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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

This report explores environmental and ecological sample data, primarily acquired from a survey of Pisces Reef Complex MPA in 2016, which are intended to serve as first point in a monitoring time series. It also includes recommendations to inform continual improvement and development of sample acquisition, analysis and data interpretation for future survey and reporting.

Pisces Reef Complex MPA is an offshore site located in the western Irish Sea, in the north-west mud basin, approximately midway between the Isle of Man and the coast of Northern Ireland. This MPA is designated for the Annex I Habitat 'Reefs' which comprises both bedrock reef and boulder-dominated stony reef. This report provides a characterisation of the attributes of the designated feature. This includes the extent and distribution of the feature across the three main reef zones of the site, named PR1, PR2 and PR3 (Objective 1), the structure and function of the biological communities on those reefs (Objective 2) and their supporting environmental processes (Objective 3). All three reef zones are morphologically distinct but with some similarities in biological communities.

The surface-dwelling biological communities at Pisces Reef Complex MPA are highly heterogenous in nature and patchy in distribution, strongly affected by local sedimentary processes and the underlying geology. Communities appear to exist along a continuum ranging from areas of high rock content, with high local diversity of reef-like communities, through areas with veneers of sediment over hard substrata and moderate diversity of reef-like communities, to areas of pure mud, no rock and dominated by burrowing infauna. To better understand these complexities, this report investigates the influence that sediment veneers on hard substrata have on reef-like communities (Objective 4), showing these communities to be different to true reef-like communities.

There are no non-indigenous species observed across the site, although there is evidence of fishing activity, mostly in the form of bottom trawling around and between the reefs of PR2 (Objectives 5 and 6).

This report makes recommendations for future monitoring at the site to ensure that optimal approaches are utilised (Objective 7). Proposed measures aim to increase the volume of data acquired through longer targeted drop camera tows with increased still image collection. They also aim to continue application of the frequency of occurrence annotation method and approaches to efficiently identify and analyse the Annex I Reef community to detect meaningful changes over time.

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Abbreviations

ANOSIM	Analysis of Similarity
ANOVA	Analysis of Variance
Cefas	Centre for Environment, Fisheries and Aquaculture Science
CHP	Civil Hydrography Programme
CP2	Charting Progress 2
Defra	Department for Environment, Food and Rural Affairs
EUNIS	European Nature Information System
FOV	Field of View
JNCC	Joint Nature Conservation Committee
NMBAQC	North East Atlantic Marine Biological Analytical Quality Control Scheme
MBES	Multibeam echosounder
MPA	Marine Protected Area
MPAG	Marine Protected Areas Survey Coordination and Evidence Group
NIS	Non-Indigenous Species
nMDS	Non-metric Multidimensional Scaling
OSPAR	The Convention for the Protection of the Marine Environment of the North-East Atlantic
PCA	Principal Components Analysis
PERMANOVA	Permutation-based Multivariate Analysis of Variance
RV	Research Vessel
SAC	Special Area of Conservation
SACFOR	Superabundant-Abundant-Common-Frequent-Occasional-Rare scale
SACO	Supplementary Advice on Conservation Objectives
SAD	Site Assessment Document
SIMPER	Similarity Percentages analysis
SIMPROF	Similarity Percentages analysis
SNCB	Statutory Nature Conservation Body
SSS	Side-scan sonar

1 Introduction

This initial monitoring characterisation report primarily explores data acquired from the CEND2316 first dedicated monitoring survey of the Pisces Reef Complex Marine Protected Area (MPA), which form the baseline for a monitoring time series against which feature condition can be assessed in the future. The specific aims of the report are discussed in detail in Section 1.3.

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

1.1 MPA site overview

Pisces Reef Complex MPA is an offshore site located in the western Irish Sea, in the north-west mud basin, approximately midway between the Isle of Man and the coast of Northern Ireland (Figure 1). Pisces Reef Complex MPA was approved by the European Commission as a designated Special Area of Conservation (SAC) in September 2017. It was designated as part of a network of Natura 2000 sites designed to meet conservation objectives under the EC Habitats Directive.

This particular site is designated for the Annex I Habitat ‘Reefs’ and comprises both bedrock reef and boulder-dominated stony reef. The area consists of an extensive mud plain, through which three areas of Annex I bedrock and boulder-dominated stony reefs protrude, forming three areas of reef (hereafter referred to as PR1, PR2 and PR3 respectively). PR1, PR2 and PR3 are situated apart from each other at distances ranging from approximately 5.5 to 14 km. The MPA encompasses the three reef sites, excluding the areas of muddy sediment in between. The site is neighboured by the North Channel MPA to the north, Strangford Lough MPA to the north-east, Murlough MPA to the east and North Anglesey Marine / Gogledd Môn Forol MPA to the south (Figure 1).

The average seabed depth within the site boundary is approximately 100 m, with a maximum of 134 m and a minimum of 70 m at the peaks of the rocky reef outcrops. The deepest depths are within the scour pits which encircle the outcropping rocky reefs.

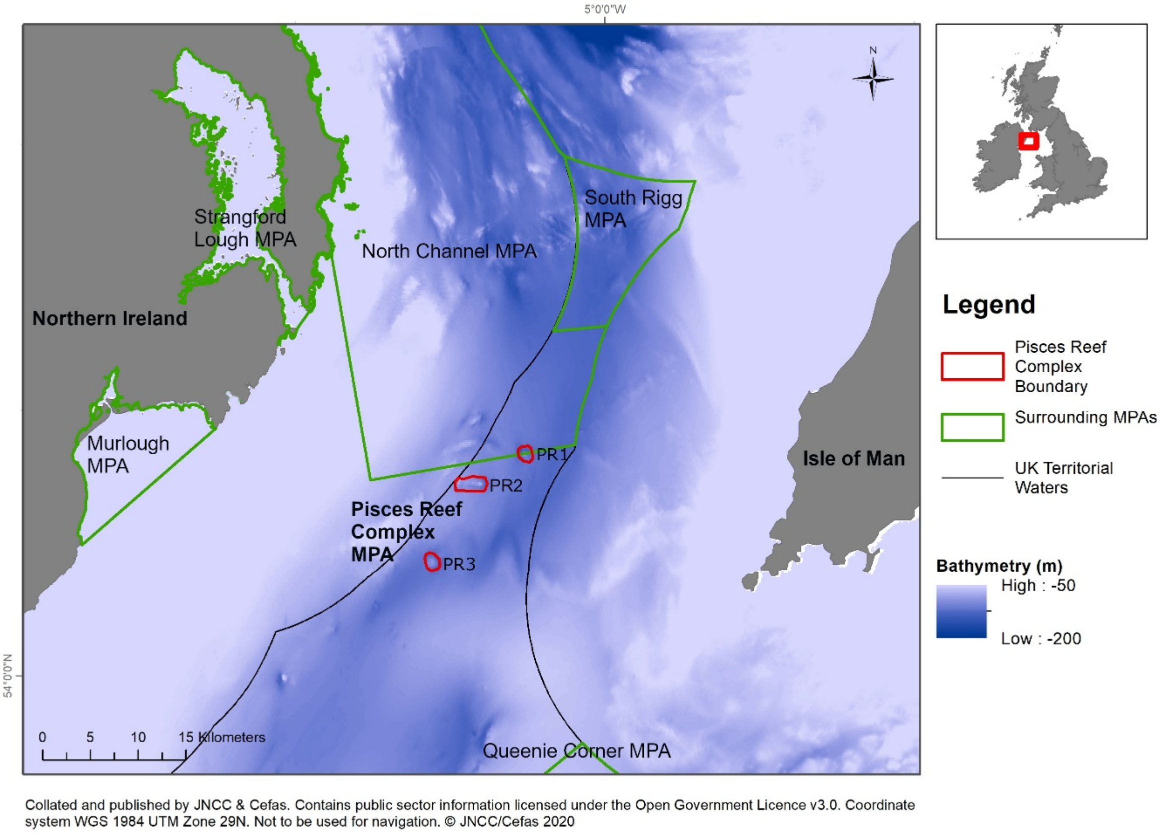


Figure 1. Location of the Pisces Reef Complex MPA in the context of other Marine Protected Areas and management jurisdictions proximal to the site. Bathymetry from Defra Astrium dataset (2016). PR1 = Pisces Reef 1, PR2 = Pisces Reef 2, PR3 = Pisces Reef 3.

1.2 Existing data and habitat maps

Seabed surveys have been carried out at the Pisces Reef Complex MPA area since 1971. During these surveys, conducted to deliver different objectives, a wide range of different samples and data types were collected, including acoustic data from multibeam echosounders (MBES), side-scan sonar (SSS) and seismic profilers, drop camera stills and video imagery, sediment grab samples and samples from the water column. A full list of these surveys and the samples and data collected is available in the 'Evidence' section of the Pisces Reef Complex MPA Site Information Centre[1].

The most relevant of these surveys, with regards to the purpose of conducting site monitoring and the planning of this study, are:

- The Strategic Environmental Assessment of the Irish Sea in 2004 (SEA6);
- The Agri-Food Bioscience Institute Mapping European Seabed Habitats survey in 2006 (AFBI MESH 2006);
- The Slieve Na Griddle rMCZ Site Verification Survey in 2012 (Service & Strong 2012; Strong 2012);
- Pisces Reef Complex MPA and Slieve Na Griddle rMCZ Drop Camera Survey in 2014 (McIlwaine 2014; Barrio Frojan *et al.* 2015).

[1] Pisces Reef Complex MPA Site Information Centre, URL: https://jncc.gov.uk/our-work/pisces-reef-complex-mpa/.

These surveys collected geophysical data and video imagery from across the Pisces Reef Complex MPA, as can be seen in Figure 2.

Data from these surveys were used to inform assessment of the distribution and extent of the Annex I Reef feature at Pisces Reef Complex MPA. This estimate was used to generate a habitat map of the site, which was used for the planning the CEND2316 monitoring survey (Figure 3)(JNCC 2012).

The drop camera and video tow data from these surveys were also used to describe the biological communities at the site, the descriptions for which can be found in the Site Information Centre[2].

[2] Pisces Reef Complex MPA Site Information Centre, URL: https://jncc.gov.uk/our-work/pisces-reef-complex-mpa/.

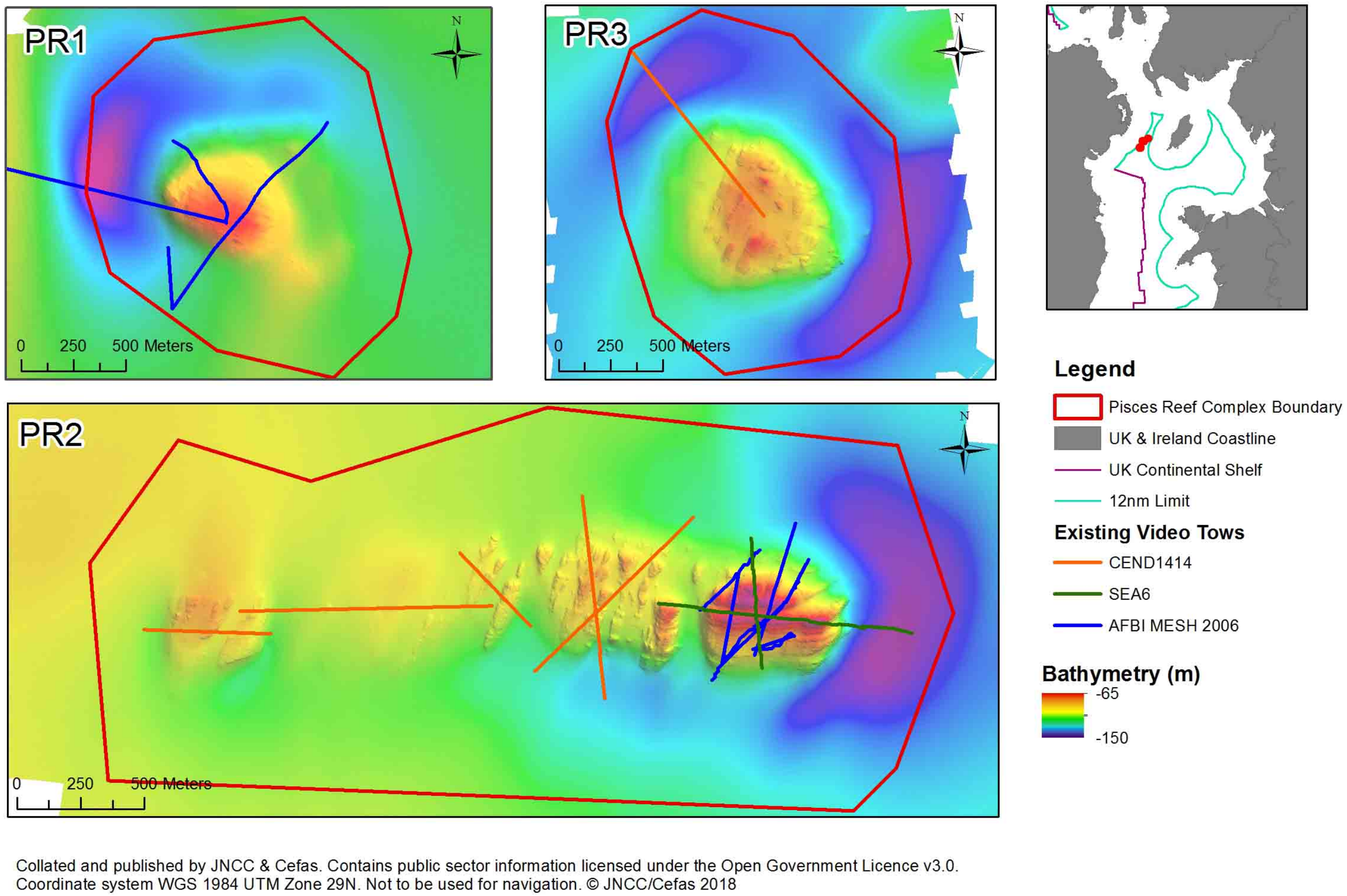


Figure 2. Positions of video tows from previous surveys to Pisces Reef Complex MPA, including Pisces Reef Complex MPA and Slieve Na Griddle rMCZ Drop Camera Survey in 2014 (CEND1414), the Strategic Environmental Assessment of the Irish Sea in 2004 (SEA6) and the Agri-Food Bioscience Institute Mapping European Seabed Habitats survey in 2006 (AFBI MESH 2006). Figure inset shows the position of the MPA within the wider Irish Sea. Bathymetry from CEND2316 survey (Jenkins & Nelson 2017).

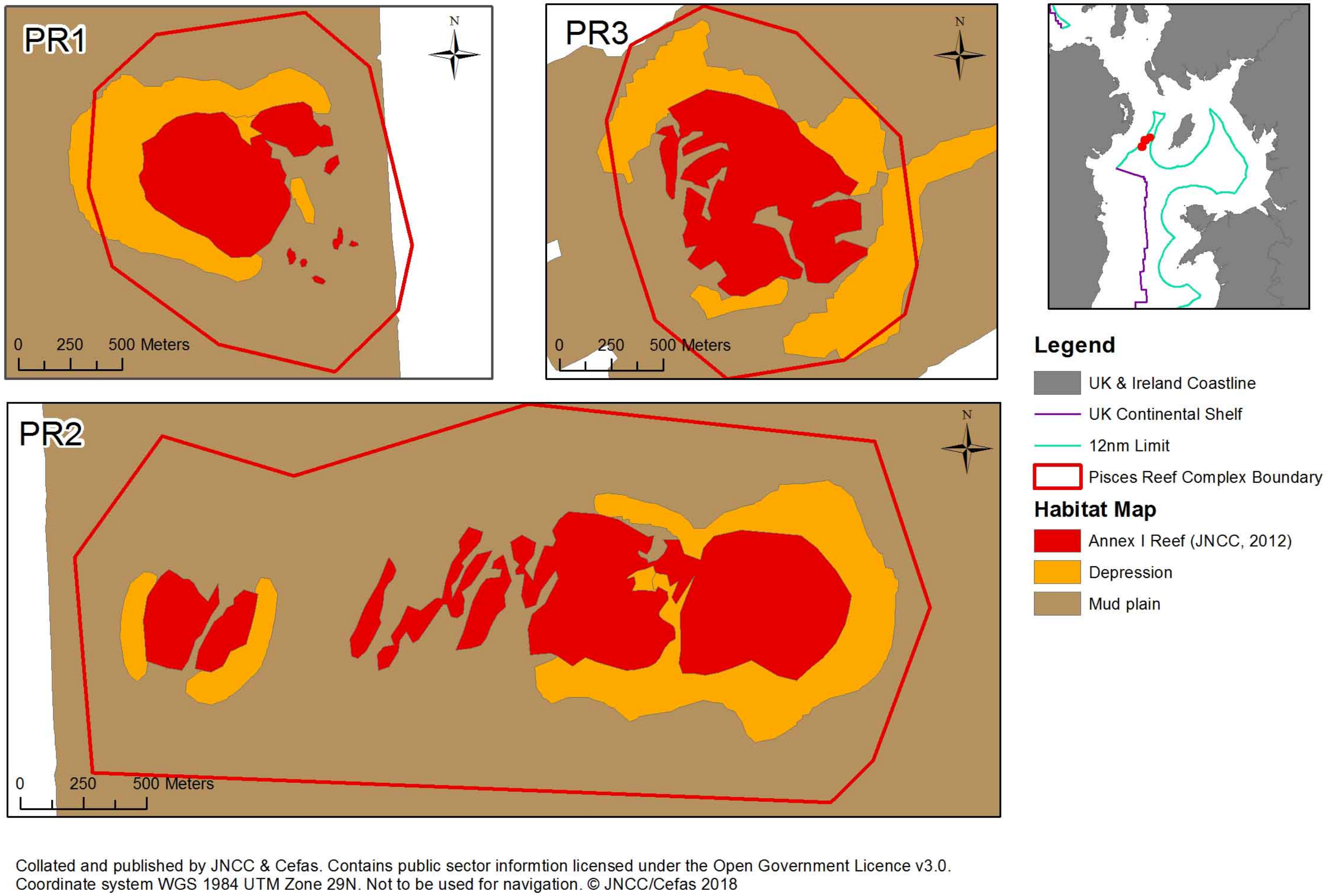


Figure 3. Estimated distribution and extent of Annex I reef feature and surrounding benthic habitat at Pisces Reef Complex MPA, based on survey data from Pisces Reef Complex MPA and Slieve Na Griddle rMCZ Drop Camera Survey in 2014 (CEND1414), the Strategic Environmental Assessment of the Irish Sea in 2004 (SEA6) and the Agri-Food Bioscience Institute Mapping European Seabed Habitats survey in 2006 (AFBI MESH 2006). Figure inset shows the position of the MPA within the wider Irish Sea.

1.3 Aims and objectives

1.3.1 Conservation objectives

Site-specific conservation objectives serve as benchmark against which to monitor and assess the efficacy of management measures in maintaining a designated Annex I habitat at, or restoring it to, 'favourable conservation status'.

Under the Habitats Directive, an Annex I habitat is considered to be in favourable conservation status when:

i) the natural environmental quality is restored;
ii) the natural environmental processes are maintained;
iii) the extent, physical structure, diversity, community structure and typical species representative of bedrock reef in the Irish Sea, are restored.

As stated in Table 1, the high-level conservation objective for Pisces Reef Complex MPA is to restore the Annex I Reef to favourable condition.

1.3.2 Report aims and objectives

The primary aim of this monitoring report is to describe the attributes of the designated features within the Pisces Reef Complex MPA, to enable future assessments of feature condition. The results presented will be used to develop recommendations for future monitoring, including the discussion of specific metrics which may indicate whether the condition of the feature has been maintained, improved or declined. The specific objectives of this monitoring report and the associated outputs are provided in Table 1.

To achieve report objectives 1, 2 and 3, selected Feature Attributes and supporting processes of the designated features are described (as defined in JNCC Supplementary Advice on Conservation Objectives[3]; see Table 1.

[3] Pisces Reef Complex SACO, URL: http://data.jncc.gov.uk/data/4f8ac443-777f-4a55-a5c8-fc44e80fa965/PiscesReef-3-SACO-V1.0.pdf

Table 1. Report objectives and outputs.

Objective		Feature attributes*	Features	Outputs
1	Describe the extent and distribution of the Annex I Reef.	Extent and distribution Physical structure and function	Annex I Reefs	Generate a habitat map to determine the extent of Annex I Reef in the MPA.
2	Describe the structures and functions and the composition of characteristic biological communities of the Annex I Reef.	Presence and spatial distribution of biological communities Presence and abundance of key structural and influential species Species composition of component communities	Annex I Reefs	Conduct multivariate analysis of epifaunal data to: - Identify patterns in biological assemblages; - Assign biotopes (where possible); - Describe variance in biological assemblage structure within the Annex I Reef; - Identify key structural and influential species; - Identify any potential indicator taxa.
3	Present information relating to supporting processes which are known to influence the designated and additional habitat and species features.	Supporting processes	Entire MPA	Conduct analysis of hydrodynamic and environmental data and present results
4	Explore the influence that sediment veneers on hard substrata have on the Annex I Reef community.	n/a	Annex I Reefs	Conduct analysis of hydrodynamic, environmental and epifaunal data and present results
5	Present any evidence of non-indigenous species and marine litter within the site.	Non-indigenous species (NIS)	Entire MPA	Point map of observations
6	Present any evidence of impact of anthropogenic activity observed within the site.	n/a	Entire MPA	Description
7	Recommend future monitoring approaches for the site, and other sites containing comparable features.	n/a	Annex I Reefs	Recommendations

2 Methods

2.1 Survey design

A survey was undertaken by JNCC and Cefas on the RV *Cefas Endeavour* between 30th October and 4th November 2016 (CEND2316). The primary objective of the survey was to collect data for Sentinel Monitoring of long-term trends (Type 1 monitoring) of the Annex I rocky and stony reef feature, to better understand long-term temporal and spatial patterns in epibenthic faunal communities across the site (Jenkins & Nelson 2017) and, more widely, across the entire UK range of the habitat type found at the site. To meet this objective, the survey also aimed to collect evidence which could be used to support the development of monitoring approaches and to begin operational monitoring of the MPA.

The survey was specifically designed to meet the monitoring aims and objectives for Pisces Reef Complex MPA (outlined in section 1.3.2). The reefs were divided into three areas according to where the main reef aggregations occurred: PR1, PR2 and PR3 (Figure 1). All sampling, including the collection of acoustic data and camera imagery, was stratified between these three areas.

The MBES survey was designed to provide 100% coverage of each reef area, to inform the distribution and extent of the reef feature at the site. This was achieved by ensuring an overlap of 20-30% between MBES lines. MBES lines were acquired along N-S (400 kHz) and E-W (200 k Hz) orientations (Jenkins & Nelson 2017). A sub-bottom profiler (SBP) was used simultaneously with the MBES, on the same lines, to inform the depth of the rock strata beneath the surface of those tracks. Owing to challenges with its acquisition and analysis, these data were not used any further in this study (see Annex 3. Data Preparation and Analysis, Habitat Map for further details).

The camera-based survey was designed for two purposes; to validate the acoustic data and to collect biological community data from the rocky and stony reefs, as well as the transition areas between them. The camera tows were targeted at each reef area using the initial seabed classification produced from the acoustic data during the survey (for details see Jenkins & Nelson 2017). The tows were targeted to sample different depths, slopes and potential substratum types, including rock strata and rock strata covered with a sediment veneer based on this initial seabed classification (Jenkins & Nelson 2017). Two lengths of tow were used: 200 m and 100 m. The 200 m lines were exploratory in design, aimed towards identifying the range of habitats, in order to improve accuracy for mapping the reef feature. The 100 m lines were placed within the reef area strata, targeted to areas suspected to be hard substrata, to provide descriptive data for the characterisation of the habitats and communities therein.

The community data extracted from extant video and stills imagery acquired at Pisces Reef Complex MPA were not of sufficient quality and consistency to conduct a power analysis (i.e., to determine the minimum number of still images required to detect a change of a given magnitude with a specified degree of power). Instead, the number of camera tows collected from each reef area was related to the amount of time available to sample each reef area: 21 camera tows for PR1, 25 camera tows for PR2 and 17 camera tows for PR3.

2.2 Data acquisition, preparation and analysis and processing

This section provides an overview of the data acquisition, preparation and analysis and processing. Table 2 summarises the data acquired by the CEND2316 survey (Jenkins & Nelson 2017). For more detail, see Annex 2 and Annex 3.

Table 2. Data acquired during CEND2316 survey to Pisces Reef Complex MPA.

Data type		Data acquired
Multibeam Echosounder	Bathymetry data at 400kHz Backscatter data at 400kHz Bathymetry data at 200kHz Backscatter data at 200kHz	391 km across predicted reefs
Sub bottom profiler (chirper)	Profile data 11.2kHz	391 km across predicted reefs
Drop camera imagery	Video imagery	63 tows at stations across predicted reefs (21, 25 and 17 at PR1, PR2 and PR3 respectively)
	Still images	2082 images (within 63 drop camera tows)
Environmental parameters	ESM2 logger with salinity, temperature, depth, suspended solids and chlorophyll sensors	63 stations across predicted reefs (21, 25 and 17 at PR1, PR2 and PR3 respectively)
	Niskin bottles	12 stations (4 at each reef area)

2.2.1 Annex I Reef community analysis

Biological and environmental data were extracted from drop camera imagery, video and still images, following North-East Atlantic Marine Biological Analytical Quality Control Scheme (NMBAQC) guidelines (Turner *et al.* 2016). Benthic habitats were classified from all imagery using the JNCC Marine Habitat Classification for Britain and Ireland scheme (Connor *et al.* 2004; Parry 2015). In only the still imagery data, biological taxa were enumerated using a frequency of occurrence method following the recommendations of Moore *et al*. (2019). For more detail of the drop camera data extraction see Annex 3.

Benthic habitat distribution maps were created using data from the video imagery. More detailed biological community analysis was conducted using the stills imagery data. The nature of the reef at Pisces Reef Complex MPA as rock outcroppings in a mud basin means the separation of the reef areas from surrounding sediments is not straightforward and cannot be done using habitat classification of imagery alone. Attached epifauna normally associated with hard substrata are observed in images where substrata are classified as sediment, due to a thin overlaying sediment veneer. As a result, the presence of rock-associated taxa, referred here as ‘reef taxa’ was chosen as the more reliable indicator of the presence or potential presence of reef.

With ‘reef taxa’ in mind, a multivariate exploratory analysis was first carried out using the CLUSTER, SIMPROF, SIMPER, nMDS, DistLM and dbRDA routines in PRIMER v7 (Clarke & Gorley 2015) using the still imagery data, to identify images which showed the reef communities at Pisces Reef Complex MPA. All data were filtered based on image quality, with only good or excellent quality imagery data being selected for analyses. Only abiotic substratum data were square-root transformed before being normalised with all the other abiotic data. Biological data were not transformed but were truncated to improve the consistency of the data set. Mobile taxa such as fish, cephalopods and large crustacea were included in this exploratory analysis to better understand the seabed community as a whole.

Once the reef communities were identified in the exploratory analyses, the mobile taxa were removed from the data before performing a second round of analysis in which the reef communities were compared across the Pisces Reef Complex MPA. Still image data was aggregated into suitable sized sampling units for the statistical comparisons in this second analysis. For more detail of these community analyses see Annex 3.

2.2.2 Annex I Reef map

The habitat map used for site designation (JNCC 2012) was compared to data collected in 2016, to assess its accuracy. The 2012 site designation map included three categories: ‘Annex I Reef’, ‘Mud plains’ and ‘Depression’. The latter two categories of the 2012 map were combined as ‘Not Reef’. Whilst the 2016 still images were classified into ‘Reef’ and ‘Reef with sediment veneer’ both were deemed to correspond to ‘Annex I Reef’ for the purposes of mapping its extent. Consequently, any still image with any ‘Reef taxa’ present was classified as ‘Annex I Reef’ for the purposes of validation of the 2012 map and producing new maps of ‘Annex I Reef’ extent (see Annex 3). A confusion matrix, with associated accuracy measures, was produced for the 2012 map tallying the mapped ‘Annex I Reef’ and ‘Not Reef’ against the observed categories for each reef location (PR1, PR2 and PR3).

Habitat maps for the Pisces Reef Complex MPA were produced by analysing the acoustic data and drop camera imagery (for data validation) to revise the extent of Annex I Reefs based on the new data alone. Mapping was done using the above two categories. The three survey areas were mapped separately, using object-based image analysis (OBIA) implemented in eCognition Developer v9. The 2016 MBES data (bathymetry, backscatter and their derivatives) were first segmented into objects (non-overlapping sections of the image with homogeneous characteristics) (Blaschke *et al.* 2010). Values were then calculated for each object from the primary acoustic data layers and their derivatives, and the substrate type was predicted (classified) using a Random Forest model. The model was trained using supporting information from the drop camera imagery, in which the presence of rock-associated taxa, referred to in this report as ‘reef taxa’, were used to provide more reliable validation of the presence of reef across the site. Final habitat maps were based on the WGS84 datum. For more detail see Annex 2 and Annex 3.

2.2.3 Additional analyses

Environmental data was aggregated for each drop camera tow to provide supporting environmental parameter data for the community analyses. Tidal models were also constructed to better understand local current flows at Pisces Reef Complex MPA. For more detail of these additional analyses see Annex 2 and 3.

3 Results

Results relating to **extent and distribution** (and selected **supporting processes**) of the habitats are presented in Section 3.1, whilst those concerning to physical and biological **structure and function** of habitat and species features are provided in Section 3.2.

3.1 Benthic and environmental overview

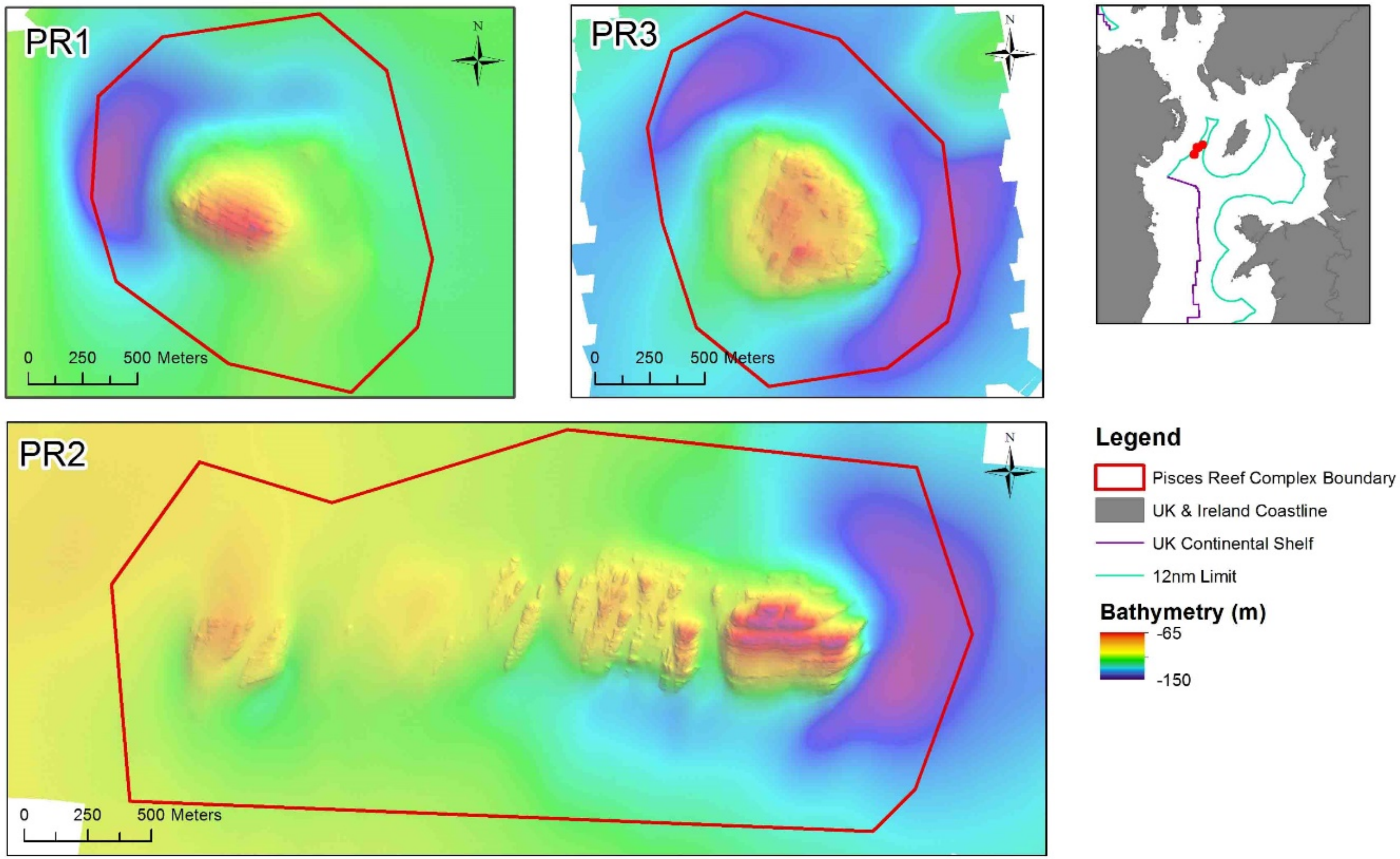
3.1.1 Bathymetry overview

The three reef areas of Pisces Reef Complex MPA, PR1, PR2 and PR3, occur across areas of elevated seabed relative to the surrounding area (Figure 4). Each of these elevated areas has a scour hollow adjacent to it, in which the depth is greater than the surrounding area. The shape and depth profile of each elevated seabed area varies from reef to reef across Pisces Reef Complex MPA. PR1 and PR3 are roughly circular reefs resembling elevated mounds, while PR2 runs along a roughly east to west orientation with more elevated areas to the far-west and far-east along that ridge (Figure 4).

Along its widest axis, the elevated area at PR1 is approximately 0.5 km across (running north-west to south-east orientation). The depth varies from approximately 82 m at the top of the elevation to approximately 143 m in the scour hollow to the south-west, west and north-western edges of the reef. Along this edge is also where the elevation has its steepest slopes.

At PR2, the elevated seabed that runs along a roughly east to west orientation is approximately 2.7 km long. The tops of the elevated areas along this ridge vary from east to west, being approximately 79 m in the far-west, shoaling to 74 m in the east-central portion of the ridge and rising to 66 m at the far-east area of the reef. This is the shallowest area of Pisces Reef Complex MPA. There are deeper ‘trough’ areas of 90 – 100 m interspersed between the elevated areas along the ridge of PR2. The largest of these ‘troughs’ occurs towards the west of the ridge, between the far-west and east-central elevations (Figure 4). The scour hollow at PR2 occurs along the eastern edge of this reef, which is also where the steepest slopes of the reef occur.

The elevated seabed at PR3 is approximately 1 km across from all orientations and is, therefore, almost circular (Figure 4). The most elevated area of the MPA is PR3, which is flatter in profile than PR1. The slopes are not as steep. This elevated area is slightly deeper than that at PR1, being 84 m depth. The scour hollow occurs to the eastern edge of the reef.



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Coordinate system WGS 1984 UTM Zone 29N. Not to be used for navigation. © JNCC/Cefas 2018

Figure 4. Bathymetry map of Pisces Reef Complex MPA (from CEND2316 survey (Jenkins & Nelson 2017)).

3.1.2 Tidal regime

According to the tidal model produced, the hydrodynamic regime surrounding the Pisces Reef Complex MPA varies across the three reef areas both spatially and temporally. The highest current flows are estimated to occur towards the north of the site, at PR1, where the model estimates that current velocities range between 0.00 $m.s^{-1}$ (during times of neap tides) and 0.30 $m.s^{-1}$ (during times of spring tides). Mean current velocities here range between 0.10-0.13 $m.s^{-1}$ (Figure 5). In the areas around PR2 and PR3, the model estimates fractionally lower current velocities, ranging between 0.01 $m.s^{-1}$ (during times of neap tides) and 0.22 $m.s^{-1}$ (during times of spring tides). The mean current velocities at PR2 and 3 are also lower than that at PR1, ranging between 0.07-0.11 $m.s^{-1}$ at PR2 (Figure 6) and between 0.06-0.09 $m.s^{-1}$ at PR3 (Figure 7).

The highest mean current flows are observed in the shallowest areas (65-90 m depth) at all three reefs and in localised areas emerging from the edges of scour features, the strongest effect was estimated along the north-eastern edge of the scour hollow at PR2 (Figure 5, Figure 6 and Figure 7).

The direction of the tide changes throughout the tidal cycle at Pisces Reef Complex MPA. There are two patterns to the tidal cycle. The first is a prolonged period in which the prevailing current flows NNE (approx. 340-355°), when the current flow is highest, followed by a sharp backing shift to a southerly flow (in an anticlockwise direction). There is then a gradual backing change to more easterly flows, ending up flowing NW (approx. 0-40°), when the current flow is at its lowest. In the second pattern, the prevailing current flow veers rapidly (in a clockwise direction) from flowing approximately NW round to NNE, increasing from the lowest to the highest velocity flow in the process (Figure 5, Figure 6 and Figure 7).

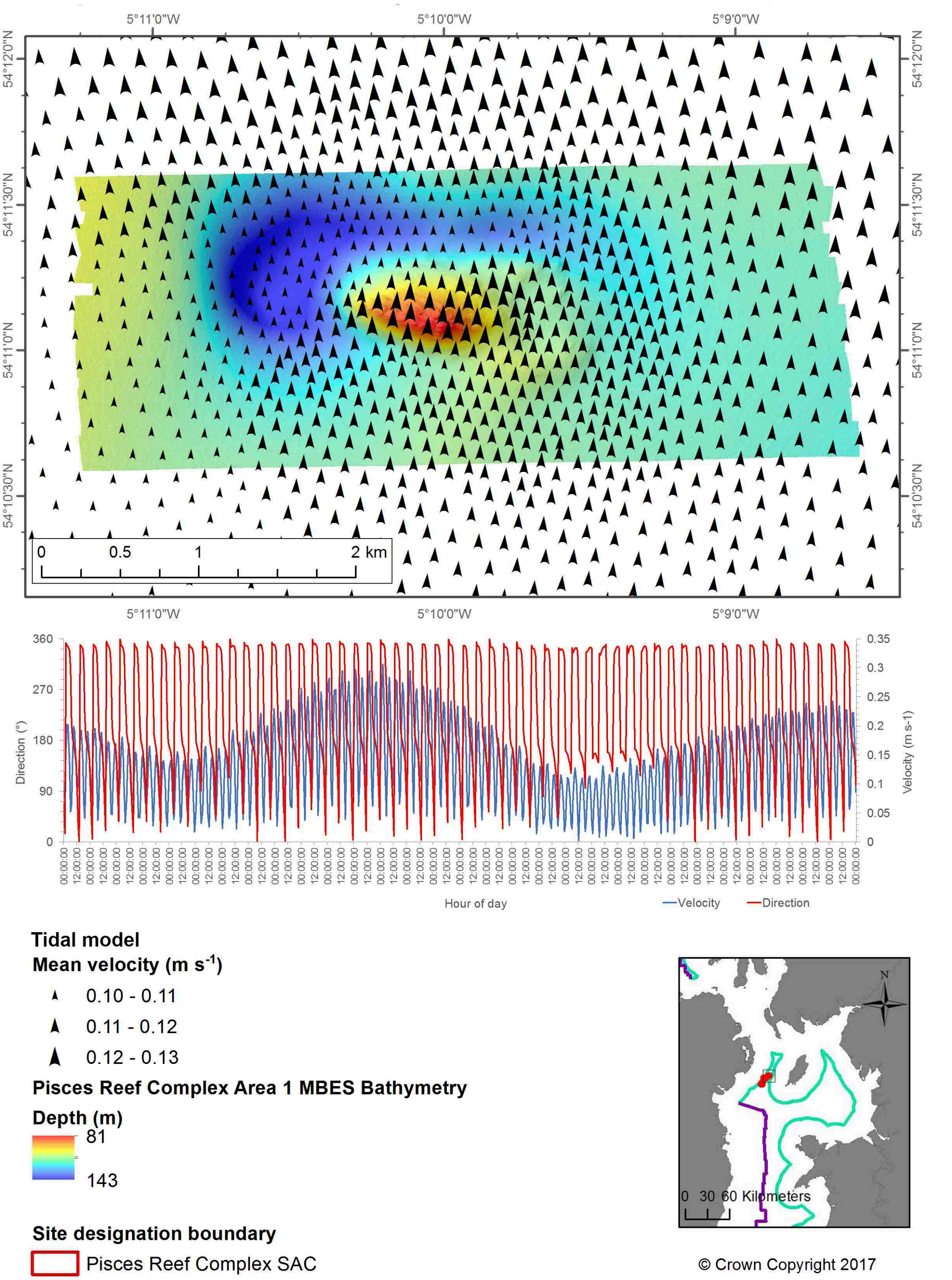


Figure 5. Tidal model output for PR1 showing the average direction and magnitude of current flows over two tidal cycles (two spring and two neap tides). These are averaged and plotted in the map. Figure inset shows the position of PR1 within the wider Irish Sea. Bathymetry from CEND2316 survey (Jenkins & Nelson 2017).

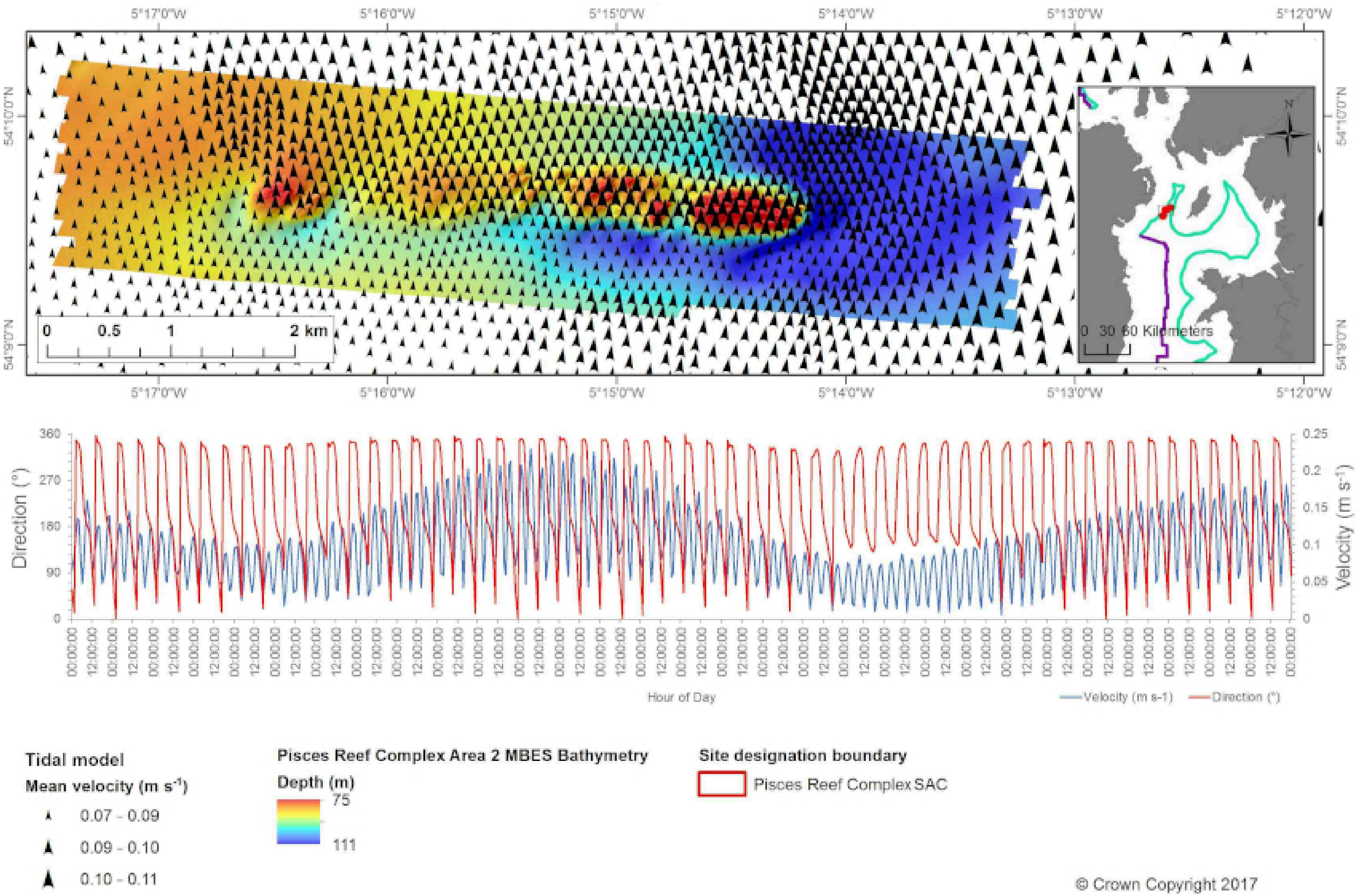


Figure 6. Tidal model output for PR2 showing the average direction and magnitude of current flows over two tidal cycles (two spring and two neap tides). These are averaged and plotted in the map. Figure inset shows the position of PR2 within the wider Irish Sea. Bathymetry from CEND2316 survey (Jenkins & Nelson 2017).

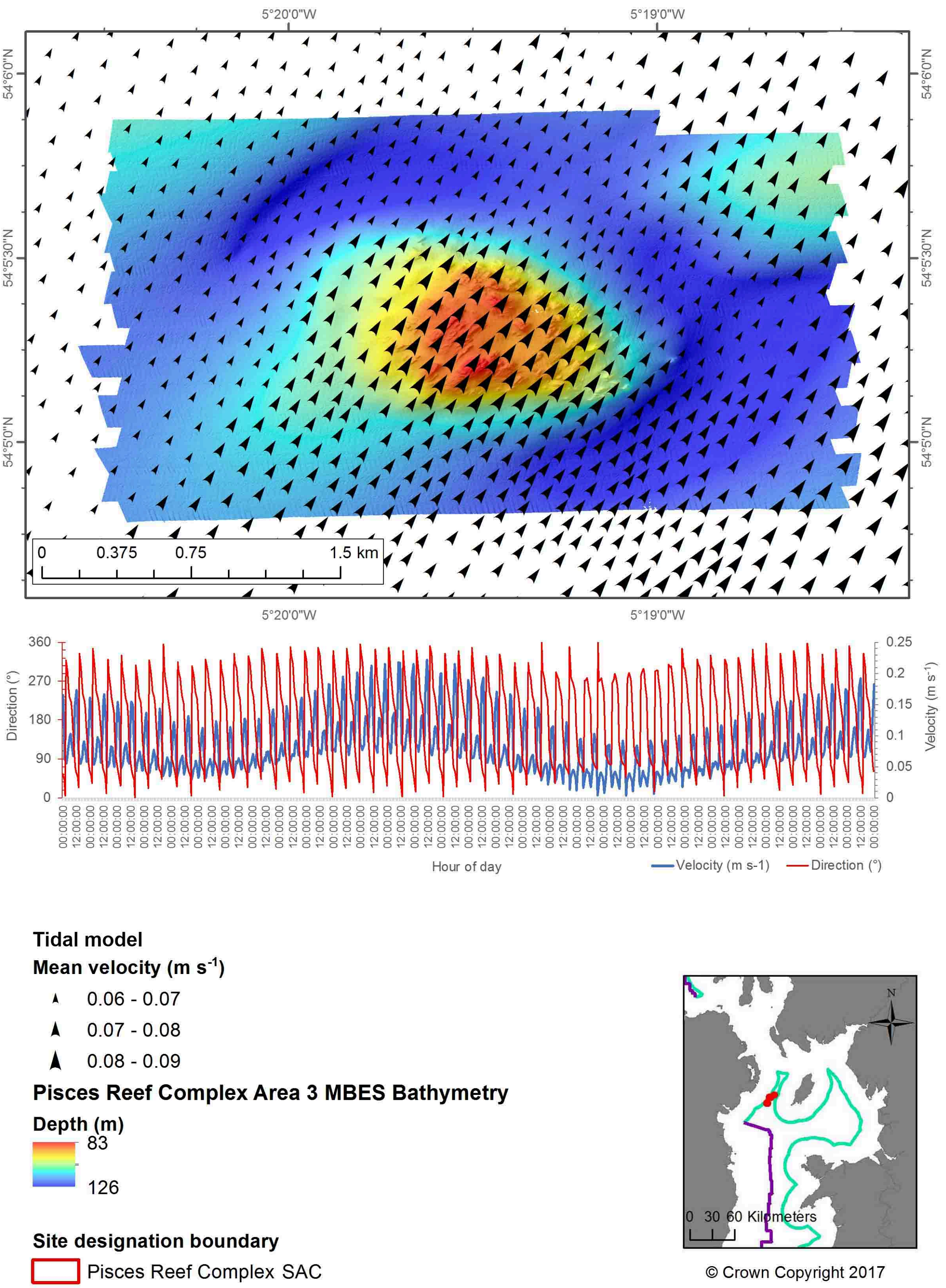


Figure 7. Tidal model output for PR3 showing the average direction and magnitude of current flows over two tidal cycles (two spring and two neap tides). These are averaged and plotted in the map. Figure inset shows the position of PR3 within the wider Irish Sea. Bathymetry from CEND2316 survey (Jenkins & Nelson 2017).

3.1.3 Environmental parameters (ESM2 logger)

The ESM2 logger recorded temperature, salinity, the concentration of oxygen, turbidity (suspended solids) and the concentration of chlorophyll A across the reefs at Pisces Reef Complex MPA. These parameters are summarised by reef area in Table 3 and by sampling station in Annex 6.

Table 3. Environmental parameters at each reef area of the Pisces Reef Complex MPA. Means and standard deviations of the means (SD) are calculated from values recorded by ESM2 logger on drop frame deployed at each sampling station.

Reef	**Temperature (°C)**		**Salinity (PSU)**		**O2 Concentration (%)**		**Turbidity (FTU)**		**Chlorophyll (µg/l)**	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
PR1 (21 stations)	13.68	0.65	32.05	7.80	256.20	19.76	76.57	292.59	0.31	0.04
PR2 (25 stations)	13.71	0.60	32.37	7.14	255.34	18.96	42.53	150.86	0.28	0.04
PR3 (17 stations)	13.49	0.73	32.05	7.73	258.84	19.47	26.02	82.84	0.29	0.05

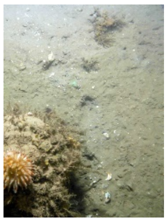
°C = degrees Celsius; PSU = Practical Salinity Unit; FTU = Formazin Turbidity Unit (determined from infrared light scattering).

The mean temperature, salinity and concentration of oxygen did not vary greatly across the three reefs (Table 3). However, the mean turbidity did vary across the three reefs. There appears to be an increase in turbidity moving from South to North across the sites, with the highest quantities present at PR1 (76.57 FTU; 292.59 SD), less at PR2 (42.53 FTU; 150.86 SD) and the lowest at PR3 (26.02 FTU; 82.84 SD). This indicates turbidity increases towards the North of the site. The concentration of chlorophyll A also varies across the three reefs but not by much, with the highest concentration recorded at PR1 (0.31 µg/l; 0.04 SD) and lower, but similar concentrations recorded at PR2 and PR3 (0.28 µg/l; 0.04 SD and 0.29 µg/l; 0.05 SD respectively).

3.1.4 Benthic habitat distribution

Video imagery captured an impression of the seabed surface across the three reefs at Pisces Reef Complex MPA (PR1, PR2 and PR3). Analysis of video imagery indicates that the seabed surface across the site is composed of a mosaic of benthic habitats. The two most dominant benthic habitats sampled across all three reefs were sublittoral mud (SS.SMu): occurring in 70%, 50% and 23% of video segments at PR1, PR2 and PR3 respectively, and sublittoral mixed sediment (SS.SMx): occurring in 28%, 33% and 72% of video segments at PR1, PR2 and PR3 respectively (Table 4). They are represented by three habitats: offshore circalittoral mud (SS.SMu.OMu), offshore circalitorral mixed sediment (SS.SMx.OMx) and circalittoral sandy mud (SS.SMu.CSaMu). Collectively, the sublittoral mud and sublittoral mixed sediment make up approximately 87% of the substratum sampled by the drop camera (video) at the site.

Table 4. Benthic habitats present at Pisces Reef Complex MPA. Video segments measure approx. 5 m in length. The percentage occurrence of each benthic habitat is calculated from the total video segments analysed across PR1, PR2, PR3 and across the site (as indicated).

Habitat (and code)	**Low energy circalittoral rock** (CR.LCR)	**Offshore circalittoral sand** (SS.SSa.OSa)	**Offshore circalittoral mud** (SS.SMu.Omu)	**Circalittoral sandy mud** (SS.SMu.CSaMu)	**Offshore circalittoral mixed sediment** (SS.SMx.OMx)
Example frame-grab					
Total video segments across site (out of 2118 segments)	142 (6.70%)	58 (2.74%)	1048 (49.48%)	85 (4.01%)	785 (37.06%)
Video segments at PR1 (out of 654 segments)	16 (2.45%)	0 (0.00%)	456 (69.72%)	42 (6.42%)	140 (21.41%)
Video segments at PR2 (out of 943 segments)	100 (10.60%)	55 (5.83%)	474 (50.27%)	9 (0.95%)	305 (32.34%)
Video segments at PR3 (out of 521 segments)	26 (4.99%)	3 (0.58%)	118 (22.65%)	34 (6.53%)	340 (65.26%)

The low energy circalittoral rock (CR.LCR), that forms the Annex I Reef at Pisces Reef Complex MPA, occurs in 2%, 11% and 5% of the video segments across PR1, PR2 and PR3 respectively (Table 4). Altogether, the low energy circalittoral rock occurs in 7% of the substratum sampled by the drop camera (video) at the site. Its spatial distribution indicates that it is mostly present on the pinnacles of subterranean rock across the site, that pierce through the surface of sediments, composed of a mosaic of sublittoral mud, sand and mixed sediment (Figure 8).

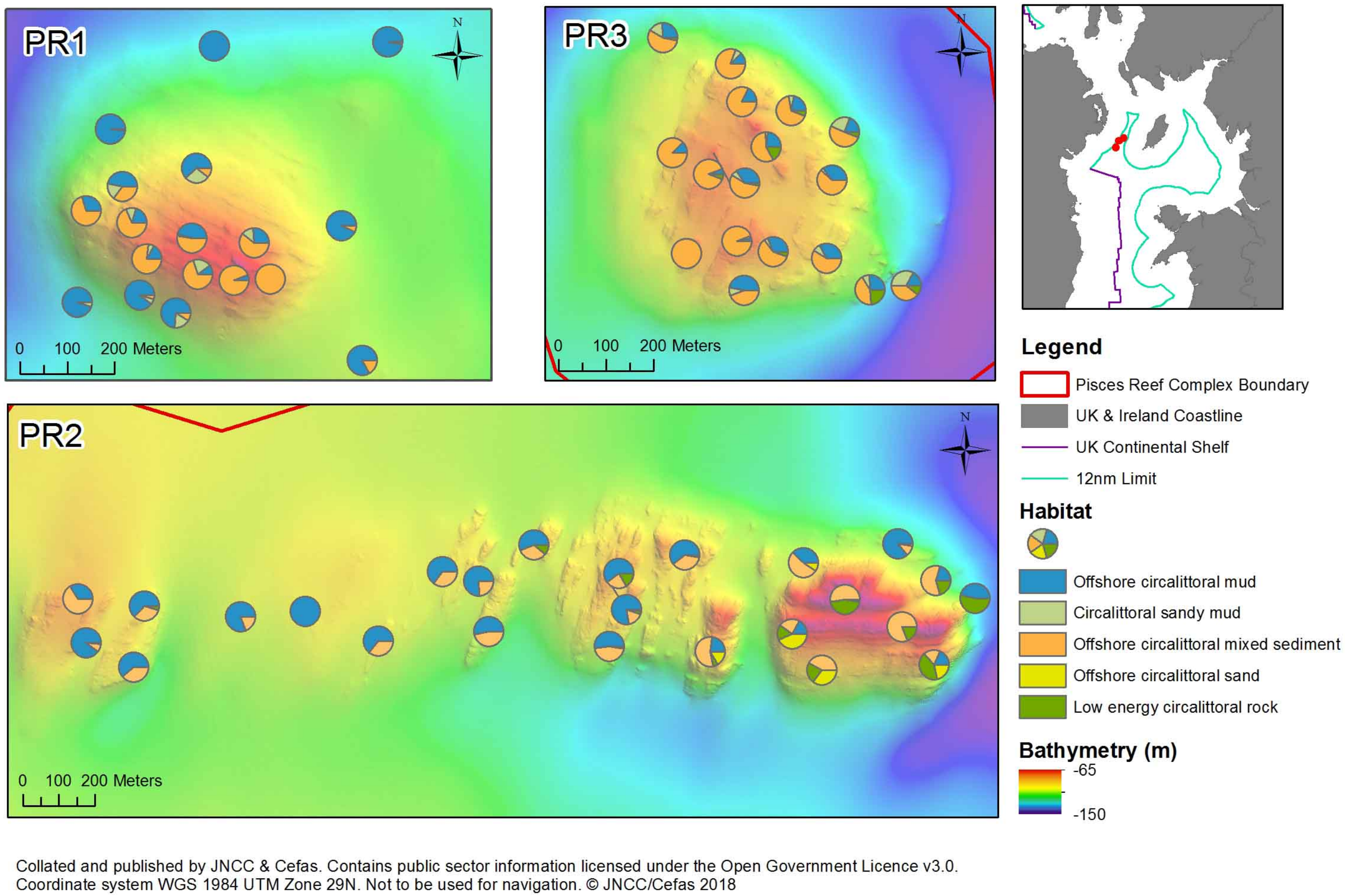


Figure 8. Map of benthic habitat composition relative to the Annex I Reef feature at Pisces Reef Complex MPA. Figure inset show position of Pisces Reef Complex MPA within the Irish Sea. Mapped extent matches extent of benthic habitat data. Figure inset shows the position of the MPA within the wider Irish Sea. Bathymetry from CEND2316 survey (Jenkins & Nelson 2017).

3.2 Annex I Reefs

3.2.1 Extent and distribution

The 2012 site designation 'Annex I Reef' map was produced based on multiple sources of acoustic and ground truth data available at the time. The reef features were delineated based on the difference observed in MBES backscatter intensity between the reef and surrounding mud, as well as the topographic prominence of the reef features. Validation with the 2016 imagery, however, shows the 2012 map overestimates reef extent (Table 5). Whilst the high Sensitivity suggests almost all observed presences of confirmed and potential 'Annex I Reef' are contained within the reef polygons, the low Specificity values indicate an inability to correctly represent the absence of reef, where the reef polygons also encompass most of the observations of sediment habitats. Consequently, the overall accuracy of the map as measured by the total proportion of observations correctly classified (PCC), the Positive Predictive Value (PPV) and the True Skill Statistic (TSS) is lowered (Table 5). The PCC conveys the general accuracy regardless of any bias towards over- or underestimation, whereas the PPV represents the probability a presence prediction is true, given the prevalence of presences in the data and the TSS is a balanced accuracy measure based on the ability to correctly predict both presences and absences.

Table 5. Validation results for the 2012 site designation map and the 2021 map produced here. N = number of observation in validation dataset (for 2012 map all observations were used, for the new map 20% of observations were kept back for validation).

Annex 1 map	Reef	N	Prev.	PCC	Sensitivity	Specificity	PPV	TSS
2012 map	**PR1**	269	0.62	0.69	0.99	0.2	0.67	0.19
	PR2	687	0.56	0.64	0.96	0.23	0.61	0.18
	PR3	339	0.8	0.79	0.97	0.06	0.8	0.03
	All	**1295**	**0.64**	**0.69**	**0.97**	**0.19**	**0.68**	**0.17**
2021 map	**PR1**	43	0.6	0.84	0.85	0.82	0.88	0.67
	PR2	117	0.58	0.81	0.81	0.82	0.86	0.63
	PR3	44	0.8	0.68	0.69	0.67	0.89	0.35
	All	**204**	**0.63**	**0.79**	**0.78**	**0.8**	**0.87**	**0.58**

Prev. = prevalence of 'Annex I Reef' observations, including potential reef with sediment veneer,
PCC = proportion correctly classified, PPV = Positive Predictive Value, TSS = True Skill Statistic.

A new Annex I Reef map of Pisces Reef Complex MPA was created from OBIA, using the acoustics data from this survey, and a Random Forest model, trained on imagery from this survey containing reef taxa (Figure 9; See Annex 3 for details of map creation). This greater detail of this 'updated' map relative to the previous map for the site (see Figure 3) is based on the availability of additional data provided by the CEND2316 survey. Validation results show improved overall map accuracy over the 2012 map (Table 5), including higher PPV and TSS scores. The general improvement does come at a cost on the Sensitivity scores, particularly for PR3. The lower Sensitivity, where presences of confirmed or potential 'Annex I Reef' have been incorrectly classified, is not unexpected given the wide representation of potential reef using all images with any 'Reef taxa', including those present on shells in otherwise burrowed sediment habitats. In these circumstances drawing the boundary between the potential reef consisting of burrowed mud, and mud that is not considered reef is challenging, and the lower Sensitivity does not indicate a significant underestimation in reef extent. The updated Annex I reef map, from 2021, shows the majority of reef features at Pisces Reef Complex MPA are distributed across the steepest slopes of the site (Figure 9). These occur in depths ranging between 66 and 100 m. At all reef areas, PR1, PR2 and PR3, the distribution of the reef feature is patchy with few large, continuous areas. In total, the extent of reef feature at all three reef areas is 0.35 km².

PR1 has the smallest total extent of reef in the Pisces Reef Complex MPA, 0.08 km², however, this is mostly made up of one continuous 'patch' of reef measuring 0.07 km² (Table 6). The reef feature at PR1 is mostly distributed across the more elevated, central area of the reef (approx. 82 to 90 m depth), running down the steep slopes along the southern, south-western and western edges of the central area to approx. 110 m depth (Figure 9). There are other patches of reef feature at PR1 but they are few, isolated and small, occurring in deeper water (approx. 100 to 120 m depth) towards the north, north-east and south-east of the elevated seabed feature.

Table 6. Summary reef extent and distribution for Pisces Reef Complex MPA. Reef 'patches' are areas of discrete reef habitat.

Reef	Total area of Annex I Reef feature		No. discrete reef 'patches'	Mean area of reef 'patches'	Largest continuous reef 'patch'
	km²	m²		m²	m²
PR1	0.08	83133	64	1299	69342
PR2	0.27	269046	291	925	145879
PR3	0.19	189214	73	2592	28015
PR Total	**0.54**	**541392**	**428**	**1605**	

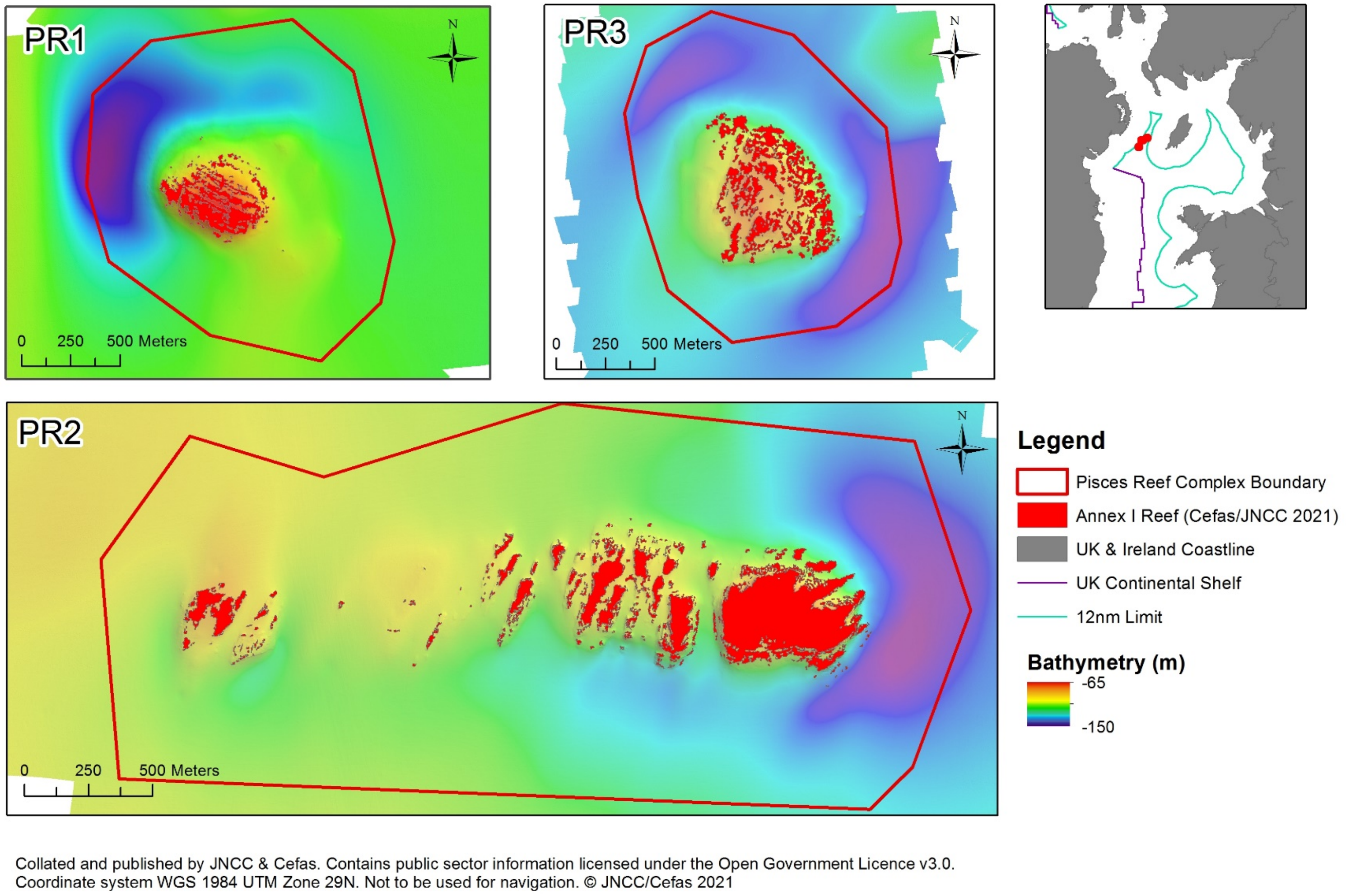


Figure 9. Distribution of Annex I Reef feature at Pisces Reef Complex MPA. Figure inset shows the position of the MPA within the wider Irish Sea. Bathymetry from CEND2316 survey (Jenkins & Nelson 2017).

PR2 has the largest extent of reef, 0.27 km², and the largest patch of continuous reef, 0.16 km², in the Pisces Reef Complex MPA. However, in general the reef patches at PR2 are the smallest and most numerous of all the reef areas in the Pisces Reef Complex MPA (Table 6). The reef feature is distributed along the ridge at PR2, occurring mostly on the steepest slopes and the shallowest elevations, at a depth of 66 m, in the far-east of the ridge (Figure 9). This far-eastern reef aggregation has the largest areas of continuous reef feature in the Pisces Reef Complex MPA and is also adjacent to the deepest scour hollow of the site.
The extent of the reef feature at PR3 is 0.19 km² (Table 6). The reef feature is widely distributed across the elevation at PR3 with the largest reef patches on average across the Pisces Reef Complex MPA (Table 6, Figure 9). Although there is a portion of the reef feature distributed along the edge of the scour hollow at PR3, the overall distribution at the site is more evenly spread than other reef areas at the site (Figure 9).

3.2.2 Annex I Reef associated biological communities

The structural and surface complexity of the substratum at Pisces Reef Complex MPA supports numerous biological communities. Multivariate cluster analysis and a SIMPROF test of the community data, derived from the stills imagery, enabled characterisation of 50 distinct cluster groups. The resulting cluster dendrogram accounts for 87% of the relationships between the data in the still images in the underlying Bray-Curtis similarity matrix (Cophenetic correlation coefficient 87%, P=0.05, N=1069, permutations=999). Each cluster group was comprised of an assemblage of taxa that shared similar occurrences in the sample images. Of the 50 cluster groups, the 26 largest, with more than 6 sample images in each group (median value for all cluster groups), are widely distributed with representation at all three reefs of the site. These may be considered as the most characteristic communities, or dominant sub-communities, of the Pisces Reef Complex MPA. A simplified nMDS plot, in which the sample images have been aggregated within each cluster group, clearly shows the similarities and dissimilarities between the 26 dominant cluster groups (Figure 10).

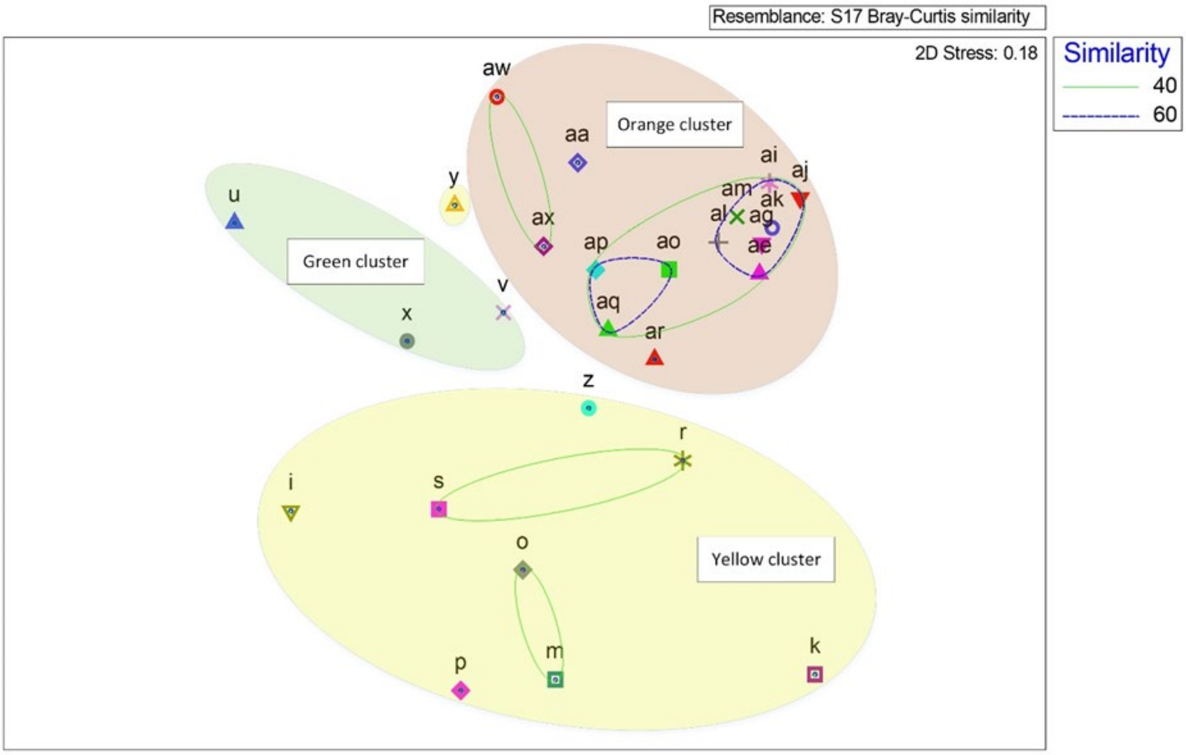


Figure 10. Non-metric, multi-dimensional scaling plot showing the similarity between the 26 dominant cluster groups identified by SIMPROF analysis (PRIMER 7). Data were ordered in a Bray-Curtis Similarity matrix. Data were also averaged for each cluster group to aid display. Similarities shown were calculated by CLUSTER analysis (PRIMER 7).

Three larger groups are visible among the 26 cluster groups based on nMDS, CLUSTER and SIMPROF analysis results. The largest of these, the ‘orange cluster’, includes 14 groups (aw, aa, ax, ap, aq, ao, ar, al, am, ai, aj, ak, ag, and ae) and some of the highest inter-group similarities within the biological data, where the sub communities within groups are over 60% similar to one another (Figure 10). When added together 765 sample images fall into this largest of the cluster groups. The second largest cluster, the ‘yellow cluster’, includes 9 groups (z, i, r, y, o, s, k, m and p) made from biological data from 202 still images in total and has lower levels of inter-group similarities than the orange cluster. The smallest cluster and lowest levels of inter-group similarity occur in the ‘green cluster’, which includes just 3 groups (x, v and u) and has 52 still images.

A closer look at the dominant taxa in the 14 cluster groups from the orange cluster indicates these sub-communities are composed mostly of sessile epifauna such as *Caryophyllia*, Hydrozoa and various growth forms of Porifera (Table 7). These sub-communities are also dominated by mixed faunal turf taxa with some mixed faunal crust taxa in places and occasional occurrences of mobile Galatheoidea. All of these taxa are usually associated with reef habitats containing rock, boulders and cobble necessary for attachment and sheltering purposes.

The abiotic substratum data generally supports the assumption that biological communities from the orange cluster are ‘reef-like’ as they tend to have relatively high proportions of boulder and cobble compositions. However, as the boulder and cobble compositions range from 0.1% (SD ±2.9) to 42.4% (SD ±0.0) within this data cluster, additional data exploration is required to better understand the structure of these communities. By arranging the mean proportion of boulder and cobble in each sub-community in order of highest to lowest composition, a continuum is created into which the sub-communities from all the clusters, including yellow and green, can be better organised and subsequently, better understood

(Figure 11). This information and the results of the SIMPER analysis enabled the sub-communities to be organised into new groupings, ‘functional communities’, intended to be indicative of their role and community function at Pisces Reef (Figure 11; Table 7).

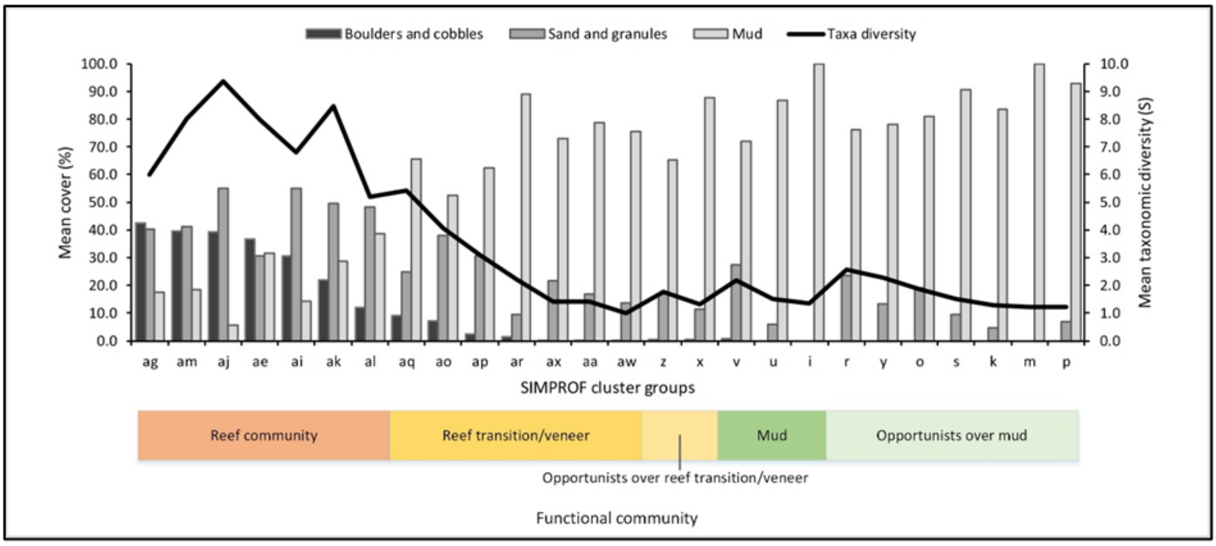


Figure 11. Relationships between taxonomic diversity and proportions of abiotic substrata across dominant SIMPROF cluster groups. Functional communities are displayed beneath the graph to indicate which larger community the cluster groups most likely fit into.

Table 7. Summary of the contributing taxa and environmental parameters of dominant cluster groups identified by SIMPROF analysis. Group similarity values for taxa are from SIMPER analysis of dominant cluster groups (untransformed data). Environmental parameter results of each cluster group were calculated from raw, untransformed data. Boulder and cobble data, and sand and gravel data were combined for added clarity. Cluster groups are grouped into functional communities, as shown, using the proportion of boulders and cobbles as a guide. Standard deviation (SD) is indicated in brackets after the means.

Functional community	Cluster Group	Sample images (N)	Cluster group community parameters (SIMPER output)			Cluster group environmental parameter means (±SD)				
			Common taxa (explain 90% group similarity)	Group similarity (%)	Mean S (±SD)	Boulders and Cobbles (%)	Sand and gravels (%)	Mud (%)	Slope	Depth (m)
Reef community	ag	19	Turf/Caryophyllia/Crusts/Hydrozoa	71.4	6 (0.5)	42.4 (0)	40.2 (12)	17.3 (26.5)	9.5 (7.5)	73.4 (10.3)
	am	32	Turf/Crusts/Hydrozoa	64.3	8 (3)	39.6 (34.9)	41.1 (36.3)	18.5 (15.9)	9.7 (4)	87.7 (5.5)
	aj	31	Turf/Hydrozoa/*Caryophyllia*/Pap.Porifera	76.0	9.4 (2)	39.3 (9.9)	55.1 (24.8)	5.6 (26.1)	6.9 (4)	68.9 (9)
	ae	6	Turf/Galatheoidea/Hydrozoa	65.0	8 (0.4)	36.6 (0)	30.5 (13.7)	31.6 (13.9)	10.5 (5.8)	91.7 (9.6)
	ai	24	Turf/Hydrozoa/*Caryophyllia*	76.5	6.8 (1.1)	30.7 (2.3)	54.9 (28.9)	14.3 (34.4)	6.8 (4.4)	68.6 (9.1)
	ak	49	Turf/Hydrozoa/Porifera.Gen	70.3	8.5 (2)	22.1 (17.8)	49.7 (30.1)	28.8 (30.3)	9.1 (3.8)	88.4 (9.9)
	al	147	Turf/Hydrozoa	69.2	5.2 (1.9)	12 (21.1)	48.2 (23.4)	38.7 (24)	6.8 (5.8)	87.7 (7.1)
Reef transition/ veneer	aq	30	Turf/Crusts/Hydrozoa/Peracarida	47.6	5.4 (2.5)	9.2 (28.1)	25 (33.2)	65.5 (23.7)	8.1 (3.5)	86.2 (5)
	ao	221	Turf/Hydrozoa	61.0	4.1 (1.3)	7.3 (0)	37.8 (16.7)	52.5 (16.5)	7 (1.4)	86.7 (3.9)
	ap	75	Turf/Hydrozoa	62.0	3.1 (0.5)	2.5 (0)	30.5 (0)	62.4 (0)	8.3 (3)	89.1 (9.7)
	ar	10	Galatheoidea/Turf	49.6	2.2 (0.4)	1.4 (0)	9.6 (0)	89 (0)	11.1 (6.2)	100 (7.8)
	ax	79	Turf	73.6	1.4 (0.5)	0.2 (0)	21.8 (15.5)	73 (15.5)	7.6 (3.9)	91.2 (3.7)
	aa	12	Hydrozoa	71.3	1.4 (1.5)	0.1 (4.4)	16.8 (27.6)	78.7 (29.1)	7.7 (5.1)	90.6 (10.4)
	aw	30	Turf	100.0	1 (0.5)	0.1 (2.9)	13.7 (15.9)	75.5 (16)	6.8 (5.3)	90.6 (11.8)
Opportunists over reef transition/ veneer	z	105	Demersal bony fish	49.2	1.8 (2.2)	0.6 (23.5)	17.2 (34.2)	65.2 (26.8)	6.3 (6.2)	87.5 (11)
	x	38	Peracarida	67.5	1.3 (1.2)	0.5 (3.6)	11.3 (15.4)	87.6 (17.1)	6.8 (5.3)	98.7 (12.3)
Mud	v	6	*Pachycerianthus multiplicatus*	60.4	2.2 (0.7)	0.8 (0.8)	27.3 (25.4)	71.9 (28.9)	6.8 (4.4)	94.5 (11.3)
	u	8	Ceriantharia	42.4	1.5 (2.2)	0 (7.4)	5.9 (11.6)	86.9 (13.3)	8.3 (4.3)	92.2 (5.3)
	i	14	*Nephrops norvegicus*	66.4	1.4 (1.2)	0 (16.6)	0 (25)	100 (20.1)	2.3 (0)	93.1 (6.8)
Opportunists over mud	r	7	Peracarida/Demersal Bony fish	66.4	2.6 (1)	0 (1.9)	23.7 (13.4)	76.3 (13.8)	7.3 (5.1)	88.1 (12.7)
	y	15	Pelagic bony fish	55.8	2.3 (0.5)	0 (0)	13.2 (12.5)	78 (23.3)	8.5 (5.4)	92 (2.1)

Functional community	Cluster Group	Sample images (N)	Cluster group community parameters (SIMPER output)			Cluster group environmental parameter means (±SD)				
			Common taxa (explain 90% group similarity)	Group similarity (%)	Mean S (±SD)	Boulders and Cobbles (%)	Sand and gravels (%)	Mud (%)	Slope	Depth (m)
	o	7	Decapoda	75.8	1.9 (0.5)	0 (0.5)	19 (17.6)	81 (17)	10.1 (4.5)	89.8 (12.4)
	s	14	Peracarida	76.0	1.5 (0)	0 (0.4)	9.4 (23)	90.6 (31.1)	6.4 (6.4)	84.6 (11.6)
	k	7	Scyliorhinidae	65.8	1.3 (1.1)	0 (0)	4.7 (37.8)	83.5 (37.8)	4.4 (6.5)	79.5 (8)
	m	9	Decapoda	87.0	1.2 (0.8)	0 (0)	0 (37.1)	100 (37.1)	6.2 (5.2)	89.4 (5.8)
	p	24	Peracarida	76.9	1.2 (3)	0 (22.1)	6.9 (31.8)	92.8 (28.2)	6.1 (4.8)	88.7 (12.9)

*Pap.Porifera = Porifera Papilate cryptic; Porifera.Gen = Porifera Sp. generic

3.2.3 Structure and function of Annex I reef-associated biological communities

The first functional community at Pisces Reef, ‘Reef community,’ comes from the orange cluster of sub-communities identified in the CLUSTER and SIMPROF analyses. All of its seven sub-communities meet the standard for Annex I Reef (Table 7). This functional community has the highest taxonomic diversity in the biological data and all sub-communities therein share similar sessile epifauna, such as *Caryophyllia*, Hydrozoa, various growth forms of Porifera and mixed encrusting and turf taxa (note this diversity is not true taxonomic diversity as it was not possible to identify all taxa in the imagery down to species level). These communities occur in the shallowest areas and the steepest slope angles sampled at Pisces Reef (Table 6).

The second functional community, ‘Reef transition/veneer’, also comes from the orange cluster of sub-communities, and meets the standard for Annex I Reef (Table 7). This functional community occurs in deeper areas with smaller slope angles relative to the Reef community areas, as well as having a higher composition of sediments, especially mud, throughout. It represents a transitional state between the reef areas and surrounding mud areas. The seven sub-communities in this functional community support mobile Peracarida (made up mostly of amphipods at Pisces Reef) and Galatheoidea, often present in mixed sedimentary habitats. It is interesting, however, that the seven sub-communities are actually dominated by mixed animal turfs and hydroids, more indicative of reef habitats. The occurrence of these sessile epifauna leads to the possibility that this functional community may occupy areas of reef that are periodically covered by a thin veneer of sediments.

Although the sub-bottom profiler used in this study was unable to provide the data resolution necessary to identify the expected resolution of sediment veneers across Pisces Reef (See 3.1.4; Objective 4), it is possible that this second functional community, ‘Reef transition/veneer’, identified by analysis of biological data from the imagery, may indeed represent reef communities that are affected by sediment veneers. In this instance, the presence of reef taxa in the stills imagery has served as a proxy indicator of underlying hard substrata that occurs beneath a sediment veneer. Assuming this to be correct, the effect of sediment veneers on the benthic community is to lower the taxonomic diversity and change the overall community structure relative to the Reef community.

Other non-reef communities are not the focus of this study. They will only be described briefly here to provide context for the general surrounding area in which the reef communities occur. All other biological communities in the dataset arise from the yellow and green cluster groups of the CLUSTER and SIMPROF analysis. Viewing these along the hard-substratum continuum shows these are predominantly mud habitats (Figure 11). The dominant taxa identified in the twelve sub-communities in these cluster groups support this notion, being largely composed of mud-dwelling anemones, such as *Pachycerianthus multiplicatus* and Ceriantharia, as well as mobile taxa strongly associated with mud habitats, such Pericarida and *Nephrops norvegicus* (Table 7). There are also many other motile taxa in these sub-communities, including ‘Demersal bony fish’, ‘Pelagic bony fish’, ‘Scyliorhinidae’ and ‘Decapod’ crabs. These may be considered as predatory opportunists that patrol the reef and surrounding mud areas for food, shelter and mates.

To improve understanding of these latter sub-communities and their relevance to the Reef and Reef transition/veneer community they may be divided into three useful functional communities. The first is the ‘Mud’ functional community composed entirely of mud dwelling benthic taxa of three sub-communities (Table 7; Figure 11). The second and third functional communities are composed of entirely motile taxa, opportunists that move around to feed over the reef and mud areas. These may be divided by whether they were observed in still images containing hard substrata or not. If hard substrata was present they may be considered as 'Opportunists over reef transition/veneer' and where absent as 'Opportunists over mud'. In reality, these motile taxa could potentially move anywhere across the Pisces Reef area and so this proposed association with these benthic habitats is just to better understand what taxa may occupy and feed in which areas. These taxa should not, however, be used for any robust statistical analysis and change detection over time as the sampling tools are not intended to monitor these taxa reliably.

3.2.4 Supporting processes of Annex I Reef-associated biological communities

A distance-based linear model (DistLM) of the environmental variables from the 26 sub-community groups (orange, green and yellow clusters from the biological data) explained 83.47% of the models fitted variation within two axes; dbRDA1 (66.56%) and dbRDA (16.91%). A Distance-based redundancy (dbRDA) plot illustrates the environmental variables and supporting processes that drive the structure of the biological communities at Pisces Reef (Figure 12). When focusing on the Reef community, grouped in orange colour, the clearest positive driver seems to be the boulder and cobble composition, and the slope to a small extent, while the clearest negative driver is the mud composition.

The plot seems to show a transition from the Reef community to the Reef transition/veneer community, grouped in peach colour, with increases in sand and gravels, pebbles, bathymetry and shells (Figure 12). Further increases in mud content and reductions in boulder and cobble composition, and the slope angle, result in the functional communities dominated by mud with many opportunistic taxa. Much like Figure 11, again a rough continuum is apparent in the data, this time showing a shift from reef- to mud-dominated communities driven by decreases in boulder and cobble, moderate increases in sand and gravels and large increases in mud composition in each still image (Figure 12).

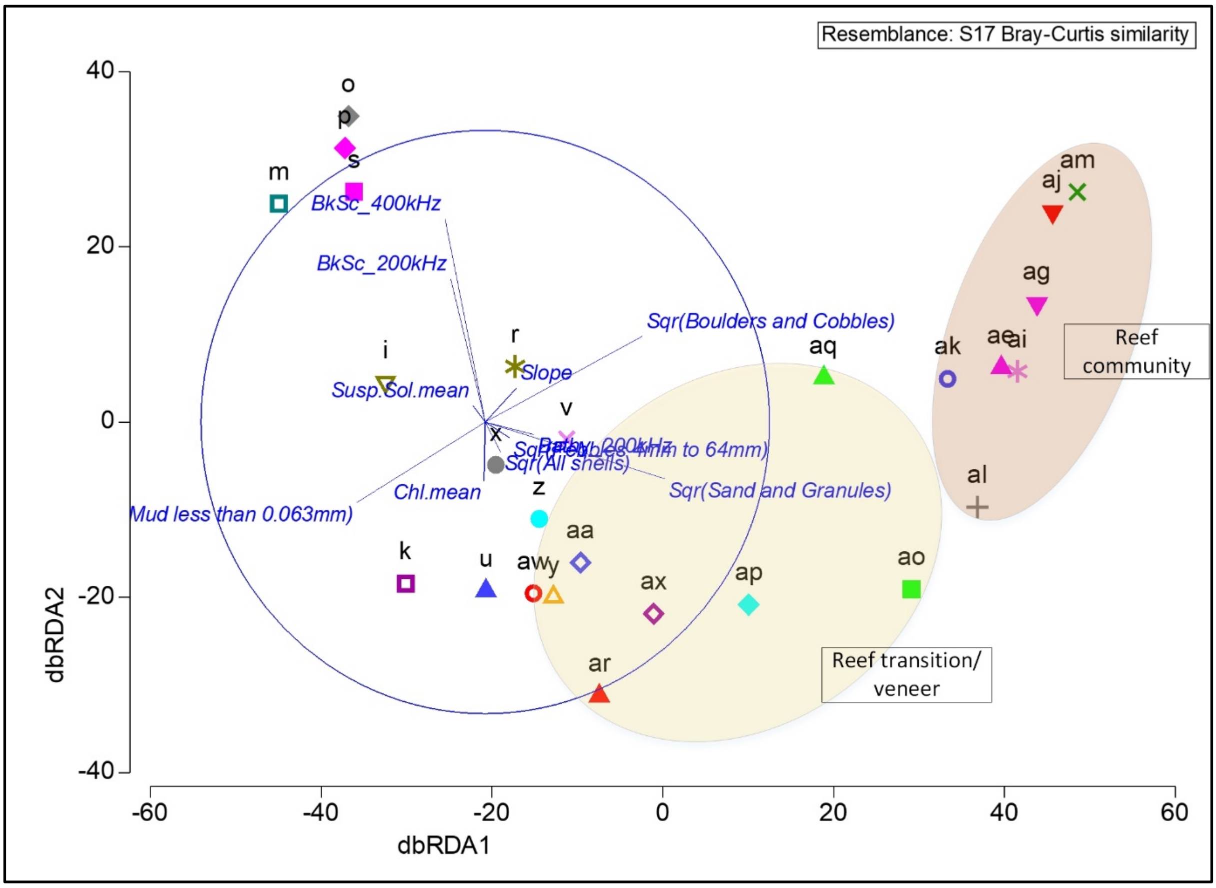


Figure 12. Distance-based redundancy (dbRDA) plot using environmental variables from the 26 dominant cluster groups identified by SIMPROF analysis (PRIMER 7). Substratum data were square root transformed (as indicated) and all abiotic data were normalised by variable, before ordering in a Euclidean distance matrix. Data were also averaged for each cluster group to aid display and improve clarity of analysis. Relevant functional communities have been circled to highlight their position in the plot. Sqr = Square root transformed variable; BkSc = backscatter; Chl. = Chlorophyll; Bathy = bathymetry; Susp.Sol. = suspended solids.

Together, all environmental variables identified by SIMPROF analysis accounted for 24% of the macrofaunal assemblage variability (Table 8). A DistLM step-wise test then identified the key drivers of macrofauna assemblage composition. The most parsimonious model deemed percentage cover of sand and gravels; boulders and cobbles; and mud useful indicators to explain macrofaunal variation (Table 8). Together, those three environmental variables explained 20% of macrofaunal assemblage variability (Table 8).

A large proportion of macrofaunal assemblage variation was not explained by the environmental drivers investigated in this work. This may indicate that additional environmental drivers are also important in shaping biotic communities.

Table 8. DISTLM analysis results using Step-wise procedure conducted on environmental variables from dominant cluster groups identified by SIMPROF analysis. Bold results indicate a significant effect (P ≤ 0.05). The environmental variables are normalised and square root transformed, where indicated, before the test. The biological data were not transformed and ordered in a Bray-Curtis Similarity matrix. The 'proportion of variation' explains the amount of variability in the Bray-Curtis Similarity matrix that each environmental variable accounts for.

Environmental variable	Sums of squares (trace)	Pseudo-F	P value	Proportion of variation	Cumulative proportion of explained variability	Residual degrees of freedom
Sqr(Sand and gravels)	307610.00	114.61	**0.001**	0.101	0.101	1017
Add - Sqr(Boulders and Cobbles)	198050.00	79.49	**0.001**	0.065	0.166	1016
Add - Sqr(Mud less than 0.063mm)	100890.00	42.13	**0.001**	0.033	0.200	1015
Add - BkSc_200kHz	42803.00	18.18	**0.001**	0.014	0.214	1014
Add - Chl.mean	23214.00	9.95	**0.001**	0.008	0.221	1013
Add - Bathy_200kHz	17138.00	7.39	**0.001**	0.006	0.227	1012
Add - Sqr(All shells)	15623.00	6.77	**0.001**	0.005	0.232	1011
Add - Slope	8298.80	3.61	**0.001**	0.003	0.235	1010
Add - Susp.Sol.mean	7460.60	3.25	**0.003**	0.002	0.237	1009
Add - BkSc_400kHz	7018.30	3.06	**0.002**	0.002	0.240	1008
Add - Sqr(Pebbles 4mm to 64mm)	3284.40	1.43	0.169	0.001	0.241	1007

Sqr = Square root transformed variable; BkSc = backscatter; Chl. = Chlorophyll; Bathy = bathymetry; Susp.Sol. = suspended solids.

3.2.5 Comparison of functional communities at Pisces Reef

The results of the exploratory analyses identified two types of reef communities at Pisces Reef, the 'Reef community', which occurs in areas of >10% hard substrata and harbours diverse communities of sessile suspension feeders, including *Caryophyllia*, Hydrozoa, various growth forms of Porifera and mixed encrusting and turf taxa. The other main community identified in the exploratory analyses also has some sessile suspension feeders but occurs in areas with little visible hard substrata (<10% per still image). This community, the 'Reef transition/veneer' community, likely occurs in areas with hard substrata just beneath a thin layer, or veneer, of sediment, which allows the attachment of hydroids and mixed encrusting and turf taxa. However, further evidence collected from these areas is needed to accurately test this assumption.

In order to compare these communities, the still imagery data representing these communities was aggregated into appropriately sized sampling units based on meaningful areas of seabed with sufficient taxonomic representation and diversity. Sampling units were made up of aggregates of data from 12 still images (see Annex 3 for more detail on process).

Although the Reef and Reef transition/veneer communities have similarities, PERMANOVA testing of their epifaunal assemblages shows that they are different to each other (Table 9). Pairwise tests reveal these differences are consistent and statistically significant across all three reefs at Pisces Reef: PR1 (P = 0.0041, permutations (perms) = 363, degrees of freedom (df) = 12), PR2 (P = 0.0001, perms = 9929, df = 31) and PR3 (P = 0.0001, perms = 8315, df = 20).

Table 9. PERMANOVA test results for a two-factor PERMANOVA model, using the fixed factors Reef and 'Functional community', with 9999 unrestricted permutations. Tests were conducted on square root-transformed data of epibenthic taxa from Pisces Reef MPA. Bold results indicate a significant effect (P ≤ 0.05). The components of variation (Comp. var.) indicate the multivariate variability of data between the fixed factors, as indicated, and between replicate sample images (Residual).

Factor	df	SS	MS	Pseudo-F	P	Unique perms	Comp. var.
Reef	2	5661.3	2830.6	7.050	**0.0001**	9890	12.1
Functional community	1	8000.2	8000.2	19.924	**0.0001**	9922	18.5
Reef x Functional community	2	1237	618.5	1.540	**0.0472**	9917	5.1
Residual	63	25296	401.5				20.0
Total	68	46435					

Df = degrees of freedom; SS = Sums of squares; MS = Mean sums of squares; perms = permutations; Comp. var. = components of variation.

PERMANOVA test results also show the epifaunal communities at three reefs, PR1, PR2 and PR3, are also different (Table 9). Pairwise testing shows the two main functional communities, Reef community and Reef transition/veneer, are different at each of the three reefs too (Table 10).

Table 10. Pairwise PERMANOVA test results for factor Reef from test for Reef x ‘Functional community’ in a two-factor PERMANOVA model, using the fixed factors Reef and ‘Functional community’, with 9999 unrestricted permutations. Tests were conducted on square root-transformed data of epibenthic taxa from Pisces Reef MPA. Bold results indicate a significant effect (P ≤ 0.05).

Functional community	Reefs	t	P	Unique perms	df
Reef community	PR1, PR2	1.962	**0.0020**	560	14
	PR1, PR3	1.418	**0.0170**	56	6
	PR2, PR3	2.141	**0.0002**	5827	16
Reef transition/ veneer	PR1, PR2	2.641	**0.0001**	9927	29
	PR1, PR3	2.018	**0.0002**	9912	26
	PR2, PR3	3.079	**0.0001**	9928	35

perms = permutations; df = degrees of freedom.

A visualisation of these results shows these differences clearly, especially between the two types of functional community across the Pisces Reefs (Figure 13). It is also clear that both functional communities at PR2 seem different from the other two reefs, PR1 and PR3, as they separate out in the nMDS plot. It is interesting that the Reef and Reef transition/veneer communities of PR1 and PR3 do not seem as separate from each other as those of PR2. Although they are statistically different, there are similarities, especially amongst the Reef communities of PR1 and PR3. Both of these reefs are smaller and more circular than those at PR2 (see sections 3.1 and 3.2.1), which may relate to this apparent similarity.

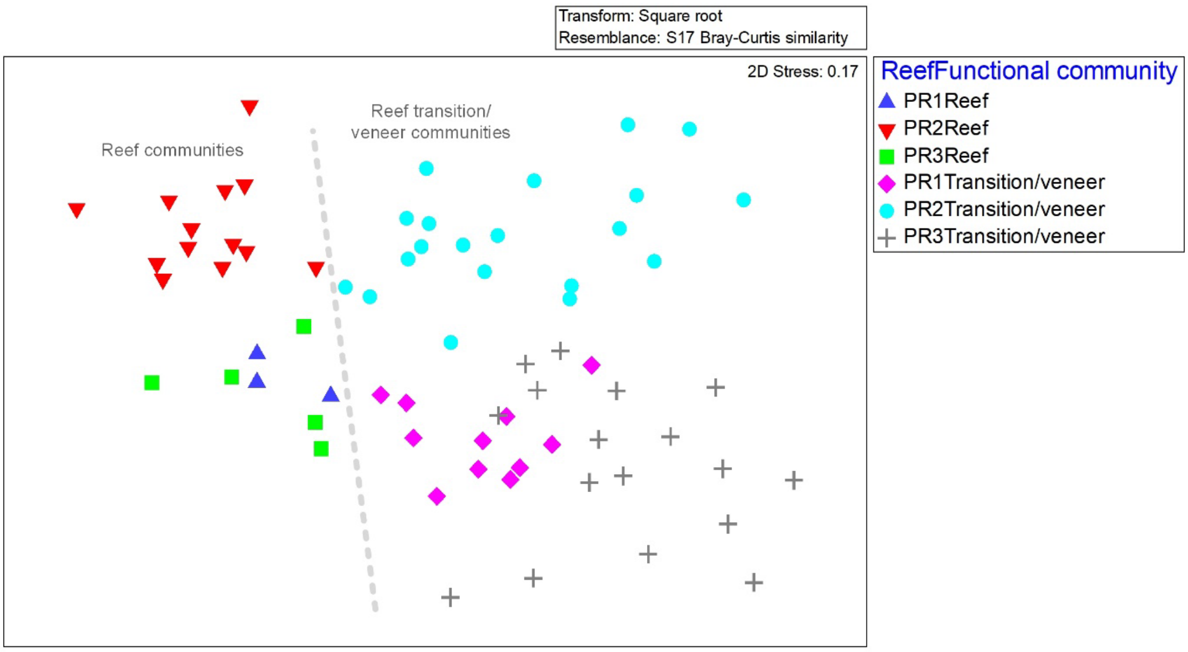


Figure 13. Non-metric multi-dimensional scaling plot showing the similarity between the main functional communities across three reefs at Pisces Reef MPA (PRIMER 7). Data were square root transformed and ordered in a Bray-Curtis Similarity matrix.

3.3 Additional monitoring requirements

3.3.1 Non-indigenous species (NIS)

No non-indigenous species were observed in the imagery at Pisces Reef Complex MPA (Annex 8).

3.3.2 Marine litter

A single item of marine litter was observed in images (Figure 14); a rubber glove (category C5, Annex 7) in camera tow station 12 at PR2 (sample image 24 at 77 m depth).

3.3.3 Evidence of anthropogenic impacts

There are trawl scars running along NNW – SSE axis between the two main reef areas within PR2 (Figure 15). Other scars are also visible around the circumference of the reef all along the eastern edges. These patterns indicate where bottom contact trawling has taken place within the boundary of the MPA.

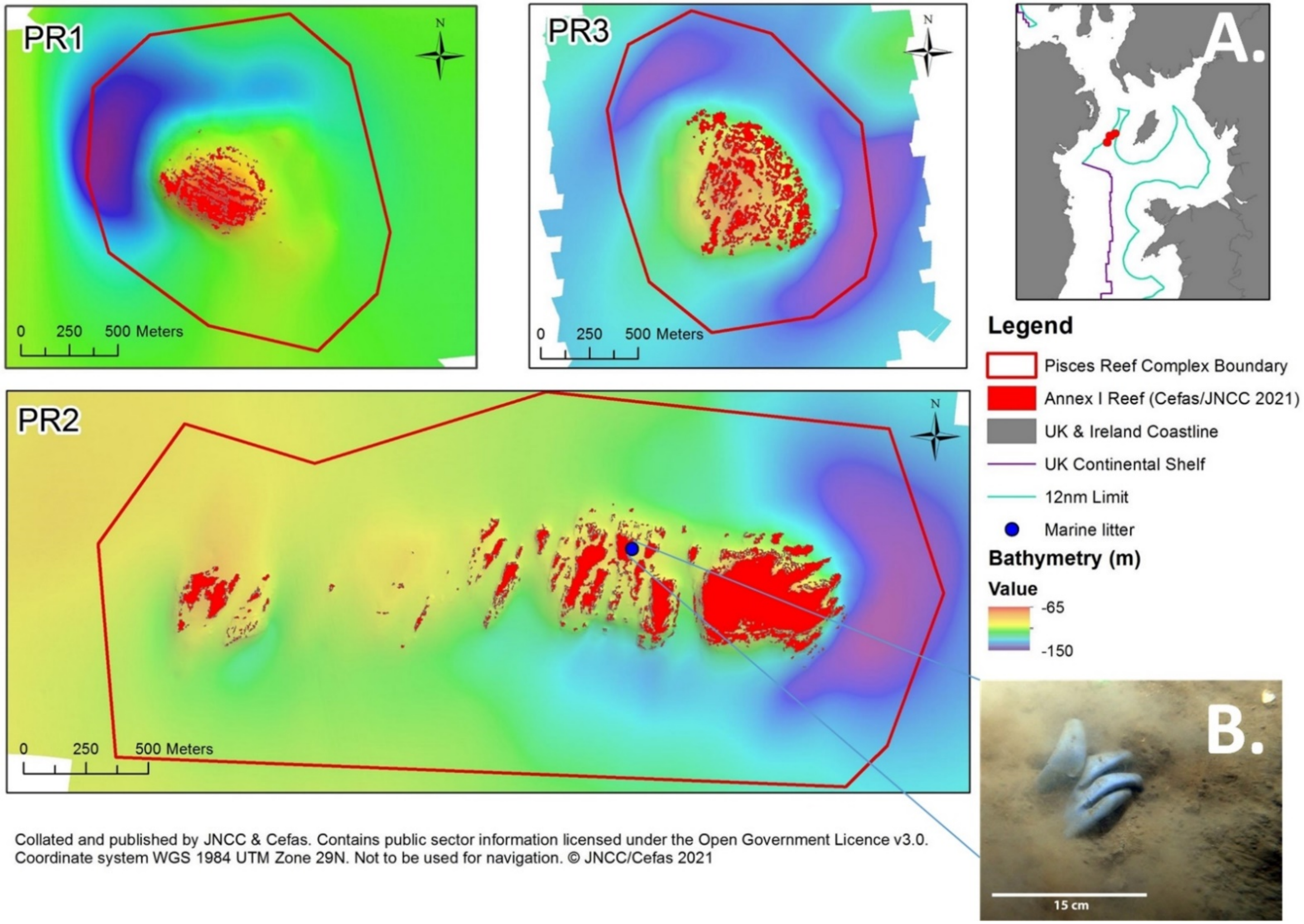


Figure 14. Location of marine litter (rubber glove) identified at PR2 within the Pisces Reef Complex MPA. Figure inset A. shows the position of the MPA within the wider Irish Sea and inset B. shows the identified rubber glove (scale as indicated). Bathymetry from CEND2316 survey (Jenkins & Nelson 2017).

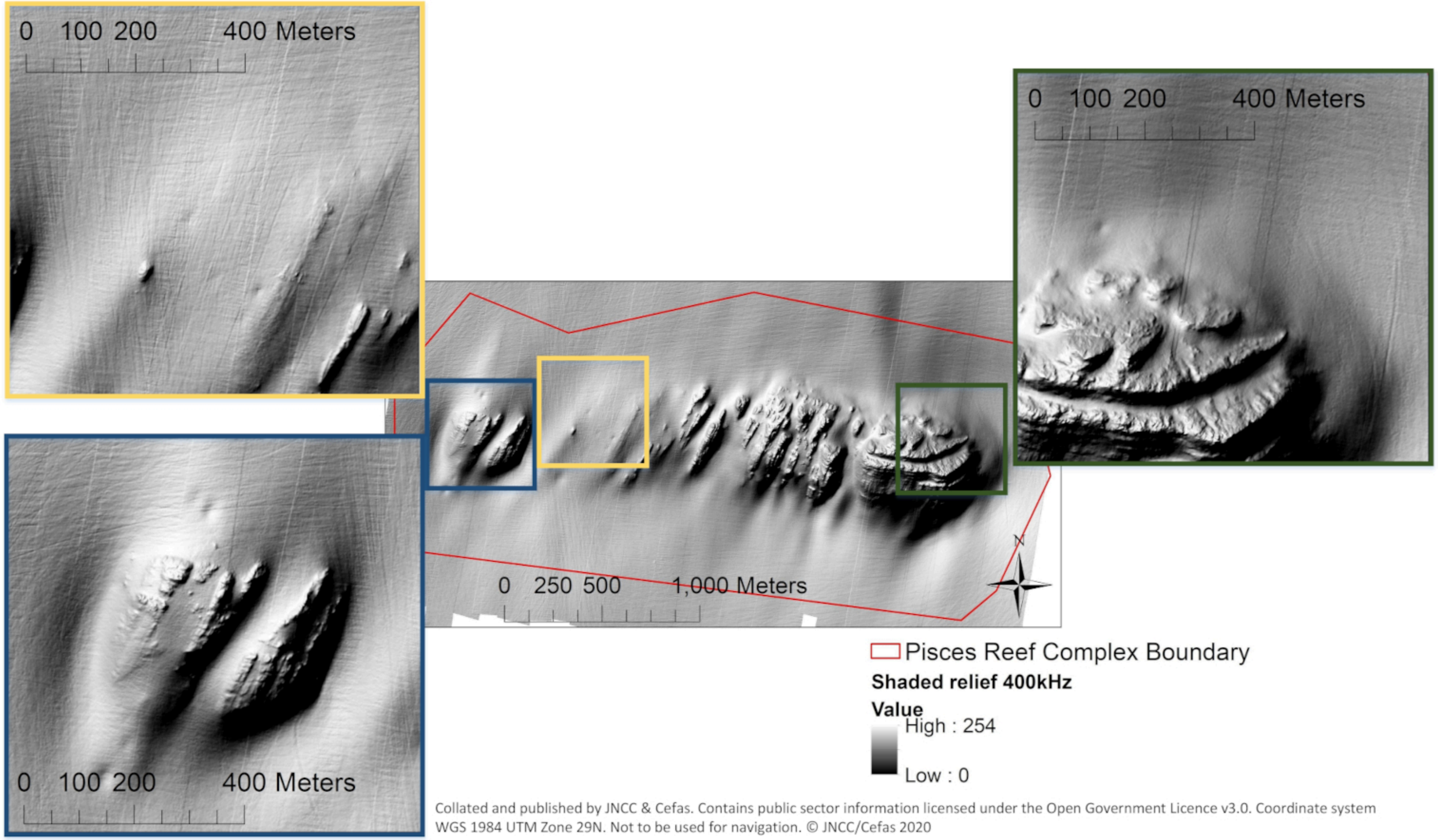


Figure 15. Shaded relief map of PR2 with colour coded close-up inserts (yellow, blue, green) showing evidence of bottom contact trawl scars within the MPA boundary. Backscatter from CEND2316 survey (Jenkins & Nelson 2017).

4 Discussion

The 2016 survey of the Pisces Reef Complex MPA provided detailed evidence of the Annex I Reef features at this site. Analysis of the different types of data collected at the site has greatly improved the understanding of the sites Annex I Reefs. In this report we have focused our interpretation of the results on the feature attributes of the site, to report on the extent and distribution (Objective 1), structure and function (Objective 2) and supporting processes (Objective 3) of the Annex I Reef at Pisces Reef Complex MPA.

The evidence supporting objectives 1, 2 and 3 indicates that Pisces Reef Complex MPA is a dynamic site characterised by groups of rocky outcrops that protrude up through the extensive mud plains of this area of the Irish Sea. Beyond the influence of surface wave energy, this area is influenced by the ebb and flow of tidal currents moving in opposite directions every tidal cycle along a north to south axis. The rocky outcrops at PR1, PR2 and PR3 occur in the shallowest parts of the site, where the tidal currents are also strongest. It is here where the Annex I Reef is more easily observed. The shallowest hard substrata, where slope angles are also steepest, support the most diverse biological sub-communities at the site, which collectively make up the Reef community. These communities are composed of mostly sessile suspension feeders, such as *Caryophyllia*, hydroids, sponges and turf taxa, although in the cracks and crevices between boulders motile taxa such as squat lobsters (Galatheoiods) are also present. The Annex I Reef maps and variability of still imagery along each drop video transect indicate these communities are extremely patchy in distribution and vary in composition across the three reefs, PR1, PR2 and PR3. The easiest way to identify them is from still images containing more than 10% hard substrate and containing any reef-like taxa. These parameters should make future monitoring of the community easier to undertake.

There were numerous challenges with Objective 4, which was “to explore the relationship that sediment veneers on hard substrata have on the Annex I Reef community”. The approach developed made use of the still imagery data. Sediment veneers were identified by selecting still images containing known reef dwelling taxa, such as *Caryophyllia*, hydroids or sponges, and where the composition of hard substratum was less than 10% in each image. In this study, the occurrence of reef dwelling taxa in the imagery collected implies hard attachment surfaces must be available for these taxa to exist. Where hard substratum was not visible in the imagery, but reef taxa were, it was assumed that a veneer of sediment must be overlying the hard substrata under the surface. In this instance these reef taxa serve as a useful proxy indicator of hard substrata. This approach could be useful to other studies with similar concerns, including the continued development of guidance on reef identification (Golding *et al.* 2020).

Assuming that reef taxa present in still imagery with less than 10% hard substratum composition are indicative of sediment veneers over underlying hard substrata, then objective 4 is addressed. Analysis of the biological data identified a ‘Reef transition/veneer community’ occurring in areas of Pisces Reef that are deeper, seabed slope angles are shallower and sediment loads are higher than areas where the main Reef community live. Physical effects such as increased sedimentation and smothering of underlying rock, boulders and communities of epifauna are to be expected in these areas. These effects will determine the type of biota most likely to inhabit these areas. This could explain why robust, smothering-resistant hydroids and turf taxa dominate these areas while smothering-sensitive cup corals and sponges are not observed much here. It is likely that the more challenging conditions have contributed to lower taxonomic diversity here than in areas where the true Reef community lives. It is also likely that the typical functions performed by the Reef community, shelter, food provision and water filtering, have also changed and are likely reduced by these sediment veneers.

Creation of a community-based continuum, shown in Figure 11, has helped to understand the relationship the sediment veneers on hard substrata have with the Annex I Reef community at Pisces Reef. It shows that with increases in sand and gravels (main factor), and mud content to a lesser extent, the Reef community can transition through an intermediate state, the Reef transition/veneer community, before becoming a completely mud-based community. These sedimentary processes are driven by local hydrodynamics and affected by underlying geology. Where there are steeper angled slopes of hard substrata at Pisces Reef it is less likely for the inhabiting reef communities to become smothered with veneers of sediment. As the slope angle shallows, however, this prospect becomes much more likely until the community changes significantly, becoming a transition/veneer community with only sparse, robust epifauna still present.

With only one sampling event in this study, it is not known for how long the transitional community state persists. Seasonal water stratification of the Western Irish Sea in the spring and summer months has been shown to create a cyclonic gyre that likely influences seabed scour and depositional processes in the area of Pisces Reef SAC (Hill *et al.* 1996; Calloway *et al.* 2009). If such changes to the seabed are possible at seasonal scales, would changes also be possible over other time scales and what is the frequency of change? Do reef communities become temporarily smothered with veneers of sediment during the slack periods of every tidal cycle and exposed again during the maximum flow periods of each flood and ebb tide? Are there areas where the transitional community state persists, where tidal forces are insufficient to significantly shift the sediment veneers from the underlying hard substrata? Only with more detailed study can these questions be tested. It seems fairly certain that there are areas long smothered with sediments that have substantially accumulated to depths greater than a metre, where no transitional community survives. These areas support burrowing taxa, such as *Pachycerianthus multiplicatus*, Ceriantharia, and *Nephrops norvegicus* and have now become entirely mud-based habitats. The continuum of community change created for Pisces Reef can at least provide conceptual understanding of the relationship the sediment veneers on hard substrata have on Annex I Reef communities at Pisces Reef.

A variety of mobile epibenthic, demersal and pelagic taxa were observed in the drop camera imagery. It is usual that such taxa are removed from statistical analyses because the sampling method, drop camera video and stills in this study, were not designed to correctly sample these taxonomic groups. Retaining these taxa in just the exploratory community analyses, however, revealed useful contextual information that improves understanding of how Pisces Reef functions. Moving around the reefs, transitional areas with sediment veneers over hard substrata and surrounding mud of PR1, PR2 and PR3 are a variety of bony fishes (of demersal and pelagic habits), catsharks (Scyliorhinidae), decapod crabs and scavenging amphipods (Peracarida). These mobile taxa are likely opportunists availing of the reef functions of shelter and food provision.

Many of the mobile taxa supported by the reefs and those from the surrounding muddy areas, especially *Nephrops norvegicus,* are targeted by the fishing industry to provide food for human consumption. Indeed, fishing activity was evident from analysis of the bathymetric data around PR2 where numerous trawl scars persist in the sediments around the edges of, and between, the reefs. Of greater importance perhaps is that this evidence enables a clear connection between the fishing industry and the habitats in this area that support populations of commercially viable taxa, such as *Nephrops norvegicus*. Although it was not an objective of this study, understanding the relationship that the reef habitats have with the commercially viable taxa, as well as the economic value of the reef habitats of Pisces Reef, may enable more sustainable management and conservation of its Annex I reef features at this site.

On the other hand, anthropogenic activities may also negatively impact reef communities itself. For example, Hinchen *et al.* (2021) observed a negative relationship between the occurrence of solitary coral genus *Caryophyllia* and cup-like sponges and resuspended sediments pressure from demersal fishing activities at Pisces Reef MPA. This negative relationship was also observed when fishing activities were infrequent (as little as five times per year) and even when fishing activities took place outside the MPA (within 1km from the MPA boundary). The environmental link between reef and surrounding soft-sediment areas, including the role of fishing activities in this, should be considered in management decisions for Pisces Reef – a site with a heterogenous seascape of Annex I reef and surrounding habitats.

5 Recommendations for future monitoring

Objective 7 of this study is to recommend future monitoring approaches that are considered useful for the site, and potentially other sites containing comparable features. These have been divided into the following four sections.

5.1 Data acquisition and analysis

The different approaches taken in this study to acquire the acoustic data at different frequencies and directions reduced their ability to be appropriately compared against one another. Whilst the decision to acquire MBES data at two frequencies was a valid one, the implementation of these data at different orientations to save survey time rendered any subsequent comparisons, particularly of backscatter data, invalid. As it has been shown that orientation of insonification can impact the utility of data (McGonigle *et al.* 2010; Lamarache & Lurton 2017) a decision was made not to utilise data from one orientation in the study.

Similarly, surface deployment of the chirper impacted the utility of the seismic data for their intended purpose. The acoustic footprint of the system resulted in large overlap of soundings which confused the signal and prevented discrimination of thin sediment layers that the system is capable of if deployed correctly. To enable discrimination of thin sediment layers, seismic systems need to be towed at depth to reduce the size of the acoustic footprint and increase the resolution of data acquired.

To enable effective modelling of Annex I features, ground truth sampling must include areas where the features are not present. Sampling outside features is of particular importance in an area such as Pisces Reef where acoustic data may indicate the presence of rock substrata but where imagery data will indicate sedimentary substrata in the same location. The acquisition of sediment samples using a grab in areas where sediments seem to be both within the reef areas and outside of the reef areas (Callaway *et al.* 2009) would have also improved the discriminatory ability of all subsequent analyses. This is due to the difficulties of accurately identifying differences in substrata from the underwater still imagery in this study. Such an approach could be beneficial in better understanding and validating veneer areas across the reef, although caution must be used when using sediment grab samplers in potentially rocky areas.

5.2 Drop camera survey design

Owing to the spatial patchiness of the Annex I Reef at Pisces Reef it will be difficult to target discrete, homogenous areas of Reef community for future monitoring effort. As shown by this study, even though areas thought to be reef were specifically targeted for drop camera tows, the resultant imagery still contained large areas of mud habitat and transitional reef habitats with sediment veneers over hard substrata. This heterogeneity must be considered in future sampling events as it is a feature of this site.

Future effort must still be focused on maximising the acquisition of video and still imagery from areas of Annex I Reef, but sampling approaches can be improved in four ways:

- Camera tows can be better targeted using the maps shown in this report. Specifically, use of the occurrence of reef taxa in imagery, especially in areas where sediment veneers are present, can be used to better focus these efforts to areas of the reef most likely to contain the Reef community;
- Longer camera tows, 500-1000 m, that traverse the entire reef area in some cases, will enable greater opportunities to sample the Reef community rather than shorter tows. This is largely because the Reef community at Pisces Reef SAC is distributed very heterogeneously. Such camera tows may also prove to be more efficient over an entire survey at Pisces Reef SAC;
- Collecting more still images from future transects is essential if the data from individual still images are to be aggregated into suitable, appropriate sample units. In this study, 12 images were aggregated into units approximately 5 m^2 in area. Taxonomic accumulation curves suggested the asymptote for the imagery data sets in this study was between 50-100 images per sample unit. If we took this lower limit of just 50 images for demonstration purposes only and estimated that a minimum of 5 replicate samples would be needed to monitor the Reef community at each reef, PR1, PR2 and PR3, then a minimum of 750 still images of good quality and a field of view less than 0.9 m^2 would be needed for future sampling efforts. This study had only 271 available for this purpose. Future surveys can reach these higher targets of successful still acquisition with improvements to surveyor training, by collecting images more frequently along a drop camera tow (e.g., every 20 seconds) and by carrying out longer, targeted tows of Annex I reef areas;
- Repeat sampling of the longer tow transects in successive monitoring events, i.e., fixed transects, will collect benthic imagery from similar areas over time. As these longer tows will be targeted to areas most likely to contain Reef communities, these areas will become indicative of the condition of the Annex I Reef feature at this site. Repeated sampling of these areas will improve the ability to detect changes in those areas over time, and by proxy, the whole reef over time.

5.3 Imagery data analysis and interpretation

Following recommendations of Moore *et al.* (2019), the frequency of occurrence method was selected to enumerate taxa in the imagery collected from Pisces Reef. The key drawback of this method, a difficulty in taxonomic truncation (Moore *et al.* 2019), was not a big issue with the dataset. Rather the benefits were more obvious, in that biological data could be analysed as one data metric rather than two, i.e., abundance counts and percentage cover, which saved time spent on the analysis while also simplifying the interpretation of results. Owing to the data range of 0-25 for the frequency of occurrence data in this study data transformations were not deemed necessary and thus more of the original data integrity was preserved in the exploratory analysis. Transformations were only made once frequency of occurrence data was aggregated into samples from sets of twelve images, i.e., data ranges now of 0-300.

Aggregating still image data into sampling units also presented no issues with this data metric, although some consideration of what was happening while this occurred is important. As the spatial area of still images varied from approximately 0.5 – 0.9 m^2 the size of each frequency ‘cell’, 25 per image, was not equal across all images. Therefore, when aggregated together into groups of twelve images, data from 300 differently sized cells were aggregated together to represent a sample unit of a biological community. The effect of this data artefact is not known on the representation of the biological community. Whatever the effect, if equally sized frequency cells had been used to enumerate each still image, e.g., 10 x 10 cm cells, the aggregation would have been standardised across all sample units. The overall sample areas of each sample unit were fairly uniform across the aggregated data set (Annex 3), improving confidence in the approach taken to aggregate the still image data.

Further use of the frequency of occurrence method is recommended for future monitoring at this site for the reasons discussed above. If there is a way to standardise the frequency cell size, perhaps using the image field of view as a guide, this is also recommended, as it will improve confidence in data aggregations and enable standardisation across data sets from other sites.

5.4 Annex I 'Reef community' detection

- It is recommended that future monitoring surveys at this site use the presence of reef taxa (see Annex 4) as guides to select those images representing the Annex I Reef. In this study, all still images with reef taxa present and containing >10 % hard substrata qualified as the Reef community. There are images in this data set in which the reef taxa are slightly covered by a veneer of sediment (potentially of the Reef transition/veneer community) but contain <10 % of visible hard substratum these can be included in assessments of the Annex I Reef. The Reef transition/veneer communities were statistically different from the Reef community in this study and so should be considered separately under the current definition of Annex I Reef.
- Future monitoring efforts at this site should aim to first identify the Reef community in the data. Appropriately sized sampling units of the community then need to be randomly aggregated from the still images for comparisons, preferably informed by the asymptote of taxonomic accumulation curves of the still image data on each reef (PR1, PR2 and PR3). If future monitoring focused efforts on key or influential taxa of the Reef community then analysis of the cup coral, *Caryophyllia* sp., is recommended. Hinchen *et al.* (2021) concluded that the solitary coral genus *Caryophyllia* show a sharp and strong negative response to pressure exposure, thus having a potential to be a starting point for a future monitoring programme of UK sublittoral rock habitats. As this taxon is sessile, long-lived (~20 years), reef building, feeds from the currents, is sensitive to smothering and occurs in the majority of Reef community images, it serves as a good proxy for the occurrence of the Reef community. The optimum data enumeration metric for detection in changes of this taxon should be also explored, i.e., frequency of occurrence vs abundance counts.

6 References

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

Anderson, M.J., Gorley, R.N. & Clarke, K.R. (2008). PERMANOVA$^+$ for PRIMER: Guide to Software and Statistical Methods. PRIMER-E: Plymouth, UK.

Astrium. (2011). Creation of a high-resolution Digital Elevation Model (DEM) of the British Isles continental shelf: Final Report. Prepared for Defra, Contract Reference: 13820. 26 pp.

Barrio Frojan, C., Brown, L. & Downie, A. (2015). Slieve na Griddle rMCZ Post-survey Site Report. Report number 37 of Defra project Marine Protected Areas Data and Evidence Co-ordination Programme. 39 pp.

Blaschke, T. (2010). Object based image analysis for remote sensing. *ISPRS journal of photogrammetry and remote sensing* 65 (1) 2-16.

Boehner, J. & Antonic, O. (2009). Land-surface parameters specific to topo-climatology. in: Hengl, T. & Reuter, H. (Eds.): 'Geomorphometry - Concepts, Software, Applications'. *Developments in Soil Science* 33, p.195-226, Elsevier.

Callaway, A., Smyth, J., Brown, C.J., Quinn, R., Service, M. & Long, D. (2009). The impact of scour processes on a smothered reef system in the Irish Sea. *Estuarine, Coastal and Shelf Science* 84, 409-418. DOI: 10.1016/j.ecss.2009.07.011

Clare, D.S., Bolam, S.G., McIlwaine, P.S.O., Garcia, C., Murray, J. & Eggleton, J.D. (in prep). Biological traits of marine benthic invertebrates.

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

Connor, D.W., Allen, J.H., Golding, N., Howell, K.L., Lieberknecht, L.M., Northen, K.O. & Reker, J.B. (2004). The Marine Habitat Classification for Britain and Ireland (version 04.05). Joint Nature Conservation Committee, Peterborough.

Conrad, O., Bechtel, B., Bock, M., Dietrich, H., Fischer, E., Gerlitz, L., Wehberg, J., Wichmann, V. & Böhner, J. (2015). System for Automated Geoscientific Analyses (SAGA) v. 2.1.4, *Geoscientific Model Development* 8, 1991-2007. https://doi.org/10.5194/gmd-8-1991-2015.

Diesing, M. & Thorsnes, T. (2018). Mapping of Cold-Water Coral Carbonate Mounds Based on Geomorphometric Features: An Object-Based Approach. *Geosciences* 8, 34.

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

Elliott, M., Nedwell, S., Jones, N., Read, S.J., Cutts, N.D. & Hemingway, K.L. (1998). Volume II: Intertidal sand and mudflats and subtidal mobile sandbanks. An overview of dynamic and sensitivity characteristics for conservation management of marine SACs. UK Marine SACs project, Oban, Scotland. English Nature.

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

Environmental Systems Research Institute (ESRI). (2017). *ArcGIS Release 10.5*. Redlands, CA.

Folk, R.L. (1954). The distinction between grain size and mineral composition in sedimentary rock nomenclature. *Journal of Geology* 62, 344-359.

Golding. N., Albrecht. J. & McBreen. F. (2020). Refining criteria for defining areas with a 'low resemblance' to Annex I stony reef; Workshop Report. JNCC Report No. 656, JNCC, Peterborough, ISSN 0963-8091. Available at: https://hub.jncc.gov.uk/assets/4b60f435-727b-4a91-aa85-9c0f99b2c596 [Accessed October 2020].

Hill, A.E., Brown, J. & Fernand, L. (1996). The western Irish Sea gyre: a retention system for Norway lobster (*Nephrops norvegicus*)? *Oceanologica Acta* 19 (3–4), 357–368.

Hinchen, H., Gallyot, J., Carter, A., Ferguson, M., Webb, K., Nelson, M., & Jenkins, C. (2021). Detecting the impacts on UK sublittoral rock communities of resuspended sediments from fishing activity. *Ecological Indicators*, 125, 107545.

Hothorn, T., Hornik, K. & Zeileis, A. (2006). Unbiased Recursive Partitioning: A Conditional Inference Framework. *Journal of Computational and Graphical Statistics* 15 (3) 651-674.

Irving, R. (2009). The identification of the main characteristics of stony reef habitats under the Habitats Directive. Summary report of an inter-agency workshop 26-27 March 2008. JNCC Report No. 432. Available at: https://hub.jncc.gov.uk/assets/21693da5-7f59-47ec-b0c1-a3a5ce5e3139 [Accessed June 2020].

Jenkins, C. & Nelson, N. (2017). CEND23/16X Cruise Report: Monitoring survey of Pisces Reef Complex cSAC/SCI. JNCC/Cefas Partnership Report Series No. 18. JNCC, Peterborough. pp. 66.

JNCC. (2004). Common standards monitoring guidance for littoral sediment habitats. Peterborough, JNCC. Available at: http://data.jncc.gov.uk/data/9b4bff32-b2b1-4059-aa00-bb57d747db23/CSM-LittoralSedimentHabitats-2004.pdf [Accessed Oct 2020].

JNCC. (2010). JNCC guidelines for minimising the risk of injury and disturbance to marine mammals from seismic surveys. https://hub.jncc.gov.uk/assets/24cc180d-4030-49dd-8977-a04ebe0d7aca.

JNCC. (2012). Offshore Special Area of Conservation: Pisces Reef Complex SAC Selection Assessment Document. Version 7.0. Available at: https://data.jncc.gov.uk/data/9fd4a46a-7d93-461a-87b1-10a0a0834ad7/PiscesReef-SAC-SelectionAssessment-V7.0.pdf [Accessed October 2021].

JNCC. (2018). Conservation Objectives for Pisces Reef Complex Special Area of Conservation. Peterborough: JNCC. Available at: https://hub.jncc.gov.uk/assets/4f8ac443-777f-4a55-a5c8-fc44e80fa965.

JNCC. (2018). Supplementary Advice on Conservation Objectives for Pisces Reef Complex Special Area of Conservation. Peterborough: JNCC, Available at: https://hub.jncc.gov.uk/assets/4f8ac443-777f-4a55-a5c8-fc44e80fa965

Kursa, M.B. & Rudnicki, W.R. (2010). Feature Selection with the Boruta Package. *Journal of Statistical Software* 36 (11) 1-13. URL http://www.jstatsoft.org/v36/i11/.

Lamarche, G. & Lurton, X. (2017). Recommendations for improved and coherent acquisition and processing of backscatter from seafloor-mapping sonars. *Marine Geophysical Research* 6, 1-18. DOI 10.1007/s11001-017-9315-6.

Liaw, A. & Wiener, M. (2002). Classification and Regression by randomForest. *R News* 2, 18–22.

Long, D. (2006). BGS detailed explanation of seabed sediment modified folk classification. Peterborough: JNCC. Available at: https://webarchive.nationalarchives.gov.uk/20101014085414/http://www.searchmesh.net/PDF/BGS%20detailed%20explanation%20of%20seabed%20sediment%20modified%20folk%20classification.pdf. [Accessed Oct 2020].

Lundblad, E.R., Wright, D.J., Miller, J., Larkin, E.M., Rinehart, R., Naar, D.F., Donahue, B.T., Anderson, S.M. & Battista, T. (2006). A Benthic Terrain Classification Scheme for American Samoa. *Marine Geodesy* 29, 89–111.

Lurton, X. & Lamarche, G. (Eds) (2015) Backscatter measurements by seafloor-mapping sonars. Guidelines and Recommendations. 200p. Available at: https://www.researchgate.net/profile/Geoffroy_Lamarche/publication/275890570_Backscatter_measurements_by_seafloor-mapping_sonars_-_Guidelines_and_Recommendations/links/5548dbbb0cf25a87816aa8c8/Backscatter-measurements-by-seafloor-mapping-sonars-Guidelines-and-Recommendations.pdf?origin=publication_detail [Accessed 25/08/2020].

Mason, C. (2011). NMBAQC's Best Practice Guidance Particle Size Analysis (PSA) for Supporting Biological Analysis. NMBAQC Best Practice Guidance. Available at: http://www.nmbaqcs.org/media/1255/psa-guidance_update18012016.pdf. [Accessed Oct 2020].

McGonigle, C., Brown, C.J. & Quinn, R. (2010). Insonification orientation and its relevance for image-based classification of multibeam backscatter. *ICES Journal of Marine Science* 67, 1010-1023.

McIlwaine, P. (2014). Pisces Reef Complex and Slieve na Griddle MCZ Drop Camera Survey – Preliminary Findings. Cefas Cruise Report (CEND1414), Issue date: 29/07/14. 25 pp.

Moore, J., van Rein, H., Benson, A., Sotheran, I., Mercer, T. & Ferguson, M. (2019). Optimisation of Benthic Image Analysis Approaches, JNCC Report, No. 641, JNCC, Peterborough, ISSN 0963-8091.

MSFD GES Technical Subgroup on Marine Litter. (2013). Guidance on Monitoring of Marine Litter in European Seas. Publications Office of the European Union. EUR 26113. http://publications.jrc.ec.europa.eu/repository/handle/JRC83985

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

Parry, M.E.V. (2015). Guidance on assigning benthic biotopes using EUNIS or the Marine Habitat Classification of Britain and Ireland JNCC report No. 546 Joint Nature Conservation Committee, Peterborough.

R Core Team. (2017). R: A language and environment for statistical computing. R Foundation for Foundation for Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

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).

Service, M. & Strong, J.A. (2012). MCZ Site Verification within the Irish Sea. AFBI Survey of Irish Sea Proposed MCA Cruise Report (CO 082012). 17 pp.

Stebbing, P., Murray, J., Whomersley, P. & Tidbury, H. (2014). Monitoring and surveillance for non-indigenous species in UK marine waters. Defra Report. 57 pp.

Strong, J. (2012). MCZ Site Verification within the Irish Sea – Slieve na Griddle. Final Report (Project Code): CO082012. 66 pp.

Turner, J.A., Hitchin, R., Verling, E. & van Rein, H. (2016). Epibiota remote monitoring from digital imagery: Interpretation guidelines. A report for the North-East Atlantic Marine Biological Analytical Quality Control Scheme.

van Rein, H.B., Brown, C.J., Quinn, R. & Breen, J. (2009). A review of sublittoral monitoring methods in temperate waters: a focus on scale. *Underwater Technology* 28, 99-113.

Wakefield, W.W. & Genin, A. (1987). The use of a Canadian (perspective) grid in deep-sea photography. *Deep-Sea Research*, 34 (3) 469-78.

Weise, C. (2012). Multiple object difference conditions-based fusion version 3. Customised Algorithm. eCognition developer.

Wilson, M.F.J., O'Connell, B., Brown, C., Guinan, J.C. & Grehan, A.J. (2007). Multiscale Terrain Analysis of Multibeam Bathymetry Data for Habitat Mapping on the Continental Slope. *Marine Geodesy* 30, 3–35.

Winkler, L.W. (1888). Die Bestimmung des in Wasser gelösten Sauerstoffen. *Berichte der Deutschen Chemischen Gesellschaft* 21, 2843–2855.

Worsfold, T.M., Hall, D.J. & O'Reilly, M. (2010). Guidelines for processing marine macrobenthic invertebrate samples: a processing requirements protocol version 1 (June 2010). Unicomarine Report NMBAQC MbPRP to the NMBAQC Committee. 33 pp.

7 Annex I. Glossary

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

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.*
Community	A general term applied to any grouping of populations of different organisms found living together in a particular environment; essentially the biotic component of an ecosystem. The organisms interact and give the community a structure (Allaby 2015).
Conservation Objective	A statement of the nature conservation aspirations for the feature(s) of interest within a site, and an assessment of those human pressures likely to affect the feature(s).*
EC Habitats Directive	The EC Habitats Directive (Council Directive 92/43/EEC on the Conservation of natural habitats and of wild fauna and flora) requires Member States to take measures to maintain natural habitats and wild species of European importance at, or restore them to, favourable conservation status.
Epifauna	Fauna living on the seabed surface.
EUNIS	A European habitat classification system, covering all types of habitats from natural to artificial, terrestrial to freshwater and marine.*
Favourable Conservation Status	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.
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 Protected Area (MPA)	A generic term to cover all marine areas that are 'A clearly defined geographical space, recognised, dedicated and managed, through legal or other effective means, to achieve the long-term conservation of nature with associated ecosystem services and cultural values' (Dudley 2008).*
Natura 2000	The EU network of nature protection areas (classified as Special Areas of Conservation and Special Protection Areas), established under the 1992 EC Habitats Directive.*
Non-indigenous Species	A species that has been introduced directly or indirectly by human agency (deliberately or otherwise) to an area where it has not occurred in historical times and which is separate from and lies outside the area where natural range extension could be expected (Eno *et al.* 1997).*
Pressure	The mechanism through which an activity has an effect on any part of the ecosystem (e.g., physical abrasion caused by trawling). Pressures can be physical, chemical or biological, and the same pressure can be caused by a number of different activities (Robinson *et al.* 2008).*
Special Areas of Conservation	Protected sites designated under the European Habitats Directive for species and habitats of European importance, as listed in Annex I and II of the Directive.*
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.
Sentinel Monitoring of long-term trends (Type 1 monitoring)	Objective: to measure rate and direction of long-term change. This type of monitoring provides the context to distinguish directional trends from short-scale variability in space and time by representing variability across space at any one time and documenting changes over time. To achieve this objective efficiently, a long-term commitment to regular and consistent data collection is necessary; this means time-series must be established as their power in identifying trends is far superior to any combination of independent studies.

Annex 2. Data Acquisition

Acoustic data

A hull-mounted Kongsberg EM2040 MBES was used to acquire bathymetry and backscatter data simultaneously across planned areas in PR1, PR2 and PR3. The MBES data were collected at frequencies of 200 kHz and 400 kHz, with each frequency being acquired perpendicular to the other.

A chirper system was used to acquire sub-bottom profiling imagery across the site at a frequency of 11.2 kHz. The JNCC guidelines for seismic surveys were followed throughout the survey, which included pre-shoot watches by a trained Marine Mammal Observer in daylight hours and using a Passive Acoustic Monitoring operator during night hours (JNCC 2010; Jenkins & Nelson 2017).

All acoustic data were processed onboard RV *Cefas Endeavour* by EGS Survey. Bathymetry data were processed using CARIS HIPS and SIPS to remove erroneous soundings and to ensure data conformed to IHO order 1a. Backscatter data were processed using QPS FMGT to Cefas standards (as per Lurton & Lamarche 2015). Following processing, the seismic data were subsequently interrogated ashore to create deliverables. All data was subjected to quality control (QC) procedures, carried out by Cefas staff.

Seabed imagery

A drop frame equipped with laser scale, digital stills and HD video camera was used to collect imagery from the benthic community across the site. Tows were conducted at a speed of 0.3 knots using the vessel's dynamic positioning capability. Video footage with overlay of ship's position were recorded during the tow, once the drop frame has reached the seabed. Still images were taken at 60 second intervals from a dedicated stills camera, together with opportunistic stills that would assist in completing the survey objectives. The drop frame was deployed from the side gantry and the Tower logging system was set up to record USBL position at 5 second intervals.

Additional environmental data

An ESM2 logger was mounted on the drop frame to record conductivity (salinity), temperature, pressure (depth), light transmission (suspended solids) and fluorescence (chlorophyll A). The ESM2 logger recorded data during every drop camera tow. Sea water samples were collected using a 10 L Niskin sample bottle to validate data collected by the ESM2 logger. Each Niskin was subsampled by siphoning off water using silicon rubber tubing for dissolved oxygen, salinity, chlorophyll A, and suspended solids. All samples were collected in four replicates per reef area (i.e., PR1, PR2 and PR3). For details on sample processing see Jenkins and Nelson (2017).

Annex 3. Data Preparation and Analysis

Tidal modelling

Mean and maximum tidal current velocities (m s-1) at the seabed and mean and maximum bed shear stress were obtained from a tidal model built for the study area. The depth-averaged model of Pisces Reef Complex MPA is nested with a larger Irish Sea model and has been built using an unstructured triangular mesh, using the hydrodynamic software Telemac2D (v7p1). The model domain extends 50.13°N – 50.13°N and 2.38°E – 7.73°W. The unstructured mesh was discretised with 195,395 nodes and 370,997 elements. The mesh had a resolution of approximately 350 m close to shore growing to a maximum of 6 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 was 1 arc second (~30 m). In the area of the MPA, the MBES bathymetry from the area was used, gridded to a 2 m resolution. 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 days, the model was run for 30 days to cover a full spring-neap cycle. Bed shear stress (N/m2) was calculated according to Soulsby (1997), based on current speed and local sediment characteristics (derived from the habitat map and sediment samples).

Environmental parameter data

Environmental parameter data were collated from the ESM2 logger, MBES data and still images. The range of parameters are tabulated in Table 11. This dataset was filtered so that only those samples that corresponded with the Reef community data were selected. Any parameter that did not have any values that corresponded with the Reef community data set was removed before the statistical analysis.

Table 11. Sources and parameters of environmental data collected from Pisces Reef Complex MPA during CEND2316 (Jenkins & Nelson 2017).

Environmental data source	Environmental parameter
ESM2 logger	Salinity, temperature, depth, suspended solids and chlorophyll A.
MBES	Bathymetry, backscatter and slope (degrees).
Still imagery	Percentage covers for bedrock, boulders (over 1024 mm), boulders (512 to 1024 mm), boulders (256 to 512 mm), cobbles (64 mm to 256 mm), pebbles (4 mm to 64 mm), shells (Empty), shells (live *Modiolus*), gravel (2 mm to 4 mm), shell (2 mm to 16 mm), dead Maerl, live Maerl, sand (0.063 mm to 2 mm), mud (less than 0.063 mm) and artificial biogenic reef.

Habitat Map

Data preparation

Bathymetry and backscatter data were re-sampled onto a common grid at 1 m resolution. A range of surface derivatives were calculated from the bathymetry data (see Table 11), using ArcMap v10.5 (Environmental Systems Research Institute 2017) and SAGA GIS tools for QGIS (v. 3.2; Conrad *et al.* 2015).

Table 12. Description of derivatives calculated for bathymetry.

Derivative	Description
Slope	The maximum slope gradient (degrees) (Wilson *et al.* 2007).
Roughness	The difference between the minimum and maximum value of each cell and the eight adjacent cells (metres) (Wilson *et al.* 2007).
Curvature (curvPL, curvPR)	The rate of change of slope. Profile curvature (curvPR) is measured parallel to the maximum slope; plan curvature (curvPL) is measured perpendicular to the slope (Wilson *et al.* 2007).
Bathymetric position index (BPI3, BPI5, BPI10, BPI25)	The vertical position of a cell relative to adjacent cells. Radii of 3, 5, 10 and 25 pixels were used (Lundblad *et al.* 2006).
Wind Effect Index (WEX)	The Wind Effect Index indicates to how exposed an area is in relation to surrounding topography (Boehner & Antonic 2009).

The nature of Pisces Reef, as rock outcropping in a mud basin, means the separation of reef from surrounding sediments is not straightforward. An estimate of surficial substrata from imagery, such as the percentage of hard substrata visible, is not a reliable indicator for presence of reef at the site. Attached epifauna normally associated with hard substrata are observed in images where substrata are classified as sediment due to a thin overlaying veneer. To capture both exposed reef and reef with a thin sediment veneer, the presence of rock-associated taxa, referred here as 'reef taxa' was chosen as the more reliable indicator of the presence or potential presence of reef. It must, however, be noted that reef taxa were also occasionally found to be present in burrowed muds in low numbers and abundance, having colonised a shell fragment or small stone (Figure 16a). Consequently, in aspiring to encompass all potential reef with sediment veneer the use of the simple metric of presence of reef taxa leads to an unavoidable overestimation of reef presence. Figure 16b shows an example of reef taxa present with a sediment veneer.

a) **b)**

Figure 16. Examples of images with 'Reef taxa' present where substrata are predominately soft sediments. (a) Image of a sedimentary 'Not reef' area containing 'Reef taxa' (outlined). (b) Image of a 'sediment veneer' containing 'Reef taxa'.

Segmentation

The eCognition multiresolution segmentation tool was used to conduct the segmentation (see Diesing & Thorsnes 2018 for further details). Image segmentation was carried out on the layers reported in Table 13 for each survey area, with a scale factor of 1, shape of 0.1 and compactness of 0.5. Different combinations of segmentation parameters were tested and examined visually to aid selection.

Selection of variables for use in segmentation and classification was done using R (R Core Team 2017) package ‘Boruta’ (Kursa & Rudnicki 2010). Boruta is a feature selection wrapper algorithm that uses Random Forest classification to identify features with higher importance values compared to the importance achievable at random. The variable weightings for segmentation were chosen based on significant variables selected by Conditional Inference (CI) trees using the R package ‘party’ (Hothorn *et al.* 2006). Variables with highly significant p values ($p < 0.001$) were given a weighting of two during segmentation and other variables included in the CI trees were weighted at one.

Table 13. Feature layers used for image segmentation and their respective weightings, and those used in the classification of each area of Pisces Reef Complex (PR1-3). See Table 2 for descriptions of acoustic derivatives (BPI, Curve_PL, Curv_PR, WEX, slope and roughness). WEX was not included in the first step for choosing variables for segmentation.

Feature layers	Segmentation			Classification		
	PR1	PR2	PR3	PR1	PR2	PR3
Backscatter 200 kHz	0	2	0		√	
Backscatter 400 kHz	0	1	1	√		√
Bathymetry 200 kHz	0	1	0	√	√	
Bathymetry 400 kHz	2	2	0			√
BPI25	2	2	2	√	√	√
BPI10	1	0	1			
BPI5	0	0	0			
BPI3	0	0	0	√	√	√
Curv_PL	0	0				
Curv_PR	0	0	0	√		
Slope	0	1	1	√	√	√
Roughness	0	2	0			
WEX	NA	NA	NA	√	√	√

An object fusion routine using the multiple object difference conditions-based fusion tool (Weise 2012) was then applied to the initial objects to create larger meaningful objects. This fuses objects based on the difference of specified features between neighbouring objects. Objects with a common border value of ≥0.1 and a difference in backscatter 200 kHz of no more than two, and slope of no more than one, were combined to form larger object units.

Classification

A Random Forest algorithm run using the ‘RandomForest’ package in R (Liaw & Wiener 2002) was used to classify the final segmentation objects into ‘Annex I Reef’ and ‘Not Reef’. Individual models were built for PR1, PR2 and PR3. Data points were filtered to remove duplicate stills from objects, leaving 1012 observations (211, 581 and 220 for PR1, PR2 and PR3, respectively; Table 14) of ‘Reef’ (including sediment veneers as potential reef) vs. ‘Not Reef’. The data was randomly split into training (80%) and test (20%) datasets to evaluate the accuracy of the map output. The Random Forest models were trained on the classified images using object mean values of the acoustic feature layers (see Table 12) as predictors. All other parameters were set to default. The layers used as predictors were selected based on variable importance in an initial run using all layers. Of highly correlated variables (>0.8) only the one with a higher importance was kept in the final model. Additionally, variables were removed on inspection of their response curves, where the curves were indicative of spurious overfitting. The models were then applied to the remaining objects to predict the probability of the 'Annex I Reef' habitat class based on their acoustic and topographic values. The probabilities were converted to 'Annex I Reef' vs. 'Not Reef' using a threshold determined by achieving equal Sensitivity and Specificity (i.e., output correctly identifies presences and absences equally well).

Table 14. Numbers of observations included in training and testing the Random Forest algorithms for mapping 'Annex I Reef'. The ratio of presences (P) to absences (A) in each dataset are given in parentheses.

	Train (P/A)	Test (P/A)	Total
PR1	168 (102/66)	43 (26/17)	211 (128/83)
PR2	464 (270/194)	117 (68/49)	581 (338/243)
PR3	176 (138/38)	44 (35/9)	220 (173/47)
Total	808 (510/298)	204 (129/75)	1012 (639/373)

Sub-bottom profiler data

The chirper system collected sub-bottom profiling imagery across the Pisces Reef Complex MPA. However, the vertical resolution of the imagery did not prove sufficient to estimate the depth of the sediment veneer over the reef to a resolution of 10 cm. The data could only be reliably resolved to 0.5 m resolution. It is believed that towing the chirper system at the surface rather than at depth reduced the capacity of the data to be processed to 10 cm horizontal resolution. This precluded the possibility of using this dataset to detect areas where hard substrata lay beneath a 10 cm thick veneer of sediment and where robust epifaunal taxa may have persisted despite the smothering of these sediment veneers.

Epifaunal data preparation

All imagery collected from the video and stills cameras was initially assessed to determine its 'image quality' according to a categorical scale recommended by the North-East Atlantic Marine Biological Analytical Quality Control Scheme (NMBAQC): 'Excellent', 'Good', 'Poor', 'Very poor' and 'Zero' (Turner *et al.* 2016). This quality score was used to filter data and improve its quality in the analysis (see next section for more details).

The main dataset extracted from the video imagery was benthic habitat data. Owing to its scale of operation, field of view (FOV) and motion capture qualities, video imagery is recommended as a good source of benthic habitat information for benthic community studies (van Rein *et al*. 2009). As the smallest unit of size for benthic habitats, biotopes, is 5 x 5 m (25 m²; Connor *et al.* 2004; Parry 2015), video imagery from each camera tow was divided up into segments approximately 5 m in length. This method enabled enumeration and quantification of benthic habitat information for the site. The JNCCs Marine Habitat Classification for Britain & Ireland scheme[4] (Connor *et al.* 2004; Parry 2015) was used to classify the benthic habitats within each segment to their lowest classification units possible, depending on the quality of the imagery.

The main dataset extracted from the stills imagery was community- and substratum-related data. Owing to its smaller scale of operation, relative to video, and capture of higher resolution imagery that facilitates the identification of benthic taxa, stills imagery is recommended as a good source of epifaunal community information for benthic community studies (van Rein *et al.* 2009). Taxa in the still images were identified to varying taxonomic levels, depending on image quality and resolution as well as the location of individuals in the image. Once identified, they were enumerated using a cell frequency of occurrence method,

[4] JNCC Marine Habitat Classification for Britain & Ireland, URL: http://jncc.defra.gov.uk/page-1584.

as recommended by Moore *et al.* (2019) for its statistical precision, high power, efficiency and levels of consistency relative to comparative methods. Using this method, the image area is first divided up by overlaying a 5 x 5 grid across it, composed of 25 equally sized 'cells'. Once taxa are identified their presence is only recorded from the cells in which they are observed. If an organism spans two cells it is recorded twice as it occurs in those two cells. The result for the image is that all taxa end up with an occurrence score up to a maximum of 25 cells: their frequency of occurrence.

There are three key advantages of this enumeration method over others:

- The analyst does not need to score colonial organisms differently from solitary individuals, a process that generates separate data sets for percentage cover and abundance data which in turn adds additional complexity to subsequent data analysis and interpretation.
- The data range of this single enumeration metric is constrained along a reduced scale relative, in this case 0 - 25. This has the dual effect of reducing data variability while also increasing the relative expression of cryptic/low occurrence taxa and reducing the dominance of spatially dominant taxa with high occurrence in the dataset. Alternatively, this effect can be achieved by carrying out square root and fourth root transformations of the data, a typical practice in marine ecological analysis studies (Clarke & Gorley 2015). These transformations are less necessary with frequency of occurrence data, thus better maintaining the original integrity of the epifaunal data.
- This enumeration method has been demonstrated to be more efficient to use relative to comparative methods (Moore *et al.* 2019).

A full list of the 53 taxa identified in the stills imagery can be seen in Annex 4. The FOV was also estimated for each still image using the laser dots in each image (for method see Wakefield & Genin 1987). The image FOV was used to filter data and improve its quality in the analysis.

Attempts were made to classify the substratum type (i.e., benthic habitat) from the still imagery, however, owing to the highly heterogenous nature of the substratum at Pisces Reef Complex MPA records were inconsistent and this data field was, therefore, removed from further analysis.

Still image quality assessment and data truncation

A number of data filtering measures were applied to improve the quality of the stills image data set. Firstly, only images of 'excellent' and 'good' quality were selected for subsequent analysis. Secondly, the remaining images were scrutinised to select only images with a FOV of less than, or equal to, 0.9 m^2. This threshold was chosen to optimise the number of still images per camera tow and the taxa to be retained for further analyses, as well as image quality (defined as the ability to identify taxa to the highest taxonomic detail possible). The remaining images were filtered by plotting them in ArcMap v10.5 (Environmental Systems Research Institute 2017) alongside existing Annex I Reef layers and selecting only images that occurred within known reef areas. From whichever images remained after these filtering measures were applied, only images that still contained biological taxa were retained for the 'structure and function' analyses. In combination, all these measures reduced the total number of image records from 2,082 to 1,249 across all three reefs (PR1, PR2 and PR3).

The list of taxa in the remaining 1,249 still images were truncated in two general steps to further improve the quality of the data before the analysis. Firstly, to provide a list of taxa that avoided grouping at low taxonomic levels to allow for a more accurate estimation of species richness and diversity. Secondly, by grouping taxa at lower taxonomic levels to maximise the taxon occurrence data retained for subsequent community analyses. All still image data that did not contain any of the truncated taxa was removed from the dataset, leaving data from 1069 still images for the analysis. A full breakdown of truncation protocol and steps taken to improve the quality of the stills data is provided in Annex 5.

As there were no reliable habitat classification information in the stills data, a taxonomic traits-driven approach was taken to determine which still images were collected from Reef areas at the site. The taxonomic traits associated with each of the truncated epifauna were assigned using the Cefas taxonomic traits database (Clare *at al.*, in prep). The traits associated with substratum attachment and how immobile (sessile) a taxon was were used to isolate those taxa most likely to occur at a Reef, in particular at a bedrock or stony reef. They formed a list of potential 'reef taxa', which was further informed using the experience and judgement of the reporting team to arrive at a final list of reef taxa from the site (Annex 4). Only still sample images that contained these reef taxa were selected for analysis of the Annex I Reef feature. Following this final data processing step, epifauna community data remained for a total of 870 still images unevenly distributed across all three reefs at Pisces Reef Complex MPA.

Numerical and Statistical Analyses

Basic summary analyses were carried out on the biotope data from the video imagery to show how many biotopes were recorded across the three reef areas. More detailed multivariate analyses were carried out using the truncated biological data from the stills imagery and environmental parameter data from the ESM2 logger and MBES. Relevant data were imported into PRIMER v7 (Clarke & Gorley 2015). These detailed analyses were divided into two parts, the first being exploratory community analysis based on the individual still image data to better understand the biological community structure, function and supporting processes of Pisces Reef. The second community analysis used aggregated still image data to make robust comparisons between the biological communities of Pisces Reef using appropriately sized sampling units (see later "Community comparison analysis" section for more details). These latter approaches aim to be repeatable for successive monitoring surveys of the Reef.

Exploratory community analysis

Owing to the heterogenous nature of the seabed at Pisces Reef, relationships in the biological data were first explored using the CLUSTER analysis with a SIMPROF test to determine if groupings existed within the stills data. A Bray-Curtis similarity matrix of the biological data was made to run these tests. No data transformations were applied before constructing this matrix because the biological data were generated using the cell frequency method. As stated before, this method creates data where the expressions of dominant taxa are already reduced and rare taxa are already increased, a similar effect to a low-level data transformation (Moore *et al.* 2019).

The SIMPROF test generated 50 distinct cluster groups although many of the groups did not contain many representative still images. Cluster groups with less than the median number of still images, 6 images, were removed from the analysis to reduce the number of groupings and improve the robustness of the analysis. This left 26 cluster groups for the analysis, now referred to as the dominant cluster groups.

To visualise the similarities in biological data between the remaining 26 dominant cluster groups they were plotted using non-metric multidimensional scaling (nMDS) ordination from another Bray-Curtis similarity matrix with no transformations. The SIMPER routine was also used to identify the taxa most responsible for the similarities within each cluster group, and dissimilarities between other groups (Clarke & Gorley 2015). Environmental parameter data were summarised, and taxonomic richness was calculated for each dominant cluster group, to better describe and understand the characteristics of each biological data cluster.

Further multivariate tests were conducted using the environmental parameter data to determine whether any or all of environmental factors were driving the patterns observed in the biological data (see Table 2 for list of environmental parameter data). A Draftsmans plot of the environmental parameter data showed similarity between the boulder and cobble categories; pebbles, sand and gravels; and shells, so these groups were merged into single categories to reduce the variability in the data set. The combined boulder and cobble data were slightly right-skewed, and in accordance with recommendations from Clarke and Gorley (2015), all substratum data, including those relating to sands, gravels and muds, were square root transformed. The resultant environmental parameter data were then normalised and the relationships with the biological data in the 26 dominant clusters were explored by constructing a distance-based linear model (DistLM; Anderson *et al.* 2008). The DistLM was tested by distance-based redundancy analysis (dbRDA), with the Stepwise procedure, to determine the optimum number of variables that explain the majority of variability in the biological data matrix. This performs a similar function to the BIOENV test in the BEST analysis routine, with the added benefit of also partitioning the proportion of data variability attributable to each environmental variable (Anderson *et al.* 2008). The most relevant results were tabulated or turned into figures to summarise the key points of the data analysis of the biological and environmental data in this study.

Community comparison analysis

After identifying the main functional communities of Pisces Reef in the exploratory analysis, sample units were assembled from the stills imagery data to enable robust comparisons between the two main functional communities: Reef community and Reef transition/veneer community. Taxonomic accumulation curves were plotted using all available still images representing each community. In total, there were 271 images for the Reef community and 590 images for the Reef transition/veneer community (Table 13).

For each community the asymptote value on each taxonomic accumulation curve was likely between 50 – 100 images. If still image data was accordingly aggregated into sample units of between 50 and 100 images each there would not be enough samples to enable comparison of communities between the three reefs at Pisces Reef, PR1, PR2 and PR3. As an alternative, it was decided to aggregate the still image data so that each analysis factor, Reef (PR1, PR2 and PR3) and Functional community (Reef and Transition/veneer) would have a minimum level of replication of three samples per design stratum. Accordingly, the still image data were aggregated into sampling units made up of randomised groups of twelve images each. A consequence of aggregating twelve individual still images, each with data ranges of 0 – 25 per taxon, was that the new aggregated sample units now had potential data ranges of 0 - 300 per taxon. Table 5 shows the parameters of these sampling units and number of replicates per design stratum.

Table 15. Sampling unit parameters for main Pisces Reef functional communities at each reef system, as indicated, when twelve still images are aggregated into one sample unit. Mean sample unit areas and standard deviations of the mean (SD) are calculated using the aggregated field of view for each sample.

Functional community	Sample information	Reef			Total
		PR1	PR2	PR3	
Reef community	Still images available	38	167	66	271
	Number of potential samples (N)	3.2	13.9	5.5	22.6
	Mean sample unit area (m^2; ±SD)	4.9 (0.1)	4.6 (0.3)	4.8 (0.5)	4.9 (0.3)
Reef transition/ veneer	Still images available	138	244	208	590
	Number of potential samples (N)	11.5	20.3	17.3	49.2
	Mean sample unit area (m^2; ±SD)	4.8 (0.3)	4.5 (0.5)	4.8 (0.3)	4.7 (0.4)
All reef communities	Still images available	176	411	274	861
	Number of potential samples (N)	14.7	34.3	22.8	71.8
	Mean sample unit area (m^2; ±SD)	4.9 (0.2)	4.5 (0.4)	4.8 (0.4)	4.7 (0.4)

A PERMANOVA test was used to compare the Reef community and Reef transition/veneer communities across the three reefs at Pisces Reef (Anderson *et al.* 2008). The PERMANOVA design had two fixed factors: Reef (PR1, PR2 and PR3) and Functional community (Reef and Transition/veneer). Only epifaunal taxa were selected for these tests. Before running the test, it was decided to square root transform the epifaunal data. Note that transformations were not needed in the exploratory analyses as data ranges were on a 0-25 scale and not a 0-300 scale, as they were once imagery data was aggregated into samples. The transformation not only reduced the expression of dominant taxa, while increasing the expression of rarer taxa (Clarke & Gorley 2015), resulting with data ranges along a 0 - 20 scale per taxon, similar to its original format as determined using the frequency of occurrence measure. A Bray-Curtis similarity matrix was constructed with these data to run the PERMANOVA test and all subsequent pair-wise tests between the experimental factors.

Annex 4. List of taxa identified in sample imagery (semi-truncated)

Table 16. Taxa identified from the still imagery collected across the Pisces Reef Complex MPA by Jenkins and Nelson (2017). No taxa have been removed from the list other than those that were merged with similar taxa, as indicated (hence the list is 'semi-truncated'). Total occurrence refers to the frequency of occurrence cells in which each taxon was recorded. Note there are 25 cells in an image and 1249 images in the dataset, therefore, 31225 cells in the dataset.

Phylum	Original Taxa	Total occurrence (1249 still images)		Reef taxa
		No. cells	Proportion (%)	
Annelida	*Chaetopterus variopedatus*	10	0.03	-
Annelida	*Sabella pavonina*	20	0.06	-
Annelida	Sabellidae	257	0.82	-
Annelida	Serpulidae	20	0.06	-
Annelida	Terebellomorpha	8	0.03	-
Arthropoda	Brachyura	17	0.05	-
Arthropoda	*Cancer pagurus*	20	0.06	-
Arthropoda	Caridea	534	1.71	-
Arthropoda	Crustacea	9	0.03	-
Arthropoda	Decapoda	133	0.43	-
Arthropoda	Galatheoidea	210	0.67	-
Arthropoda	Inachidae	54	0.17	-
Arthropoda	*Necora puber*	14	0.04	-
Arthropoda	*Nephrops norvegicus*	52	0.17	-
Arthropoda	Paguroidea	28	0.09	-
Arthropoda	Peracarida	419	1.34	-
Chordata	Ascidiacea	63	0.20	Reef taxon
Chordata	Demersal bony fish	765	2.45	-
Chordata	Pelagic bony fish	136	0.44	-
Chordata	Scyliorhinidae	48	0.15	-
Cnidaria	Actiniaria	64	0.20	Reef taxon
Cnidaria	*Adamsia palliata*	2	0.01	-
Cnidaria	Anthozoa	57	0.18	Reef taxon
Cnidaria	*Arachnanthus sarsi*	3	0.01	-
Cnidaria	*Caryophyllia*	910	2.91	Reef taxon
Cnidaria	Ceriantharia	45	0.14	-
Cnidaria	Diphasia	60	0.19	Reef taxon
Cnidaria	Hydrozoa	3081	9.87	Reef taxon
Cnidaria	*Pachycerianthus multiplicatus*	45	0.14	-
Cnidaria	Zoantharia	14	0.04	Reef taxon
Echinodermata	*Asterias rubens*	14	0.04	-

Phylum	Original Taxa	Total occurrence (1249 still images)		Reef taxa
		No. cells	Proportion (%)	
Echinodermata	Asteroidea	28	0.09	-
Echinodermata	Crinoidea	85	0.27	Reef taxon
Echinodermata	Ophiuroidea	17	0.05	-
Echinodermata	Porania	13	0.04	-
Mollusca	Anomiidae	32	0.10	Reef taxon
Mollusca	Cephalopoda	6	0.02	-
Mollusca	Loliginidae	14	0.04	-
Mollusca	Nudibranchia	2	0.01	Reef taxon
Mollusca	Pectinidae	2	0.01	-
Porifera	Porifera Arborescent branching	170	0.54	Reef taxon
Porifera	Porifera Cuplike cups	111	0.36	Reef taxon
Porifera	Porifera Encrusting encrusting	219	0.70	Reef taxon
Porifera	Porifera Erect erect forms	268	0.86	Reef taxon
Porifera	Porifera Foliose laminar	30	0.10	Reef taxon
Porifera	Porifera Globular balls	15	0.05	Reef taxon
Porifera	Porifera Massive massive forms	227	0.73	Reef taxon
Porifera	Porifera Papilate cryptic	353	1.13	Reef taxon
Porifera	Porifera Pedunculate stalked	16	0.05	Reef taxon
Porifera	Porifera Solitary simple	136	0.44	Reef taxon
Porifera	Porifera Sp. generic	343	1.10	Reef taxon
NA	Animalia Crust/Meadow Faunal crust	1011	3.24	Reef taxon
NA	Animalia Massive/Turf Faunal turf	9275	29.70	Reef taxon

Reef taxa: show traits associated with attaching to substrata and being sessile, as well as being recognised as a reef-dweller by the reporting group (expert judgement applied).

Annex 5. Epifauna data truncation protocol applied to seabed imagery data

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

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

Specific details of the data preparation and truncation protocols applied to the epifaunal datasets acquired at Pisces Reef Complex MPA, conducted ahead of the analyses, are provided below:

- Where there are records of one named genus together with records of members of the same family (but the latter not identified to genus level) the entries are merged and the resulting entry retains only the name of the family (e.g., 'Marcopodia' was merged with 'Inachidae', now just one taxon to represent the whole family).
- Finfish records are usually removed from drop camera records. In this study they were retained to provide greater ecological context for the results. To improve the consistency of the taxa records all demersal bony fish were merged into one taxon, 'Demersal bony fish', all pelagic bony fish were also merged into one taxon, 'Pelagic bony fish', and the remaining finfish records, from the 'Scyliorhinidae', were merged under that taxon.
- Records of rare taxa, i.e., 2 records of 'Bryozoa', were merged with general growth forms to improve consistency, i.e., 'Animalia Crust/Meadow Faunal crust'.
- Where records carried very poor taxonomic information they were removed (e.g., 'Animalia Indet. non identifiable).
- Where taxa occurrences are low merge taxa with higher taxonomic level to improve data quality and consistency (e.g., 2 records of 'Octopoda' merged with 'Cephalopoda' class).
- Where there were no higher taxonomic levels to raise a taxon to in the taxa matrix records of one very rare taxon were removed (e.g., 2 occurrences of 'Sedentaria' were removed to improve consistency throughout the dataset).

Annex 6. Environmental parameter data

Table 17. Summaries of environmental parameters recorded across PR1 at Pisces Reef Complex MPA. Means and standard deviations of the means (SD) are calculated from values recorded by ESM2 logger on drop frame deployed at each sampling station (as indicated).

Reef	Station	Temperature (°C)		Salinity (PSU)		O2 Concentration (%)		Suspended solids (FTU)		Chlorophyll (µg/l)	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
PR1	1	13.71	0.75	32.60	6.68	256.09	18.54	55.91	306.01	0.34	0.03
PR1	2	13.72	0.77	32.50	6.95	253.38	18.81	13.84	2.90	0.33	0.03
PR1	3	13.73	0.64	32.69	6.51	255.92	19.17	71.03	325.96	0.31	0.03
PR1	4	13.81	0.38	32.68	6.55	251.96	16.76	25.00	124.11	0.41	0.03
PR1	5	13.70	0.74	31.66	8.58	253.86	22.78	62.81	262.53	0.32	0.03
PR1	6	13.70	0.77	31.85	8.24	254.18	21.61	43.40	174.13	0.32	0.03
PR1	7	13.69	0.85	32.35	7.24	254.40	18.24	34.78	148.86	0.32	0.03
PR1	8	13.51	1.03	30.58	10.19	260.77	28.20	189.83	560.69	0.31	0.04
PR1	9	13.69	0.76	32.55	6.83	255.17	18.47	88.62	375.48	0.31	0.03
PR1	10	13.73	0.59	32.29	7.41	257.33	21.22	12.14	3.11	0.43	0.05
PR1	11	13.62	0.90	31.74	8.42	256.78	22.93	155.06	545.75	0.32	0.03
PR1	12	13.74	0.67	32.58	6.80	253.49	17.89	54.26	275.04	0.35	0.03
PR1	13	13.78	0.49	32.70	6.49	254.19	16.66	45.87	250.55	0.39	0.03
PR1	14	13.63	0.53	31.14	9.43	259.17	21.47	178.02	559.97	0.25	0.04
PR1	15	13.65	0.54	31.95	8.08	257.86	18.25	68.55	248.79	0.26	0.04
PR1	16	13.62	0.60	31.49	8.85	259.10	21.67	116.72	419.29	0.26	0.04
PR1	17	13.66	0.55	31.92	8.13	256.95	18.07	101.19	410.72	0.25	0.04
PR1	18	13.67	0.49	32.26	7.46	256.59	16.84	85.63	339.72	0.26	0.04
PR1	19	13.64	0.60	31.66	8.55	258.23	20.81	31.73	122.83	0.27	0.04
PR1	20	13.67	0.50	32.09	7.80	257.05	18.04	61.35	293.94	0.25	0.04
PR1	21	13.64	0.54	31.68	8.56	257.72	18.60	112.29	394.02	0.26	0.04

Units: °C = degrees Celsius; PSU = Practical Salinity Unit; FTU = Formazin Turbidity Unit.

Table 18. Summaries of environmental parameters recorded across PR2 at Pisces Reef Complex MPA. Means and standard deviations of the means (SD) are calculated from values recorded by ESM2 logger on drop frame deployed at each sampling station (as indicated).

Reef	Station	Temperature (°C)		Salinity (PSU)		O2 Concentration		Suspended solids (FTU)		Chlorophyll (µg/l)	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
PR2	1	13.75	0.51	32.40	7.15	254.81	19.73	12.92	3.49	0.29	0.05
PR2	2	13.74	0.51	32.46	7.02	255.47	19.59	13.19	6.83	0.28	0.03
PR2	3	13.71	0.61	32.20	7.58	254.87	19.53	13.49	3.89	0.30	0.04
PR2	4	13.73	0.58	32.34	7.26	256.40	18.86	12.43	3.69	0.32	0.04
PR2	5	13.72	0.67	32.29	7.35	255.10	20.27	12.87	6.66	0.31	0.04
PR2	6	13.73	0.61	32.41	7.11	255.55	19.46	12.36	3.07	0.30	0.03
PR2	7	13.72	0.53	32.21	7.54	255.53	20.44	28.48	91.12	0.29	0.04
PR2	8	13.62	0.99	31.64	8.57	255.87	22.37	12.02	3.42	0.31	0.05
PR2	9	13.78	0.38	32.60	6.70	254.63	17.54	12.62	2.67	0.30	0.04
PR2	10	13.74	0.51	32.34	7.29	254.93	20.48	13.13	3.06	0.29	0.04
PR2	11	13.68	0.70	32.27	7.43	255.72	19.98	101.76	411.20	0.29	0.04
PR2	12	13.74	0.52	32.43	7.07	255.19	20.59	67.55	251.32	0.30	0.04
PR2	13	13.74	0.57	32.88	5.99	254.10	16.95	69.02	328.61	0.28	0.04
PR2	14	13.74	0.50	32.55	6.82	255.20	18.04	64.15	255.37	0.28	0.03
PR2	15	13.71	0.60	32.46	7.03	255.25	18.97	58.42	221.53	0.29	0.04
PR2	16	13.73	0.58	32.57	6.77	254.89	18.08	51.62	192.81	0.29	0.04
PR2	17	13.75	0.54	32.72	6.40	254.14	16.60	57.12	300.50	0.27	0.03
PR2	18	13.69	0.67	32.06	7.85	255.73	20.73	60.08	202.35	0.27	0.04
PR2	19	13.74	0.55	32.30	7.26	255.92	19.51	83.27	335.80	0.28	0.05
PR2	20	13.68	0.66	31.55	8.71	258.17	22.81	115.03	410.15	0.27	0.04
PR2	21	13.69	0.66	31.75	8.36	256.54	20.08	108.71	427.06	0.27	0.04
PR2	22	13.61	0.80	31.68	8.51	257.28	21.11	17.55	64.94	0.23	0.04
PR2	23	13.75	0.42	33.05	5.52	253.60	13.96	22.14	104.52	0.24	0.03
PR2	24	13.70	0.63	32.98	5.73	254.14	14.37	28.77	123.65	0.24	0.03
PR2	25	13.69	0.65	33.05	5.57	254.37	13.93	14.47	13.73	0.25	0.04

Units: °C = degrees Celsius; PSU = Practical Salinity Unit; FTU = Formazin Turbidity Unit.

Table 19. Summaries of environmental parameters recorded across PR3 at Pisces Reef Complex MPA. Means and standard deviations of the means (SD) are calculated from values recorded by ESM2 logger on drop frame deployed at each sampling station (as indicated).

Reef	Station	Temperature (°C)		Salinity (PSU)		O2 Concentration		Suspended solids (FTU)		Chlorophyll (µg/l)	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
PR3	1	13.45	0.87	31.74	8.42	259.24	19.22	79.02	254.34	0.29	0.05
PR3	2	13.57	0.58	32.43	7.05	257.58	18.99	61.76	333.45	0.30	0.04
PR3	3	13.33	0.91	30.31	10.53	263.69	27.09	11.64	4.54	0.28	0.05
PR3	4	13.49	0.82	32.14	7.66	258.24	18.17	12.37	5.34	0.28	0.05
PR3	5	13.46	0.82	31.91	8.11	259.45	21.32	12.40	3.72	0.28	0.04
PR3	6	13.36	0.99	31.18	9.32	261.09	22.66	11.89	3.63	0.29	0.05
PR3	7	13.48	0.78	31.59	8.66	259.46	21.14	12.10	4.23	0.28	0.05
PR3	8	13.57	0.58	32.64	6.58	257.13	18.23	27.65	121.22	0.29	0.04
PR3	9	13.45	0.75	31.97	7.99	259.27	19.81	12.24	3.25	0.29	0.05
PR3	10	13.51	0.69	32.41	7.12	258.23	18.55	12.43	2.80	0.28	0.04
PR3	11	13.50	0.79	32.26	7.45	258.15	17.68	41.33	173.94	0.29	0.05
PR3	12	13.59	0.52	32.71	6.38	257.18	16.41	29.05	120.68	0.30	0.04
PR3	13	13.41	0.81	31.26	9.20	261.28	23.21	17.32	42.70	0.28	0.05
PR3	14	13.49	0.69	32.35	7.22	258.52	18.19	12.56	2.96	0.29	0.05
PR3	15	13.54	0.68	32.39	7.14	258.16	19.59	63.68	326.57	0.31	0.05
PR3	16	13.56	0.62	32.56	6.78	257.09	17.14	12.45	2.73	0.28	0.04
PR3	17	13.57	0.46	32.94	5.82	256.56	13.52	12.41	2.25	0.28	0.04

Units: °C = degrees Celsius; PSU = Practical Salinity Unit; FTU = Formazin Turbidity Unit.

Annex 7. Marine litter categories

Table 20. Categories and sub-categories of litter items for sea floor from the OSPAR/ICES/IBTS for North East Atlantic and Baltic. Guidance on Monitoring of Marine Litter in European Seas, a guidance document within the Common Implementation Strategy for the Marine Strategy Framework Directive, MSFD Technical Subgroup on Marine Litter 2013.

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

Related size categories

A: ≤ 5*5 cm = 25 cm^2

B: ≤ 10*10 cm = 100 cm^2

C: ≤ 20*20 cm = 400 cm^2

D: ≤ 50*50 cm = 2500 cm^2

E: ≤ 100*100 cm = 10000 cm^2

F: ≥ 100*100 cm = 10000 cm^2

Annex 8. Non-indigenous species (NIS) lists

The epifaunal taxon lists generated from 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 (Stebbing *et al.* 2014) and identified as significant by the GB Non-Native Species Secretariat (Table 21; Table 22).

Table 21. 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		

Species name	List	Species name	List
Watersipora subatra	Present		

Table 22. Additional taxa listed as non-indigenous species in the JNCC 'Non-native marine species in British waters: a review and directory' report by Eno *et al.* (1997) which have not been selected for assessment of Good Environmental Status in GB waters under MSFD Descriptor 2.

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

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

Cefas

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