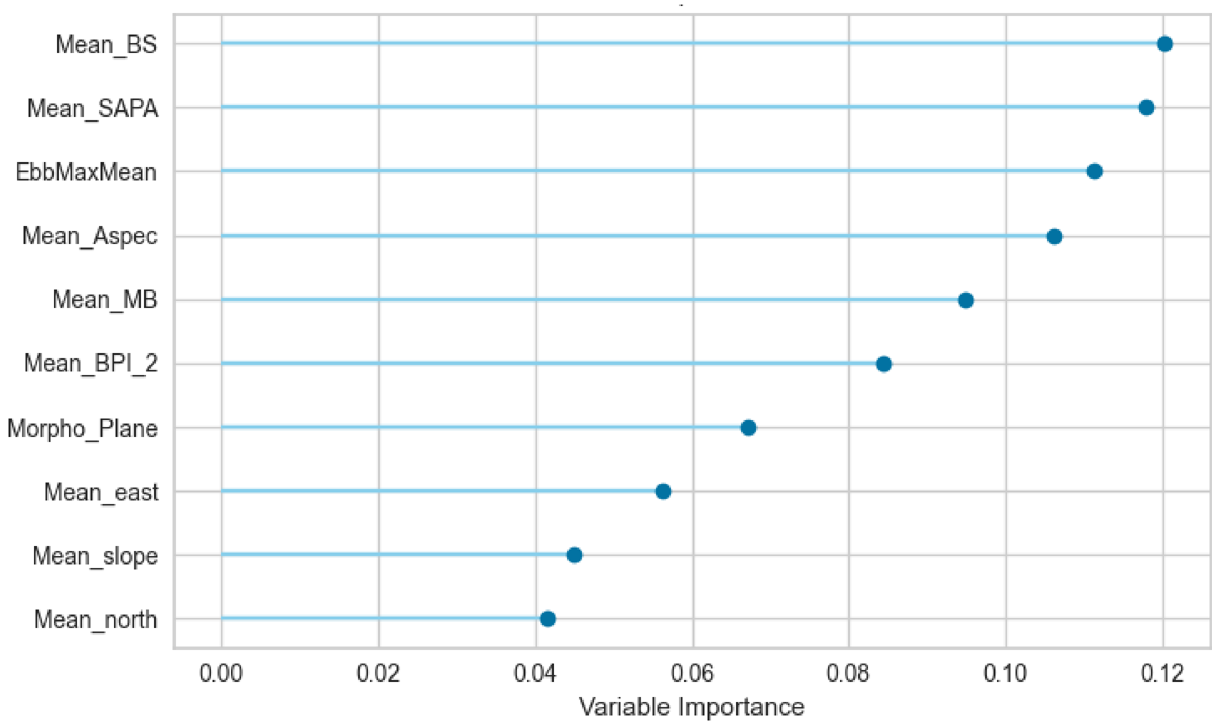

Figure 24. Feature importance (*post hoc*) following selection of the tuned 'Extreme Gradient-boosted Classifier' (XGBC) classifier model. BS =Backscatter, SAPA = Surface area to Planar Area Aspect = Aspect, BPI = Bathymetric Position Index.

3.6.4 Assemblage map

The assemblage map is presented in Figure 25. The limited extent of assemblage class 5 corresponds very closely to the observed morphological class 'Moving Bedforms'. Assemblage class 1 is limited in spatial extent to the north-west half of the site, corresponding to the mapped morphological extent of sub-cropping 'Wealdon group' rock and the flank of the escarpment leading up from the palaeovalley floor (the 'Platform' morphological class). Patterns in the separation between the extents of classes 3 and 4 are clearer in the context of palaeovalley morphology, with class 3 noted on the plain to the north of the palaeovalley and on the raised lemniscate mounds. Association between class 4 and the northern arm of the palaeovalley floor is noteworthy. Similarly, comparison with the ebb tidal magnitude (Figure 5), alongside feature importance (Figure 24) indicates that the highest tidal magnitudes are broadly associated with the extent of class 4.

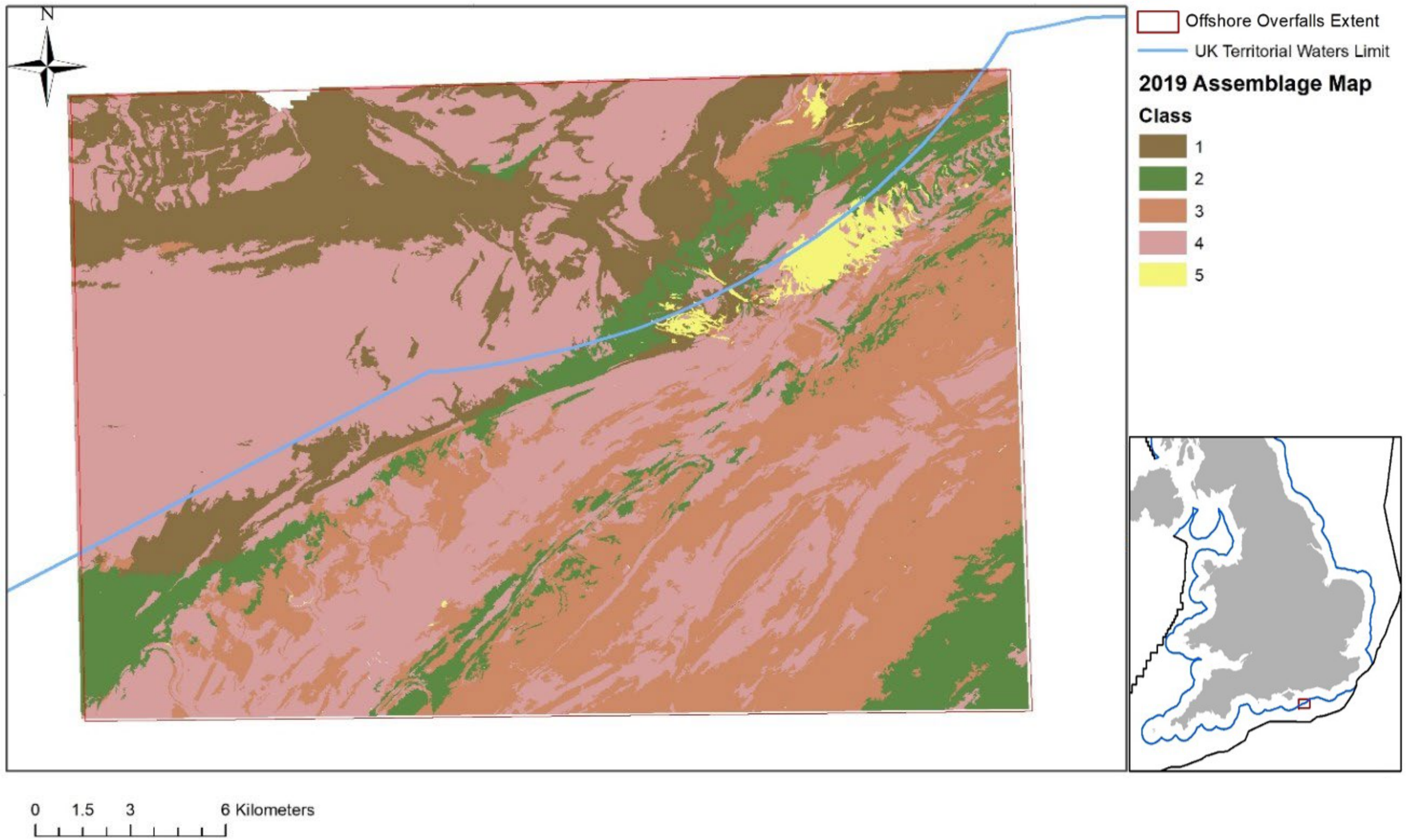

Figure 25. Predicted extents of the five mapped habitat classes at Offshore Overfalls MCZ, as derived from epifaunal and infaunal assemblage analysis alongside particle size data.

3.6.5 Cross-validation and accuracy

Results from the 10-fold cross-validation of the final (tuned) XGBC model are presented in Table 12, which shows an overall high level of both producers and user's accuracies (as defined in Annex 3) and a high overall accuracy of 76%.

Table 12. Cross-validation derived accuracy metrics for final Extreme Gradient Boosting (XGBC) model used to predict habitat extent at Offshore Overfalls MCZ**.**

Model	Accuracy	AUC	Recall	Prec.	F1	Kappa	MCC
Extreme Gradient Boosting	0.76	0.953	0.7659	0.7788	0.7641	0.6907	0.6933

Figure 26 indicates that there was limited confusion (where the model misclassifies a known class during cross-validation) between all classes, with classes 3 and 4 (the most spatially extensive) showing a small amount of cross-confusion, and with class 4 also showing slight confusion with classes 2 and 1. Figure 27 shows the class prediction error for the model.

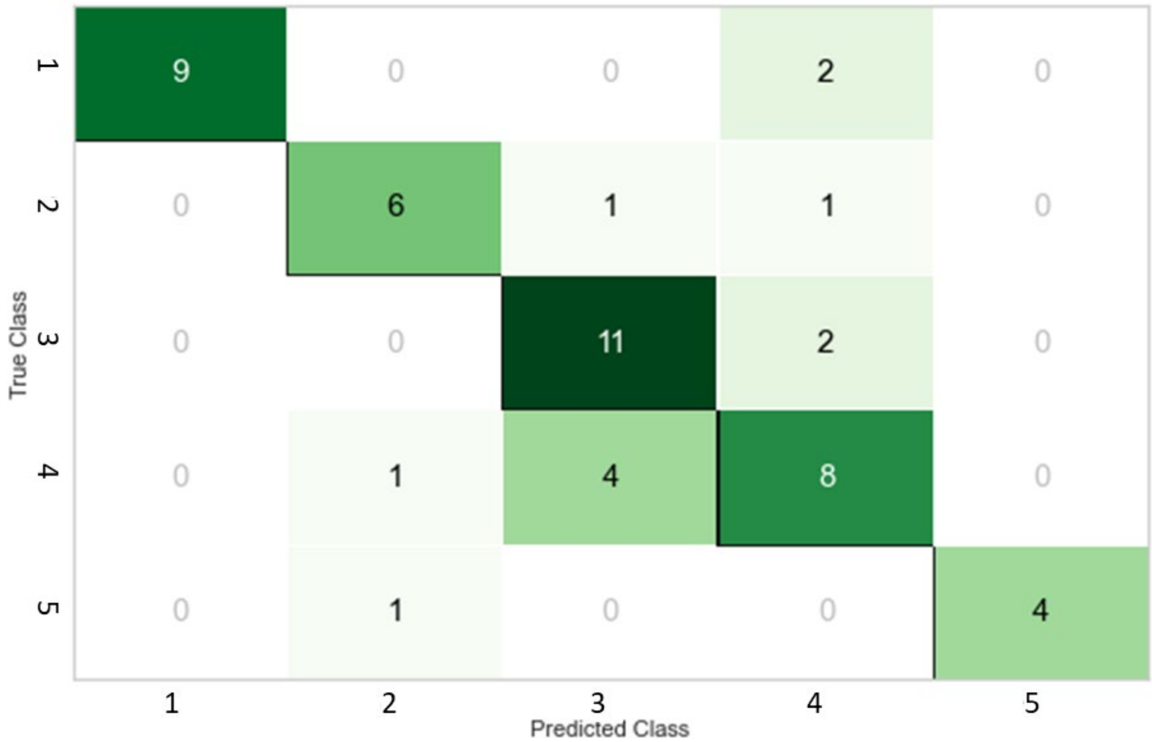

Figure 26. Confusion matrix for the five predicted habitat classes at Offshore Overfalls MCZ as per the final tuned Extreme Gradient Boosting (XGBC) classifier model.

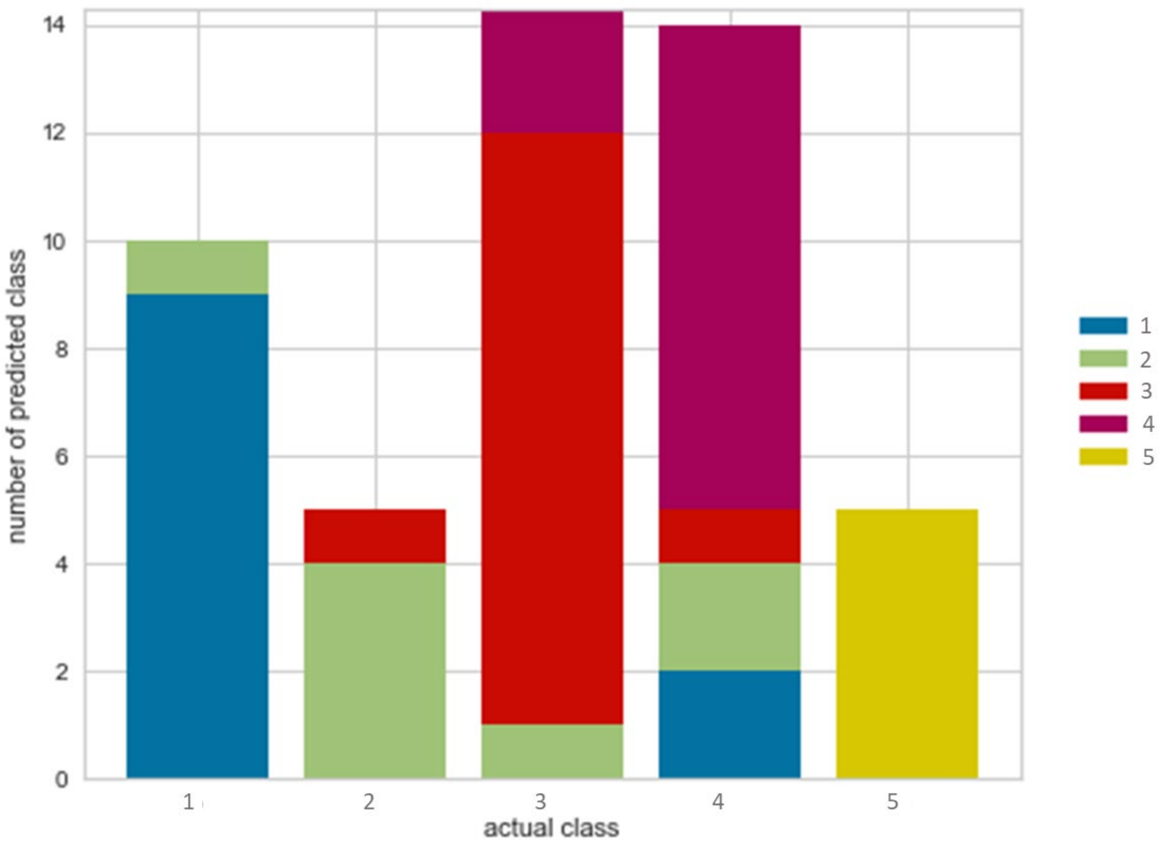

Figure 27. Class prediction error for XGBC classifer model used to predict habitat class at Offshore Overfalls MCZ.

3.7 Biotopes

The closest biotope matches from the Marine Habitat Classification of Britain and Ireland (MHCBI, Version 15.03) to the five mapped habitat classes (Figure 25 are described below. Apparent changes between biotopes over time should be interpreted with a high degree of caution. Such changes should not be assumed to indicate a change in condition, or trigger changes to management measures or conservation advice without additional robust evidence.

The five identified habitat classes have been mapped (Figure 25) based on the four epifaunal assemblages and the presence of the BSH ‘Subtidal sand’, as identified from limited sediment sampling. There were too few successful infaunal samples to provide for a robust, site-wide infaunal assemblage assessment fit for monitoring purposes. As such, the assignment of relevant biotopes to the four coarse sediment-dominated habitat classes has been undertaken primarily using assemblage information provided by the epifaunal analyses and informed by the infaunal assemblages of the limited co-located sediment samples. Four of the five mapped habitat classes have co-located epifaunal and infaunal sampling, with only habitat class 3 being not sampled for infauna and PSD.

Habitat class 1 was characterised by the presence of live *Sabellaria spinulosa* crust on exposed sub-cropping chalk or Wealdon group rock, usually in the presence of a coarse sand and gravel veneer. Further characterising taxa were encrusting sponges (orange and yellow colour-morphs), solitary ascidians and the bryozoan *Flustra foliacea*. Also of note is the association of Ross coral (*Pentapora folicea*) to class 1, as shown in Figure 28.

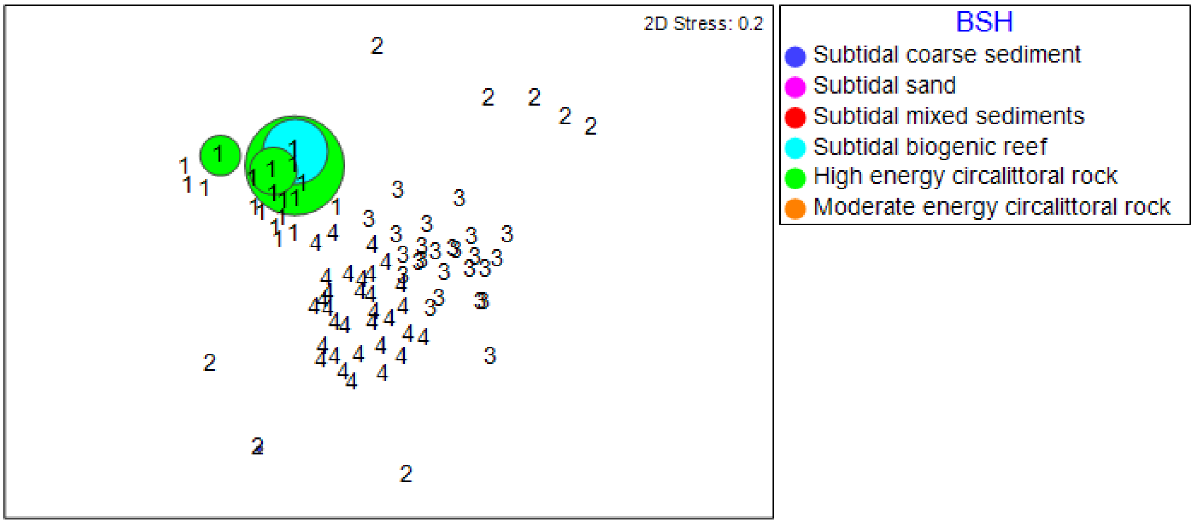

Figure 28. nMDS of epifaunal assemblages at Offshore Overfalls MCZ, with relative abundance of Ross coral (*Pentapora foliacea*) overlain as bubble plots.

The characterising taxa are indicative of the biotope CR.MCR.CSab.Sspi (*Sabellaria spinulosa* encrusted circalittoral rock), or possibly the coarse sediment dominated SS.SBR.PoR.SspiMx (*Sabellaria spinulosa* on stable circalittoral mixed sediments). It is considered likely that the assemblage is a mixture of both these biotopes, grading according to particle size and tidal exposure. Co-location of two infaunal samples with class 1 epifaunal stations indicates that the heavily veneered component habitats of this class comprise mixed substrate elements representative of infaunal cluster 'b' (*Sabellaria spinulosa* dominated) and more mobile sand communities represented by infaunal cluster 'd'.

The low within-group similarity of epifaunal assemblage 2 makes for low confidence in the assignation of a biotope to habitat class 2. Given the dominance of *Ophiocomina nigra* and *Ophiothrix fragilis,* and the association with finer particle sizes, this assemblage has been assigned to the possibly ephemeral biotope SS.SMx.CMx.OphMx (*Ophiothrix fragilis* and/or *Ophiocomina nigra* brittlestar beds on subtidal mixed sediments).

Habitat class 3 had no co-located infaunal sampling. It is possible to determine that this more mixed (yet coarse gravel-dominated) habitat is characterised by the scallop species *Aequipecten opercularis*, branching hydroids and the infaunal species *Ampharete lindstroemi*, *Lumbrineris cingulata* and *Echinocyamus pusillus*. This habitat appears to be a local variant of the biotope SS.SCS.CCS.MedLumVen (*Mediomastus fragilis*, *Lumbrineris* spp. and venerid bivalves in circalittoral coarse sand or gravel), lacking in *Mediomastus fragilis* and venerid bivalves, and perhaps grading into the sediment veneer-dominated components of habitat class 1.

Habitat class 4 was co-located with a single successful sediment sampling station (which was classified as infaunal cluster 'b') and is a coarse substrate habitat, impoverished in faunal diversity compared with class 3 and associated with large particle sizes such as pebbles and cobbles. Characterising epifauna comprise serpulid worms, and a higher proportion of indeterminable true anemone taxa (large cereid and sagartid species) compared to class 3. This habitat class is best associated at the habitat level and is matched with SS.SCS.CCS ('Circalittoral coarse sediment').

The mobile sand-dominated habitat of class 5 was targeted only through sediment sampling (the ad-hoc samples located over two areas of acoustically identified megaripple features); only infaunal assemblage information has been used to characterise this habitat class. The infaunal samples used to define this class comprise the infaunal clusters d and f, which correspond to the biotopes SS.SCS.ICS.Glap (*Glycera lapidum* in impoverished infralittoral mobile gravel and sand) and SS.SSa.CFiSa.EpusOborApri (*Echinocyamus pusillus*, *Ophelia borealis* and *Abra prismatica* in circalittoral fine sand). The distribution of these two biotopes within this class is likely driven by depth (SS.SCS.ICS.Glap being associated with shallower waters) and substrate particle size, as SS.SSa.CFiSa.EpusOborApri is associated with finer mobile sands.

3.8 Assessment of within-station variation

Objective 2.a set out to '*explore and discuss the efficacy of the sampling strategy for future monitoring surveys*'. This objective aimed to investigate the efficacy of each of the two survey approaches, single transects and the increased replication approach (five replicates), in quantitatively characterising the epifaunal assemblage of a single station. Two sub-objectives were specified, as follows:

I. Comparison of Species Accumulation Curves and univariate metrics to assess optimum seabed sample area.

II. Comparison of epifaunal assemblages by four treatments (number and spatial distribution of seabed photographs used to create the sample) of epifaunal assemblage – multivariate analysis of variance.

These sub-objectives were investigated using two separate statistical approaches with the same null hypothesis:

Overall H_0 = *a single transect will characterise epifaunal assemblage as effectively as multiple transects (high rep stations) when controlled for cumulative seabed area*

3.8.1 Comparison of Species Accumulation Curves and univariate metrics

The first of the two sub-objectives aimed to assess the optimum seabed sample area required to ensure that a complete understanding of the univariate metrics of an assemblage is achieved. Species Accumulation Curves (SpACs) were created using species richness (total number of taxa, 'S') as the univariate metric, as described in Annex 3. These are presented in Figure 29 (all SpAC curves from each of the eight increased replication stations on a single plot) and in Figure 30 (separately).

Figure 30 shows the variation in the SpAC profiles (species richness against cumulative image area in m^2) for the eight increased replication stations, with slope and asymptote showing clear variation between the stations. This variation is to be expected, as each station is in an area of differing substrate (BSH) or topography, as per the experimental design. From these curves, an estimation of the optimum (cumulative) seabed sample area is possible by observing the predicted asymptote value (the cumulative sampled area at which the curve flattens). These are presented in Table 13 alongside the cumulative sample and mean image Field of View (FoV) values, and BSH / *k*-means assemblage group.

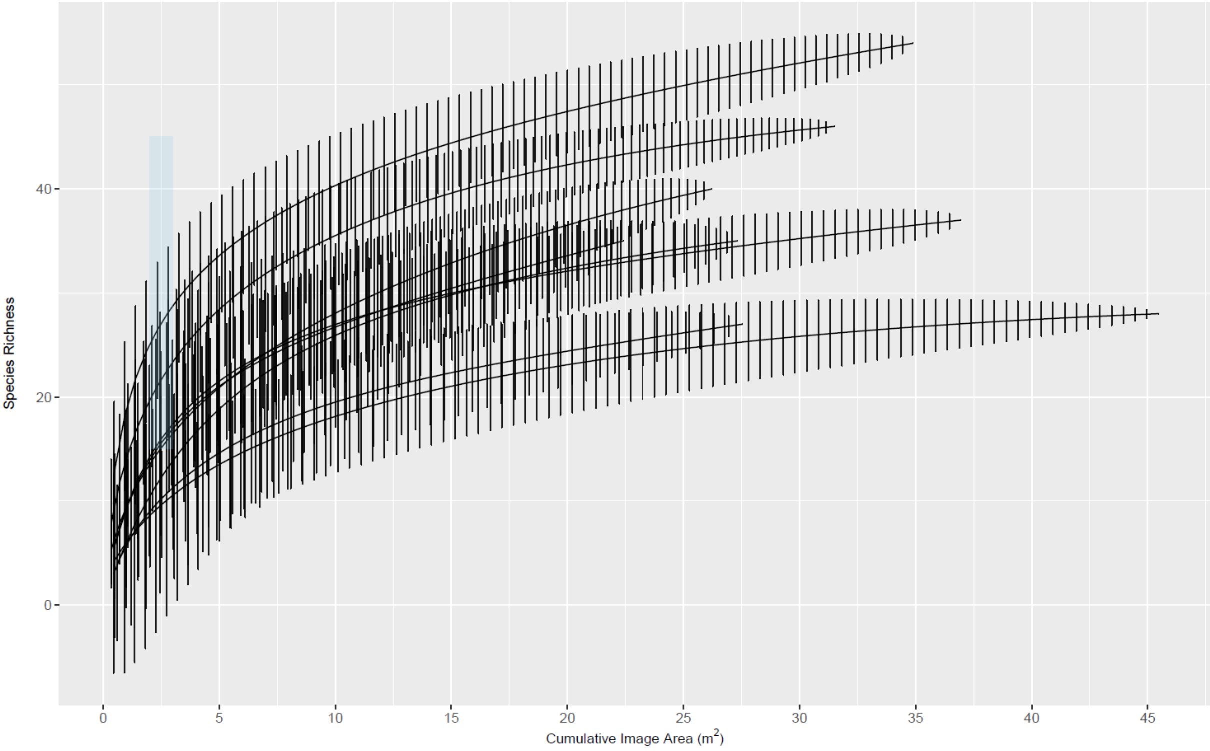

Figure 29. Species Accumulation Curves showing cumulative image area (m^2) against species richness for each of the increased replication stations at Offshore Overfalls MCZ, 2019.The areas shaded blue represent a cumulative area of 2.5 m^2 for reference**.**

Table 13. Results of the Species Accumulation Curves statistics for the increased replication stations at Offshore Overfalls MCZ in 2019. Multiple BSHs refers to the inclusion or removal of still images which were assigned (by video segment) as being of a differing BSH type to the dominant BSH of the transect; see Annex 3 for further details.

STN	Dominant BSH (Video)	Dominant *K*-means Group	Sum FoV (m^2)	Mean Still FoV (m^2)	Asymptote Multiple BSHs Inc. (m^2)	Asymptote No Multiple BSHs (m^2)
OOVR016	Subtidal coarse sediment	2	45.47	0.51	20.19	20.19
OOVR018	Subtidal coarse sediment	4	26.23	0.47	42.56	42.56
OOVR020	Subtidal coarse sediment	1	31.50	0.35	21.93	21.93
OOVR039	Subtidal coarse sediment	4	36.95	0.61	29.88	29.88
OOVR041	Subtidal coarse sediment and Subtidal biogenic reef	1	33.57	0.46	23.85	20.39
OOVR043	Subtidal coarse sediment	3	34.90	0.35	31.39	31.39
OOVR044	High energy circalittoral rock	1	39.52	0.50	68.66	30.06
OOVR045	Subtidal coarse sediment and High energy circalittoral rock	1	41.72	0.49	32.46	19.97

Whilst the ‘bullring’ methodology aimed to reduce the probability of one station (100 m diameter area) covering more than one BSH, this did occur at two of the stations (OOVR41 and OOVR45), for five of the 10 replicates. To ensure the accuracy of the predicted asymptote values, these ‘multiple BSH’ replicates were controlled for by comparing the asymptote values of stations including ‘multiple BSH’ replicates with the asymptote values of the same stations with multiple BSH replicates removed. The two sets of asymptote values were tested for significant differences using an independent two-sample t-test (assuming unequal variances), with the following null hypothesis tested:

H_0 = No observable difference in asymptote value between within-station replicates

No significant difference was found between the mean asymptotes of the with ‘multiple BSH’ and the without ‘multiple BSH’ groups, t (7) = 1.09, $p = 0.15$. The asymptote values from the samples including ‘multiple BSH’ replicates were investigated further. The average cumulative sample area estimated to effectively capture species richness for the BSH ‘Subtidal coarse sediment’ was 29.2 m^2, compared with an average of 41.7 m^2 for matrices of ‘Subtidal coarse sediment’ and ‘High energy circalittoral rock’/ ‘Subtidal biogenic reef’. The average cumulative seabed area (pooled between all five replicates) sampled at the increased replication stations was 36.2 m^2, in comparison to the average cumulative area sampled at the grid stations, which was 6.9 m^2.

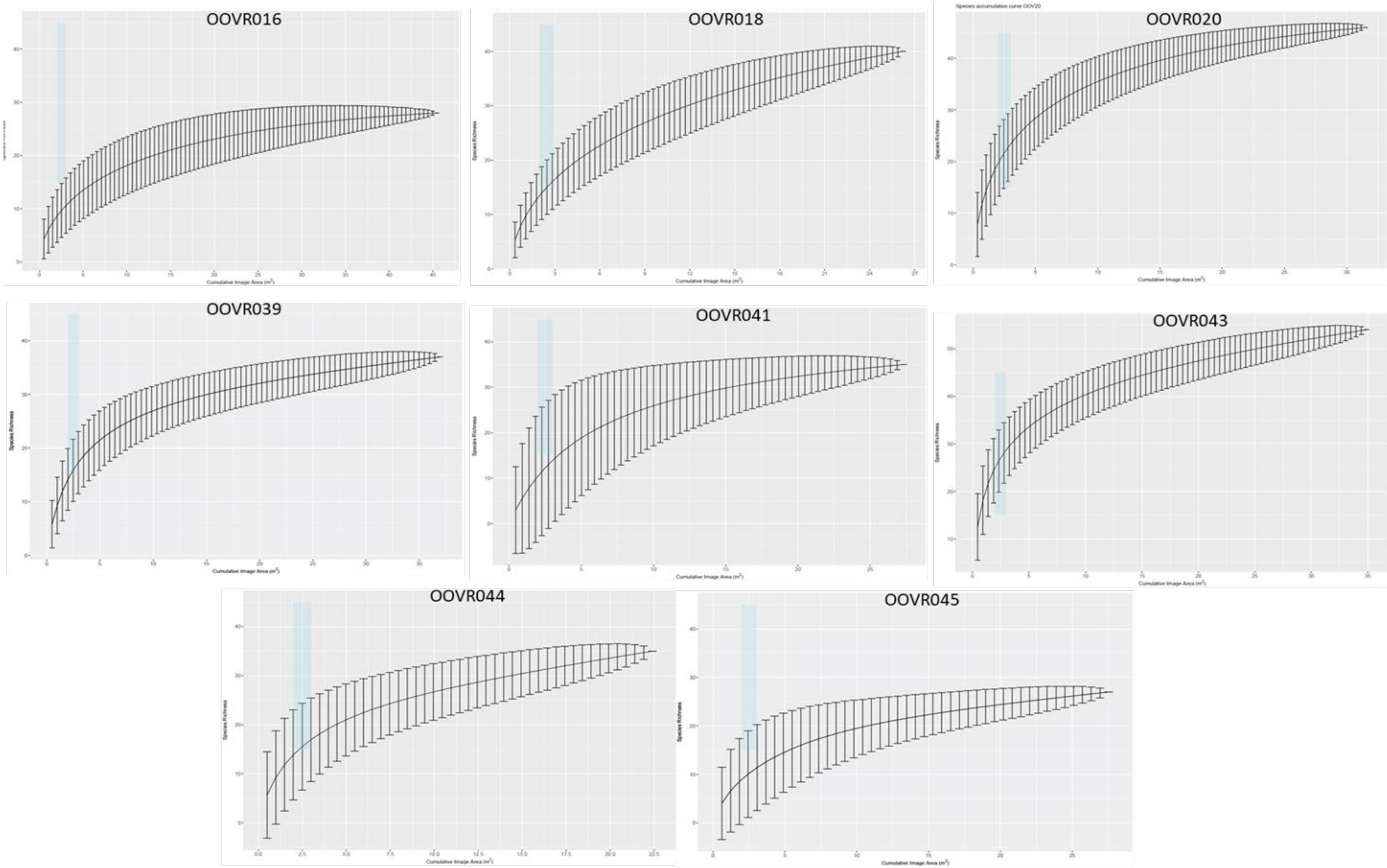

Figure 30. Individual Species Accumulation Curves (SpACs) (Species richness) for each increased replication station at Offshore Overfalls MCZ presented individually, with the area shaded blue representing a cumulative area of 2.5 m^2 for reference.

3.8.2 Comparison of epifaunal assemblages by four spatially different treatments of epifaunal samples

This sub-objective (2.a.ii) aimed to ascertain what influence, if any, spatial distribution of the component still images across the 'bullring' sample area (100 m diameter) may have on univariate descriptors and multivariate assemblage of the stations. To determine this, the increased replication stations were separated into four treatments ('Trt x'; Table 14), as described in Annex 3 and summarised as:

Table 14. List and description of treatments used in analysis for Objective 2.a.ii.

Treatment	Description	No. of Replicates	No. Stills	Average Area (m^2)
Trt1	15 stills chosen randomly from C transect (central)	8	15	6.68
Trt2	5 stills chosen randomly from C, A and E transects	8	15	7.42
Trt3	3 stills chosen randomly from A, B, C, D and E transects	8	15	6.64
Trt4	All stills from all transects at the station	8	75	34.81

The optimum 29.2-41.7 m^2 cumulative area (derived in Section 3.8.1) is greater than the average cumulative FoV acquired from any single replicate transect (i.e. the grid stations). The decision was therefore taken to aggregate 15 images as the sample unit for each station, as this was the maximum number of still images analysed for each replicate transect. Treatment 4 (75 images) was included as a control, as this is approximately equivalent to or above the optimum cumulative seabed area defined in Section 3.11.1.

Univariate metrics (Shannon's 'H' and Simpsons 'λ' diversity metrics, species richness, Pielou's 'J' evenness metric) of each treatment were then calculated and their distributions tested for normality using the Shapiro-Wilk test (H_0 = *diversity metric scores are normally distributed*).

Table 15 presents the results of the ANOVA testing of the three univariate metrics. The *p* values were all greater than 0.05, and thus the null hypothesis of no significant difference between the treatments was accepted.

Table 15. Results from ANOVA testing of the univariate metrics calculated for each of the increased replication treatments.

Treatment / Metric	DF	MSE	F Statistic	p
Pielou's 'J'	2	0.00127	0.158	0.855
Species Richness (S)	2	0.14680	0.146	0.865
Simpsons 'L'	2	0.00121	0.25	0.781

Figure 31 presents box plots resulting from the ANOVA testing of the univariate metrics for the three spatial treatments (Trt1, Trt2 and Trt3). The testing of Pielou's 'J' presented the highest degree of variation in score between the three treatments and associated extrema.

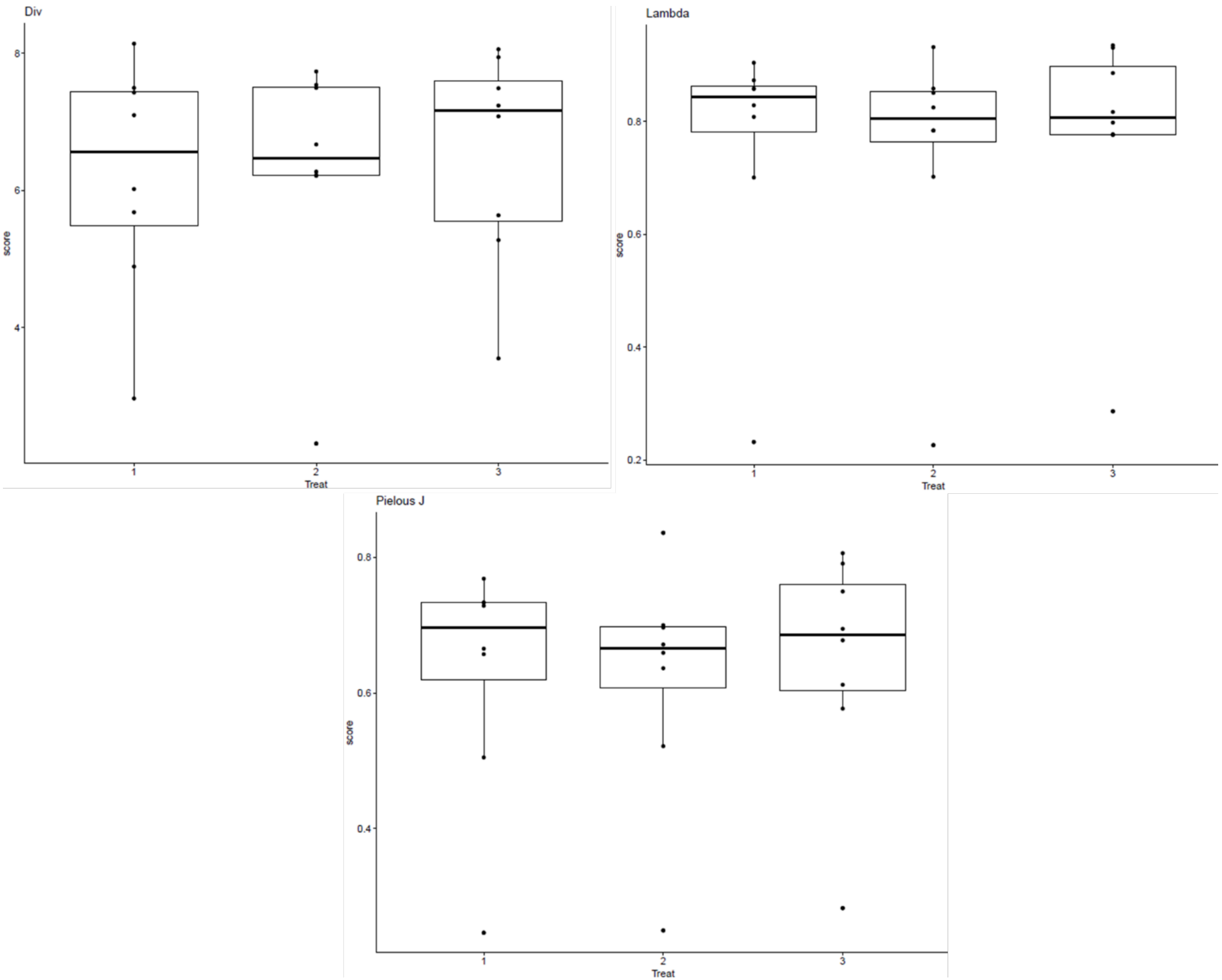

Figure 31. Box plots resulting from ANOVA testing of the univariate metrices for the three spatial treatments (Trt1, Trt2 and Trt3).

Variation in assemblage composition was investigated using PERMANOVA, specifically with the *adonis*() function in the R package 'vegan', as described in Annex 3. The multivariate assemblages of all four treatments were presented in non-metric MDS plot, in Figure 32.

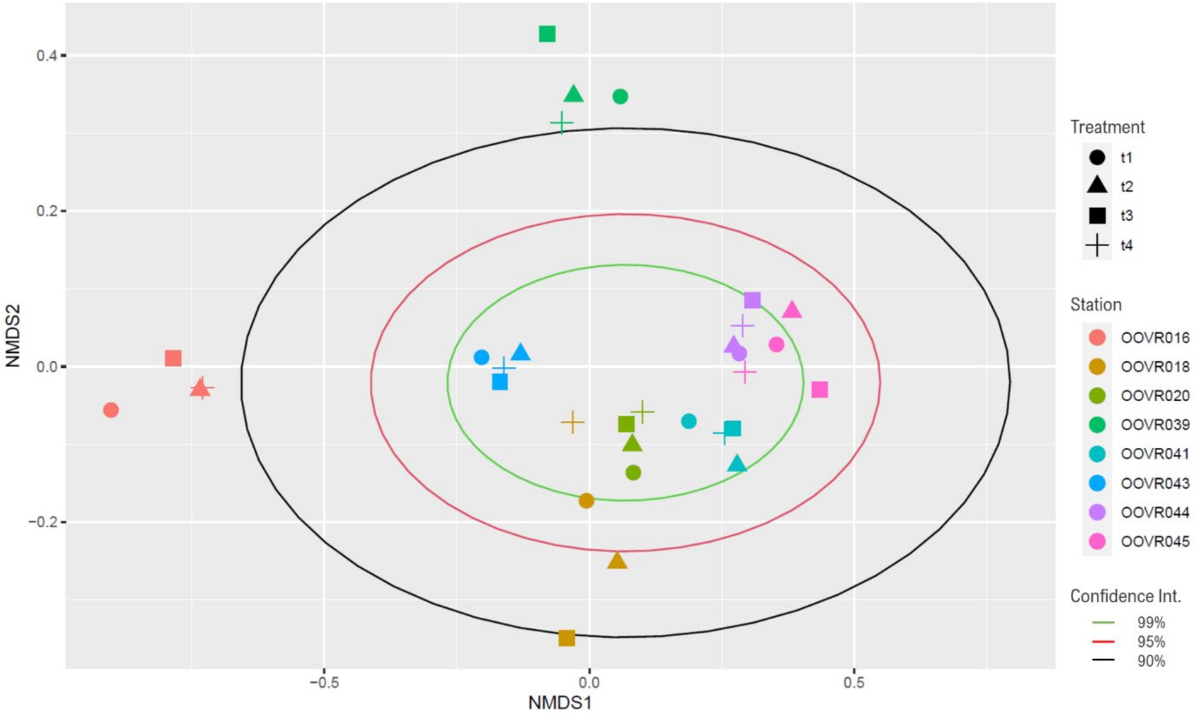

Figure 32. nMDS ordination of the assemblages observed at each of the eight 'Increased replication' stations, across each of the four treatments. Elipses represent confidence intervals.

Review of Figure 32 indicates that there is substantial variation between the stations, with stations OOVR16, OOV018 and OOVR39 notably set apart from the central cluster. Whilst there is some within-station separation noted from the ordination (most obviously at the three outlier stations), the significance of this separation must be tested statistically using PERMANOVA. The first of these tests included Treatment 4 (all images comprising the sample) as a control, the results of which are displayed in Table 16.

Table 16. PERMANOVA results between all four treatments of increased replication stations.

	Df	SumsOfSqs	MeanSqs	F	R^2	*p*
Treatment	3	1.23	0.41	1.62	0.15	0.04
Residuals	28	7.10	0.25	0.85		
Total	31	8.33	1.00			

Table 16 presents the results of the first PERMANOVA test, indicating a significant difference between the assemblages of the four treatments at the 5% significance level (F = 1.61, p = 0.037). As the levels of treatment and station are unbalanced (Trt4 being composed of all 45 images from each station), a nested two-way PERMANOVA could not be undertaken. A second one-way PERMANOVA was therefore undertaken, with Trt4 removed to ascertain if the significant difference observed in the first test was associated with Trt4 samples having many more still images. The results of this second PERMANOVA test can be seen in Table 17, which shows that no significant difference between the assemblages could be determined (F = 0.35, p = 0.99).

Table 17. Results of PERMANOVA (*adonis*() function) between the three main treatments of increased replication stations at Offshore Overfalls MCZ (Treatment 4 removed).

	Df	SumsOfSqs	MeanSqs	F	R^2	*p*
Treatment	2	0.18	0.09	0.35	0.03	0.99
Residuals	21	5.48	0.26	0.97		
Total	23	5.66	1.00			

To further visualise the differences between the treatments, *post-hoc* testing of the homogeneity of multivariate dispersions (how dispersed the assemblages are) for each treatment was undertaken using the *betadisper*() function within vegan (analogous to PERMDISP). No obvious difference can be seen in the homogeneity of dispersions of the three main treatments (Table 18), indicating that the source of significant difference in assemblage composition between treatments is Trt4.

Table 18. Dispersion statistic for each of the four treatments, tested using the *betadisper*() test.

	T1	T2	T3	T4
Average distance to median	0.4605	0.4576	0.4765	0.4349

Permutational testing for the significance of the difference in these dispersion statistics was undertaken using the *permutest*() function on the *betadisper*() results. This test found no significant difference ($F = 0.13$, $p = 0.93$) between the dispersions of each treatment, indicating homogeneity of dispersion in each treatment and thus conforming to a central assumption of the PERMANOVA test undertaken using the *adonis*() function above.

The results of the *betadisper*() testing (dispersion of each of the four treatments) are presented in Figure 33. Plate A (ordination of multivariate dispersions) highlights how homogeneity of dispersion is relatively comparable across all treatments, with Treatment 4 showing the smallest dispersion. However, there is a marked difference between the location of these assemblages on the ordination, with Treatment 4 noticeable separate from the Treatments 1 to 3. This is confirmed in the box plots (Plate B). When all are overlaid on the same plot, the centroid and sites of Treatment 4 can be seen to be well above (in non-metric space) the three main treatments.

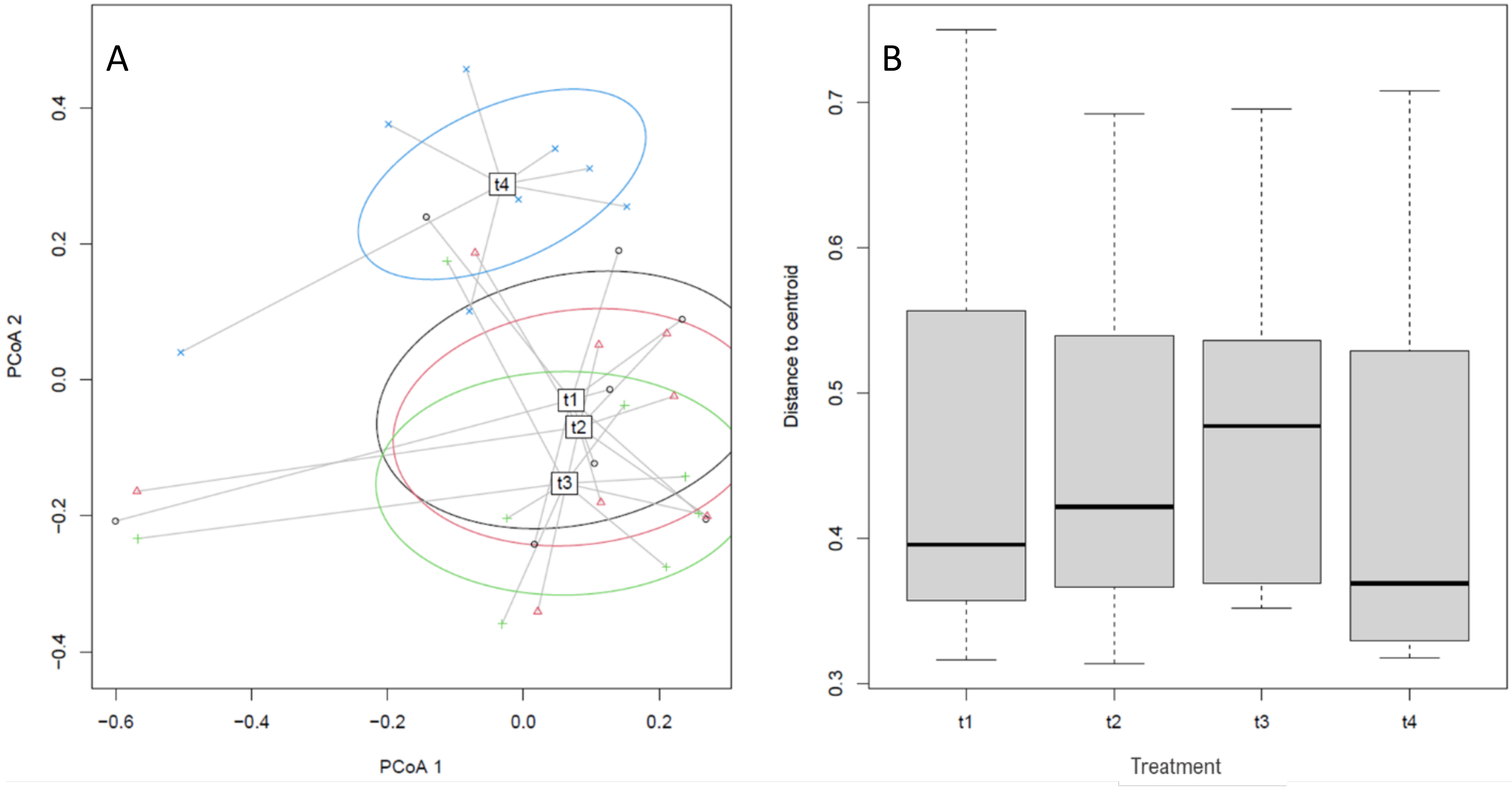

Figure 33. nMDS ordinations of the *betadisper*()-derived multivariate dispersions from each of the increased replication stations (Treatments 1 to 4 and all overlaid), from Offshore Overfalls MCZ.

3.9 Habitat Features of Conservation Importance (FOCI)

3.9.1 Designated habitat FOCI

Offshore Overfalls MCZ has not been designated for any habitat FOCI.

3.9.2 Undesignated habitat FOCI

Ross worm aggregations (*Sabellaria spinulosa)* have been noted across the site, particularly in the north-western section (associated with the sub-cropping Wealdon group rock). Ross worm aggregations are characteristic of *k*-means epifaunal assemblage 1. Two video transects, located over areas predicted to be the BSH 'Subtidal coarse sediment' were classified by the analysts to be the BSH 'Subtidal biogenic reef'. A full 'reefiness' assessment (as per Gubbay 2007) was beyond the scope of this report. Further stations also showed moderate to extensive presence of *S. spinulosa* crusts; these were not assessed as having sufficient spatial extents to be classified as habitat FOCI. Example images of *S. spinulosa* crust are presented in Figure 34.

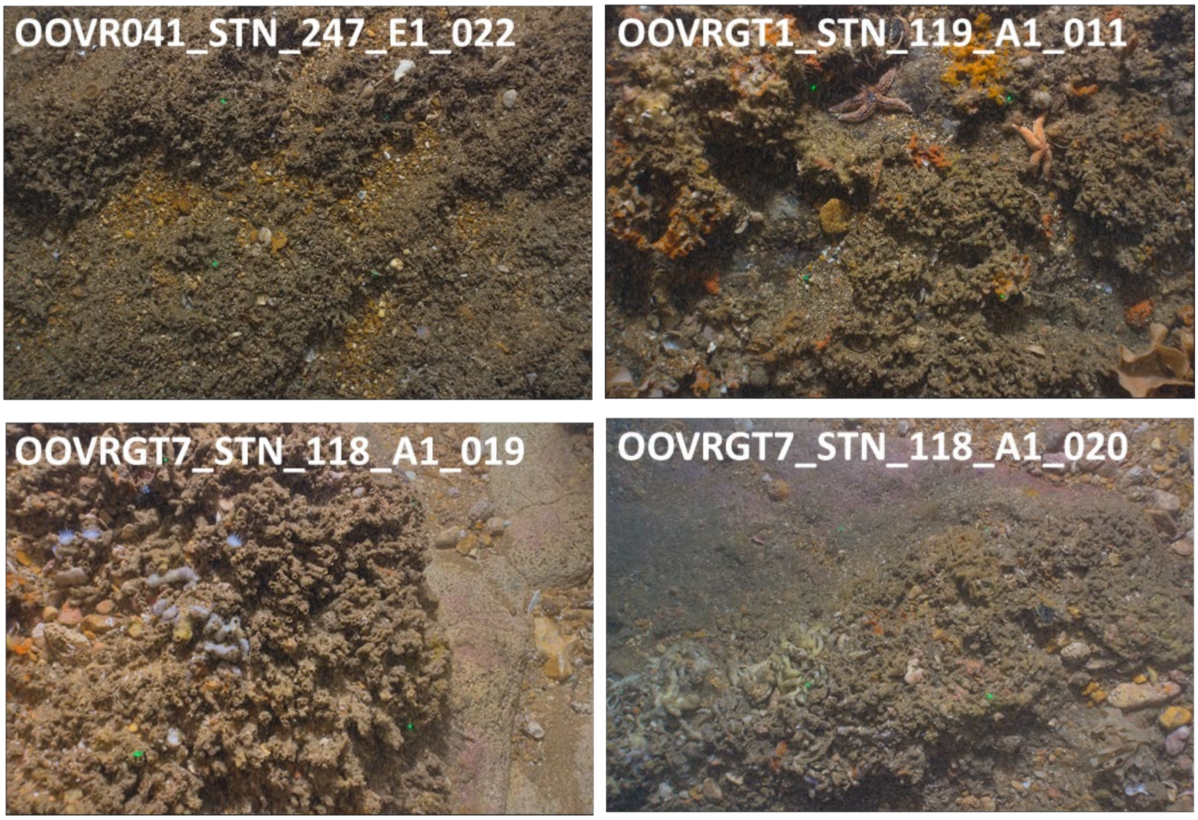

Figure 34. Example images of Ross Worm (*Sabellaria spinulosa)* crust observed at Offshore Overfalls MCZ.

The geographic extent of the observed *Sabellaria spinulosa* occurrences is presented in Figure 35.

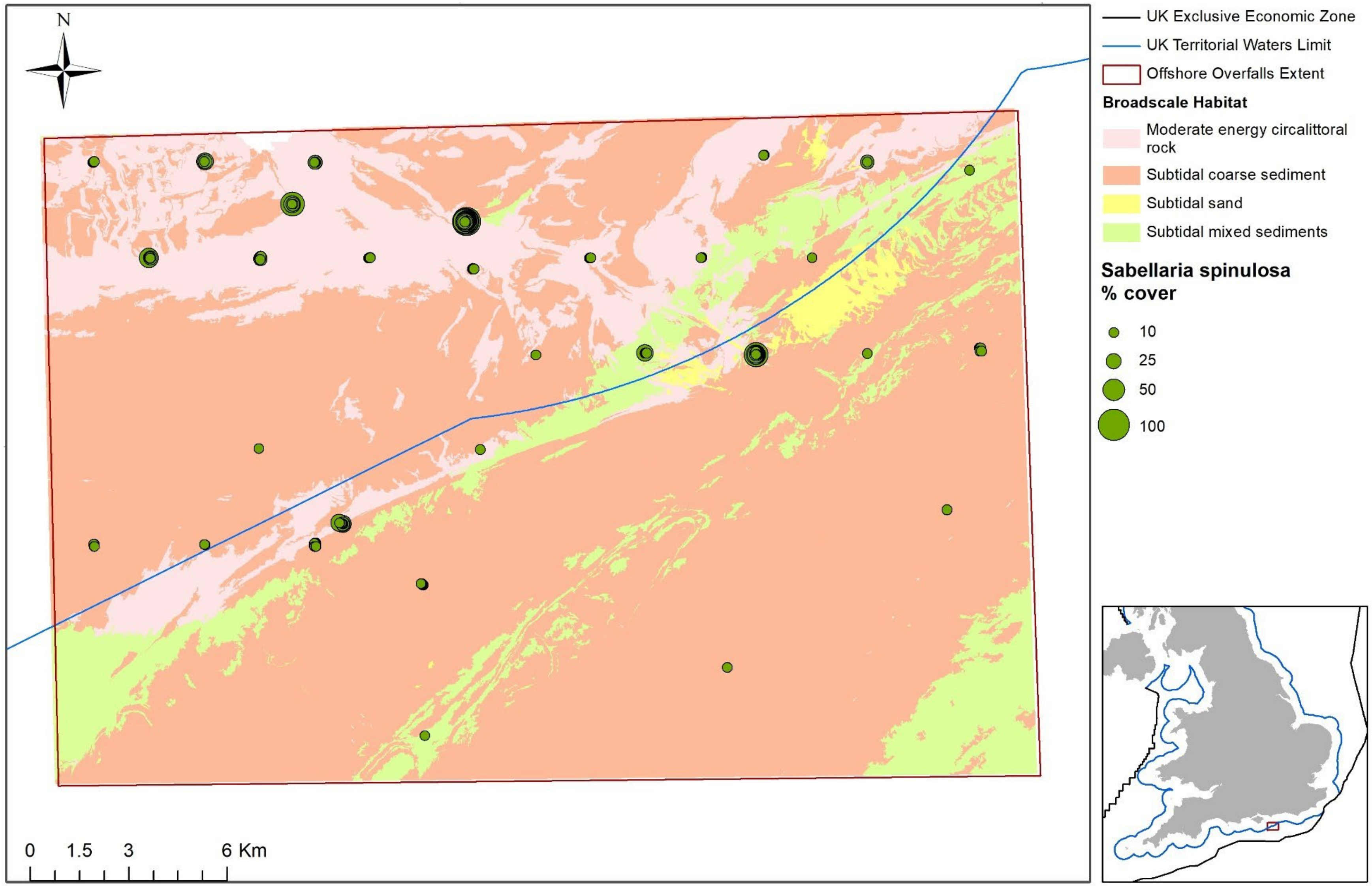

Figure 35. 2019 habitat map with *S. spinulosa* occurrences per still image where observed (size of circle indicates abundance in percent cover of image) overlain at Offshore Overfalls MCZ.

Figure 35 shows that *S. spinulosa* was more prevalent in the north of the site and is to some degree apparently associated with the BSH ‘Moderate energy circalittoral rock’.

3.10 Species FOCI

Offshore Overfalls MCZ is not designated for any species FOCI, and none were observed at Offshore Overfalls MCZ in 2019.

3.11 Non-indigenous species (NIS)

Two of the non-indigenous species (NIS) in Table 26 (Annex 6) were observed from the seabed imagery (26 still images) across 11 stations, presented in Figure 36. These species were the slipper limpet *Crepidula fornicata* and tunicate *Styela clava*. No records of NIS were observed from the video footage, nor the sediment sampling.

3.12 Marine litter

Marine litter (classified as detailed in Table 25, Annex 5) was observed from 12 still images across eight stations, alongside three observations from three video transects (Figure 37). The majority of litter items observed were forms of plastic, with none being larger than category B (10 cm x 10 cm). Three pieces of glass / ceramics were observed, alongside two pieces of metal (one of which was classed as size category C, see Annex 5).

3.13 Observed anthropogenic activities and pressures

As well as the marine litter findings presented in Section 3.12 three observations of potential unexploded ordnance (UXO) were made at Offshore Overfalls MCZ.

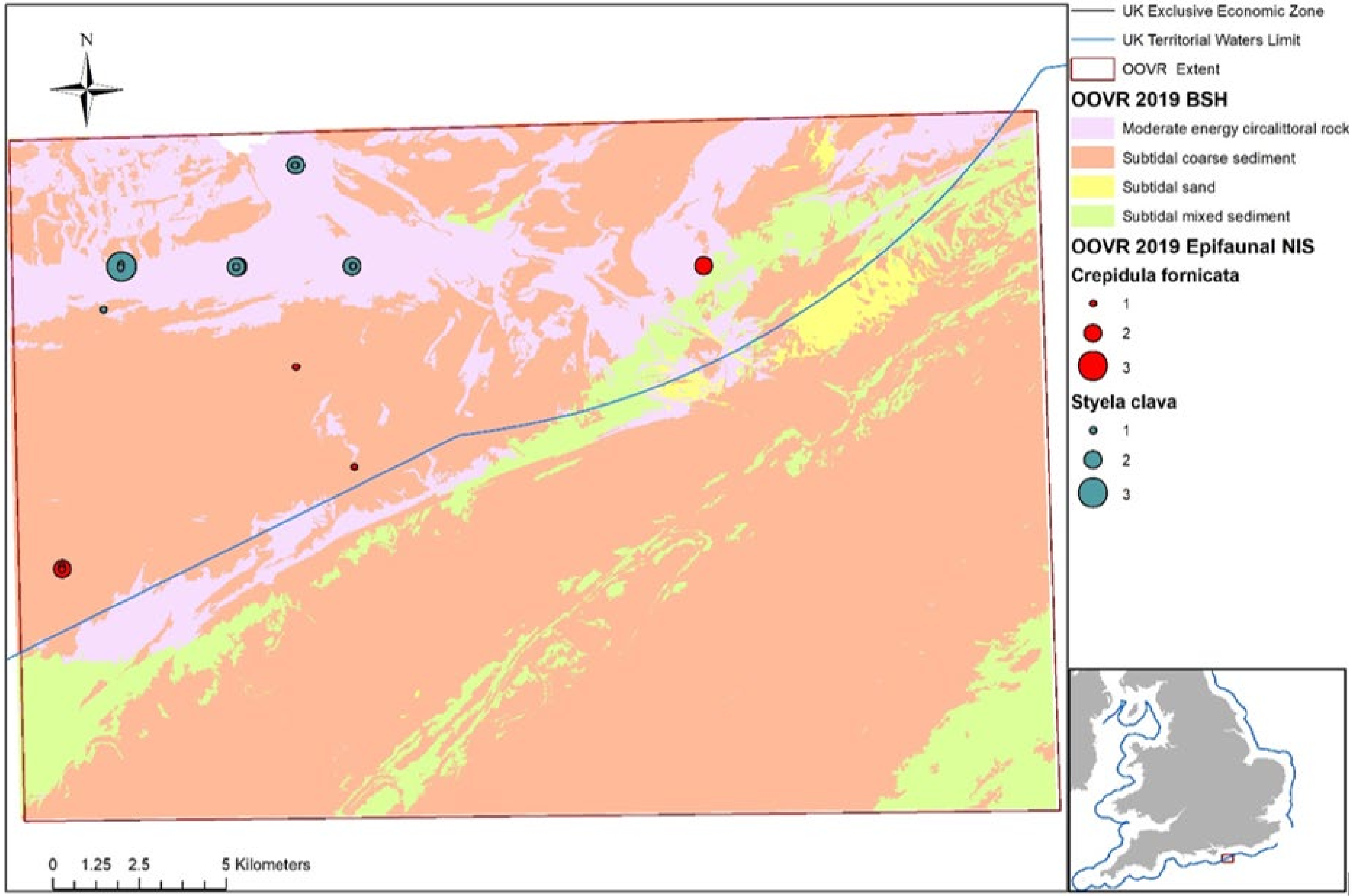

Figure 36. 2019 habitat map with Non Indigenous Species occurrences per still image where observed (size of circle indicates number of individuals) overlain at Offshore Overfalls (OVVR) MCZ.

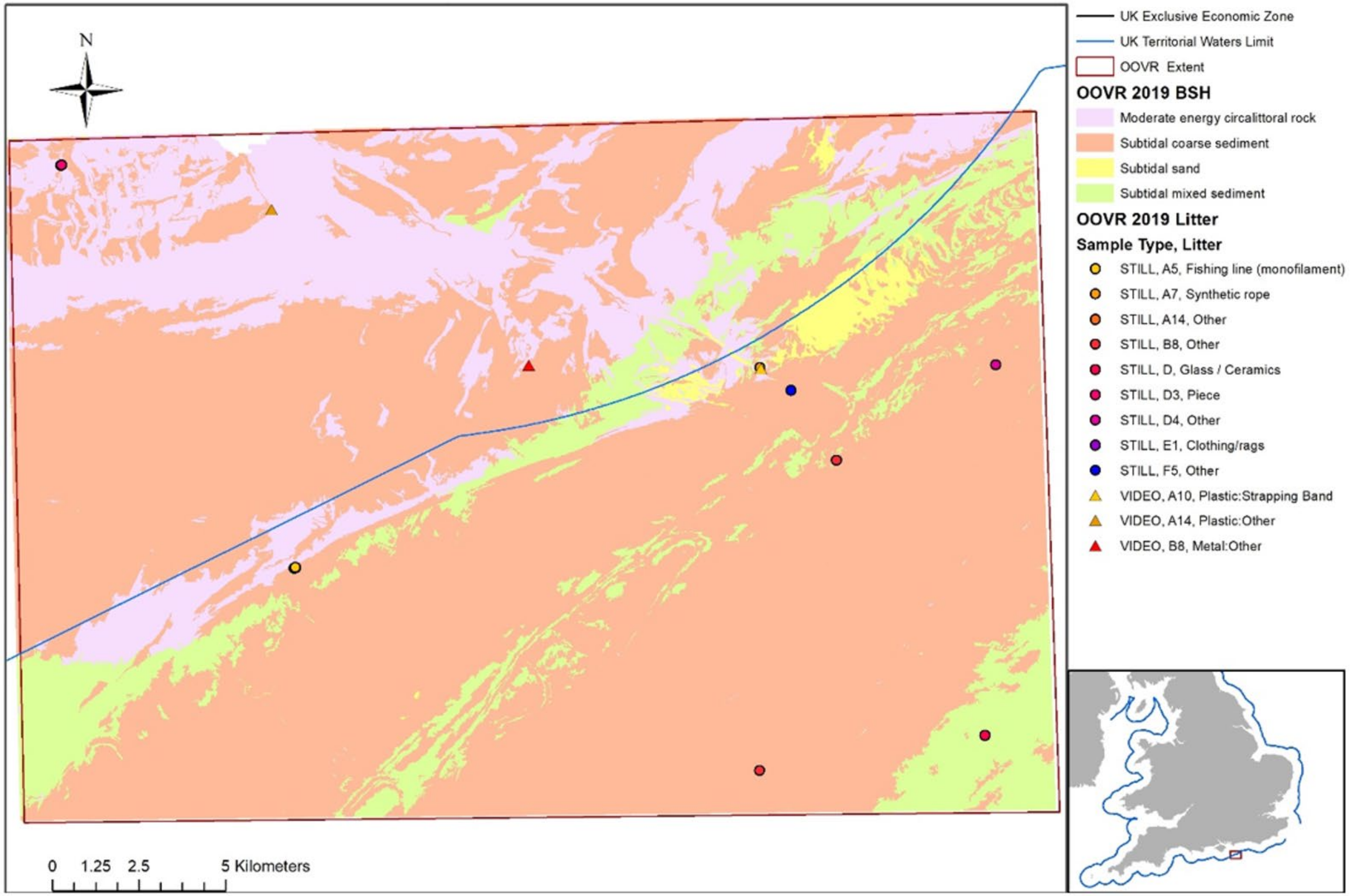

Figure 37. 2019 habitat map with litter occurrences overlain at Offshore Overfalls (OOVR) MCZ.

4 Discussion

This monitoring report has achieved objectives 1 to 5 (Table 1). The following sections discuss the evidence pertaining to the report objectives and provide monitoring recommendations for the designated features.

4.1 Objective 1. Extent, distribution, and structural attributes of the designated features

4.1.1 Fine scale topography and geomorphology

This data exploration and the geomorphological classification undertaken in this report have provided the most detailed study to date of the English Channel outburst flood features (Quaternary fluvio-glacial erosion features). Specific morphological forms have been identified from this site for the first time, and are detailed in a peer-reviewed publication (Arosio *et al.* 2021). The geomorphological forms identified as a result of this investigation into fine scale topography, such as bedrock streamlined mounds and tear-drop shaped hills, the over-deepened basin and deep (>1 m) scour marks in the bedrock, have presented evidence in favour of erosion by catastrophic flooding as the primary creative process for the Northern Palaeovalley feature.

The geomorphological classification of these features at high resolution has resulted in a greater diversity of geomorphological classes than previously thought to occur at the site. These classes have been incorporated into the predictive habitat model as potential environmental drivers of faunal assemblage extent and distribution.

4.1.2 Physical structure, extent, and distribution of BSH

Investigating the physical structure of the BSH features at Offshore Overfalls MCZ presented significant (although not unexpected) challenges, given the difficulty of acquiring sediment samples of sufficient quality from coarse gravel habitats. This resulted in a limited number of samples and very limited success in acquiring replicate samples from the subset of increased replication stations.

Sediment samples indicated a largely gravel-dominated substrate with varying proportions of sand. Several sediment samples were successfully acquired from the grid stations, which indicated limited proportions of fine (mud) fractions, supporting the predictive mapping result of a larger area of ‘Subtidal mixed sediments’ than previously predicted based on the 2015 map.

4.1.3 Biological structure

Initial investigations into biological structure using the epifaunal data and the ANOSIM routine (with BSH as *a-priori* factor) presented no discernible structure. This is in keeping with expectations, considering the prevalence of ‘Subtidal coarse sediment’ across the site, and that this BSH class can include multiple and distinctly varied habitats. This is due to the gradation of particle sizes this BSH contains and the functional differences between the associated habitats, i.e. mobile gravelly sand through to stable pebbles and cobbles. Particle size estimation from video and still imagery (a crucial part of ‘Subtidal coarse sediment’ classification) has previously been subject to a degree of subjectivity, further exacerbating the difficulties in assessing assemblage structure within this BSH. These issues, and the consequent lack of structure identified indicate that BSH classes are not ecologically meaningful monitoring units for this MCZ.

The analysts' assessment of the predominant BSH 'High energy circalittoral rock' in 2019 versus 'Moderate energy circalittoral rock' in the 2015 map supports the finding of BSH limitations in monitoring, owing to the subjective nature of BSH designation from seabed imagery (as also observed at East of Haig Fras MCZ; Clare *et al.* 2020). Upon review of the characterising taxa resolved using the indicator value metric and the subsequent biotope assignment, it is possible to confirm that the 2015 assessment of 'Moderate energy circalittoral rock' is likely more representative of the rocky habitat present.

Following the BSH-partitioned epifaunal assemblage analysis, *k*-means clustering was used to determine epifaunal assemblages. This made ecological sense, aligning with the approximate number of habitats observed by eye whilst on survey and in review of the seabed images. These assemblages were further investigated through use of the indicator value metric and the 'Multipattern Analysis' routine. Redundancy analysis (RDA) proved a useful method of relating *k*-means classified assemblages with observed environmental variables.

These approaches allowed for delineation between the potentially difficult to distinguish assemblages of epifaunal assemblages 3 and 4. This separation appears to be strongly associated with particle size, with larger particles, specifically cobbles, being associated with assemblage 4. Furthermore, the spatial distribution of assemblage 3 is limited to the south-east of the site, co-located with the Northern Palaeovalley channel floor and the area of the site with the largest variation in tidal magnitude. Conversely, the distribution of assemblage 4 is centred on the 'plain' feature to the north-east of the site, which is shallower than the palaeovalley floor and subject to a constant moderate tidal exposure (with a uniformity of direction owing to an amphidromic point predicted by the tidal model).

One of the primary characterising taxa of epifaunal assemblage 3 is the bivalve *Aequipecten opercularis* (Queen Scallop). The abundance of the species was high across assemblage 3 samples. *A. opercularis* is a low motility species, which is long lived (up to six years; Heilmayer *et al.* 2004) and is targeted by fishers (Carter, 2008).

Epifaunal assemblage 1 appeared to be associated with bedrock and sand; given the prevalence of *Sabellaria spinulosa* this is likely to be a sand veneer in locations adjacent to more continuous sandy patches. This is in keeping with the nature of the sub-cropping Wealdon assemblage rock associated with the north-west of the site. Epifaunal assemblage 2, however, is a difficult assemblage for monitoring purposes, owing to the mixed nature of the assemblage (low within-assemblage similarity) and ephemeral nature of ophiuroid beds. Owing to the close association observed between assemblage classes, and observed and modelled physical parameters across the site, it is considered likely that the areas mapped as assemblage class 2 are areas which either have ophiuroid beds present or are areas where the environmental conditions are correct for these beds to occur.

The building of mappable habitat classes from the epifaunal assemblage groups involved the addition of a class which was not identifiable from seabed imagery: class '5'. This class comprised mobile coarse to fine sands and was determined through sediment sampling and PSD analysis from stations which targeted two areas of megaripple features, identified from the MBES data (the 'Ad-hoc' stations).

The assignation of associated biotopes to the five mapped classes confirmed their ecological coherence, with no class (including the difficult to interpret class 2) found to be un-relatable to the MHCBI. The limitations of sediment sampling at this coarse gravel-dominated site presented a challenge in obtaining co-located infaunal and epifaunal samples. The agreement between infaunal assemblages derived from the two sediment samples co-located with epifaunal stations classed as assemblage 1 was unexpected. That sediment samples of greater than 4L were acquired from these stations indicates that a substantial depth of soft sediment veneer or proximity to large patches of mobile sand is associated with some assemblage 1 stations, and the confirmed presence of live *Sabellaria spinulosa* in the infaunal samples (indeed, being characteristic of the infaunal assemblage) confirms the indicative nature of this species for habitat class 1.

4.1.4 Biological extent

The final assemblage-driven habitat map, based on the above, described five habitat classes and had a high degree of both users and producers accuracy, resulting in an overall accuracy of 76%, and a Kappa statistic (observed vs. expected accuracy) of 0.69, considered "substantially accurate" (Landis & Koch 1977). The assemblage-driven habitat map provides insight into the distribution of coarse habitats, which are primarily associated with the BSH 'Subtidal coarse sediment'. Without the assemblage analysis and subsequent mapping, this structure and extent would have remained unexplored.

Owing to dominance across the site of the 'Subtidal coarse sediment' BSH (as identified from the video segments), predictive modelling of initial BSH was not possible (due to the imbalance of the training data). As such, the epifaunal samples were back-translated to BSH type based on their assemblage, with the resulting map having a validation accuracy (back-translated predicted BSH vs. initial 2019 video BSH) of 65%, indicating good alignment between the translated BSH types and initial video derived BSH assignations.

Habitat mapping based upon the prediction of assemblage extent does have inherent uncertainties associated with it. One can never be certain that one has fully sampled the assemblages present at the site, or that some unmeasured environmental parameter is not driving a large degree of the variation in assemblage. Given the strength of the link between assemblage class and environmental parameters, this association provides assurances as to the confidence placed in the final habitat map. The previously generated BSH map (2015) used very limited Hamon grab grain size data for groundtruthing, over partial coverage MBES bathymetry, producing a manually derived (expert judgement based) habitat map. Given the cross-validation results of this 2019 habitat map, the process presented in this report can be considered more reliable and informative.

4.2 Objective 2. Within-station variability

The 2019 survey strategy was designed to acquire the first quantified dataset in a monitoring time series, to be used for future comparison and assessment of condition. As the coarse substrate dominated habitats preclude effective sediment sampling, the survey required acquisition of seabed imagery data. Previously employed methodologies for acquisition and analysis of seabed imagery allowed for, at best, a semi-quantitative epifaunal abundance dataset. The 2019 survey and analysis strategy focused on the acquisition of seabed imagery from well controlled and comparable areas, whilst minimising the geographical spread of still images which comprised individual samples (by using 50 m transects as opposed to traditionally used 15-minute tows). This resulted in the 'bullring' acquisition methodology presented here. This was based on the theory that, with prior knowledge in the form of OBIA segmentation of high resolution MBES and backscatter data, small areas with similar properties (depth, reflectance intensity, topography etc.) could serve as sampling locations with consistent conditions across their areas. This was thought to reduce the chances of acquiring imagery from multiple habitats within a single transect, and thus (in conjunction with downward facing imagery and tightly controlled, altitude based FoVs) provide an accurate, standardised, and quantitative assemblage dataset for future monitoring.

To test this theory, the increased replication subset of eight stations was subjected to further analysis in two parts. The first of these, the calculation of Species Accumulation Curves (SpACs), aimed to assess the area of seabed that might best constitute a ‘sample’ at this site (i.e. through aggregation of still images to represent a single station). The second part aimed to ascertain whether any difference in derived epifaunal assemblage composition might be detectable if component images were distributed not on a single, central transect, but more randomly across the entire bullring, giving a more accurate indication of micro-variability within the defined area of the station.

4.2.1 Assessment of Species Accumulation Curves

The primary finding of part 1 of objective 2.a, the SpAC curves, was that the mean area required to effectively capture univariate diversity (the asymptote of each curve) at a station within Offshore Overfalls MCZ varied between BSH. The analysis showed that the minimum seabed area required for the BSH ‘Subtidal coarse sediment’ was 29.2 m^2, compared with an average of 41.7 m^2 for the BSH ‘Moderate energy circalittoral rock’. These values are comparable with the summed area covered by all five 50 m transect replicates at an increased replication station (the mean value was 36.23 m^2). These values are significantly higher than the average area covered by the 15 selected images from grid (single transect) stations at the site (6.87 m^2).

This indicates that samples from the grid monitoring stations are unlikely to cover a sufficient area to accurately assess within-station epifaunal diversity.

4.2.2 Assessment of within-station variation in epifaunal assemblages

In summary, these findings show that for a limited dataset of 15 still images within a 100 m radius bullring, a single transect is all that is required to describe the within-station variation of the epifaunal assemblage. However, the findings of this section also provide further support to the finding of the above section (4.2.1); namely that 15 still images is not a sufficient number of still images for a fully accurate characterisation of the epifaunal assemblage of a station. The number required is likely to vary by habitat type, however the final number will be closer to 75 images per station (depending on field of view of each image).

4.3 Operational and survey strategy recommendations

- The ‘bullring’ and 50 m transect approach to grid stations has been successfully tested and can be recommended for future use.
- Future surveys at this site should look to ensure that a minimum of 30 m^2 seabed area is sampled per station for BSH ‘Subtidal coarse sediment’, and 42 m^2 for BSH ‘Moderate energy circalittoral rock’. This will likely equate to ~75 images at the standard altitudes of ~0.5 to 1.5 m (swell and operator dependent).
    - Should 50 m transects be considered too short for acquisition of ~75 high quality images these may be extended to 75 m.
    - If weather conditions (sea state and winds) do not permit the acquisition of sufficient quality (non-overlapping) still images from such 75 m transects, then a three replicate transect approach within the bullring is recommended.

  - Still images taken at a frequency of ~10 second intervals would allow for a sufficient seabed area per station. The findings of this report indicate that this could, where possible, be increased to ~5 second intervals.
  - If budgetary constraints are a limiting factor, the findings of this report support the sampling of fewer stations to increase the number of still images available for each station.
  - Eight increased replication stations from a single study are not considered sufficient to inform a universal verdict on the use of this approach in the wider MPA monitoring programme. A minimum of five increased replication stations per feature is recommended, with five being considered a robust number of replicates according to expert judgement.
  - Future within-station variation experiments should ensure that sufficient still images from the central transect are analysed to provide for the optimum seabed area. This will enable more conclusive results in the PERMANOVA testing.

- Acquisition of high frequency altimetry data proved invaluable for selection of still imagery for analysis and should be acquired wherever possible.
- Orthogonal (top-down) orientation of the stills camera resulted in no significant identification issues and, crucially, made quantitative assessment of seabed area possible. This is recommended for future surveys at any site where seabed imagery will be used as the principal means of developing a quantified monitoring dataset.
- Optimum altitude for acquisition of high-quality seabed still images is context dependent (i.e. will depend on the communities present and physical factors such as turbidity at the time of survey). It is recommended that this should not exceed 1.5 m. Images acquired at 1 m altitude appeared to be best suited at Offshore Overfalls, based on retaining consistent ground resolution for identification of the taxa present.
- High numbers of replicate grab sampling failures may indicate the unsuitability of a particular sampling methodology for the sediments encountered. This report recommends review of failure to success ratios for different BSH classes across the whole of the MPA programme, to ascertain better ‘attempt number thresholds’ for specific sediment types.
  - Where grab sampling is ineffective, trawling is an effective means of epifaunal sampling.
  - Channel gravels may be the best-case study site for a trawl vs. camera gear comparison.

4.4 Analysis and interpretation recommendations

- Not all still images were analysed due to budgetary constraints (15 out of between 35-45 per station), and one of the key findings was that the recommended summed FoV per station for adequate characterisation was considerably greater than that analysed. It is therefore recommended that the remaining stills are analysed to improve baseline characterisation of the grid stations.
- The use of the BIIGLE platform in annotation of the still images was invaluable for ensuring extraction of reliable, repeatable, and quantitative abundance data from the still imagery. This is highly recommended for future monitoring projects.
- Creation of a relative abundance matrix through the normalisation of the ground cover and point count datasets increased the amount of data available for the multivariate

analyses. As this report relied so heavily on epifaunal data acquired from seabed imagery of relatively species-poor coarse gravel habitats, maximising the number of entries in the matrix is of singular importance in assessing biological structure. This approach is therefore recommended for future studies which rely on still or video imagery and use a mixed enumeration approach (abundance and ground cover taxa) as opposed to cell frequency.

- The derivation of epifaunal assemblages using the *k*-means clustering approach proved invaluable in this report and should be considered a standard analytical technique for imagery data in future reporting.
- The use of the 'indicator value' metric and the *multipatt*() algorithm produced a list of characterising morpho-taxa which were much easier to interpret than the traditional SIMPER routine (which tends to be obscured by high abundance).This should also be considered a standard analytical technique for imagery data in future reporting.
- Consideration should be paid to the potential of using abundance of the Queen Scallop, *Aequipecten opercularis,* as an indicator of site condition for Offshore Overfalls MCZ. The life history and ease of sampling of this species conforms to the nine requirements set out by OSPAR (2012) and presented in Annex 3.

5 References

Allaby, M. (2015). A dictionary of ecology (5th edition). Oxford University Press, UK.

Anderson, M.J. (2006). Distance-based tests for homogeneity of multivariate dispersions. *Biometrics* 62, 245–253. https://doi.org/10.1111/j.1541-0420.2005.00440.x

Arosio, R., Collier, J.S., Hawes, J., Gupta, S. and Sperry, J. (2021). New perspectives on the English Channel megaflood hypothesis: High-resolution multibeam and seabed camera imaging of submarine landforms in the Northern Palaeovalley. *Geomorphology* 382, 107692. https://doi.org/10.1016/j.geomorph.2021.107692

Blaschke, T. (2010) Object Based Image Analysis for Remote Sensing. *ISPRS Journal of Photogrammetry and Remote Sensing*, 65, 2-16.

Caliński, T. and Harabasz, J. (1974). A dendrite method for cluster analysis. *Communications in Statistics* 3, 1–27. https://doi.org/10.1080/03610927408827101

Carter, M.C. (2008). *Aequipecten opercularis* Queen scallop. In Tyler-Walters H. and Hiscock K. Marine Life Information Network: Biology and Sensitivity Key Information Reviews, [on-line]. Plymouth: Marine Biological Association of the United Kingdom. [cited 08-12-2021]. Available from: https://www.marlin.ac.uk/species/detail/1997

Clare, D., Downie, A., Hawes, J. & Langton, B. (2020). East of Haig Fras Marine Conservation Zone (MCZ) Monitoring Report. JNCC/Cefas Partnership Report No. 29. JNCC, Peterborough, ISSN 2051-6711, Crown Copyright. https://hub.jncc.gov.uk/assets/0a1035b1-3b40-4ada-a47a-7c8771aff3f1 (Accessed 29/08/2022).

Clarke, K.R., Chapman, M.G., Somerfield, P.J. & Needham, H.R. (2006). Dispersion-based weighting of species counts in assemblage analyses. *Marine Ecology Progress Series*, 320, 11-27.

Clarke, K.R. & Gorley, R.N. (2015). PRIMER v7: User Manual/Tutorial. PRIMER-E, Plymouth, 296pp

Clarke, K.R., Gorley, R.N., Somerfield, P.J. & Warwick, R.M. (2014). Change in marine communities: an approach to statistical analysis and interpretation, 3rd edition. PRIMER-E: Plymouth.

Coggan, R., Mitchell, A., White, W. & Golding, N. (2007). Recommended operating guidelines (ROG) for underwater video and photographic imaging techniques V11.2 (http://www.emodnet-seabedhabitats.eu/default.aspx?page=1915) [Accessed 24/07/20]

De Cáceres, M., Legendre, P. & Moretti, M. (2010). Improving indicator species analysis by combining groups of sites. *Oikos* 119 (10) 1674–1684. https://doi.org/10.1111/j.1600-0706.2010.18334.x

De Cáceres, M. (2019). How to use the indicspecies package (ver. 1.7.8). R supporting document.

Dietrich, H. & Böhner, J. (2006). Cold air production and flow in a low mountain range landscape in Hessia (Germany). Contrib. to *Phys. Geogr. Landsc. Ecol*. - Hambg. 37–48.

Dove, D., Nanson, R., Bjarnadóttir, L.R., Guinan, J., Gafeira, J., Post, A., Dolan, M.F.J., Stewart, H., Arosio, R. & Scott, G. (2020). A two-part seabed geomorphology classification scheme (v.2); Part 1: morphology features glossary 22. https://doi.org/10.5281/ZENODO.4075248

Downie, A., Arosio, R. & McBreen, F. 2022. Wight-Barfleur Reef Special Area of Conservation (SAC) Characterisation Report 2017. JNCC/Cefas Partnership Report 41. JNCC, Peterborough, ISSN 2051-6711, Crown Copyright. https://hub.jncc.gov.uk/assets/bdcaa728-6738-47ab-9de9-dd1589ab6316

Dufrene, M. & P. Legendre. (1997). Species assemblages and indicator species: The need for a flexible asymmetrical approach. *Ecological Monographs*, 67: 345-366.

Dudley, N. (2008). Guidelines for applying Protected Area management categories. IUCN, Gland.

Eno, N.C., Clark, R.A. & Sanderson, W.G. (1997). Non-native marine species in British waters: a review and directory. Joint Nature Conservation Committee, Peterborough, UK.

Folk, R.L. (1954). The Distinction between Grain Size and Mineral Composition in Sedimentary-Rock Nomenclature. *Journal of Geology*. https://doi.org/10.1086/626171

Gubbay, S. (2007). Defining and managing *Sabellaria spinulosa* reefs: Report of an inter-agency workshop 1-2 May 2007. JNCC Report No. 405. JNCC, Peterborough, ISSN 0963-8091. https://hub.jncc.gov.uk/assets/ecdbc5ba-e200-47e3-b7c6-adf464287712

Heilmayer, O., Brey, T., Storch, D., Mackensen, A. & Arntz, W.E. (2004). Population dynamics and metabolism of *Aequipecten opercularis* (L.) from the western English Channel (Roscoff, France). *Journal of Sea Research* 52, 33–44. https://doi.org/10.1016/j.seares.2003.07.005

Hill, N., Althaus, F., Rees, T., Jordan, A., Industries, P., Colquhoun, J., Sch, C., Case, M., Scott, F., Edgar, G., Legorreta, R.F., Fromont, J., Museum, W.A., Przeslawski, R., Hayes, K., Marzinelli, Z., Friedman, A., Toohey, L., Hibberd, T., Hovey, R., Kendrick, G., Wyatt, M., Edwards, L. & Centre, P.S. (2014). CATAMI classification scheme for scoring marine biota and substrata in underwater imagery 1–24. Available from CATAMI_Pictorial Guide (researchgate.net) (Accessed 23/08/2022).

Jones, R.E., Unsworth, R.K.F., Hawes, J. & Griffin, R.A. (2020). Improving benthic biodiversity assessments in turbid aquatic environments. *Aquatic Conservation: Marine and Freshwater Ecosystems,* 31 (6) 1379-1391. https://doi.org/10.1002 /aqc.3509

Kindt, R. & Coe, R. (2005). Tree diversity analysis. A manual and software for common statistical methods for ecological and biodiversity studies. http://apps.worldagroforestry.org/downloads/Publications/PDFS/MN08242.pdf (Accessed 23/08/2022).

Landis, J.R. & Koch, G.G. (1977). An application of hierarchical Kappa-type statistics in the assessment of majority agreement among multiple observers. *Biometrics*, 33 (2) 363–374. https://doi.org/10.2307/2529786

Langenkämper, D., Zurowietz, M., Schoening, T. & Nattkemper, T.W. (2017). BIIGLE 2.0 - Browsing and annotating large marine image collections. *Frontiers in Marine Science* 4, 1–10. https://doi.org/10.3389/fmars.2017.00083

Legendre, P. & Legendre, L. (2018). Numerical Ecology, 3rd ed. Elsevier B.V.

Legendre, P. & Gallagher, E.D. (2001). Ecologically meaningful transformations for ordination of species data. *Oecologia* 129, 271–280. https://doi.org/10.1007/s004420100716

Lim, A., Kane, A., Arnaubec, A. & Wheeler, A.J. 2018. Seabed image acquisition and survey design for cold water coral mound characterisation. *Marine Geology* 395, 22–32.

Long, D. (2006). BGS detailed explanation of seabed sediment modified Folk classification Available at https://www.researchgate.net/publication/284511408_BGS_detailed_explanation_of_seabed_sediment_modified_folk_classification (Accessed 23/08/2022).

Lucieer, V. & Lamarche, G. (2011). Unsupervised fuzzy classification and object-based image analysis of multibeam data to map deep water substrates, Cook Strait, New Zealand. *Continental Shelf Research* 31 (11) 1236–1247.https://doi.org/10.1016/j.csr.2011.04.016

Mason, C. (2011). NMBAQC's Best Practice Guidance Particle Size Analysis (PSA) for Supporting Biological Analysis.

Mellett, C. & Green, S. (2015). Offshore Overfalls rMCZ Post-survey Site Report 54. Available at https://randd.defra.gov.uk/ProjectDetails?ProjectId=18983 (Accessed 22/01/24).

Natural England & JNCC. (2010). The Marine Conservation Zone Project: Ecological Network Guidance. Sheffield and Peterborough, UK.

Oksanen, J. (2008). Vegan: an introduction to ordination. Management 1, 1–10. https://doi.org/intro-vegan.Rnw 1260 2010-08-17 12:11:04Z jarioksa processed with vegan 1.17-6 in R version 2.12.1 (2010-12-16) on January 10, 2011.

Oksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H.H., Szoecs, E. & Wagner, H. (2019). Package "vegan" - Community Ecology Package. Available at https://cran.r-project.org/web/packages/vegan/index.html (Accessed 23/08/2022).

Olofsson, P., Foody, G.M., Herold, M., Stehman, S. V., Woodcock, C.E. & Wulder, M. (2014). Good practices for estimating area and assessing accuracy of land change. *Remote Sensing of Environment* 148, 42–57. https://doi.org/https:/doi.org/10.1016/j.rse.2014.02.015itle

OSPAR Commission. (2012). MSFD Advice Manual and Background Document on Biodiversity: Approaches to determining good environmental status, setting of environmental targets and selecting indicators for Marine Strategy Framework Directive descriptors 1, 2, 4 and 6. Version 3.2. Prep.

Phinn, S.R., Roelfsema, C.M. & Mumby, P.J. (2012). Multi-scale, object-based image analysis for mapping geomorphic and ecological zones on coral reefs. *International Journal of Remote Sensing* 33, 3768–3797.

Prentice, I.C. (1980). Multidimensional scaling as a research tool in quaternary palynology: A review of theory and methods. *Review of Palaeobotany and Palynology*. 31, 71–104. https://doi.org/10.1016/0034-6667(80)90023-8

R Development Team. (2019). R: A language and environment for statistical computing.

Rao, C. (1995). A review of canonical coordinates and an alternative to correspondence analysis using Hellinger distance. Questiió Quad. d'Estadística, Sist. Inform. i Investig. Oper.

Robinson, L.A., Rogers, S. & Frid, C.L.J. (2008). A marine assessment and monitoring framework for application by UKMMAS and OSPAR – Assessment of pressure and impacts (Contract No. C-08-0007-0027 for JNCC). University of Liverpool and the Centre for the Environment, Fisheries and Aquaculture Science (Cefas).

Roelfsema, C., Phinn, S., Jupiter, S., Comley, J. & Albert, S. (2013). Mapping coral reefs at reef to reef-system scales, 10s–1000s km^2, using object-based image analysis. *International Journal of Remote Sensing* 34 (18) 6367-6388 https://doi.org/10.1080/01431161.2013.800660.

Roelfsema, C., Kovacs, E., Ortiz, J. C., Wolff, N. H., Callaghan, D., Wettle, M., Ronan, M., Hamylton, S. M., Mumby, P. J. & Phinn, S. (2018). Coral reef habitat mapping: A combination of object-based image analysis and ecological modelling. *Remote Sensing of Environment* 208, 27–41. https://doi.org/10.1016/j.rse.2018.02.005.

Stebbing, P., Murray, J., Whomersley, P. & Tidbury, H. (2014). Monitoring and surveillance for non-indigenous species in UK marine waters. Defra Report.

Stephens, D. & Diesing, M. (2014). A comparison of supervised classification methods for the prediction of substrate type using multibeam acoustic and legacy grain-size data. *PloS One* 9 (4) e93950.

Van Rein, H., Downie, A-L. & Bluemel, J. (2022). Pisces Reef MPA Monitoring Report 2016. JNCC/Cefas Partnership Report No. 40. JNCC, Peterborough, ISSN 2051-6711, Crown Copyright. https://hub.jncc.gov.uk/assets/4065f4d8-1e35-48c0-bc99-b7511034ca7e (Accessed 26/08/2022).

Ware, S.J. & Kenny, A.J. (2011). Guidelines for the Conduct of Benthic Studies at Marine Aggregate Extraction Sites (2nd Edition). Marine Aggregate Levy Sustainability Fund, 80 pp.

Warton, D.I., Wright, S.T. & Wang, Y. (2012). Distance-based multivariate analyses confound location and dispersion effects. *Methods in Ecology and Evolution* 3, 89–101. https://doi.org/10.1111/j.2041-210X.2011.00127.x

Wasson B, Núñez J. The first records of Rullierinereis ancornunezi (Polychaeta: Nereididae) from British waters. *Marine Biodiversity Records*. 2013;6:e26. doi:10.1017/S1755267213000031

Weiss, A. (2001). Topographic position and landforms analysis. Poster Present. ESRI User Conf. San Diego, CA 64, 227–245. http://www.jennessent.com/downloads/TPI-poster-TNC_18x22.pdf

Wood, D., Albrecht, J., Hawes, J. & Sperry, J. (2020). CEND0119 Survey Report: Offshore Overfalls MCZ and Offshore Brighton MCZ. JNCC/Cefas Partnership Report No. 35, JNCC, Peterborough, ISSN 2051-6711. https://hub.jncc.gov.uk/assets/d96bbe8e-915c-48db-9472-3c64669b52e6 (Accessed 23/08/2022)

Worsfold, T.M., Hall, D.J. & O'Reilly, M. (2010). Guidelines for processing marine macrobenthic invertebrate samples: a processing requirements protocol version 1. Unicomarine Report NMBAQCMbPRP to the NMBAQC Committee.

Annex 1. Glossary

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

Term	Description
Activity	A human action which may have an effect on the marine environment; e.g. fishing, energy production (Robinson *et al.* 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).*
Biotope	The physical habitat with its associated, distinctive biological communities. A biotope is the smallest unit of a habitat that can be delineated conveniently and is characterised by the community of plants and animals living there.*
Broadscale Habitats	Habitats which have been broadly categorised based on a shared set of ecological requirements, aligning with level 3 of the EUNIS habitat classification. Examples of Broadscale Habitats are protected across the MCZ network.
Community	A general term applied to any grouping of populations of different organisms found living together in a particular environment, essentially the biotic component of an ecosystem. The organisms interact and give the community a structure (Allaby 2015).
Conservation Objective	A statement of the nature conservation aspirations for the feature(s) of interest within a site, and an assessment of those human pressures likely to affect the feature(s).*
Epifauna	Fauna living on the seabed surface.
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.*

Term	Description
Habitats of Conservation Importance (HOCI)	Habitats that are rare, threatened, or declining in Secretary of State waters.*
Impact	The consequence of pressures (e.g. habitat degradation) where a change occurs that is different to that expected under natural conditions (Robinson *et al.* 2008).
Infauna	Fauna living within the seabed sediment.
Joint Nature Conservation Committee (JNCC)	JNCC is the public body that advises the UK Government and devolved administrations on UK-wide and international nature conservation. JNCC has responsibility for nature conservation in the offshore marine environment, which begins at the edge of territorial waters and extends to the UK Continental Shelf (UKCS).
Marine Strategy Framework Directive (MSFD)	The MSFD (EC Directive 2008/56/EC) aims to achieve Good Environmental Status (GES) of EU marine waters and to protect the resource base upon which marine-related economic and social activities depend.
Marine Conservation Zone (MCZ)	MPAs designated under the Marine and Coastal Access Act (2009). MCZs protect nationally important marine wildlife, habitats, geology and geomorphology, and can be designated anywhere in English and Welsh inshore and UK offshore waters.*
Marine Protected Area (MPA)	A generic term to cover all marine areas that are ‘A clearly defined geographical space, recognised, dedicated and managed, through legal or other effective means, to achieve the long-term conservation of nature with associated ecosystem services and cultural values’ (Dudley 2008).*
Natural England	The statutory conservation advisor to Government, with a remit for England out to 12 nautical miles offshore.
Non-indigenous Species	A species that has been introduced directly or indirectly by human agency (deliberately or otherwise) to an area where it has not occurred in historical times and which is separate from and lies outside the area where natural range extension could be expected (Eno *et al.* 1997).*
Pressure	The mechanism through which an activity influences any part of the ecosystem (e.g. physical abrasion caused by trawling). Pressures can be physical, chemical or biological, and the same pressure can be caused by a number of different activities (Robinson *et al.* 2008).*
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.

Annex 2. Data Acquisition

A2.1 Acoustic data

Acoustic data were collected using a Kongsberg EM2040 multibeam echosounder (MBES) at 300 kHz. Survey lines were placed to achieve full coverage (minimum 30% overlap) with the MBES. Ship motion and position were recorded using the SBG Systems Motion Reference Unit (MRU) and CNAV 3050 high precision GPS. Bathymetry data were processed using CARIS HIPS and MBES backscatter data with the QPS FMGT software package. Layback was applied using High Precision Acoustic Positioning (HiPAP). A more detailed description of the acoustic data collection is given in Wood *et al*. (2020).

A2.2 Grab sampling

Seabed sediment samples for particle size distribution and benthic infauna analyses were collected using a 0.1 m^2 Hamon Grab (also known as a 'mini' Hamon Grab).

A 500 ml sub-sample was taken from each grab sample and stored at -20°C prior to determining the particle size distribution. Sediment samples were processed by Cefas following the recommended methodology of the North-East Atlantic Marine Biological Analytical Quality Control (NMBAQC) scheme (Mason 2011). The less than 1 mm sediment fraction was analysed using laser diffraction and the greater than 1 mm fraction was dried, sieved, and weighed at 0.5 phi (ϕ) intervals. Sediment distribution data were merged and used to classify samples into sediment Broadscale Habitats.

The faunal fraction was sieved over a 1 mm mesh, photographed, then fixed in buffered 4% formaldehyde. Faunal samples were processed by Ocean Ecology Limited to extract all fauna present in each sample. Fauna were identified to the lowest taxonomic level possible, enumerated and weighed (blotted wet weight) to the nearest 0.0001 g following the recommendations of the NMBAQC scheme (Worsfold *et al.* 2010).

A2.3 Seabed imagery

The modified approach was centred on the 'bullring' survey strategy described above with transect length and replication dependent on station type ('grid', 'increased replication' and 'ground truth' stations). For all stations the camera frame was towed at a speed of 0.3 km for the required distance. Still images were acquired every ~10 seconds. Slight variation timing (~2 seconds either side) was allowed to account for swell, with the operator endeavouring to wait for the camera to be below 2 m altitude before taking the still. Full details of acquisition and processing methodologies can be found in the survey report (Wood *et al.* 2020).

Position was recorded continuously at five second intervals throughout the tow, for both the side gantry steer point and the position derived from HiPAP.

Additional environmental data

The depth and altitude of the camera frame were measured with a 250 khz precision altimeter and logged once a second.

Salinity, chlorophyll-a, dissolved oxygen, and turbidity were recorded along each video tow using a micrologger (Ecosystem Monitor 2; ESM2). Discrete water samples were collected using the continuous flow 'ferrybox' system for salinity, oxygen, suspended particulate matter (SPM) and chlorophyll-a to calibrate the ESM2 sensors. The drop camera frame was held at 4 m below sea level (level of the 'ferrybox' intake) for a few minutes whilst taking the water sample. The ESM2 logger and water sampling protocols are described in more detail in Wood *et al.* (2020).

Annex 3. Data preparation and analysis

A3.1 Tidal modelling

The tidal model referenced in Section 1.2.1 has a domain which extends 48.01°N to 52.48°N and 2.23°E to 9.51°W. The unstructured mesh was discretised with 292, 630 nodes and 571, 260 elements. The mesh has a resolution of approximately 3 km along the open boundary. In the area of interest, 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 is 1 arc second (~30 m). The hydrodynamics were forced along the open boundaries using 11 tidal constituents (M2, S2, N2, K2, K1, O1, P1, Q1, M4, MS4 and MN4) from the OSU TPXO European Shelf 1/30° regional model. After a spin up period of 5 d, the model was run for 30 d to cover a full spring-neap cycle.

A3.2 Sediment particle size distribution

Sediment particle size distribution data (half phi classes) were grouped into the percentage contribution of gravel, sand and mud derived from the classification proposed by Folk (1954). In addition, each sample was assigned to one of four sediment Broadscale Habitats (BSH) using a modified version of the classification model produced during the Mapping European Seabed Habitats (MESH) project (Long 2006).

A3.3 Infaunal data preparation

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. 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. Therefore, prior to analysis, the data were checked and truncated to ensure that each row represented a legitimate taxon, and that they were consistently recorded within the dataset.

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

Details of the data preparation and truncation protocols applied to the infaunal datasets acquired at Offshore Overfalls 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 (except for some well-studied molluscs and commercial species). Many truncation methods involve the removal of all taxa with the 'juvenile' qualifier. 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 the Offshore Overfalls 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 was removed when no adults of the same species had been recorded.

Meiofauna (i.e. nematodes), vertebrate species (i.e. fish), records of animals in larval or reproductive stages (e.g. crustacean larvae) and plants were removed.

A3.3 Epifaunal data preparation

Annotation

Data extraction from seabed imagery within the MPA programme has previously been undertaken by means of sub-contracted expert analysts using Microsoft Excel-based proformas to record abundance of motile taxa, and estimated percentage cover (or semi-quantitative SACFOR scores, more frequently). With greater control of image acquisition parameters, these CEND0119 data are considered more quantifiable than previous imagery derived abundance data, therefore the decision was taken to trial the BIIGLE online image annotation platform. This platform allows for instant collaboration and review between contactors and Cefas / JNCC, alongside controllable label hierarchies and accurate point placement. Alongside this, polygon tools allow for much greater accuracy in percentage cover annotations.

The greater control of the label hierarchy enables each entry to be nested within a morpho-taxonomic tree as discussed in Jones *et al*. (2020), which in turn is derived from the CATAMI structure (Hill *et al.* 2014).

Truncation

It is often the case that some taxa must be merged to a level in the taxonomic hierarchy that is higher than the level at which they were identified. See Table 24, Annex 4 for complete list of truncations undertaken for the epifaunal (imagery derived) dataset.

Combination of percentage cover and point count data (Normalisation)

Normalisation and sample selection of the data, described here, were undertaken using Excel.

In relatively species-poor habitats, such as the coarse gravels observed at Offshore Overfalls, the choice of either percentage cover or point count matrices to provide a robust overview of epifaunal assemblages was considered very restrictive (two separate matrices of limited taxa entries). These two datasets of mixed measurement scales were combined through normalisation (each entry was divided by the sum of the row totals, then multiplied by a constant of 1000). This method was employed on the advice of Kerry Howell (University of Plymouth) and Bob Clarke (University of Plymouth / Primer-e Ltd.) as described in chapter 5–19 of the PRIMER 7 user manual (Clarke *et al.* 2014). The result is a 'relative abundance' matrix, which was then further transformed through division by the summed FoV of each sample, resulting in a 'relative density' matrix.

Sample selection

Recent studies by Cefas and JNCC (Downie *et al.,* in press) and van Rein *et al*. (2022) have demonstrated the issues around the use of a single still image as a sample in multivariate analysis. Species accumulation curves developed as part of these projects show that species richness / diversity indices find their asymptote at >20 still images (or >10 m^2 of coverage). Sample selection should include as many non-overlapping still images as possible from an ecologically contiguous area (the 50 m 'bullring' survey rationale used at Offshore Overfalls was designed to aid in this). This presented a difficulty in determining how many still images should be analysed, as a balance must be struck between the number of images per station and total number of stations analysed, given that analysis budget is a restricting factor.

The decision was taken to analyse 15 still images from each station, selected from the total stills acquired (usually ~ 45; 1 taken every 10 seconds). These were chosen at random, after an altitude filter was applied (>0.7 m and <1.2 m) as a means of standardisation. This decision was reached through a balance between previous work, such the Pisces Reef Monitoring Report indicating a minimum of 12 still images to be used (van Rein *et al.* 2022) and budgetary constraints.

A single sample, with data extracted and normalised as above, would then comprise the summed relative density for each morpho-taxonomic entry across those 15 still images analysed per transect (noting that there are 5 replicate transects at each 'Increased replication' station). As the imagery at ground truth stations were acquired over a larger geographical area (planned to be ~ 250 m in length), data from these stations were used in the assemblage analysis for mapping purposes only. Sample selection for ground truth stations was achieved by random selection of 15 still images from each of distinct segment of the ground truth transect (a segment is an area of continuous BSH type). Varying numbers of segments were noted across the ground truth stations, with the result that each analysed ground truth sample had an approximately equivalent summed FoV, the still images comprising these samples by nature covered varying geographical areas.

Transformation

Transformation and the further statistical testing routines described here were undertaken using the R statistical environment (R Development Team 2019) using R Studio v1.3.1056.

Abundance data derived from seabed imagery are often skewed strongly by the prevalence of a limited number of highly abundant (and/or easily observed) taxa, contrasted against the zero-inflation typical of faunal community matrices. As such, transformations such as Log^{x+1}, square root or fourth root are often performed.

The option of using *k*-means clustering routines meant that the relative density data required further transformation into a metric distance matrix: the Hellinger Distance matrix (Legendre & Gallagher 2001; Legendre 2018). This is a two part calculation, where the data are first transformed using the Hellinger transformation (known as D17 in Primer 7), using the *decostand*() function in the R package 'vegan'; (Oksanen *et al.* 2019), followed by the calculation of a Euclidean distance matrix from these transformed data (Legendre and Gallagher, 2001), relationships between derived assemblages and environmental variables can be investigated through constrained ordination approaches such as Redundancy Analysis (RDA). A Bray-Curtis similarity (also known as 'Percentage Difference / D14') matrix was also computed for use in hierarchical agglomerative clustering (the 'Standard Pathway' for comparison against the revised method). This matrix is non-metric, meaning it cannot be used in constrained ordinations such as RDA.

The Hellinger distance has been highly recommended for clustering or ordination of species abundance data by authors such as Prentice (1980) and Rao (1995), alongside Legendre and Gallagher (2001).. Hellinger distance also has the lowest coefficient of variation (and thus is considered the optimum resemblance coefficient for metric ordination), despite the fact that it is strongly non-linear (Legendre 2018).

Hellinger distance is asymmetric (meaning it handles double zeros as effectively as Bray-Curtis), however it provides further standardisation of the data by sample vector. This means that it assesses sample importance (relative to the sum of the individuals along the row / sample vectors) and if samples are of equal importance, then rare or abundant taxa will contribute the same amount to the distance between those samples. If the samples are of differing importance, then rare taxa will contribute more.

Treatments for within-station analysis

As discussed in Section 2.1, a subset of eight stations (the increased replication stations) were sampled with five replicates of 50 m transects. Analyses using these data were conducted to address Objective 2.

Objective 2.a.i

Species Accumulation Curves were used to assess the optimum seabed area needed to effectively characterise the diversity of a station. This required that an assessment of the effect of multiple BSH occurrence along any of the five replicate transects be undertaken. Where more than one BSH was identified along each 50 m transect, these were classified as ‘Multiple BSH’ replicates. The data were then separated into two sets, one containing ‘Multiple BSH’ replicates, and one with these ’Multiple BSH’ replicates removed.

Objective 2.a.ii

An understanding of the influence of spatial distribution of component images on the final sample was required to assess any within-station assemblage variation (univariate and multivariate). The question which forms the basis of these analyses is as follows:

If limited to a certain amount of imagery per station, the observed assemblage changes if all component images are from a single, straight line transect or randomly distributed across the ‘bullring’.

First, the decision was taken to require a ‘sample’ to be comprised of 15 images from a station (the maximum number available). This rationale was informed by the results of Objective 2.a.i (the SpAC curves), which indicated that the optimum FoV required to adequately characterise the diversity of a station was > 25 m^2; as such analysis of all 15 images from a replicate was the best possible option.

The normalised, transformed density matrix was then separated into four treatments:

Trt1 = 15 stills from A replicate (central)

Trt2 = 5 stills each from C, A and E replicates

Trt3 = 3 stills each from A, B, C, D and E replicates

Trt4 = All images from all replicates (control 75 images)

A3.4 Numerical and statistical analyses and normality testing

Infaunal analysis

Full methodology is provided in Section 2.4.1.

Epifaunal analysis

To address Objectives 1.a and 1.b, multivariate assemblage analysis of epifaunal abundance data was undertaken on data extracted from the seabed imagery acquired from the grid and ground truth stations. These analyses provide a T0 characterisation of the grid stations, and furthermore provided the classes used to inform the predictive habitat mapping.

Objectives 1.a and 1.b

Clustering / partitioning

The choice of a hierarchical versus a non-hierarchical clustering approach is based on the requirement not just to derive discrete assemblages for monitoring purposes, but also to map the distribution of the habitats associated with them. Hierarchical agglomerative methods, such as Unweighted Pair-Group Method Average (UPGMA) used in the PRIMER CLUSTER routine clustering, are easier to compute than non-hierarchical methods and useful for summarising the relationships between groups of taxa. However, monitoring and mapping are perhaps best aided by single partitions which most accurately describe the direct relationship among samples, using non-hierarchical methods where samples are not fixed in a branch and can therefore be swapped between clusters as the process progresses (Legendre 2018). These non-hierarchical methods are more computationally intensive than hierarchical clustering.

K-means partitioning (a non-hierarchical method) was undertaken for the Offshore Overfalls epifaunal relative density data (Hellinger distance matrices), using the ‘cascadeKM’ package in R. *k*-means algorithms are divisive clustering methods which minimise an objective function (total error sum of squares; TESS) by permutationally re-ordering samples (Legendre 2018), resulting in a set number of partitions. To avoid the issue of local minima in *k*-means clustering (the issue of the random initial position of centroids in *k*-means partitioning which then influences the final partitioning) the ‘cascadeKM’ package in R assigns random starting centroids (between values set to between 2 and 20) and permutationally cycles through a specified number of iterations (100 in this example) for each value of *k*. Selection of the optimum partition is then performed using the Caliński-Harabasz criterion (Caliński & Harabasz 1974), a method of assessing TESS.

Representation of the assemblages in non-metric space was undertaken using the *ordiplot*() function in the R package ‘vegan’ (Oksanen *et al.* 2019).

Assemblage composition

Previous MPA reporting has applied statistical methods used to analyse infaunal data to imagery data. This analysis calls for interrogation of the composition using Similarity Percentage contribution assessment, most often undertaken using the SIMPER routine in Primer 7. The outputs of the SIMPER routine are often very difficult to interpret; the routine does not account for the mean-variance relationship of the taxa, as the variance increases with the mean abundance / relative density. Taxa with smaller variances (but with known between-group effects) tend not to be identified as important contributors by the SIMPER routine (Warton *et al.* 2012). The prevalence of taxa with high variance is especially true for imagery data, as there is sporadic high frequency of occurrence for generic low-resolution entries such as ‘Faunal turf’ and ‘Serpulidae’, and such entries often dominate the SIMPER output.

In order to better investigate taxa which characterise the defined assemblages as derived from imagery data (inherently very different to quantified infaunal data), the *multipatt*() function of the R package ‘indicspecies’ (De Caceres *et al.* 2019) was used. This is a permutational testing routine which permutes the input clusters and compares these combinations against presence of a taxon in the raw matrix, using the IndVal index as a test statistic (Dufrêne & Legendre 1997) to measure associations between individual clusters. For each taxon the routine chooses the combination with the highest association value per cluster. The patterns which best match are tested for statistical significance (permutational testing) of the associations, providing the IndVal test statistic and a *p*-value for each taxon within each cluster. Higher values of the test statistic indicate a greater value of the taxon as an indicator of that cluster. The two components of the IndVal index, A and B, provide information on the specificity and fidelity (respectively) of each taxon as characteristic of that assemblage (De Caceres *et al.* 2010). The specificity (IndVal A) is the probability that the sample belongs to the assemblage, given the presence of the taxon in question. The fidelity (IndVal B) is the probability of finding the taxon in samples belonging to the assemblage. A more intuitive description of these components is that the specificity is the total percentage of all individuals (or biomass) of one taxon that are found in each class. Those across all classes add up to 1. The fidelity is the percentage of all the samples in a cluster that include the taxon in question. Hence that does not add up to 1 over all classes. The indicator value is the product of those two. Hence if prevalence in a group is low, the indicator value will be low even if a species is only found in that group, because it is not present regularly.

Redundancy Analysis – association with environmental variables

Redundancy Analysis (RDA) was used to investigate any association between the measured environmental variables and the assemblages defined using *k*-means partitioning. Transformation-based RDA (tb-RDA) is an asymmetric form of canonical analysis (Legendre & Gallagher 2001), with an explanatory dataset (the environmental variables) and a response dataset (the Hellinger-transformed density matrix). The RDA was undertaken using the *rda*() function of the R package 'vegan' (Oksanen 2008), whilst the RDA results were plotted in a tri-plot using the *plot.rda*() function in the same package. Permutational testing of the significance of the constraints was undertaken using the *anova.rda*() function of the R package 'vegan' (Oksanen 2008).

Objectives 2. a.1 and 2.a.ii

Species accumulation curves

To assess the efficacy of a single 50 m transect (within a 50 m radius 'bullring') to characterise a station, an understanding of within-station assemblage variability was required. Prior to this, an assessment of the 'optimum' seabed area was needed. This was undertaken to address Objective 2.a.i, though creation and comparison of Species Accumulation Curves (SpACs). Two sets of SpACs were created as per the two treatments described above ('no 'Multiple BSH' images and including 'Multiple BSH' images). SpACs were created using two functions within two separate R packages; the non-metric (sample accumulation by number of stills) using the *specaccum*() function in the R package 'vegan' (Oksanen *et al.* 2019). The metric SpACs (accumulation by summed field of view) was undertaken using the *accumcomp*() function in the R package 'BiodiversityR' (Kindt & Coe 2005).

Univariate metrics

Univariate metrics (Shannon's 'H' and Simpsons 'λ' diversity metrics, species richness, Pielou's 'J' evenness metric) of each spatial treatment (Table 19; Trt1, Trt2 and Trt3 – not Trt4 as this was the control) were then calculated and their distributions tested for normality using the Shapiro-Wilk test (H_o= *diversity metric scores are normally distributed*).

Table 19. Results from Shapiro-Wilk test for normality of the distribution of the univariate metrics calculated for each of the increased replication treatments (non-normality indicated by an asterisk).

Treatment / Metric	**W Statistic**	***p***
Shannon 'H'		
Trt1*	**0.633***	**0.000661***
Trt2	0.98	0.96
Trt3	0.828	0.0774
Pielou's 'J'		
Trt1	0.823	0.0684
Trt2	0.929	0.542
Trt3	0.946	0.691
Species Richness		
Trt1	0.942	0.656
Trt2	0.815	0.0578
Trt3	0.87	0.184
Simpsons 'L'		
Trt1	0.87	0.187
Trt2	0.976	0.936
Trt3	0.847	0.116

All distributions were found to be normally distributed aside from Shannon's 'H', the three alternate univariate metrics ('Species Richness', 'Simpsons L' and 'Pielou's J') were assessed for difference between the three treatments (Trt4 not included owing to the large difference in sampled seabed area) using Analysis of Variance (ANOVA), with the following null hypothesis tested:

H_0 = No observable difference in the means of univariate metrics between the three treatment levels at all stations.

Multivariate analysis of variance

The development of non-parametric MANOVA techniques, based on permutation testing, has allowed for the variances of two ecological datasets to be tested for significant differences. This test was used to determine whether statistically significant differences exist between the assemblages of the four treatments described above (Trt1 to Trt4). Permutational MANOVA testing was undertaken using the *adonis*() function in the R package 'vegan' (Oksanen *et al.* 2019). The following null hypothesis was tested:

H_0 = No significant difference exists in the variance of assemblage composition between stations and between treatments

Homogeneity of multivariate dispersions

Investigation into the homogeneity of the variances (dispersions) by treatment was then undertaken. Significant differences in multivariate dispersion between replicates within the station (i.e. within the bullring) could be understood to indicate variation in assemblage composition across the station, owing to undetected environmental parameters (substrate, relief, bottom currents etc) or ecological interactions (predation, competition etc). The method chosen was a non-parametric analogue to Levene's test, PERMDIST2 (Anderson 2006). This test was distance based and permutational, and implemented by the *betadisper*() function in the R package 'vegan' (Oksanen *et al.* 2019).

A3.5 Assemblage mapping

Object-Based Image Analysis (OBIA)

OBIA is a two-step process consisting of segmentation and classification. The aim of segmentation is to divide an image into meaningful objects based on their spectral and spatial characteristics (Blaschke 2010). It has also been increasingly used in seabed mapping, either for interpreting satellite imagery in shallow environments (Phinn *et al.* 2012; Roelfsema *et al.* 2013, 2018), or for interpreting bathymetry and other remotely sensed data (Lucieer & Lamarche 2011; Stephens & Diesing 2014). In comparison to working at the pixel level, OBIA allows further characterisation based on layer values (such as mean or standard deviation), geometry and neighbour relationships. OBIA also reduces computational intensity by reducing millions of pixels into a smaller number of more meaningful objects. When using bathymetric variables, seabed mapping is more analogous to super-pixels which represent areas of homogenous values as opposed to meaningful topographic objects visible in spectral data.

Raw and derived environmental data layers used here included a composite multibeam bathymetry dataset (CHP and CEND0119 acquired data) at 1 m resolution. Alongside the MBES data, a composite backscatter data layer (CHP and CEND0119) and several topographic derivative layers (calculated using ArcMap 10.5 and QGIS 3.8). See Table 20 for a full list of derivatives.

A multi-resolution segmentation algorithm was applied to the data layers using eCognition v9.3.5. The segmentation is a bottom-up approach with image-objects iteratively merged until the homogeneity criterion is reached (termed the scale parameter) incorporating limitations based on object shape (compactness and shape parameters). The optimal parameters and weightings were determined through an iterative process by visually assessing different segmentations to find the settings which most closely reflected real world objects.

The segmentation was bathymetrically driven, with a weight of 1 applied to the bathymetry layer and 0.5 applied to the slope derivative (both layers were included at each level for each mound). Through a process of trial and error, to optimise object boundaries, a segmentation routing using a scale parameter (SP) of 5 with compactness (C) value of 0.9 and a shape (S) value of 0 was decided upon and used.

Derivatives were calculated using ESRI ArcMap 10.5 and the geoprocessing tools of System for Automated Geoscientific Analyses (SAGA) in QGIS v3.4. Although several derivatives were considered, the final suite of layers and scales used (Table 20) in either the segmentation or classification were determined based on an iterative approach based on visually assessing the mapped products using different variables.

Table 20. Bathymetric derivative layers used in the segmentation and classification of the 2015 MBES data for Offshore Overfalls MCZ.

Derivative	Description
Bathymetric Position Index (5–10; 10–20; 40–50)	Vertical position of cell relative to neighbourhood (identifies topographic peaks and troughs). Calculated with two neighbourhood sizes of 5–10, 10–20 and 40–50 cells (inner and outer annulus respectively) to capture topographical elevation at different spatial scales (Weiss 2001). Positive values represent features higher than surrounding area and negative values represent lower features, and values near zero are either flat or areas of constant slope.
Standardised height	First normalised height is calculated, which considers the extension of a catchment area of a specific terrain point. Normalised height allots a value of 1 to the highest and value 0 to the lowest position within a respective reference area. Standardised height is the product of normalised height multiplied with absolute height (Dietrich & Böhner 2006).
Slope	The incline, or steepness, of a bathymetric surface. Measured in degrees from the horizontal. The slope for a cell in a raster is the steepest slope of a plain defined by the cell and its eight surrounding neighbours.
Aspect	Aspect identifies the downslope direction of the maximum rate of change in value from each cell to its neighbours. It can be thought of as the slope direction. The values of each cell in the output raster indicate the compass direction that the surface faces at that location. It is measured clockwise in degrees from 0 (due north) to 360 (again due north), coming full circle. Flat areas having no downslope direction are given a value of -1.

Morphological classification

The acquisition of high resolution MBES data from across the entire MCZ has allowed for a much greater level of detail in mapping of the Quaternary paleovalley feature. To map the morphology of this and other seabed features within the site, the segmentation (as derived using OBIA, described above) was then classified semi-autonomously within eCognition v9.3.5, using a combination of a custom-built ruleset and manual delineation. The classification scheme of the MIM-GA working group (Dove *et al.* 2020) was used, noting that only part 1 (morphology) could be applied, as full geological investigation of the site has not been undertaken, and geomorphological classification required information on the underlying lithology. The resulting morphological classes are presented in Table 21.

Table 21. Morphological classes used to broadly classify the mapped areas within Offshore Overfalls MCZ.

Morphological class	Description
Ridge	Outcropping and or sub-cropping chalk or Wealdon group rock, trending approximately east-west, gently folded and fractured, in the southern part of the mapped area. Possible sub-cropping rock (bumpy and irregular seabed) in the north-western part of the mapped area.
Plain	Broad and flat-topped area to the north-west of the paleovalley which occupies a large portion of the mapped area.

Morphological class	Description
Moving bedform	Large (>1 m) sedimentary structures that indicate sediment agitation by water current or waves (or wind). They consist of repeating wavelike forms with symmetrical/asymmetrical slopes, sharp peaks, and rounded troughs, and occur in the northern portion of the map or on the sandbank.
Slope	Irregular and gently sloping seabed that does not fall within any of the other classes.
Depression	Incised channels and wider shallower depressions.
Platform	Flat areas raised above surrounding features, i.e. lemniscate mounds.
Floor	Paleovalley bed.

Predictive (classification) model generation

For predictive classification, the segmentation was then exported from eCognition v9.3.5 as a shapefile, with the attribute table including the mean value, per object, of each of the data layers included and a 'class' attribute. Where ground truth samples were located entirely within an object, those objects were classified based on that sample's assigned assemblage class (as derived from *k*-mean partitioning of the seabed imagery data). If no ground truth sample was found within that object, the object was classed as 'Unclassified'. Due to the lack of seabed imagery acquired from the region of sand in the north-east of the site, this habitat was under-sampled in the imagery derived ground truth data. As such the sediment samples taken from that area were used as ground truth samples for this habitat (provided they showed a Folk classification of sand, muddy sand, or gravelly sand). This resulted in a total of 165 classified segments (the training dataset), out of a total of 12,215 objects.

Predictive modelling was undertaken using the Python 3 machine learning library PyCaret 2.2.2, performed in Jupyter Notebook 6.1.4. The *pycaret.classification* module *setup*() function was used to first separate the data into training and testing datasets (70 / 30 %) using random sampling with proportional allocation based on class type (Olofsson *et al.* 2014). The *setup*() function allows for mixed numerical and categorical predictor classes to be incorporated into the same model testing environment and allows for unbalanced training class data (which is often the case in seabed habitat mapping with limitations on ground truth sample acquisition). Cross-validation was set to 10 folds using the 'stratifiedKfold' strategy. The *compare_models*() function was then used to run an iterative model creation and performance assessment process across all valid classification models within the PyCaret library. This allows for quick identification of the most applicable and accurate model type (using a variety of cross-validated performance metrics such as: producers accuracy (quality of the classification), users accuracy (probability that the prediction represents reality), AUC, recall, precision, and Kappa) allowing the user to then create the most accurate model.

Following setup of the model building and testing algorithm (using the PyCaret *setup*() function), the *best*() function was used to train (using the specified hyperparameters) and compare a suite of all suitable classification models held within the PyCaret library. The cross-validation accuracy (10 fold) of these models was compared to determine which model was most suited to the task.

A3.6 Derived habitat map

Broadscale Habitats were assigned to each of the five classes derived from epifaunal assemblage analysis and infaunal samples of cluster group 'd', as shown in Table 8. The habitats to assemblage associations were determined based upon review of the characterising substrata associated with each of the four imagery derived assemblages, as identified through the RDA (Figure 21). The fifth mapped habitat class (class '5') was determined through particle size analysis to be 'Subtidal sand'.

The habitat extents were then mapped based upon this back-correlation with epifaunal assemblage, as opposed to using predictive mapping techniques based upon only the initial BSH values assigned by the analyst to the 129 video segments. This was undertaken due to the low number of non 'Subtidal sand' segments, which would have resulted in too little balance in class training data and thus produced a map of lower accuracy.

A3.6 Evaluating potential indictors

Where potential indicators were identified for future monitoring of feature condition within the site (e.g. a metric, or a specific taxon or group of taxa), they were evaluated against the criteria provided in Table 22. These criteria were set out by the OSPAR Commission (2012) in advice on the selection of indicators for descriptors of marine biodiversity under the MSFD, however they can be broadly applied outside of this context, including in the selection of site or feature-specific indicators.

Table 22. OSPAR Commission (2012) state indicator selection criteria (adapted from ICES and UK scientific indicator evaluation).

Criterion	Specification
Sensitivity	Does the indicator allow detection of change against background variation or noise?
Specificity	Does the indicator respond primarily to a particular human pressure, with low responsiveness to other causes of change?
Accuracy	Is the indicator measured with a low error rate?
Simplicity	Is the indicator easily measured?
Responsiveness	Is the indicator able to act as an early warning signal?
Spatial applicability	Is the indicator measurable over a large proportion of the geographical area to which it is to apply?
Management link	Is the indicator tightly linked to an activity which can be managed to reduce its negative effects on the indicator (i.e. are the quantitative trends in cause and effect of change well known?)
Validity	Is the indicator based on an existing body or time series of data (either continuous or interrupted) to allow a realistic setting of objectives?
Communication	Is the indicator relatively easy to understand by non-scientists and those who will decide on their use?

A3.7 Non-indigenous species (NIS)

The infaunal and epifaunal taxon lists generated from the infaunal samples and seabed imagery data were cross-referenced against lists of non-indigenous target species which have been selected for assessment of Good Environmental Status in GB waters under MSFD Descriptor 2 and identified as significant by the GB Non-Native Species Secretariat. These taxa are listed in Annex 6.

Annex 4. Faunal dataset truncations

Table 23. Infaunal truncation protocol matrix.

Taxon Name	Qualifier	Abundance Units	Action
Capitellidae	Fragment	Presence / Absence	remove
Mediomastus fragilis	-	Count	
Notomastus	-	Count	
Pseudonotomastus southerni	-	Count	
Maldanidae	Fragment	Presence / Absence	remove
Nicomachinae	Damaged	Count	
Clymenella cincta	-	Count	
Euclymene oerstedii	Aggregate	Count	
Euclymene oerstedii	Aggregate, Fragment	Presence / Absence	add to count entry
Heteroclymene robusta	-	Count	
Leiochone	-	Count	
Nicomache	-	Count	
Notoproctus	-	Count	
Petaloproctus	-	Count	
Petaloproctus	Fragment	Presence / Absence	add to count entry
Praxillella affinis	-	Count	
Ophelia borealis	-	Count	
Orbiniidae	Juvenile	Count	include
Orbiniidae	Fragment	Presence / Absence	add to count entry
Cirrophorus furcatus	-	Count	
Paradoneis ilvana	-	Count	
Paradoneis lyra	-	Count	
Paradoneis lyra	Fragment	Presence / Absence	add to count entry
Sabellaria spinulosa	-	Count	
Asclerocheilus	-	Count	
Scalibregma celticum	-	Count	
Scalibregma inflatum	-	Count	
Scalibregma inflatum	Fragment	Presence / Absence	add to count entry

Taxon Name	Qualifier	Abundance Units	Action
Scalibregma stenocerum	-	Count	
Sclerocheilus	-	Count	
Travisia forbesii	-	Count	
Euphrosine foliosa	-	Count	
Dorvillea rubrovittata	-	Count	
Schistomeringos rudolphi	-	Count	
Schistomeringos rudolphi	Fragment	Presence / Absence	add to count entry
Eunicidae	Fragment	Presence / Absence	remove
Eunice vittata	-	Count	
Lysidice ninetta	-	Count	
Lysidice unicornis	-	Count	
Paucibranchia	Damaged	Count	remove
Paucibranchia bellii	-	Count	
Paucibranchia totospinata	-	Count	
Lumbrineridae	Fragment	Presence / Absence	remove
Lumbrineriopsis paradoxa	-	Count	
Lumbrineris cingulata	Confer	Count	
Lumbrineris futilis	-	Count	
Scoletoma magnidentata	-	Count	
Arabella iricolor	-	Count	
Drilonereis	-	Count	
Aphroditidae	Juvenile	Count	remove
Aphrodita aculeata	-	Count	
Glycera	Juvenile	Count	remove
Glycera	Fragment	Presence / Absence	remove
Glycera alba	-	Count	
Glycera fallax	-	Count	
Glycera lapidum	Aggregate	Count	
Glycera lapidum	Aggregate, Fragment	Presence / Absence	add to count entry
Glycera oxycephala	-	Count	
Glycera unicornis	-	Count	
Glycinde nordmanni	-	Count	

Taxon Name	Qualifier	Abundance Units	Action
Goniada	Fragment	Presence / Absence	remove
Goniada emerita	-	Count	
Goniada maculata	-	Count	
Hesiospina aurantiaca	-	Count	
Syllidia armata	-	Count	
Nephtys	Juvenile	Count	remove
Nephtys	Fragment	Presence / Absence	remove
Nephtys caeca	-	Count	
Nephtys cirrosa	-	Count	
Nephtys cirrosa	Fragment	Presence / Absence	
Nereididae	Juvenile	Count	remove
Nereididae	Fragment	Presence / Absence	remove
Eunereis longissima	-	Count	
Rullierinereis ancornunezi	-	Count	
Rullierinereis ancornunezi	Fragment	Presence / Absence	
Pholoe baltica	-	Count	
Pholoe inornata	-	Count	
Phyllodocidae	Fragment	Presence / Absence	remove
Eteone longa	Aggregate	Count	
Eulalia expusilla	-	Count	
Eulalia mustela	-	Count	
Eulalia tripunctata	-	Count	
Eumida bahusiensis	-	Count	
Eumida sanguinea	Aggregate	Count	
Mysta picta	-	Count	
Notophyllum foliosum	-	Count	
Paranaitis kosteriensis	Fragment	Presence / Absence	treat as count
Phyllodoce groenlandica	-	Count	
Phyllodoce maculata	-	Count	
Pseudomystides limbata	-	Count	

Taxon Name	Qualifier	Abundance Units	Action
Pterocirrus macroceros	-	Count	
Harmothoe	Damaged	Count	remove
Harmothoe extenuata	-	Count	
Harmothoe impar	Aggregate	Count	
Lepidonotus squamatus	-	Count	
Malmgrenia	Damaged	Count	remove
Malmgrenia andreapolis	-	Count	
Malmgrenia arenicolae	-	Count	
Malmgrenia darbouxi	-	Count	
Malmgrenia ljungmani	-	Count	
Malmgrenia mcintoshi	-	Count	
Polynoe scolopendrina	-	Count	
Subadyte pellucida	-	Count	
Fimbriosthenelais zetlandica	-	Count	
Fimbriosthenelais zetlandica	Fragment	Presence / Absence	add to count entry
Pelogenia arenosa	-	Count	
Ephesiella abyssorum	-	Count	
Sphaerodorum gracilis	-	Count	
Dioplosyllis cirrosa	-	Count	
Epigamia alexandri	-	Count	
Eurysyllis tuberculata	-	Count	
Eusyllis assimilis	-	Count	
Eusyllis lamelligera	-	Count	
Haplosyllis spongicola	-	Count	
Myrianida rubropunctata	-	Count	
Odontosyllis ctenostoma	-	Count	
Odontosyllis fulgurans	-	Count	
Odontosyllis gibba	-	Count	
Odontosyllis gibba	Fragment	Presence / Absence	add to count entry
Palposyllis prosostoma	-	Count	
Paraehlersia dionisi	-	Count	
Paraehlersia ferrugina	-	Count	
Parexogone hebes	-	Count	
Proceraea	-	Count	

Taxon Name	Qualifier	Abundance Units	Action
Prosphaerosyllis tetralix	-	Count	
Sphaerosyllis bulbosa	-	Count	
Sphaerosyllis taylori	Confer	Count	
Syllides	-	Count	
Syllis armillaris	Aggregate	Count	
Syllis garciai	-	Count	
Syllis garciai	Fragment	Presence / Absence	add to count entry
Syllis pontxioi	-	Count	
Syllis variegata	-	Count	
Trypanosyllis zebra	-	Count	
Galathowenia oculata	-	Count	
Galathowenia oculata	Fragment	Presence / Absence	add to count entry
Owenia	-	Count	
Sabellidae	Damaged	Count	remove
Sabellidae	Fragment	Presence / Absence	remove
Acromegalomma	-	Count	
Jasmineira	-	Count	
Parasabella	-	Count	
Pseudopotamilla	-	Count	
Serpulidae	Damaged	Count	remove
Spirobranchus lamarcki	-	Count	
Spirobranchus triqueter	-	Count	
Poecilochaetus serpens	-	Count	
Spionidae	Fragment	Presence / Absence	remove
Aonides oxycephala	-	Count	
Aonides paucibranchiata	-	Count	
Dipolydora	Damaged	Count	remove
Dipolydora caulleryi	-	Count	
Dipolydora coeca	-	Count	
Dipolydora saintjosephi	-	Count	
Laonice	Juvenile	Count	remove
Laonice bahusiensis	-	Count	
Pseudopolydora pulchra	-	Count	

Taxon Name	Qualifier	Abundance Units	Action
Spio arndti	-	Count	
Spio symphyta	-	Count	
Spiophanes bombyx	-	Count	
Ampharete lindstroemi	Aggregate	Count	
Amphicteis midas	-	Count	
Cirratulidae	Fragment	Presence / Absence	remove
Aphelochaeta	Species A	Count	
Caulleriella alata	-	Count	
Caulleriella alata	Fragment	Presence / Absence	add to species count entry
Chaetozone zetlandica	-	Count	
Cirratulus	Juvenile	Count	treat as adult
Dodecaceria	-	Count	
Kirkegaardia	-	Count	
Diplocirrus stopbowitzi	-	Count	
Flabelligera affinis	-	Count	
Lagis koreni	-	Count	
Terebellidae	Fragment	Presence / Absence	remove
Amphitritides gracilis	-	Count	
Eupolymnia nesidensis	-	Count	
Lanassa venusta	-	Count	
Lanice conchilega	-	Count	
Loimia medusa	-	Count	
Lysilla loveni	-	Count	
Lysilla nivea	-	Count	
Nicolea venustula	-	Count	
Phisidia aurea	-	Count	
Pista	-	Count	
Polycirrus	-	Count	
Thelepus	Damaged	Count	remove
Thelepus cincinnatus	-	Count	
Thelepus setosus	-	Count	
Terebellides	-	Count	
Copepoda	Parasite	Count	remove

Taxon Name	Qualifier	Abundance Units	Action
Scalpellum scalpellum	-	Count	
Sessilia	Damaged	Count	remove
Verruca stroemia	-	Count	
Ampelisca	Damaged	Count	remove
Ampelisca	Juvenile	Count	remove
Ampelisca diadema	-	Count	
Ampelisca spinipes	-	Count	
Nototropis vedlomensis	-	Count	
Bathyporeia elegans	-	Count	
Cheirocratus	-	Count	
Leptocheirus hirsutimanus	-	Count	
Iphimedia perplexa	-	Count	
Ericthonius	-	Count	
Leucothoe procera	-	Count	
Animoceradocus semiserratus	-	Count	
Maerella tenuimana	-	Count	
Maerella tenuimana	Fragment	Presence / Absence	add to species count entry
Othomaera othonis	-	Count	
Melitidae	Damaged	Count	remove
Melitidae	Fragment	Presence / Absence	remove
Abludomelita obtusata	-	Count	
Megamphopus cornutus	-	Count	
Photis longicaudata	-	Count	
Harpinia antennaria	-	Count	
Unciola crenatipalma	-	Count	
Urothoe elegans	-	Count	
Bodotria scorpioides	-	Count	
Alpheus macrocheles	-	Count	
Athanas nitescens	-	Count	
Axius stirhynchus	-	Count	
Callianassa subterranea	-	Count	
Callianassa subterranea	Fragment	Presence / Absence	add to species count entry
Galathea intermedia	-	Count	

Taxon Name	Qualifier	Abundance Units	Action
Hippolyte varians	-	Count	
Inachus	Juvenile	Count	remove
Inachus dorsettensis	-	Count	
Ebalia	Juvenile	Count	remove
Ebalia tuberosa	-	Count	
Eurynome	Juvenile	Count	remove
Eurynome spinosa	-	Count	
Paguridae	Fragment	Presence / Absence	remove
Anapagurus hyndmanni	-	Count	
Anapagurus laevis	-	Count	
Pagurus	Juvenile	Count	remove
Pagurus cuanensis	-	Count	
Pandalina brevirostris	-	Count	
Pilumnus hirtellus	-	Count	
Pinnotheres pisum	-	Count	
Liocarcinus	Juvenile	Count	keep (name as juv)
Pisidia longicornis	-	Count	
Processa canaliculata	-	Count	
Eualus pusiolus	-	Count	
Upogebia	Juvenile	Count	remove
Upogebia deltaura	-	Count	
Anthura gracilis	-	Count	
Astacilla	Juvenile	Count	keep (name as juv)
Conilera cylindracea	-	Count	
Eurydice inermis	-	Count	
Eurydice truncata	-	Count	
Gnathia	-	Count	remove
Gnathia dentata	-	Count	
Gnathia oxyuraea	-	Count	
Ione thoracica	-	Count	
Janira maculosa	-	Count	
Nebaliidae	Fragment	Presence / Absence	remove
Nebalia	Damaged	Count	keep

Taxon Name	Qualifier	Abundance Units	Action
Mysidae	Fragment	Presence / Absence	keep
Rissoides desmaresti	-	Count	
Apseudes talpa	-	Count	
Leptognathia paramanca	-	Count	
Tanaopsis graciloides	-	Count	
Achelia echinata	Aggregate	Count	
Callipallene brevirostris	-	Count	
Callipallene tiberi	-	Count	
Nymphon brevirostre	-	Count	
Anoplodactylus petiolatus	-	Count	
Reptadeonella insidiosa	-	Presence / Absence	
Reptadeonella violacea	-	Presence / Absence	
Aetea	-	Presence / Absence	
Schizomavella	-	Presence / Absence	
Porella concinna	-	Presence / Absence	
Bicellariella ciliata	-	Presence / Absence	
Amphiblestrum auritum	-	Presence / Absence	
Amphiblestrum flemingii	-	Presence / Absence	
Crassimarginatella solidula	-	Presence / Absence	
Scrupocellaria scrupea	-	Presence / Absence	
Scrupocellaria scruposa	-	Presence / Absence	
Cellepora pumicosa	-	Presence / Absence	
Lagenipora lepralioides	-	Presence / Absence	
Hagiosynodos latus	-	Presence / Absence	

Taxon Name	Qualifier	Abundance Units	Action
Chorizopora brongniartii	-	Presence / Absence	
Cribrilaria innominata	-	Presence / Absence	
Figularia figularis	-	Presence / Absence	
Puellina bifida	-	Presence / Absence	
Aspidelectra melolontha	-	Presence / Absence	
Conopeum reticulum	-	Presence / Absence	
Electra pilosa	-	Presence / Absence	
Escharella immersa	-	Presence / Absence	
Escharella variolosa	-	Presence / Absence	
Escharella ventricosa	-	Presence / Absence	
Neolagenipora collaris	-	Presence / Absence	
Escharina johnstoni	-	Presence / Absence	
Phaeostachys spinifera	-	Presence / Absence	
Escharoides coccinea	-	Presence / Absence	
Flustra foliacea	-	Presence / Absence	
Hincksina flustroides	-	Presence / Absence	
Celleporella hyalina	-	Presence / Absence	
Hippothoa divaricata	-	Presence / Absence	
Hippothoa flagellum	-	Presence / Absence	
Microporella ciliata	-	Presence / Absence	
Rhynchozoon bispinosum	-	Presence / Absence	

Taxon Name	Qualifier	Abundance Units	Action
Schizotheca fissa	-	Presence / Absence	
Schizoporella	-	Presence / Absence	
Scruparia	-	Presence / Absence	
Phylactella labrosa	-	Presence / Absence	
Prenantia cheilostoma	-	Presence / Absence	
Smittoidea reticulata	-	Presence / Absence	
Alcyonidioides mytili	-	Presence / Absence	
Alcyonidium diaphanum	-	Presence / Absence	
Triticella flava	-	Presence / Absence	
Amathia	-	Presence / Absence	
Amathia lendigera	-	Presence / Absence	merge to genus entry
Vesicularia spinosa	-	Presence / Absence	
Crisia	-	Presence / Absence	
Crisidia cornuta	-	Presence / Absence	
Disporella hispida	-	Presence / Absence	
Microeciella suborbicularis	-	Presence / Absence	
Oncousoecia dilatans	-	Presence / Absence	
Entalophoroecia deflexa	-	Presence / Absence	
Plagioecia patina	-	Presence / Absence	
Plagioecia sarniensis	-	Presence / Absence	
Stomatoporina incurvata	-	Presence / Absence	

Taxon Name	Qualifier	Abundance Units	Action
Tubulipora	-	Presence / Absence	
Chaetognatha	-	Count	remove
Diplecogaster bimaculata bimaculata	-	Count	remove
Ascidiacea	Juvenile	Count	remove
Didemnidae	-	Presence / Absence	
Ascidiella	-	Count	
Pyura tessellata	-	Count	
Dendrodoa grossularia	-	Count	
Polycarpa fibrosa	-	Count	
Folliculinidae	-	Presence / Absence	
Actiniaria	-	Count	
Edwardsiidae	-	Count	merge to actiniaria
Alcyonium digitatum	-	Presence / Absence	
Cerianthidae	Juvenile	Count	remove
Cerianthus lloydii	-	Count	
Epizoanthus couchii	-	Count	
Anthoathecata	-	Presence / Absence	
Eudendrium	-	Presence / Absence	merge to anthoethecata
Campanulariidae	-	Presence / Absence	
Clytia hemisphaerica	-	Presence / Absence	merge to capanuliadriidae
Halecium	-	Presence / Absence	
Nemertesia antennina	-	Presence / Absence	
Sertularella	-	Presence / Absence	
Abietinaria abietina	-	Presence / Absence	
Amphisbetia distans	-	Presence / Absence	

Taxon Name	Qualifier	Abundance Units	Action
Diphasia	-	Presence / Absence	
Hydrallmania falcata	-	Presence / Absence	
Sertularia	-	Presence / Absence	
Psammechinus miliaris	-	Count	
Echinocyamus pusillus	-	Count	
Echinocyamus pusillus	Fragment	Presence / Absence	add to species count entry
Leptosynapta bergensis	-	Count	
Leptosynapta inhaerens	-	Count	
Cucumariidae	Juvenile	Count	keep as juv
Amphiuridae	Fragment	Presence / Absence	remove
Amphipholis squamata	-	Count	
Amphiura (Ophiopeltis) securigera	-	Count	
Ophiothrix fragilis	-	Count	
Ophiuridae	Juvenile	Count	remove
Ophiuridae	Fragment	Presence / Absence	remove
Ophiura albida	-	Count	
Barentsia	-	Presence / Absence	
Loxosomella	-	Presence / Absence	
Pedicellina	-	Presence / Absence	
Enteropneusta	-	Count	
Mollusca	Fragment	Presence / Absence	remove
Thracia villosiuscula	-	Count	
Glycymeris glycymeris	-	Count	
Gari tellinella	-	Count	
Abra	Juvenile	Count	remove
Abra	Fragment	Presence / Absence	remove
Abra alba	-	Count	

Taxon Name	Qualifier	Abundance Units	Action
Abra prismatica	-	Count	
Moerella donacina	-	Count	
Kurtiella bidentata	-	Count	
Sphenia binghami	-	Count	
Mytilidae	Juvenile	Count	remove
Modiolus adriaticus	-	Count	
Musculus discors	-	Count	
Nucula hanleyi	-	Count	
Nucula nucleus	-	Count	
Anomiidae	Juvenile	Count	keep as juv
Pectinidae	Juvenile	Count	remove
Pectinidae	Fragment	Presence / Absence	remove
Aequipecten opercularis	-	Count	
Spisula	Juvenile	Count	remove
Spisula elliptica	-	Count	
Diplodonta rotundata	-	Count	
Chamelea striatula	-	Count	
Polititapes rhomboides	-	Count	
Timoclea ovata	-	Count	
Venus casina	-	Count	
Brachystomia carrozzai	-	Count	
Eulimella acicula	-	Count	
Philinidae	Juvenile	Count	keep as juv
Emarginula fissura	-	Count	
Emarginula rosea	-	Count	
Calyptraea chinensis	-	Count	
Tornus subcarinatus	-	Count	
Buccinidae	Juvenile	Count	remove
Buccinum undatum	-	Count	
Cuthona	-	Count	
Doto	-	Count	
Onchidorididae	-	Count	
Limacia clavigera	-	Count	
Calliostoma zizyphinum	-	Count	
Gibbula tumida	-	Count	

Taxon Name	Qualifier	Abundance Units	Action
Polyplacophora	Juvenile	Count	remove
Leptochiton asellus	-	Count	
Leptochiton cancellatus	-	Count	
Nematoda	-	Count	remove
Nemertea	-	Count	
Nemertea	Fragment	Presence / Absence	treat as count
Phoronis	-	Count	
Phoronis	Fragment	Presence / Absence	treat as count
Platyhelminthes	-	Count	
Porifera	-	Presence / Absence	
Sycon	-	Presence / Absence	
Cliona	-	Presence / Absence	
Grania	-	Count	remove
Corallinaceae	-	Presence / Absence	remove
Sipuncula	Juvenile	Count	remove
Golfingia (Golfingia) elongata	-	Count	
Golfingia (Golfingia) vulgaris vulgaris	-	Count	
Nephasoma (Nephasoma) minutum	-	Count	
Phascolion (Phascolion) strombus strombus	-	Count	
Gyptis	-	Count	

Epifaunal truncation protocol

Table 24. Epifaunal matrix truncation protocol matrix.

Morpho-taxon Entry	Annotation Type	Sum	Action	Final Code
Biota > Ascidians > Stalked > Colonial**	Percentage Cover	0.1	**Keep**	B_Asc_St_Col_Indet
Biota > Ascidians > Unstalked > Colonial**	Percentage Cover	68.3	**Keep**	B_Asc_UnSt_Col_Indet
Biota > Ascidians > Unstalked > Colonial** > cf. *Botryllus schlosseri* > *Botryllus schlosseri*	Percentage Cover	0.1	**Keep**	B_Asc_UnSt_Col_ cf. B.schlosseri
Biota > Ascidians > Unstalked > Colonial** > cf. *Diplosoma sp.*	Percentage Cover	2.3	**Keep**	B_Asc_UnSt_Col_cf. Diplosoma sp.
Biota > Bacterial mats**	Percentage Cover	0.2	**Keep**	B_Bac.mat
Biota > Bryozoa > Hard > Branching**	Percentage Cover	1.0	**Remove**	
Biota > Bryozoa > Hard > Branching** > *Cellaria sp.***	Percentage Cover	180.7	**Keep**	B_Bry_Hard_Brn_Cellaria sp.
Biota > Bryozoa > Hard > Branching** > cf. *Palmiskenea skenei***	Percentage Cover	80.0	**Keep**	B_Bry_Hard_Brn_cf. P.skenei
Biota > Bryozoa > Hard > Encrusting** > Bry_Hard_Enc_Grey	Percentage Cover	0.1	**Keep**	B_Bry_Hard_Brn_Enc_Grey
Biota > Bryozoa > Hard > Encrusting** > Bry_Hard_Enc_Orange**	Percentage Cover	0.3	**Keep**	B_Bry_Hard_Brn_Enc_Orange
Biota > Bryozoa > Hard > Encrusting** > Bry_Hard_Enc_Pink**	Percentage Cover	0.2	**Keep**	B_Bry_Hard_Brn_Enc_Pink

Morpho-taxon Entry	Annotation Type	Sum	Action	Final Code
Biota > Bryozoa > Hard > Encrusting** > Bry_Hard_Enc_Red**	Percentage Cover	1.7	**Keep**	B_Bry_Hard_Brn_Enc_Red
Biota > Bryozoa > Hard > Encrusting** > Bry_Hard_Enc_White**	Percentage Cover	393.4	**Keep**	B_Bry_Hard_Brn_Enc_White
Biota > Bryozoa > Hard > Encrusting** > Bry_Hard_Enc_Yellow**	Percentage Cover	0.2	**Keep**	B_Bry_Hard_Brn_Enc_Yellow
Biota > Bryozoa > Hard > Massive** > cf. *Pentapora foliacea*** > *Pentapora foliacea***	Percentage Cover	162.8	**Keep**	B_Bry_Hard_Massive_cf. P.foliacea
Biota > Bryozoa > Soft > Dendroid** > cf. *Bugula sp.***	Percentage Cover	0.7	**Keep**	B_Bry_Soft_Dendroid_cf. Bugula sp.
Biota > Bryozoa > Soft > Dendroid** > cf. *Crisia sp.***	Percentage Cover	33.9	**Keep**	B_Bry_Soft_Dendroid_cf. Crisia sp.
Biota > Bryozoa > Soft > Dendroid** > cf. *Vesicularia spinosa***	Percentage Cover	1373.3	**Keep**	B_Bry_Soft_Dendroid_cf. V.spinosa
Biota > Bryozoa > Soft > Foliaceous** > cf. *Flustra foliacea*** > *Flustra foliacea***	Percentage Cover	3090.1	**Keep**	B_Bry_Soft_Foliaceous_cf. F.foliacea
Biota > Cnidaria > Colonial anemones** > Corallimorphs** > *Corynactis viridis***	Percentage Cover	80.2	**Keep**	B_Cn_ColAn_Corallomorphs_cf. C.viridis
Biota > Cnidaria > Colonial anemones** > Zoanthids > cf. *Epizoanthus couchii***	Percentage Cover	40.8	**Keep**	B_Cn_ColAn_Corallomorphs_cf. E.couchii
Biota > Cnidaria > Corals > Soft corals** > cf. *Alcyonium digitatum*** > *Alcyonium digitatum***	Percentage Cover	86.6	**Keep**	B_Cn_Cor_SoftCor_cf. A.digitatum
Biota > Cnidaria > Hydroids > Bushy**	Percentage Cover	118.4	**Merge**	B_Cn_Hyd_Bushy
Biota > Cnidaria > Hydroids > Bushy** > cf. *Hydrallmania falcata*** > *Hydrallmania falcata***	Percentage Cover	1934.6		

Morpho-taxon Entry	Annotation Type	Sum	Action	Final Code
Biota > Crustacea > Barnacles** - NB USE TIER 1 LABEL > Barnacles_Acorn**	Percentage Cover	2910.0	**Merge**	B_Cr_Barnacles
Biota > Crustacea > Barnacles** - NB USE TIER 1 LABEL > Barnacles_Stalked**	Percentage Cover	10.0		
Biota > Echinoderms > Feather stars** > Unstalked crinoids**	Percentage Cover	241.3	**Keep**	B_Ecr_FStar_UnSt_Crin
Biota > Echinoderms > Ophiuroids > Brittle stars**	Percentage Cover	0.1	**Remove**	
Biota > Echinoderms > Ophiuroids > Brittle stars** > cf. *Ophiura albida*** > *Ophiura albida*	Percentage Cover	124.3	**Merge**	B_Ecr_Brit_Ophiura sp.
Biota > Echinoderms > Ophiuroids > Brittle stars** > *Ophiura sp.***	Percentage Cover	3.8		
Biota > Echinoderms > Ophiuroids > cf. *Ophiothrix fragilis / Ophiocomina nigra*	Percentage Cover	37212.0	**Keep**	B_Ecr_Brit_cf. O.Fragilis/O.nigra
Biota > Faunal turf	Percentage Cover	41350.0	**Keep**	Faunal_Turf
Biota > Macroalgae** > Encrusting > Red > Calcareous	Percentage Cover	253.0	**Keep**	B_Mac_Encr_Red_Calc
Biota > Macroalgae** > Erect coarse branching > Brown	Percentage Cover	5.5	**Keep**	B_Mac_ErrCB_Brown
Biota > Macroalgae** > Filamentous / filiform	Percentage Cover	10.0	**Keep**	B_Mac_Fili
Biota > Macroalgae** > Sheetlike / membraneous > Red	Percentage Cover	0.4	**Keep**	B_Mac_ShtMemb_Red
Biota > Sponges > Crusts** > Bioeroding** > cf. *Cliona celata*** > *Cliona celata***	Percentage Cover	1.0	**Keep**	B_Sp_Cr_Bio_cf. C.celata

Morpho-taxon Entry	Annotation Type	Sum	Action	Final Code
Biota > Sponges > Crusts** > Encrusting** > cf. *Dercitus bucklandi***	Percentage Cover	4.0	**Keep**	B_Sp_Cr_Enc_cf. D.bucklandi
Biota > Sponges > Crusts** > Encrusting** > cf. *Hemimycale columella*** > *Hemimycale columella***	Percentage Cover	2.2	**Keep**	B_Sp_Cr_Enc_cf. H.columella
Biota > Sponges > Crusts** > Encrusting** > Sp_Cr_Enc_Blue**	Percentage Cover	20.4	**Keep**	B_Sp_Cr_Enc_Blue
Biota > Sponges > Crusts** > Encrusting** > Sp_Cr_Enc_Brown**	Percentage Cover	5.3	**Keep**	B_Sp_Cr_Enc_Brown
Biota > Sponges > Crusts** > Encrusting** > Sp_Cr_Enc_Cream**	Percentage Cover	47.2	**Keep**	B_Sp_Cr_Enc_Cream
Biota > Sponges > Crusts** > Encrusting** > Sp_Cr_Enc_Green**	Percentage Cover	278.1	**Keep**	B_Sp_Cr_Enc_Green
Biota > Sponges > Crusts** > Encrusting** > Sp_Cr_Enc_Grey**	Percentage Cover	193.0	**Keep**	B_Sp_Cr_Enc_Grey
Biota > Sponges > Crusts** > Encrusting** > Sp_Cr_Enc_Orange**	Percentage Cover	763.7	**Keep**	B_Sp_Cr_Enc_Orange
Biota > Sponges > Crusts** > Encrusting** > Sp_Cr_Enc_Peach**	Percentage Cover	76.3	**Keep**	B_Sp_Cr_Enc_Peach
Biota > Sponges > Crusts** > Encrusting** > Sp_Cr_Enc_Pink**	Percentage Cover	89.2	**Keep**	B_Sp_Cr_Enc_Pink
Biota > Sponges > Crusts** > Encrusting** > Sp_Cr_Enc_Purple**	Percentage Cover	29.5	**Keep**	B_Sp_Cr_Enc_Purple
Biota > Sponges > Crusts** > Encrusting** > Sp_Cr_Enc_Red**	Percentage Cover	36.9	**Keep**	B_Sp_Cr_Enc_Red
Biota > Sponges > Crusts** > Encrusting** > Sp_Cr_Enc_White**	Percentage Cover	97.9	**Keep**	B_Sp_Cr_Enc_White

Morpho-taxon Entry	Annotation Type	Sum	Action	Final Code
Biota > Sponges > Crusts** > Encrusting** > Sp_Cr_Enc_Yellow**	Percentage Cover	844.8	**Keep**	B_Sp_Cr_Enc_Yellow
Biota > Sponges > Massive forms** > Balls**	Percentage Cover	1.3	**Keep**	B_Sp_Mas_Balls
Biota > Sponges > Massive forms** > Cryptic** > cf. *Ciocalypta penicillus* > *Ciocalypta penicillus*	Percentage Cover	0.4	**Keep**	B_Sp_Mass_Crypt_cf. C.penicillus
Biota > Sponges > Massive forms** > Cryptic** > cf. *Polymastia boletiformis* > *Polymastia boletiformis*	Percentage Cover	3.2	**Keep**	B_Sp_Mass_Crypt_cf. P.boletiformis
Biota > Sponges > Massive forms** > Cryptic** > cf. *Polymastia penicillus*	Percentage Cover	0.2	**Keep**	B_Sp_Mass_Crypt_cf. P.penicillus
Biota > Sponges > Massive forms** > Simple** > cf. *Dysidea fragilis***	Percentage Cover	73.9	**Keep**	B_Sp_Mass_Simp_cf. D.fragilis
Biota > Sponges > Massive forms** > Simple** > Sp_M_S_Brown**	Percentage Cover	13.9	**Keep**	B_Sp_M_Simp_Brown
Biota > Sponges > Massive forms** > Simple** > Sp_M_S_Cream**	Percentage Cover	80.4	**Keep**	B_Sp_M_Simp_Cream
Biota > Sponges > Massive forms** > Simple** > Sp_M_S_Grey**	Percentage Cover	0.1	**Keep**	B_Sp_M_Simp_Grey
Biota > Sponges > Massive forms** > Simple** Sp_M_S_Orange**	Percentage Cover	42.9	**Keep**	B_Sp_M_Simp_Orange
Biota > Sponges > Massive forms** > Simple** > Sp_M_S_Peach**	Percentage Cover	8.3	**Keep**	B_Sp_M_Simp_Peach
Biota > Sponges > Massive forms** > Simple** > Sp_M_S_White**	Percentage Cover	36.6	**Keep**	B_Sp_M_Simp_White
Biota > Sponges > Massive forms** > Simple** > Sp_M_S_Yellow**	Percentage Cover	146.7	**Keep**	B_Sp_M_Simp_Yellow

Morpho-taxon Entry	Annotation Type	Sum	Action	Final Code
Biota > Worms > Horseshoe worms**	Percentage Cover	0.0	**Keep**	B_Wrms_Hshoe
Biota > Worms > Polychaetes > Tube worms > Sabellariidae** > *Sabellaria spinulosa*	Percentage Cover	12330.0	**Keep**	B_Wrms_Poly_Tube _S.spinulosa
Biota > Worms > Polychaetes > Tube worms > Serpulidae** - NB USE TIER 1 LABEL > cf. *Salmacina dysteri***	Percentage Cover	560.0	**Merge**	B_Wrms_Poly_Tube _Serpulidae
Biota > Worms > Polychaetes > Tube worms > Serpulidae** - NB USE TIER 1 LABEL > cf. *Spirobranchus sp.***	Percentage Cover	25860.4		
Biota > Worms > Polychaetes > Tube worms > Serpulidae** - NB USE TIER 1 LABEL > Serpulidae**	Percentage Cover	4634.0		
Biota > Ascidians > Stalked > Solitary > *Styela clava* (NIS)	Count	28.0	**Keep**	B_Asc_St_Sol_S.Clava
Biota > Ascidians > Unstalked > Solitary	Count	624.2	**Keep**	B_Asc_UnSt_Sol_Indet
Biota > Bioturbation > Waste casts	Count	2.0	**Remove**	
Biota > Bryozoa > Soft > Gelatinous > cf. *Alcyonidium diaphanum*	Count	1.0	**Remove**	
Biota > Cnidaria > Corals > Cup corals > cf. *Caryophyllia smithii* > *Caryophyllia smithii*	Count	0.0	**Remove**	
Biota > Cnidaria > Hydroids > Branching	Count	2414.9	**Keep**	B_Cn_Hyd_Brn_Indet
Biota > Cnidaria > Hydroids > Branching > Sertularidae	Count	407.0	**Keep**	B_Cn_Hyd_Brn_Sertularidae
Biota > Cnidaria > Hydroids > Erect	Count	146.0	**Keep**	B_Cn_Hyd_Err_Indet
Biota > Cnidaria > Hydroids > Erect > cf. *Nemertesia sp*	Count	27.0	**Merge**	B_Cn_Hyd_Err_cf. Nemertesia sp.
Biota > Cnidaria > Hydroids > Erect > cf. *Nemertesia sp* > *Nemertesia antennina*	Count	81.0		
Biota > Cnidaria > Hydroids > Erect > cf. *Nemertesia sp* > *Nemertesia ramosa*	Count	1.0		
Biota > Cnidaria > True anemones	Count	1289.0	**Keep**	B_Cn_TA_Indet

Morpho-taxon Entry	**Annotation Type**	**Sum**	**Action**	**Final Code**
Biota > Cnidaria > True anemones > cf. *Actinothoe sphyrodeta*	Count	308.0	**Keep**	B_Cn_TA_cf. A.sphyrodeta
Biota > Cnidaria > True anemones > cf. *Anthopleura sp.*	Count	11.0	**Keep**	B_Cn_TA_cf. Anthopleura sp.
Biota > Cnidaria > True anemones > cf. *Capnea sanuinea*	Count	4.0	**Keep**	B_Cn_TA_cf. C.sanuinea
Biota > Cnidaria > True anemones > cf. *Cereus sp.*	Count	19.0	**Keep**	B_Cn_TA_cf. Cereus sp.
Biota > Cnidaria > True anemones > cf. *Mesacmaea mitchellii* > *Mesacmaea mitchellii*	Count	1.0	**Remove**	
Biota > Cnidaria > True anemones > cf. *Metridium senile*	Count	1.0	**Remove**	B_Cn_TA_cf. M.senile
Biota > Cnidaria > True anemones > cf. *Sagartia sp.*	Count	23.0	**Keep**	B_Cn_TA_ cf. Sagartia sp.
Biota > Cnidaria > True anemones > cf. *Urticina sp.*	Count	205.0	**Merge**	B_Cn_TA_ cf. Urticina sp.
Biota > Cnidaria > True anemones > cf. *Urticina sp.* > *Urticina felina*	Count	25.0		
Biota > Crustacea	Count	5.0	**Remove**	
Biota > Crustacea > Crabs	Count	24.0	**Keep**	B_Cr_Crabs_Indet
Biota > Crustacea > Crabs > Nutcrabs > cf. *Ebalia sp.*	Count	139.0	**Keep**	B_Cr_Crabs_Nut_cf. Ebalia sp
Biota > Crustacea > Crabs > Porcelain crabs	Count	0.0	**Remove**	
Biota > Crustacea > Crabs > Small spider crabs	Count	36.0	**Merge**	B_Cr_Crabs_Sm_Spider
Biota > Crustacea > Crabs > Small spider crabs > cf. Hyas sp.	Count	8.0		
Biota > Crustacea > Crabs > Small spider crabs > cf. Inachus sp.	Count	11.0		
Biota > Crustacea > Crabs > Small spider crabs > cf. Macropodia sp.	Count	2.0		
Biota > Crustacea > Crabs > Spider crabs > cf. *Maja brachydactyla*	Count	1.0	**Keep**	B_Cr_Crabs_Spider_cf. M.brachydactyla

Morpho-taxon Entry	Annotation Type	Sum	Action	Final Code
Biota > Crustacea > Crabs > True crabs	Count	1.0	**Remove**	
Biota > Crustacea > Crabs > True crabs > cf. *Necora puber* > *Necora puber*	Count	1.0	**Merge**	B_Cr_Crabs_Portunidae
Biota > Crustacea > Crabs > True crabs > Portunidae	Count	2.0		
Biota > Crustacea > Hermit crabs	Count	352.0	**Merge**	B_Cr_Crabs_Hermit
Biota > Crustacea > Hermit crabs > Paguridae	Count	1.0		
Biota > Crustacea > Isopoda > *Aega sp.*	Count	0.0	**Remove**	
Biota > Crustacea > Lobsters > Squat lobsters	Count	2.0	**Keep**	B_Cr_Lob_SqtLob
Biota > Crustacea > Prawns / shrimps / mysids	Count	21.0	**Keep**	B_Cr_Prn
Biota > Echinoderms	Count	0.0	**Remove**	
Biota > Echinoderms > Ophiuroids > Brittle stars > cf. *Ophiura albida*	Count	0.2	**Remove**	
Biota > Echinoderms > Sea stars	Count	3.0	**Remove**	
Biota > Echinoderms > Sea stars > *Anseropoda placenta*	Count	2.0	**Keep**	B_Ecr_SS_cf. A.placenta
Biota > Echinoderms > Sea stars > cf. *Asterias rubens* > *Asterias rubens*	Count	136.0	**Keep**	B_Ecr_SS_cf. A.rubens
Biota > Echinoderms > Sea stars > cf. *Crossaster papposus* > *Crossaster papposus*	Count	32.0	**Keep**	B_Ecr_SS_cf. C.papposus
Biota > Echinoderms > Sea stars > cf. *Henricia sp.* > *Henricia sp.*	Count	5.0	**Keep**	B_Ecr_SS_Henricia sp.
Biota > Echinoderms > Sea urchins > Regular urchins > cf. *Psammechinus sp.* > *Psammechinus miliaris*	Count	62.0	**Keep**	B_Ecr_Urch_RegUrch_cf. Psammechinus sp.
Biota > Egg masses > Eggs_*Buccinum undatum*	Count	8.0	**Remove**	
Biota > Egg masses > Eggs_Elasmobranch	Count	3.0	**Remove**	
Biota > Fishes > Bony fishes	Count	5.0	**Remove**	

Morpho-taxon Entry	Annotation Type	Sum	Action	Final Code
Biota > Fishes > Bony fishes > cf. *Callionymus sp.*	Count	5.0	**Remove**	
Biota > Fishes > Bony fishes > cf. *Mullus surmuletus* > *Mullus surmuletus*	Count	2.0	**Remove**	
Biota > Fishes > Bony fishes > Cottidae	Count	0.0	**Remove**	
Biota > Fishes > Bony fishes > Gadidae	Count	2.0	**Remove**	
Biota > Fishes > Bony fishes > Gobiesocidae	Count	0.0	**Remove**	
Biota > Fishes > Bony fishes > Gobiidae	Count	4.0	**Remove**	
Biota > Fishes > Bony fishes > Labridae	Count	1.0	**Remove**	
Biota > Fishes > Bony fishes > Pleuronectidae > cf. *Pleuronectes platessa*	Count	0.0	**Remove**	
Biota > Fishes > Bony fishes > Pleuronectidae > cf. *Pleuronectes platessa* > *Pleuronectes platessa*	Count	2.0	**Remove**	
Biota > Fishes > Bony fishes > Pleuronectiformes	Count	3.0	**Remove**	
Biota > Fishes > Bony fishes > Pleuronectiformes > Soleidae	Count	2.0	**Remove**	
Biota > Fishes > Bony fishes > Trachinidae > cf. *Echiichthys vipera* > *Echiichthys vipera*	Count	1.0	**Remove**	
Biota > Fishes > Bony fishes > Triglidae	Count	1.0	**Remove**	
Biota > Fishes > Elasmobranchs > Rays & skates > cf. *Raja clavata*	Count	2.0	**Remove**	
Biota > Fishes > Elasmobranchs > Sharks > cf. *Scyliorhinus canicula* > *Scyliorhinus canicula*	Count	1.0	**Remove**	
Biota > Molluscs > Bivalves	Count	16.0	**Keep**	B_Mol_Bi_Indet
Biota > Molluscs > Bivalves > Anomiidae	Count	9.0	**Keep**	B_Mol_Bi_Anomiidae

Morpho-taxon Entry	Annotation Type	Sum	Action	Final Code
Biota > Molluscs > Bivalves > cf. *Aequipecten opercularis* > *Aequipecten opercularis*	Count	904.3	**Keep**	B_Mol_Bi_cf. A.oppercularis
Biota > Molluscs > Bivalves > cf. *Glycymeris glycymeris* > *Glycymeris glycymeris*	Count	9.0	**Keep**	B_Mol_Bi_cf. G.glycymeris
Biota > Molluscs > Bivalves > cf. *Mimachlamys varia*	Count	0.0	**Remove**	
Biota > Molluscs > Bivalves > cf. *Pecten maximus* > *Pecten maximus*	Count	7.0	**Keep**	B_Mol_Bi_cf. P.maximus
Biota > Molluscs > Bivalves > *Spisula sp*.	Count	3.0	**Keep**	B_Mol_Bi_Spisula sp.
Biota > Molluscs > Bivalves > Venerida	Count	7.0	**Keep**	B_Mol_Bi_Venerida
Biota > Molluscs > Cephalopods > Cuttlefish	Count	0.0	**Remove**	
Biota > Molluscs > Cephalopods > Squid	Count	1.0	**Remove**	
Biota > Molluscs > Chitons	Count	53.0	**Keep**	B_Mol_Chit
Biota > Molluscs > Gastropods	Count	1118.1	**Keep**	B_Mol_Gast_Indet
Biota > Molluscs > Gastropods > cf. *Buccinum undatum* > *Buccinum undatum*	Count	126.0	**Keep**	B_Mol_Gast_cf. B.undatum
Biota > Molluscs > Gastropods > cf. *Calliostoma zizyphinum*	Count	1.0	**Merge**	B_Mol_Gast_cf. C.zizyphinum
Biota > Molluscs > Gastropods > cf. *Calliostoma zizyphinum* > *Calliostoma zizyphinum*	Count	218.0		
Biota > Molluscs > Gastropods > cf. *Calyptraea chinensis* > *Calyptraea chinensis*	Count	0.0	**Remove**	
Biota > Molluscs > Gastropods > cf. *Crepidula fornicata* > *Crepidula fornicata* (NIS)	Count	10.0	**Keep**	B_Mol_Gast_cf. C.fornicata
Biota > Molluscs > Gastropods > cf. *Gibbula sp.*	Count	961.0	**Keep**	B_Mol_Gast_cf. Gibbula sp.
Biota > Molluscs > Gastropods > cf. *Tritia sp.*	Count	21.0	**Merge**	B_Mol_Gast_Tritia sp.

Morpho-taxon Entry	Annotation Type	Sum	Action	Final Code
Biota > Molluscs > Gastropods > cf. *Tritia sp.* > *Tritia sp.*	Count	1.0		
Biota > Molluscs > Gastropods > cf. *Trivia monacha*	Count	3.0	**Keep**	B_Mol_Gast_cf. T.monacha
Biota > Molluscs > Gastropods > Muricidae	Count	119.0	**Keep**	B_Mol_Gast_Muricidae
Biota > Molluscs > Gastropods > Patellogastropoda	Count	12.0	**Keep**	B_Mol_Gast_Patellogasteropoda
Biota > Molluscs > Nudibranchs	Count	0.0	**Remove**	
Biota > Molluscs > Nudibranchs > Aeolidia	Count	3.0	**Keep**	B_Mol_Nud_Aeolidia
Biota > Molluscs > Nudibranchs > cf. *Doris pseudoargus* > *Doris pseudoargus*	Count	0.0	**Remove**	
Biota > Molluscs > Nudibranchs > Polycera	Count	2.0	**Keep**	B_Mol_Nud_Polycera
Biota > Sponges > Erect forms > Branching	Count	51.0	**Keep**	B_Sp_Err_Brn
Biota > Sponges > Erect forms > Branching > cf. *Raspailia ramosa* > *Raspailia ramosa*	Count	0.0	**Remove**	
Biota > Sponges > Erect forms > Branching > cf. *Stelligera rigida* > *Stelligera rigida*	Count	2.0	**Keep**	B_Sp_Err_Brn_cf. S.rigida
Biota > Sponges > Erect forms > Laminar	Count	0.0	**Remove**	
Biota > Sponges > Erect forms > Palmate	Count	0.0	**Remove**	
Biota > Sponges > Erect forms > Simple	Count	0.0	**Remove**	
Biota > Sponges > Erect forms > Simple > cf. *Sycon ciliatum*	Count	4.0	**Keep**	B_Sp_Err_Simple_cf. S.ciliatum
Biota > Sponges > Erect forms > Simple > cf. *Sycon ciliatum* > *Sycon ciliatum*	Count	0.0	**Remove**	
Biota > Worms > Nemertea	Count	0.0	**Remove**	
Biota > Worms > Polychaetes > Other polychaetes	Count	1.0	**Keep**	B_Wrms_Poly_Other

Morpho-taxon Entry	Annotation Type	Sum	Action	Final Code
Biota > Worms > Polychaetes > Tube worms	Count	71.0	**Keep**	B_Wrms_Poly_Tube
Biota > Worms > Polychaetes > Tube worms > Sabellidae	Count	9.0	**Keep**	B_Wrms_Poly_Tube_Sabellidae
Non-Identifiable Taxa	Count	3.0	**Remove**	

Annex 5. Marine litter categories

Table 25. Categories and sub-categories of litter items for Sea-Floor (European Commission 2013).

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

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

Annex 6. Non-indigenous species lists

Table 26. Taxa listed as non-indigenous species (present and horizon) which have been selected for assessment of Good Environmental Status in GB waters under MSFD Descriptor 2 (Stebbing *et al.* 2014).

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

Table 27. 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.

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

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

JNCC/Cefas Partnership Report Series 44. *Offshore Overfalls Marine Conservation Zone (MCZ) Monitoring Report 2019.* Hawes, J. Arosio, R., Bolam, S. & McCabe, C. October 2024. ISSN 2051-6711