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East of Haig Fras Marine Conservation Zone (MCZ) Monitoring Report 2015

Clare, D., Downie, A., Hawes, J. & Langton, B.

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For further information please contact:

Joint Nature Conservation Committee
Monkstone House
City Road
Peterborough PE1 1JY
www.jncc.gov.uk

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

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London
SW1P 3JR

Please Note:

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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).

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Abbreviations

BSH	Broadscale Habitat
Cefas	Centre for Environment, Fisheries and Aquaculture Science
CP2	Charting Progress 2
CTD	Conductivity, Temperature and Depth
Defra	Department for Environment, Food and Rural Affairs
DEM	Digital Elevation Model
EA	Environment Agency
EUNIS	European Nature Information System
FOCI	Feature of Conservation Interest
GES	Good Environmental Status
IFCA	Inshore Fisheries and Conservation Authority
JNCC	Joint Nature Conservation Committee
NMBAQC	North East Atlantic Marine Biological Analytical Quality Control Scheme
MBES	Multibeam echosounder
MCZ	Marine Conservation Zone
MPA	Marine Protected Area
MPAG	Marine Protected Areas Survey Coordination and Evidence Group
MSFD	Marine Strategy Framework Directive
NE	Natural England
NIS	Non-Indigenous Species
OSPAR	The Convention for the Protection of the Marine Environment of the North-East Atlantic
PSA	Particle Size Analysis
PSD	Particle Size Distribution
RV	Research Vessel
SAC	Special Area of Conservation
SNCB	Statutory Nature Conservation Body
SSS	Sidescan sonar

Glossary

Definitions signified by an asterisk (*) have been sourced from Natural England and JNCC Ecological Network Guidance (NE & JNCC 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.*
Broadscale Habitats	Habitats which have been broadly categorised based on a shared set of ecological requirements, aligning with level 3 of the EUNIS habitat classification. Examples of Broadscale Habitats are protected across the MCZ network.
Community	A general term applied to any grouping of populations of different organisms found living together in a particular environment; essentially the biotic component of an ecosystem. The organisms interact and give the community a structure (Allaby 2015).
Conservation Objective	A statement of the nature conservation aspirations for the feature(s) of interest within a site, and an assessment of those human pressures likely to affect the feature(s).*
EC Habitats Directive	The EC Habitats Directive (Council Directive 92/43/EEC on the Conservation of natural habitats and of wild fauna and flora) requires Member States to take measures to maintain natural habitats and wild species of European importance at, or restore them to, favourable conservation status.
Epifauna	Fauna living on the seabed surface.
EUNIS	A European habitat classification system, covering all types of habitats from natural to artificial, terrestrial to freshwater and marine.*
Favourable Condition	When the ecological condition of a species or habitat is in line with the conservation objectives for that feature. The term ‘favourable’ encompasses a range of ecological conditions depending on the objectives for individual features.*
Feature	A species, habitat, geological or geomorphological entity for which an MPA is identified and managed.*

Feature Attributes	Ecological characteristics defined for each feature within site-specific Supplementary Advice on Conservation Objectives (SACO). Feature Attributes are monitored to determine whether condition is favourable.
Features of Conservation Importance (FOCI)	Habitats and species that are rare, threatened or declining in Secretary of State waters.*
Habitats of Conservation Importance (HOCI)	Habitats that are rare, threatened, or declining in Secretary of State waters.*
Impact	The consequence of pressures (e.g. habitat degradation) where a change occurs that is different to that expected under natural conditions (Robinson *et al.* 2008).*
Infauna	Fauna living within the seabed sediment.
Joint Nature Conservation Committee (JNCC)	The statutory advisor to Government on UK and international nature conservation. Its specific remit in the marine environment ranges from 12 - 200 nautical miles offshore.
Marine Strategy Framework Directive (MSFD)	The MSFD (EC Directive 2008/56/EC) aims to achieve Good Environmental Status (GES) of EU marine waters and to protect the resource base upon which marine-related economic and social activities depend.
Marine Conservation Zone (MCZ)	MPAs designated under the Marine and Coastal Access Act (2009). MCZs protect nationally important marine wildlife, habitats, geology and geomorphology, and can be designated anywhere in English and Welsh inshore and UK offshore waters.*
Marine Protected Area (MPA)	A generic term to cover all marine areas that are 'A clearly defined geographical space, recognised, dedicated and managed, through legal or other effective means, to achieve the long-term conservation of nature with associated ecosystem services and cultural values' (Dudley 2008).*
Natura 2000	The EU network of nature protection areas (classified as Special Areas of Conservation and Special Protection Areas), established under the 1992 EC Habitats Directive.*
Natural England	The statutory conservation advisor to Government, with a remit for England out to 12 nautical miles offshore.
Non-indigenous Species	A species that has been introduced directly or indirectly by human agency (deliberately or otherwise) to an area where it has not occurred in historical times and which is separate from and lies outside the area where natural range extension could be expected (Eno *et al.* 1997).*

Pressure	The mechanism through which an activity has an effect on any part of the ecosystem (e.g. physical abrasion caused by trawling). Pressures can be physical, chemical or biological, and the same pressure can be caused by a number of different activities (Robinson *et al.* 2008).*
Special Areas of Conservation	Protected sites designated under the European Habitats Directive for species and habitats of European importance, as listed in Annex I and II of the Directive.*
Species of Conservation Importance (SOCI)	Habitats and species that are rare, threatened or declining in Secretary of State waters.*
Supplementary Advice on Conservation Objectives (SACO)	Site-specific advice providing more detailed information on the ecological characteristics or 'attributes' of the site's designated feature(s). This advice is issued by Natural England and/or JNCC.

Executive Summary

This report is one of a series of Marine Protected Area (MPA) characterisation and monitoring reports delivered to Defra by the Marine Protected Areas Survey Coordination and Evidence Group (MPAG). The purpose of the report series is to provide the necessary information to allow Defra to fulfil its obligations in relation to MPA assessment and reporting, in relation to current policy instruments, including the Oslo-Paris (OSPAR) Convention, the UK Marine and Coastal Access Act (2009) and Community Directives (e.g. the Habitats and Birds Directives and the Marine Strategy Framework Directive). This monitoring report is informed by data acquired during a dedicated survey carried out at the East of Haig Fras Marine Conservation Zone (MCZ) in 2015 and will form part of the ongoing monitoring time series for this MPA.

The East of Haig Fras MCZ is an offshore MPA located 67km northwest of Land's End, in the 'Western Channel and Celtic Sea' Charting Progress 2 (CP2) area. Five Broadscale Habitats (BSHs) were designated for protection in 2013 and 2016; 'A4.2 Moderate energy circalittoral rock', 'A5.1 Subtidal coarse sediment / A5.4 Subtidal mixed sediments mosaic', 'A5.2 Subtidal sand' and 'A5.3 Subtidal mud'. In 2019, the following additional features were designated; the habitat Feature of Conservation Importance (FOCI) 'Sea-Pen and Burrowing Megafauna Communities', the species FOCI *Atrina fragilis* (Fan Mussel) and the BSH 'A4.1 High energy circalittoral rock'. This report provides a characterisation of BSHs that were designated in 2013 and 2016 and presents additional evidence on the presence and distribution of the FOCI designated in 2019.

There was substantial overlap in both epifaunal and infaunal community composition across sediment BSHs, with assemblages from different BSHs often clustering together. This variability within and between BSHs appears to be partly due to species responding to variation in sediment components (i.e. gravel, sand and mud contents for infauna; boulder, cobble and pebble contents for epifauna) in a manner that is inconsistent with how the proportions of sediment components are used to classify BSH types. The clustering together of assemblages from different sediment BSHs precluded the identification of biotopes in most cases. For rock habitat, all assemblages were matched to the biotope 'Echinoderms and crustose communities.'

Taxa indicative of the habitat FOCI 'Sea-Pen and Burrowing Megafauna Communities' (i.e. *Callianassa subterranea*, *Goneplax rhomboides* and *Virgularia mirabilis*) were directly observed in the western half of the site. Burrows were also observed in densities sufficient for the classification of this FOCI at eight stations, and at three stations the Sea-Pen *Virgularia mirabilis* was observed. In most instances, these taxa were not found in the fine muddy substrate with which they are typically associated. However, this may be because 'A5.3 Subtidal mud' was not targeted with the camera during the survey. The species FOCI *A. fragilis* was observed in 37 images taken across 29 stations (out of 162 stations, 18%).

Operational and sampling design recommendations for future monitoring within the East of Haig Fras MCZ (and other comparable sites) are provided.

1 Introduction

The East of Haig Fras Marine Conservation Zone (MCZ) is part of a network of sites designed to meet conservation objectives under the UK Marine and Coastal Access Act (2009). These sites will also contribute to an ecologically coherent network of Marine Protected Areas (MPAs) across the North-east Atlantic agreed under the Oslo-Paris (OSPAR) Convention and other international commitments to which the UK is a signatory.

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

This report primarily explores data acquired from the first dedicated monitoring survey of the East of Haig Fras MCZ, conducted in 2015. These data form the initial point in a monitoring time series, against which feature (and site) condition can be assessed in the future. The specific aims of the report are described in detail in Sections 1.3.3 and 1.3.4.

1.1 Site overview

The East of Haig Fras MCZ is an offshore site located approximately 67 km northwest of Land's End in the Celtic Sea, which covers an area of 409km^2 (Figure 1). The site is located within the 'Charting Progress 2' (CP2) area 9 'Western Channel and Celtic Sea' and is neighboured by the Haig Fras Special Area of Conservation (SAC) and various other offshore and coastal MPAs (Figure 1). East of Haig Fras was recommended as an MCZ by the 'Finding Sanctuary' regional stakeholder group project (Lieberknecht *et al.* 2011).

The seabed within the East of Haig Fras MCZ is heterogeneous, with small patches of different habitats blending into each other (Eggleton & Downie 2017). Ridges comprising a mosaic of coarse and mixed sediments run through the site and are separated by mobile sand or mud. The tops of the sediment ridges consist of cobbles and boulders, providing hard substrata for attached fauna, including hydroids and bryozoans, sponges and cup corals, as well as providing niches for crevice-dwelling animals such as squat lobsters. Pea Urchins (*Echinocyamus pusillus*), small sea urchins which reach 1cm in diameter, are among the most common species living in the sediments, while other echinoderms such as brittle stars and cushion stars are present in lower numbers (Allen *et al*. 2016). The sediments are also home to diverse worm species.

The Broadscale Habitats (BSHs) and Features of Conservation Importance (FOCI) designated in East of Haig Fras MCZ are shown in Table 1 (with corresponding EUNIS habitat codes). This report was written prior to the designation of three additional features in 2019, and primarily focuses on the features specified in the 2016 designation ('A4.2 Moderate energy circalittoral rock', 'A5.1 Subtidal coarse sediment / A5.4 Subtidal mixed sediments mosaic', 'A5.2 Subtidal sand' and 'A5.3 Subtidal mud').

Table 1. East of Haig Fras MCZ site overview.

Charting Progress 2 Region[1]	Western Channel and Celtic Sea			
Spatial Area (km²)	409			
Water Depth Range (m)	50-100			
Year of Designation		**2013**[2]	**2016**[3]	**2019**[4]
Broadscale Habitat (BSH) Features	**EUNIS code**			
High energy circalittoral rock	A4.1	✗	✗	✓
Moderate energy circalittoral rock	A4.2	✓	✓	✓
Subtidal coarse sediment / Subtidal mixed sediments mosaic	A5.1 / A5.4	✓	✓	✓
Subtidal sand	A5.2	✓	✓	✓
Subtidal mud	A5.3	✗	✓	✓
Habitat FOCI				
Sea-Pen and Burrowing Megafauna Communities		✗	✗	✓
Species FOCI				
Atrina fragilis (Fan Mussel)		✗	✗	✓

[1] http://webarchive.nationalarchives.gov.uk/20141203170558tf_/http://chartingprogress.defra.gov.uk/ [Accessed 19/06/2018]
[2] The East of Haig Fras MCZ Designation Order 2013 http://www.legislation.gov.uk/ukmo/2013/7/pdfs/ukmo_20130007_en.pdf [Accessed 08.08.19]
[3] The East of Haig Fras Designation (Amendment) Order 2016 http://www.legislation.gov.uk/ukmo/2016/26/pdfs/ukmo_20160026_en.pdf [Accessed 08.08.19]
[4] The East of Haig Fras Designation (Amendment) Order 2019 http://www.legislation.gov.uk/ukmo/2019/14/pdfs/ukmo_20190014_en.pdf [Accessed 08.08.19]

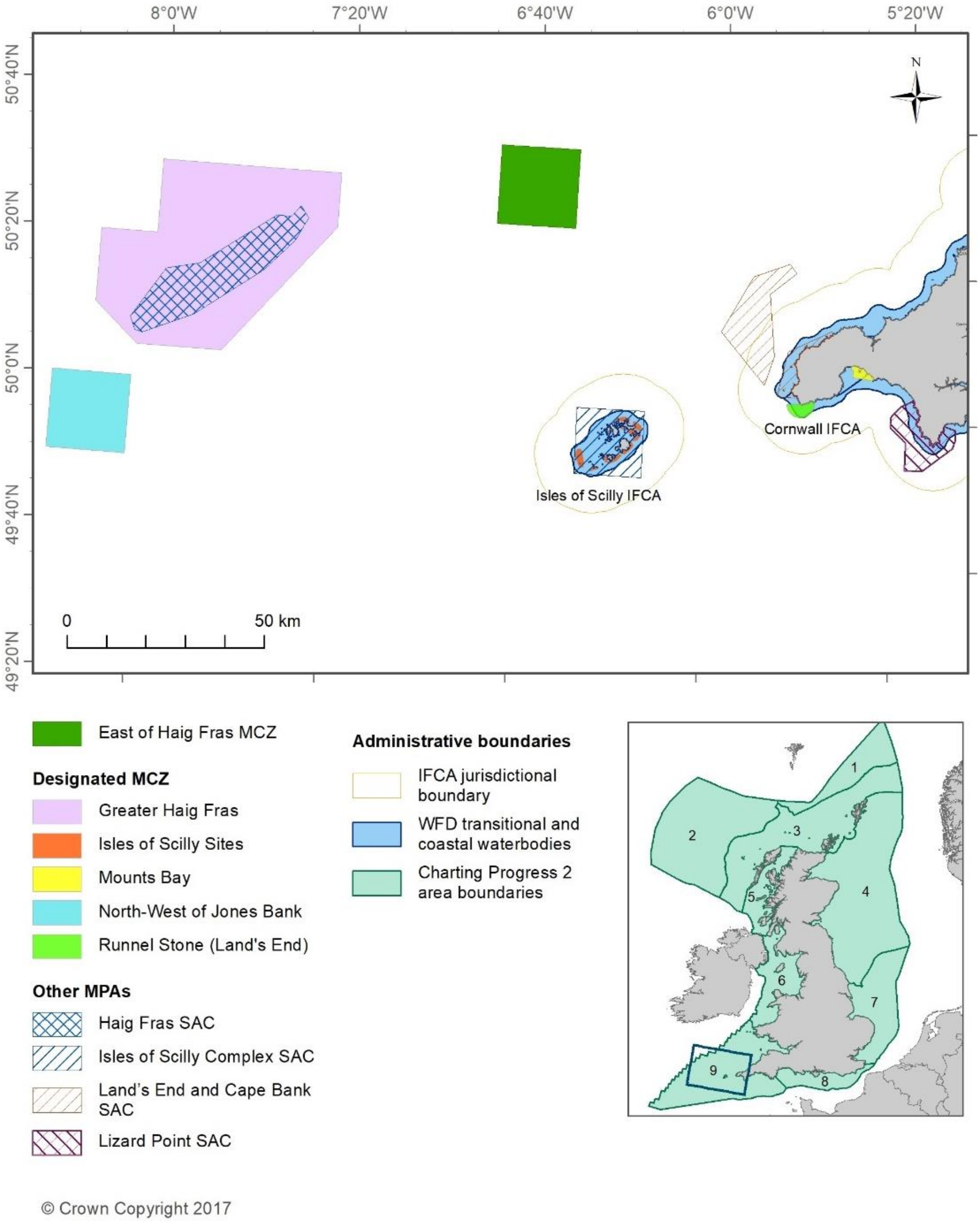

Figure 1. Location of the East of Haig Fras MCZ in the context of other Marine Protected Areas and management jurisdictions proximal to the site.

1.2 Existing data and habitat maps

Multibeam echosounder (MBES) bathymetry data, backscatter data, sidescan sonar (SSS) data and ground truth samples were collected at the East of Haig Fras MCZ between February 2012 and April 2013. MBES data were collected by Gardline Geosurvey Ltd, whereas SSS data and groundtruthing samples were collected by Cefas and JNCC on the RV *Cefas Endeavour*. Groundtruthing was conducted using a 0.1m^2 Hamon Grab (48 samples in 2012) and drop-camera/camera sledge (22 tows in 2012 and 19 tows in 2013). Visual interpretation of seabed imagery and the results of particle size analysis were used to classify stations according to their BSH (Allen *et al*. 2016).

The BSH map used in this report (presented in section 3.1.2) was produced using data collected during site verification surveys. The map is a result of a combination of object-based image analysis (OBIA) and statistical modelling of acoustic and ground truth data (Eggleton & Downie 2017).

1.3 Aims and objectives

1.3.1 High-level conservation objectives

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

As detailed in the East of Haig Fras MCZ site designation order from 2013[2] and 2016[3], the conservation objectives for the site are that the designated features;

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

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

1.3.2 Definition of favourable condition

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

a) Its extent and distribution are stable or increasing;

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

c) Its natural supporting processes[5] are unimpeded.

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

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[5] http://jncc.defra.gov.uk/pdf/EastHaigFras_SACO_V1.0.pdf [accessed 29/11/2018]

Structure encompasses the physical components of a habitat type and the key and influential species present. Physical structure refers to topography, sediment composition and distribution. Physical structure can have a significant influence on the hydrodynamic regime operating at varying spatial scales in the marine environment, as well as influencing the presence and distribution of associated biological communities (Elliott *et al*. 1998). The function of habitat features includes processes such as: sediment reworking (e.g. through bioturbation) and habitat modification, primary and secondary production and recruitment dynamics. Habitat features rely on a range of supporting processes (e.g. hydrodynamic regime, water quality and sediment quality) which act to support their functioning as well as their resilience (e.g. the ability to recover following impact).

1.3.3 Report aims and objectives

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

The broad objectives of this monitoring report are provided below:

1) Provide a description of the extent[6] and distribution and structural attributes of the designated features within the site (see section 1.3.4, Table 2 for more detail);

2) Present any available evidence on the supporting processes of the designated features of the site (see section 1.3.4, Table 2 for more detail);

3) Note observations of any habitat or species FOCI present in the site;

4) Present evidence relating to the presence and distribution of non-indigenous species (Descriptor 2), to satisfy requirements of the MSFD;

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

1.3.4 Reporting sub-objectives

To achieve report objectives 1 and 2, the report will present evidence on several feature attributes and supporting processes, as defined in supplementary advice on conservation objectives (SACOs)[5] developed by JNCC for the designated BSH features within the East of Haig Fras MCZ. It should be noted that it was not possible to address all feature attributes in the monitoring survey design, given the comprehensive nature of the attribute lists for each feature. The feature attributes were therefore rationalised and prioritised, resulting in a smaller sub-set. The sub-objectives relating to feature attributes (report objective 1) and supporting processes (report objective 2) considered in this report are presented in Table 2.

[6] Note that where current habitat maps are not available extent is described within the limits of available data.

Table 2. Survey elements and report outputs aligned with the feature attributes and supporting processes.

Sub-objective	Feature attribute	Features
Generate a habitat map to determine the spatial distribution of designated BSHs within the MCZ.	Extent and distribution	A4.2 Moderate energy circalittoral rock A5.1 Subtidal coarse sediment A5.2 Subtidal sand A5.3 Subtidal mud A5.4 Subtidal mixed sediments
Assess the composition and distribution of rock within the MCZ	Physical structure: rock composition and distribution	A4.2 Moderate energy circalittoral rock
Assess the composition and distribution of sediments within the MCZ	Physical structure: sediment composition and distribution	A5.1 Subtidal coarse sediment A5.2 Subtidal sand A5.3 Subtidal mud A5.4 Subtidal mixed sediments
Assess variation in composition, density and diversity of biological communities within and between Broadscale Habitats Assign biotopes (where possible) Identify spatial patterns in biological communities Identify key structural and influential species	Biological structure: characteristic communities Biological structure: key and influential species	A4.2 Moderate energy circalittoral rock A5.1 Subtidal coarse sediment A5.2 Subtidal sand A5.3 Subtidal mud A5.4 Subtidal mixed sediments
Produce a tidal model for the site.	Supporting processes: energy and exposure	East of Haig Fras MCZ

2 Methods

2.1 Survey design

The monitoring survey was jointly conducted by Cefas and JNCC onboard the RV *Cefas Endeavour* in May 2015 (CEND0915; Callaway 2015). A drop camera was used to target epifauna associated with rocky substrate and a Hamon Grab and Day Grab (both $0.1m^2$) were used to sample infauna in soft sediments. A Conductivity, Temperature and Depth (CTD) probe was used to measure depth at all stations.

Using data collected within the MCZ during the habitat verification survey (Eggleton & Downie 2017), a power analysis was performed to predict how many grab samples of each BSH are required to have an 80% chance of detecting a 20% change over time in various biotic indices, at a statistical significance of $p < 0.05$ (thresholds were selected as per Noble-James *et al*. 2017). Indices included total abundance, total number of species (species richness) and other diversity measures. The BSHs 'A5.1 Subtidal coarse sediment' and 'A5.4 Subtidal mixed sediments' were merged together in the power analysis due to the difficulty in distinguishing between these habitats using the available acoustic data. This prevented these two habitats being targeted separately in the survey and is why they are considered together as a habitat mosaic in the site Designation Order (see Table 1). Results of the power analysis suggested that more samples were required for total abundance than for other indices due to the relatively high variation in taxa abundances across samples. Results for total abundance were therefore used to inform the sampling strategy and ensure that the ability to detect change over time is high for all indices considered, should the same survey design be repeated in the future.

A total of 138 stations were sampled using the drop camera and 257 were sampled using the Hamon Grab (Figure 2). The fine-scale spatial variability in substrate at the site typically resulted in camera transects crossing several BSHs. Therefore, individual transects often produced 'sampling stations' for more than one BSH. In some cases, transects yielded insufficient still images of acceptable quality to allow quantitative epifaunal analysis for any BSH. Those stations (shown as white crosses in Figure 2) were excluded from community analysis but were included in the reporting of visually assigned biotopes. The procedure for selection and sub-setting camera transects is explained in detail in section 2.3.3 and Annex 1. Of the 257 stations sampled with the Hamon Grab, 34 were located on 'A5.1 Subtidal coarse sediment', 77 on 'A5.4 Subtidal mixed sediments', 93 on 'A5.2 Subtidal sand' and 53 on 'A5.3 Subtidal mud' based on Particle Size Analysis (PSA) of the sediment sub-samples taken from each grab sample (see sections 2.2.2 and 2.3.2). Fifteen additional samples were collected using the Day Grab from stations where the sediment appeared to be 'A5.3 Subtidal mud' upon visual inspection of the Hamon Grab sample, to allow the efficacy of the two grabs at sampling this habitat to be compared (see sections 2.3.2 and 2.3.4) A detailed description of the survey design is provided in Callaway (2015).

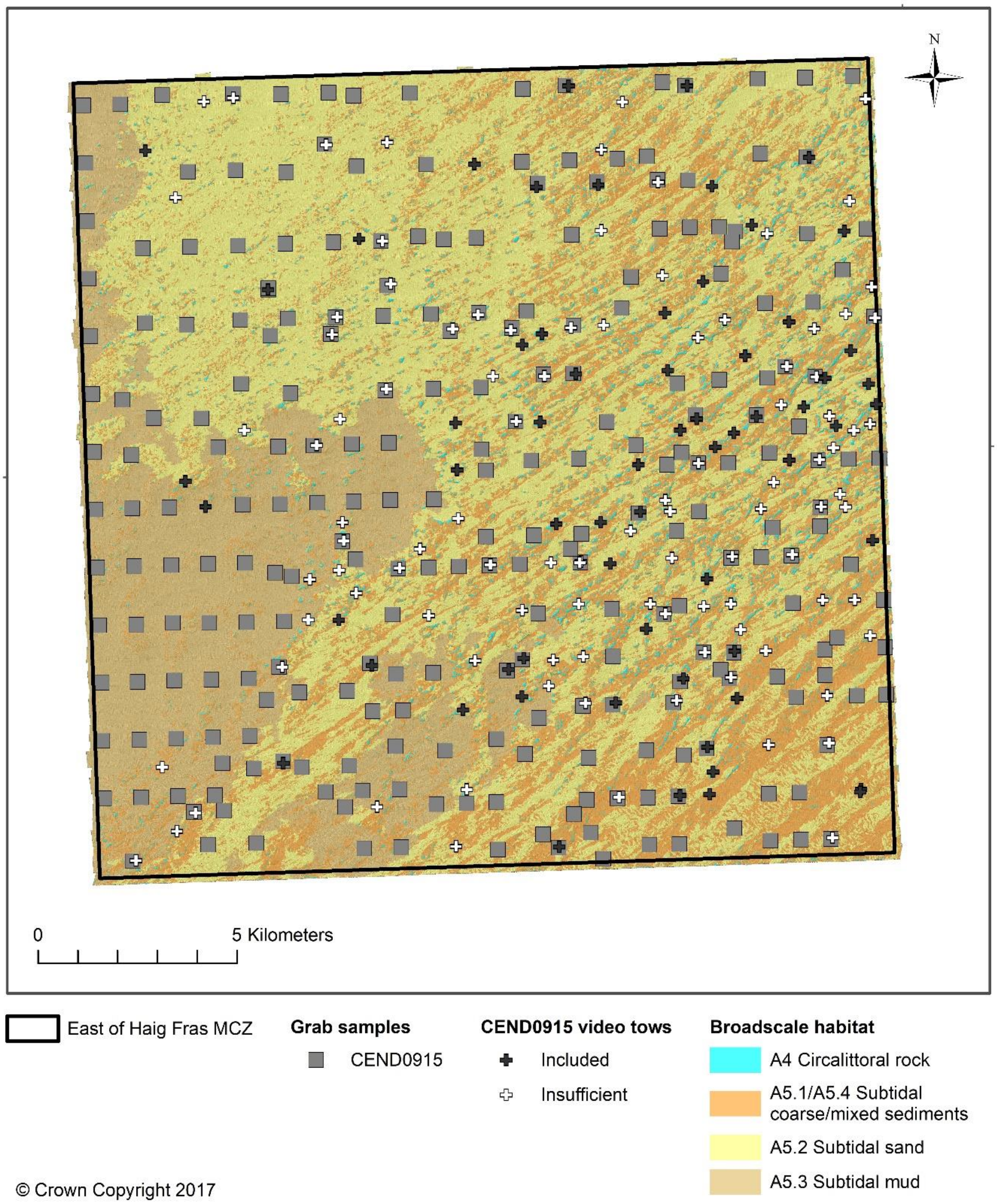


Figure 2. Location of ground truth samples collected at East of Haig Fras MCZ in 2015 (CEND0915). Camera transects included in quantitative analysis are shown in black, whereas camera transects with an insufficient number of still images for quantitative analysis are shown in white. The underlying Broadscale Habitat (BSH) map is from Eggleton and Downie (2017).

2.2 Data acquisition and processing

2.2.1 Seabed imagery

Seabed imagery data were collected using a Kongsberg drop camera system mounted on a frame. Seabed imagery data (videos and stills) were collected to contribute to the characterisation of epifaunal communities associated with both the rock and sediment habitat features. All data were collected following MESH Recommended Operating Guidelines (ROG) (Coggan *et al*. 2007). Full details on the camera system can be found in the East of Haig Fras survey report (Callaway 2015). Images of the seabed were acquired every 10-15m over ~150m transect. Additional images were collected in heterogeneous areas (comprising multiple BSHs) and where any habitat FOCI or species FOCI were observed to ensure, as far as possible, that habitats and species were adequately sampled and accurately identified.

2.2.2 Grab sampling

Grab samples (collected using a 0.1m^2 Hamon Grab or Day Grab) were used to acquire data for sediment Particle Size Analysis (PSA) and benthic infaunal communities. For the Hamon Grab, a 500ml sediment sub-sample was taken from each sample after gentle homogenisation. For the Day grab, which unlike the Hamon Grab maintains an intact vertical profile of the sample upon recovery, a 3cm diameter corer was used to extract sediment sub-samples for the full vertical profile. Sediment samples were then hand stored at -20°C and later processed and analysed for their Particle Size Distribution (PSD) by Cefas, following the recommended methodology of the North-east Atlantic Marine Biological Analytical Quality Control (NMBAQC) scheme (Mason 2011). The less than 1mm sediment fraction was analysed using laser diffraction and the greater than 1mm fraction was dried, sieved and weighed at 0.5 phi (ϕ) intervals. PSD data were then merged and used to classify samples into sediment BSHs (see section 2.3.2).

The remaining sediment for the infaunal fraction was sieved over a 5mm and 1mm mesh and the residue photographed and fixed in buffered 4% formaldehyde. During subsequent processing, samples were washed to remove the formaldehyde and all individuals were extracted from each sample, identified to the lowest taxonomic level possible (i.e. to species, where possible), enumerated and weighed (blotted wet weight) to the nearest 0.0001 g following the recommendations of the NMBAQC scheme (Worsfold *et al*. 2010). All infaunal samples were processed and identified by Thomson Ecology Ltd.

2.3 Data preparation and analysis

2.3.1 Tidal model production

To assess the level of exposure experienced by designated features (report objective 2; see section 1.3.3 and Table 2), mean and maximum tidal current velocities (ms^{-1}) at the seabed and mean and maximum bed shear stress were obtained from a hydrodynamic model built for the study area. The depth-averaged model of East of Haig Fras MCZ is nested with a larger English Channel model and has been built using an unstructured triangular mesh using the hydrodynamic software Telemac2D (v7p1). The model domain extends 48.01°N–52.48°N and 2.23°E–9.51°W. The unstructured mesh was discretised with 292,630 nodes and 571,260 elements and has a resolution of approximately 3km along the open boundary. In the area of interest, the resolution is refined to approximately 25m. Bathymetry for the model was sourced from the Defra Digital Elevation Model (DEM) (Astrium 2011). The resolution of the dataset is 1 arc second (~30m). Within the MCZ area, the MBES bathymetry from the area was used, gridded to a 2m resolution. The hydrodynamics are forced along the open boundaries using 11 tidal constituents (M2, S2, N2, K2, K1, O1, P1, Q1, M4, MS4 and MN4) from the OSU TPXO European Shelf 1/30° regional model. 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/m^2) was calculated according to Soulsby (1997), based on current speed and local sediment characteristics (derived from the habitat map and sediment samples).

2.3.2 Sediment particle size distribution

Sediment particle size distribution (PSD) data (half phi classes) acquired during the 2015 survey were used to indicate sediment composition of each grab sample and thus each sampling station, based on the percentage contribution of gravel (> 2mm diameter), sand (0.063–2mm) and mud (<0.063mm) according to the classification proposed by Folk (1954). Each station was also assigned to one of four sediment BSHs with respect to sediment composition (i.e. ‘A5.1 Subtidal coarse sediment’, ‘A5.2 Subtidal sand’, ‘A5.3 subtidal mud’ and ‘A5.4 Subtidal mixed sediments’) using a version of the classification model produced during the Mapping European Seabed Habitats (MESH) project (Long 2006). For seafloor imagery, sediment composition was estimated by visual inspection and used to assign BSH type. Here, the gravel category was split across granules (2–4mm), shells (2–16mm) and pebbles (4–64mm), with three additional categories – cobbles (64–256mm), boulders (>256mm) and bedrock – also considered. The substrate was classified as a form of ‘A4 Circalittoral rock’ when the contribution of these latter categories exceeded 30%.

The BSHs and percent contributions of each sediment component (gravel, sand and mud) at each station were mapped and compared to a habitat map derived from data collected during habitat verification surveys (Eggleton & Downie 2017) to assess the extent and distribution of designated BSHs and describe spatial variation in sediment composition (report objective 1; see section 1.3.3 and Table 2).

For the fifteen muddy stations where grab samples were collected using the Hamon Grab and Day Grab, the resulting PSD data were compared to assess whether the two gears give different impressions of sediment composition and therefore determine whether the choice of gear is likely to have implications for monitoring of the physical structure of ‘A5.3 Subtidal mud’ (report objective 5; see section 1.3.3). The focus of the analysis was percent mud content, partly because ‘A5.3 Subtidal mud’ is defined by this variable but also because gravel content is necessarily low within this habitat, meaning that mud and sand contents are inevitably strongly correlated and thus effectively provide the same information. Differences between gears were tested using a general linear model in R version 3.4.1 (R Core Team 2017). ‘Sampling station’ was included in the model to account for any spatial variation in mud content before testing the effect of gear type. Assumptions of homogenous variance and normality of residuals were checked by inspection of plots of residuals against fits and normal quantile plots, respectively. Mud content data were transformed by $\log_e(x+1)$ to meet test assumptions.

2.3.3 Biological data preparation

Epifauna

Epifaunal communities were investigated using still image data collected during camera transects (see section 2.2.1).

Image analysis was completed by Seastar Survey Ltd. Each image was assigned a biotope and broadscale habitat (BSH) type based on the characterising features (substrate and biota). Taxa were identified to the lowest possible level and a SACFOR score and count or percent coverage was estimated for each taxon as appropriate.

As stills were acquired using a drop camera, with images taken at a wide range of heights above the seabed (and hence, with variable field of view (FOV) and pixel ground resolution), a representative and comparable subset of images, better suited to quantitative analysis, was selected for each habitat in each transect. FOV (m^2) was calculated for each image using a semi-automated procedure. Images with a FOV above $0.6m^2$ or an NMBAQC image quality score (Turner *et al.* 2016) 'Poor' or below given by the image analyst were excluded from analysis.

The unit of analysis was set at station level, with data from individual images pooled within individual BSHs. For brevity, for the remainder of the report each of these 'station–habitat' combinations are referred to as a 'transect'. Images for each transect were randomly sub-sampled until a cumulative maximum area of $2m^2$ was achieved. All transects that did not reach a minimum area of $1m^2$ were rejected. The range of area between $1\text{-}2m^2$ was considered sufficiently consistent for quantitative analysis. The FOV and data sub-setting procedure is explained in detail in Annex 1.

A total of 68 transects, from 62 stations, were included for analysis in the final dataset. The final epifaunal taxon matrix was truncated according to the protocol described in Annex 2. SACFOR abundance from individual images in each transect were pooled into one abundance value per taxon by taking the median numeric SACFOR value across all images selected per transect.

Infauna

Infaunal taxa were checked for compliance with up-to-date nomenclature using the WORMS 'match taxa' tool (http://www.marinespecies.org/aphia.php?p=match, accessed 19/06/2018). Any recorded taxa not invertebrates (e.g. fish) were removed from the dataset. Juveniles were generally retained in the dataset and their abundances merged with those of adults of the same species; only juveniles identified to a lower taxonomic resolution than adults were removed from the dataset (*sensu* Callaway *et al.* 2018; Downie *et al.* 2018). This obviated the need to reduce the taxonomic resolution of adult records. In cases where it was not possible to determine whether one or more individuals of a taxon were present (e.g. with small colonial taxa) an abundance of '1' was assigned (*sensu* Callaway *et al.* 2018; Downie *et al.* 2018). A full description and rationale for this truncation process is provided in Annex 3.

2.3.4 Biological data analysis

The composition, density and diversity of biological communities associated with each designated BSH, and variation in biological communities across BSHs, were assessed using the epifaunal (drop camera) and infaunal (Hamon Grab) datasets (report objective 1; see section 1.3.3 and Table 2). While 'A5.1 Subtidal coarse sediment' and 'A5.4 Subtidal mixed sediments' are grouped together in the site designation order (Table 1) and had to be considered together during survey design as they could not be distinguished using the available acoustic data, these BSHs were considered separately in the analysis to assess whether this grouping is ecologically meaningful (i.e. whether the similar assemblages are supported by the two BSHs). Such information is necessary to inform survey design and the analytical approach to future monitoring surveys (report objective 5; see section 1.3.3), with similarity in assemblages implying that these BSHs can be grouped together and dissimilarity implying that they cannot.

To assess variation in benthic community composition, epifaunal and infaunal taxa abundance datasets were imported into the statistical package PRIMER (version 6; Clarke & Gorley 2006). Infaunal taxa abundance data were transformed by $\log_e(x+1)$ to downweigh the influence of dominant species and allow variation in less abundant taxa to be detected. As epifauna abundances were recorded on the SACFOR scale, which is already scaled by taxon size and approximates a logarithmic transformation, no further transformations were made to these data (Connor & Hiscock 1996). For each faunal dataset, a resemblance matrix was created from the Bray-Curtis similarities of each pair of stations for infauna and transects for epifauna. Variation in community composition within and between BSHs was depicted visually using non-metric multidimensional scaling (nMDS) ordinations and tested using the analysis of similarities (ANOSIM) R-statistic, which ranges from 0 (similarities within and between sites are the same on average) to 1 (similarities within groups are higher than those between groups; Clarke 1993). Communities in different BSHs were considered statistically distinguishable when $p < 0.05$. However, as small differences in communities can result in statistically 'significant' differences, especially when the sample size is large (as is the case in the survey reported on here), focus was placed on the R-statistic as a measure of the degree of compositional difference in communities across BSHs. SIMPER was used to indicate average sample similarity and reveal which taxa characterised each BSH and distinguished different BSHs.

Univariate indices that reflect the density and diversity of the benthos were calculated in PRIMER. Total abundance per sample, total number of species per sample (i.e. 'species richness'), the Margalef Diversity Index (Margalef 1958; hereafter 'Margalef Index') and the Shannon Diversity Index (Shannon 1948; hereafter 'Shannon Index') were calculated for infauna. Total abundance and species richness are used as they are fundamental and commonly used measures of density and diversity. The Margalef Index – species richness relative to the log of total abundance – is used because there is evidence that it could be a good general indicator for physical, organic and chemical disturbance (van Loon *et al.* 2018) and therefore useful for condition monitoring. The Shannon Index is an integrated measure of both species richness and evenness (i.e. how evenly total abundance is distributed across species) and is used for its ability to respond to changes in either aspect of biodiversity (increases in richness or evenness lead to a larger Shannon Index). As epifauna abundances were recorded on the SACFOR scale, thus precluding the calculation of indices that incorporate total abundance, only the total number of species was calculated for this component of the benthos.

Mean values for univariate biotic indices were determined for each BSH and ANOVA (performed in R version 3.4.1; R Core Team 2017) was used to test whether these values varied significantly ($p < 0.05$) across BSHs ('A4.2 Moderate energy circalittoral rock', 'A5.1 Subtidal coarse sediment', 'A5.2 Subtidal sand', 'A5.3 Subtidal mud' and 'A5.4 Subtidal mixed sediments'). Assumptions of homogenous variance and normality of residuals were checked by inspection of plots of residuals against fits and normal quantile plots, respectively. Data were transformed by $\log_e(x+1)$, where necessary, to meet test assumptions. For the set of univariate biotic indices considered, the means and 95% confidence for each sediment BSH were depicted using bar charts. Differences in these indices between infauna samples collected using different gear types (Hamon Grab *vs* Day Grab) were also tested using models analogous to the one used to test variation in mud content (see section 2.3.2), to determine whether either gear was more effective at sampling 'A5.3 Subtidal mud' (e.g. recorded significantly more individuals or species) and may therefore be preferable for future monitoring of this BSH (report objective 5; see section 1.3.3).

To further inspect patterns in biological structure within the MCZ (report objective 1; see section 1.3.3 and Table 2), hierarchical cluster analyses were performed on the epifaunal and infaunal datasets in PRIMER. SIMPROF was used in association with the cluster analyses to determine which groups of stations were significantly different ($p < 0.05$) from others in terms of epifaunal and infaunal community composition (based on Bray-Curtis similarities). For infauna, the resulting cluster groups were plotted onto a BSH map and inspected for spatial patterns. The SIMPER routine was then used to indicate which taxa characterised the benthos of each cluster. This information was used to match infaunal community clusters to biotopes, where possible, using the procedure described in Parry (2015). Epifauna were matched to biotopes visually during image analysis.

The results of the above mentioned analyses (i.e. that benthic communities were spatially variable and not clearly structured with respect to BSH type; see sections 3.3.2 and 3.3.3) prompted additional analyses to investigate the roles of sediment components (e.g. cobbles, gravel, mud) in shaping variation in biological communities within and across designated BSHs (report objective 1; see section 1.3.3 and Table 2). The intention was to identify more precise and fauna–sediment associations than can be achieved by considering substrate type at the BSH-level, which can then be used to inform the design of future monitoring surveys aimed at assessing temporal changes in feature condition (report objective 5; see section 1.3.3 and Table 2); for example, by monitoring a set of stations that cover a range of sediment compositions (both within and across BSHs) that support distinct biological communities.

General linear models, produced in R, were used to test how univariate biotic indices vary in relation to percent gravel, sand and mud contents for infauna analyses and percent boulder, cobble, pebble, shell, gravel, sand and mud contents for epifauna analyses. For infaunal analyses, all two-way interactions between sediment components (i.e. tests of whether the association with one sediment component depends on the content of another sediment component) were also included in the models. Assumptions were checked and data transformed, where necessary, to meet test assumptions, as described above. Sums of squares were calculated using the Type II approach (i.e. the relationship that the response variable has with each explanatory variable was tested after accounting for its relationship with all other explanatory variables). Relationships were considered significant when $p < 0.05$. Significant relationships were depicted using 3D plots.

To analyse multivariate community data in a manner consistent with the analysis of univariate indices, infauna taxa abundances were Hellinger-transformed (i.e. the square root of relative taxa abundances) and these data were imported into PRIMER to create principal components that capture variation in community composition. Hellinger transformation is particularly suitable for creating Euclidean distance-based principal components using the studied infaunal data, as it gives low weights to variables with many zeros and many species were absent from most stations (Legendre & Gallagher 2001). The first two principal components were then analysed using general linear models, as described above, to determine how subsets of taxa with correlated abundances vary in relation to sediment composition. As each principal component explained only a small proportion of total community variation (< 12%), overall infaunal community composition was also analysed by correlating the Bray-Curtis similarity matrix of $\log_e(x+1)$-transformed taxa abundances with a Euclidean distance matrix of normalised sediment components using RELATE in PRIMER. The BEST routine was then used to determine which combination of sediment components best explained variation in infaunal community composition, and this output was compared to general linear model outputs for principal components. BEST was also used to determine which combination of sediment components best explained variation in epifaunal community composition.

Finally, all infaunal and epifaunal species were cross-referenced against lists of species FOCI and habitat FOCI-defining taxa (report objective 3; see section 1.3.3 and Table 2) and non-indigenous species (NIS) that have been selected for assessment of GES in GB waters under MSFD Descriptor 2 (report objective 4; see section 1.3.3 and Table 2). The list of NIS includes two categories: species which are known to be present within the assessment area (present) and species which are not yet thought to be present but have a perceived risk of introduction and impact (horizon) (Stebbing *et al.* 2014; Annex 4). An additional list of NIS, identified as invasive in the ‘Non-native marine species in British waters: a review and directory’ (Eno *et al.*1997), was also referenced against all taxa observed (Annex 4). The distributions of any species FOCI, habitat FOCI-defining taxa or NIS recorded in grab samples and/or seabed imagery were plotted onto a habitat map of the MCZ.

3 Results and Interpretation

3.1 Benthic and environmental overview

3.1.1 Hydrodynamics: energy and exposure

The site has weak (< 0.5ms^{-1}) to moderate (0.5-1.5ms^{-1}) tidal currents flowing on a west-east axis (Figure 3). The hydrodynamic model of the East of Haig Fras MCZ area shows there is very little variation in current strength across the site, with the maximum velocities over the spring-neap tidal cycle at 0.52ms^{-1}.

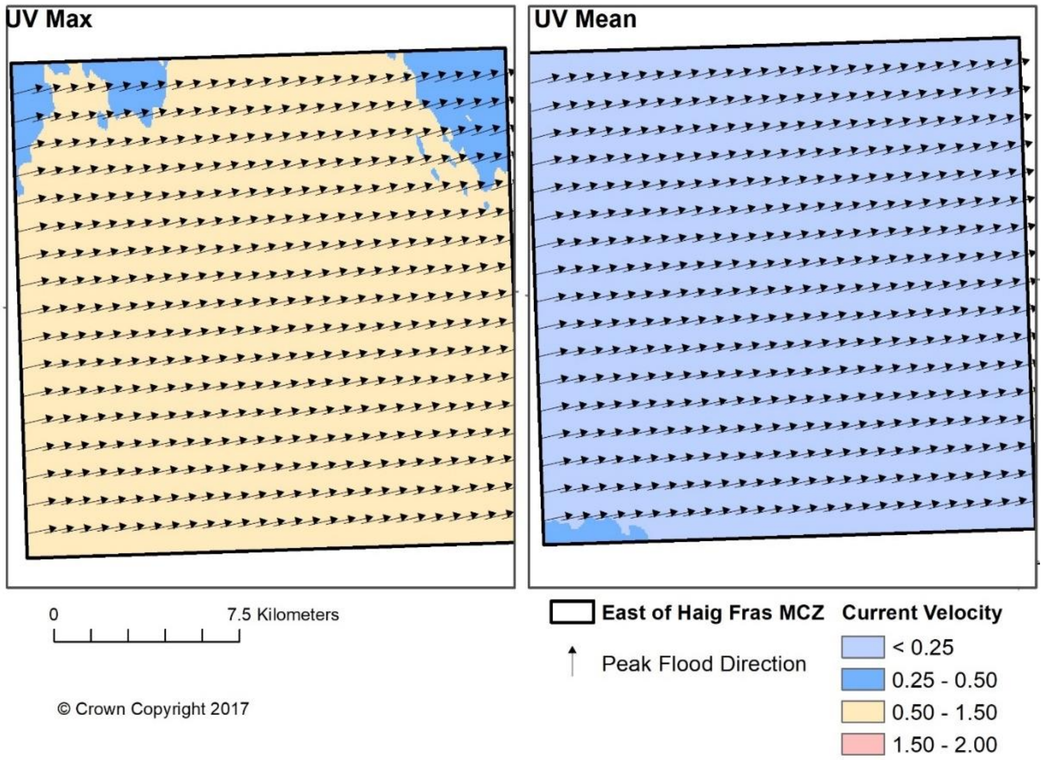


Figure 3. Hydrodynamic environment at the East of Haig Fras MCZ. Arrows illustrate the main direction of tidal flow during the peak flood phase. The base map colour indicates the maximum (left panel) and mean (right panel) current velocity over a spring-neap tidal cycle within the MCZ.

3.1.2 Broadscale Habitat (BSH): extent and distribution

Subtidal habitats within the East of Haig Fras MCZ were mapped using data collected in 2012 and 2013 as part of the MCZ's habitat verification process (Eggleton & Downie 2017). The seabed at the site was found to consist of an intricate mosaic of the BSHs 'A5.1 Subtidal coarse sediment', 'A5.2 Subtidal sand', and 'A5.4 Subtidal mixed sediments', which is highly spatially variable on a scale of tens of metres with ridges formed of coarse and mixed sediments interspersed by sand (Figure 4). The highest ridges of boulders and cobbles form regular small patches of 'A4 Circalittoral rock' within the coarse/mixed sediments matrix. Rock is presented in the map as 'A4 Circalittoral rock', as it was not possible to map energy class or sediment sizes with acceptable confidence at the time the map was produced. Large areas of 'A5.3 Subtidal mud' were mapped in the deeper, western part of the site (Figure 4).

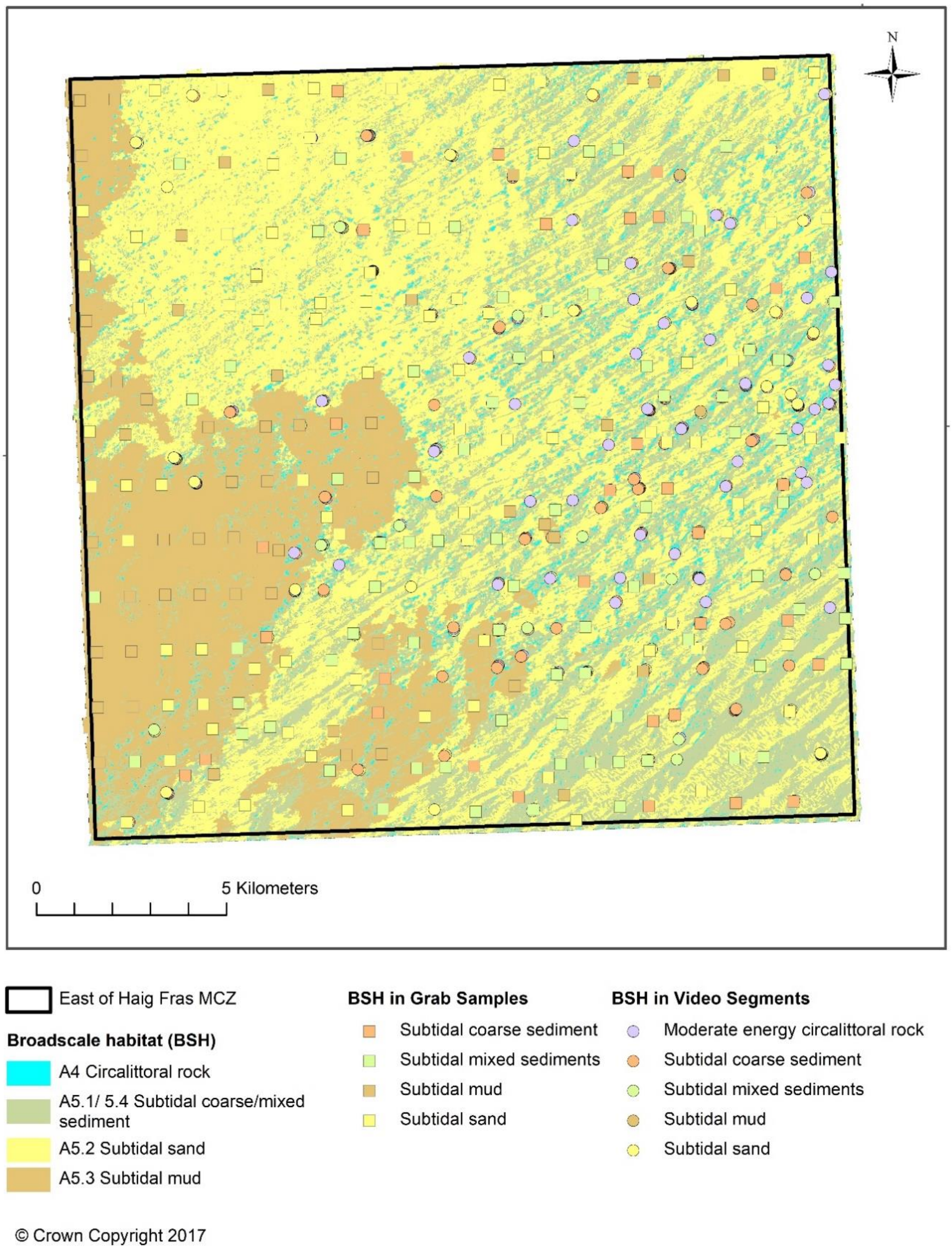


Figure 4. Broadscale Habitat (BSH) map for the East of Haig Fras MCZ (from Eggleton & Downie 2017) overlaid with BSHs derived from grab sample and seabed imagery data collected in 2015 (CEND0915).

In order to provide some quantitative assessment of the resemblance between the BSH classes assigned in the habitat map based on data collected in 2012 and 2013 and the sample data collected in 2015 and reported here, confusion matrices are given in Table 3. Sampling gears employed in 2015 were not deployed across all BSH habitat types and therefore the results are split by sampling gear type. Accuracy estimates have not been made where a gear type was intentionally not deployed within areas of certain predicted BSHs. Considering the sampling bias introduced by the different distributions of sampling gears it is not considered appropriate to produce a single overall accuracy score however accuracy scores for each BSH class and for both sampling gears are given and illustrate the levels of agreement between the mapped BSH classes and the 2015 samples.

Table 3. Confusion matrices examining resemblance between BSH classes mapped based on 2012 and 2013 data and a. 2015 grab sampling and b. 2015 video sampling.

a.

		2015 Sampling - Grab				Total	User accuracy (%)	Error commission
		A4	A5.1 / 5.4	A5.2	A5.3			
BSH Map	A4							
	A5.1 / 5.4	0	66	18	3	87	76	24
	A5.2	0	33	54	20	107	50	50
	A5.3	0	12	21	44	77	57	43
Total		0	111	93	67	271		
Producer accuracy %			59	58	66			
Error Omission %			41	42	34			
Overall accuracy of BSH categories (2015 sampling grab)					61 %			

b.

		2015 Sampling - Video				Total	User accuracy (%)	Error commission
		A4	A5.1 / 5.4	A5.2	A5.3			
BSH Map	A4	47	21	6	0	74	64	36
	A5.1 / 5.4	49	117	43	3	212	55	45
	A5.2	4	27	33	2	66	50	50
	A5.3							
Total		100	165	82	5	352		
Producer accuracy (%)		47	71	40				
Error Omission (%)		53	29	60				
Overall accuracy of BSH categories (2015 video sampling)					56 %			

The drop camera was used in 2015 to target areas predicted to be 'A4 Circalittoral rock' in the habitat map. There was generally good agreement between the predictions and observations of this BSH (Table 3, b., Figure 4). The new survey data indicate that rock habitat within the MCZ is appropriately classified as 'A4.2 Moderate energy circalittoral rock'. All sediment BSHs were sampled extensively using grabs throughout the site in the 2015 survey (Figure 4). As with rock habitat, predictions of the extent and distribution of sediment BSHs from the habitat map were broadly supported the data acquired in 2015. The correspondence was strongest for 'A5.3 Subtidal mud' (Table 3, a.), with this BSH concentrated in the relatively deep areas within the western section of the MCZ (Figure 5) and forming a relatively contiguous feature compared to other BSHs (Figure 4). The 2015 data do show a possible eastward extension of ‘A5.3 Subtidal mud’, with a series of samples (on a linear north–south axis) classified as this BSH in the northwest of the site, where ‘A5.2 Subtidal sand’ was predicted. Similarly, ‘A5.2 Subtidal sand’ was recorded at various stations predicted to be ‘A5.3 Subtidal mud’ in the southwest of the site, particularly near the MCZ boundaries. Throughout the remainder of the MCZ, the distribution of stations identified as ‘A5.2 Subtidal sand’ in 2015 varied, as expected, given the small-scale heterogeneity of the substrate associated with the ridge system described above. There was broad agreement between 2015 samples and 2013 prediction for ‘A5.1 Subtidal coarse sediment’ / ‘A5.4 Subtidal mixed sediments’ (Table 3, a.), with confirmation of the association between these BSHs and the flanks of ridges topped by cobbles and boulders, i.e. ‘A4.2 Moderate energy circalittoral rock’. However, assessment of the correspondence between map predictions and 2015 grab samples for ‘A5.1 Subtidal coarse sediment’ and ‘A5.4 Subtidal mixed sediments’ is hindered by the requirement for these two BSHs to be merged to produce the habitat map.

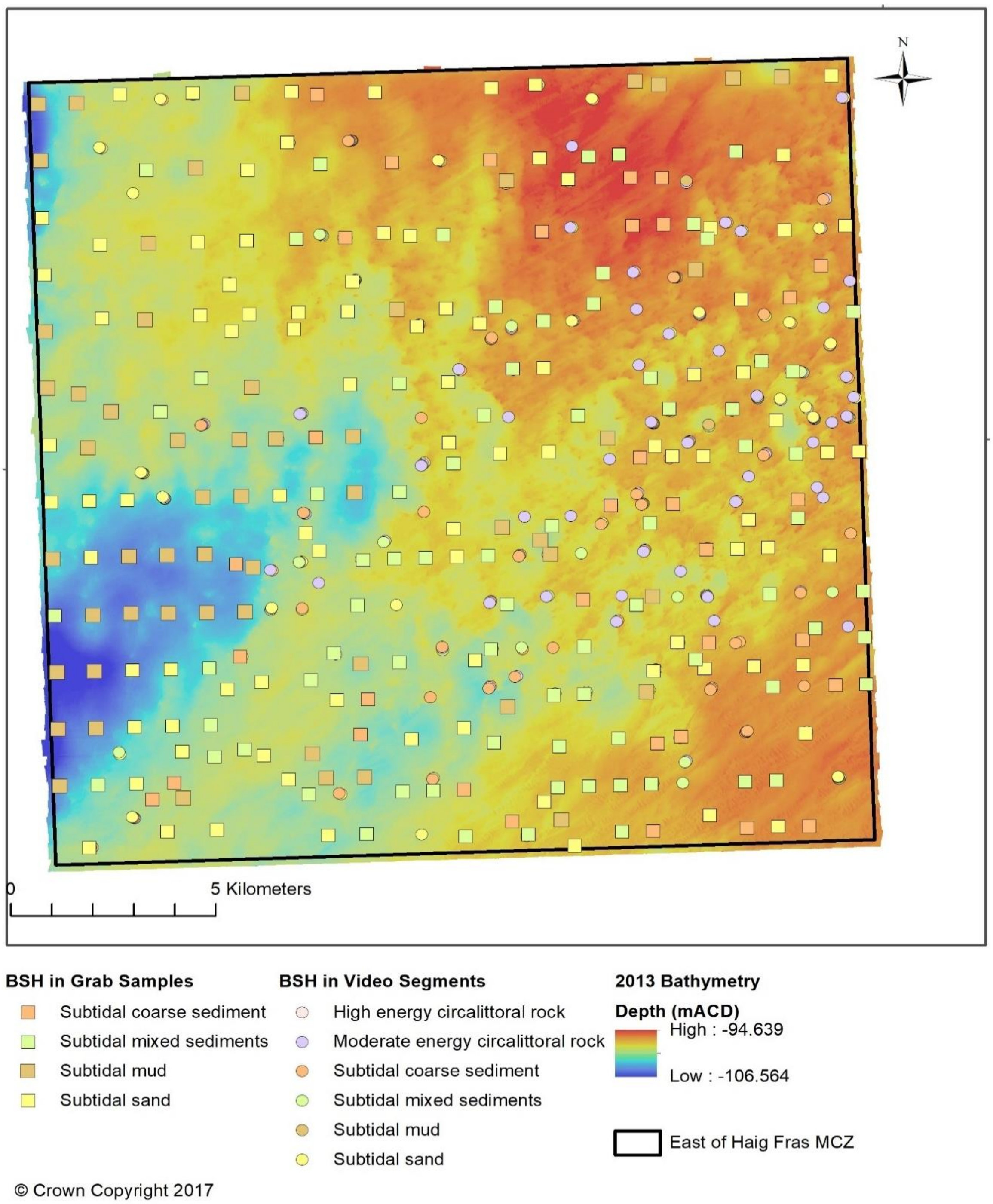


Figure 5. Broadscale Habitats (BSHs) derived from grab samples and camera transects segments during a survey of the East of Haig Fras MCZ in 2015 (CEND0915) in relation to water depth.

3.2 Subtidal rock BSH: Physical and biological structure

3.2.1 Rock composition and distribution

All rock habitat within the East of Haig Fras MCZ was identified as 'A4.2 Moderate energy circalittoral rock'. The rock feature is formed by elevated ridges of boulders and cobbles, located mainly in the eastern part of the site (Figure 6). These hard substrata are interspersed by mixtures of gravel, shells and sand, with approximately 40% of the area sampled along transects covered in sediment. Figure 7 shows an example of the substrata observed along a transect where ‘A4.2 Moderate energy circalittoral rock’ was observed. The topography of the rock is low relief, with an average elevation of 0.18m within mapped patches of rock (range 0–2.6m) and 1m within camera transects (range 0–3.3m).

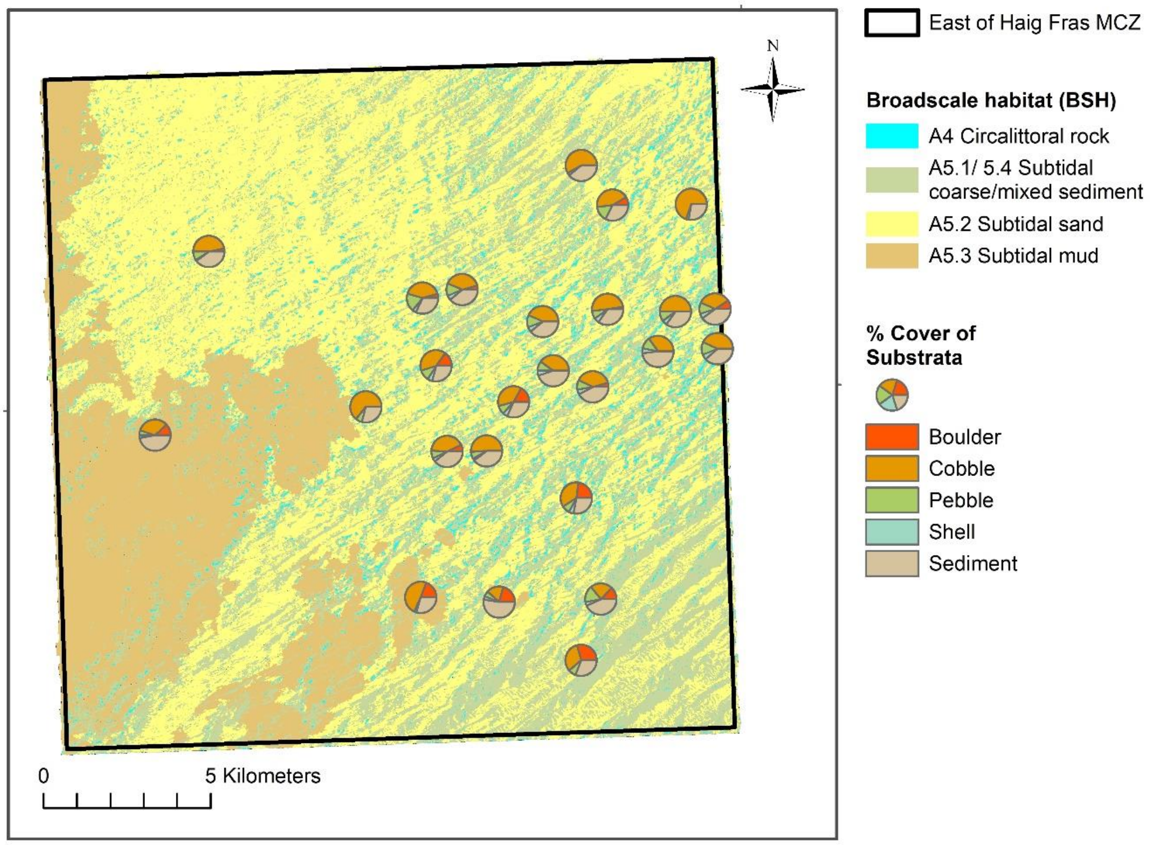


Figure 6. Substrate composition of stations classified as ‘A4.1 Moderate energy circalittoral rock’ within the East of Haig Fras MCZ in 2015 (CEND0915) based on video transect data. The underlying Broadscale Habitat (BSH) map is from Eggleton and Downie (2017).

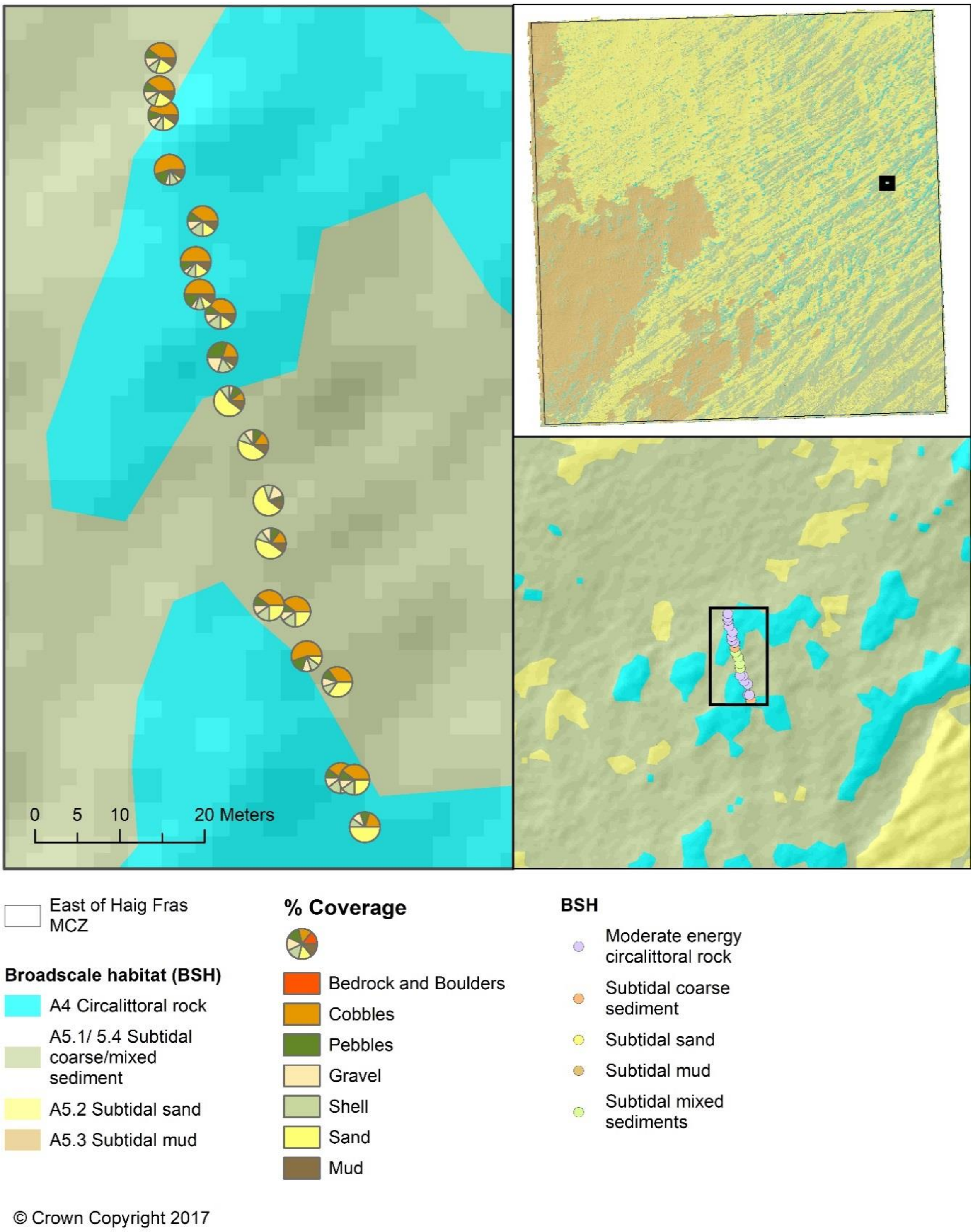


Figure 7. Close-up example of a rock feature and surrounding sediments at the East of Haig Fras MCZ. The underlying Broadscale Habitat (BSH) map is from Eggleton and Downie (2017).

3.2.2 Biological structure of epifauna

'A4.2 Moderate energy circalittoral rock' was characterised by hydroid turf, encrusting bryozoans and sponges, along with brachiopods, several species of the hydroid *Nemertesia*, colonies of the bryozoan *Porella* sp., cup corals (*Caryophyllia* spp.), tubiculous worms of the family Serpulidae and squat lobsters. The only biotope identified in association with rock habitat was ‘Echinoderms and crustose communities’ (CR.MCR.EcCr). A cluster analysis using only rock transects revealed three epifaunal sub-groups at 55% dissimilarity (Figure 8). The sub-groups share the most common characterising taxa but show different variations of the full epifaunal community. Rock sub-group (A) had a higher proportion of sand mixed with cobbles and was the only sub-group with the burrowing brittle star *Amphiura* sp., tube-dwelling anemones of the family Cerianthidae and crabs of the family Inachidae. The Rosy Starfish (*Stichastrella rosea*) was found in rock sub-group (A) as well as rock sub-group (B), which comprises the stations with the greatest proportion of boulders. Another taxon indicative of rock sub-group (B) was the common sea urchin (*Echinus esculentus*). Rock sub-group (C) had the highest cover of gravel, shell gravel and shell fragments and had a higher abundance of Edwardsiid anemones than the other sub-groups, as well as being the only sub-group harbouring globular sponges. Figure 9 shows example images from each of the three sub-groups of ‘A4.2 Moderate energy circalittoral rock’.

Despite the dissimilarities across the three sub-groups, ‘A4.2 Moderate energy circalittoral rock’ had the highest internal similarity for epifaunal communities of all BSHs (57.8%), indicating that biological structure was relatively stable in this BSH. ‘A4.2 Moderate energy circalittoral rock’ also had the highest epifaunal diversity of all BSHs, with an average of 21.4 (± 3.5; standard deviation) taxa observed in the 1-2m^2 sample area.

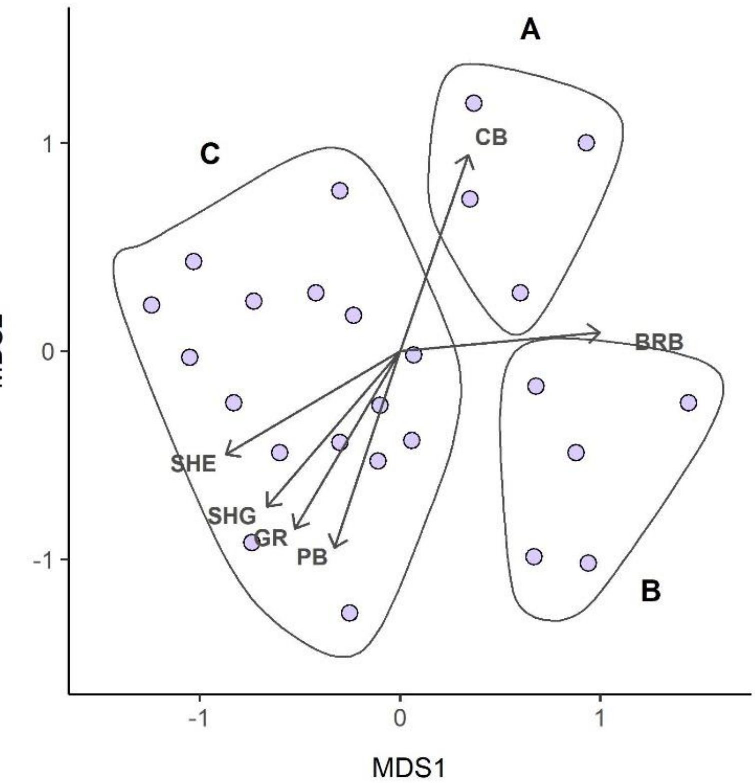
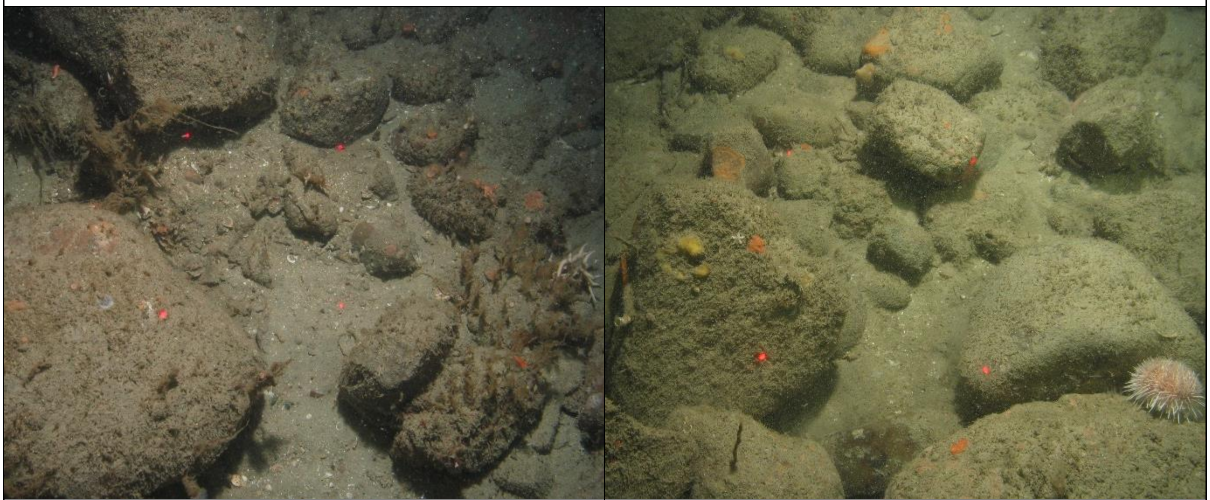


Figure 8. ‘A4.2 Moderate energy circalittoral rock’ sub-groups of epifaunal communities identified by cluster analysis, with correlation vectors for percent cover of sediment categories. BRB = bedrock and boulders, CB = cobbles, PB = pebbles, SHE = Shell, SHG = shell gravel, GR = gravel.

Moderate energy circalittoral rock (A)

Moderate energy circalittoral rock (B)



Moderate energy circalittoral rock (C)

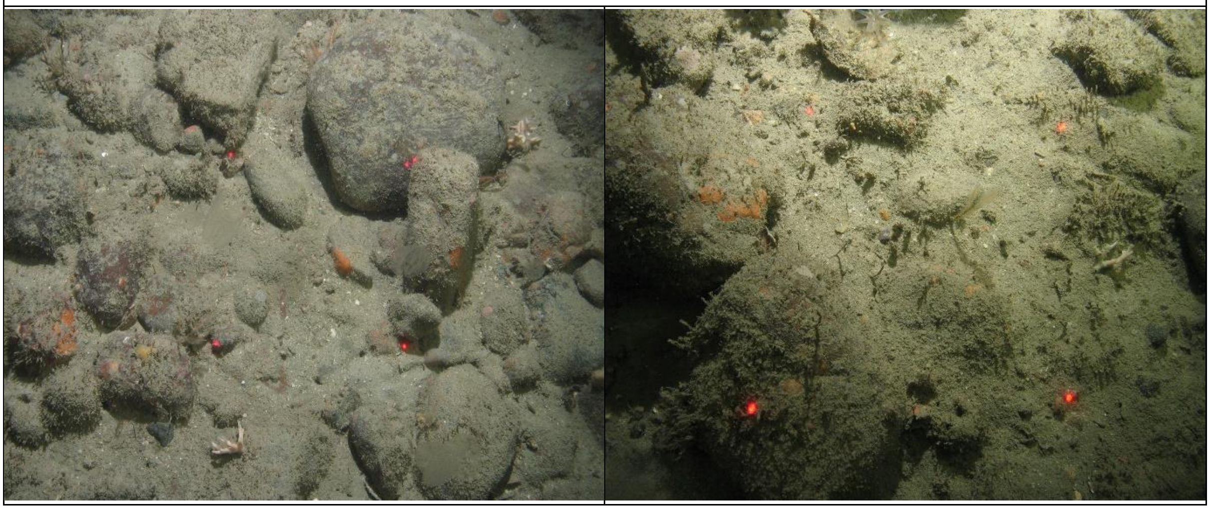


Figure 9. Example images of the 'A4.2 Moderate energy circalittoral rock' features acquired at the East of Haig Fras MCZ in 2015. Examples of each of the three sub-groups of rock epifauna identified through cluster analysis are given.

3.3 Subtidal sediment BSH: Physical and biological structure

3.3.1 Sediment composition and distribution

Sediment composition (percent gravel, sand and mud contents) and the resulting BSH for each Hamon Grab sample are presented in Figure 10. The proportions of gravel, sand and mud at each station sampled with a Hamon Grab is illustrated in Figure 11.

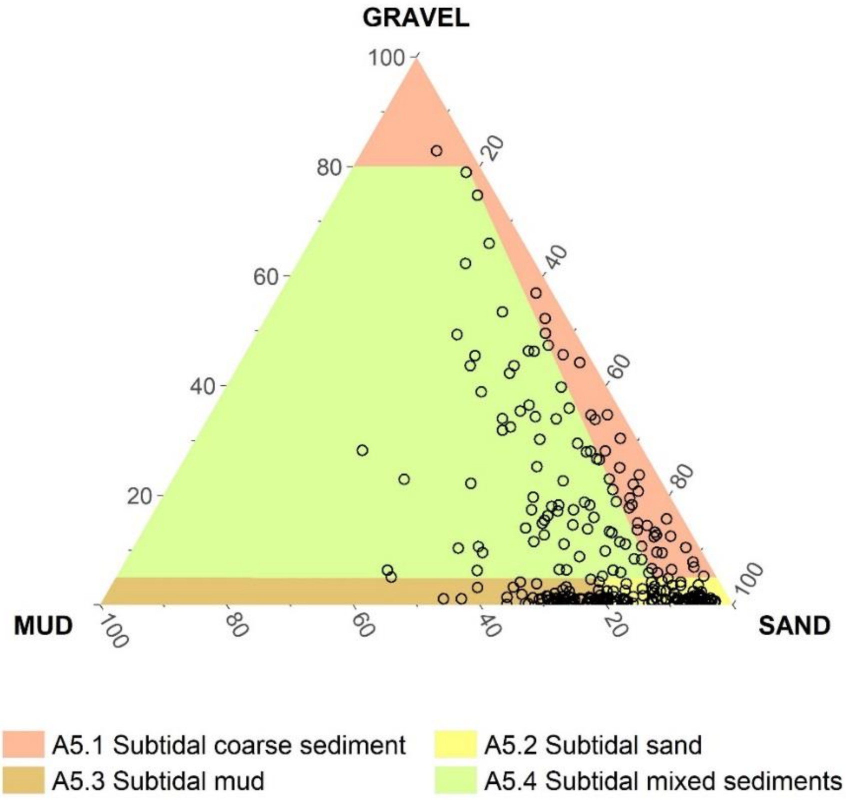


Figure 10. Classification of particle size distribution (half phi) information for each 0.1m² Hamon Grab sample (hollow black circles) into one of the Sediment Broadscale Habitats (BSHs) (coloured areas) plotted on a true scale subdivision of the Folk triangle into the simplified classification for UKSeaMap (Long 2006; Folk 1954). Particle size distribution data were collected during the East of Haig Fras MCZ survey in 2015.

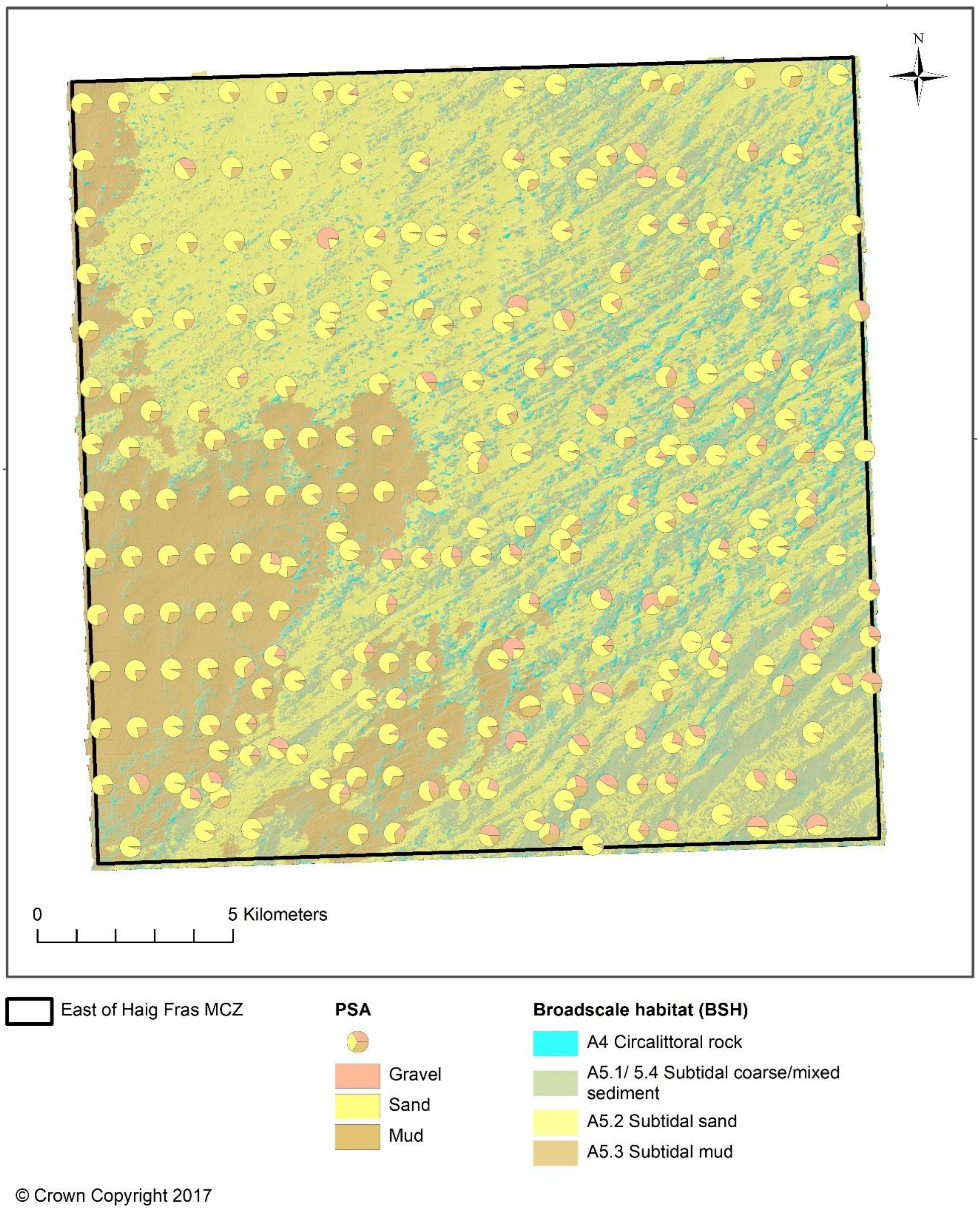


Figure 11. Sediment fractions of gravel, sand, and mud recorded at each station sampled with a 0.1m^2 Hamon Grab at the East of Haig Fras MCZ in 2015. The underlying Broadscale Habitat (BSH) map is from Eggleton and Downie (2017).

3.3.2 Biological structure of epifauna

Epifaunal communities were variable across sediment BSHs and this was largely driven by the proportion of gravel and hard substrata available for attached sessile fauna. Only one transect where the BSH 'A5.3 Subtidal mud' was observed had a sufficient number of images for analysis following the image selection process described in Annex 1 (note that the expansive areas of 'A5.3 Subtidal mud' were not targeted for the collection of seabed imagery data; see section 2.1). Therefore, ‘A5.3 Subtidal mud’ was excluded from analysis of epifauna. ANOSIM indicated that there were no large differences (R > 0.5) between sediment BSHs in their associated epifaunal communities. ‘A5.1 Subtidal coarse sediment’ and ‘A5.4 Subtidal mixed sediments’ were not significantly different ($p > 0.05$). All still images of ‘A5.4 Subtidal mixed sediments’ were assigned into the parent biotope ‘Circalittoral mixed sediment’ (SS.SMx.CMx). Similarly, no biotopes more detailed than ‘Circalittoral coarse sediment’ (SS.SCS.CCS) were identified in the ‘A5.1 Subtidal coarse sediment’ images. Epifaunal taxa characteristic of ‘A5.4 Subtidal mixed sediments’ were very similar to those in ‘A5.1 Subtidal coarse sediment’, with tube worms of the family Serpulidae, the cup coral *Caryophyllia* spp. and turf-forming and clumped hydrozoans present on cobbles and pebbles, and tube-dwelling polychaetes on finer sediment (Figure 12 and Figure 13). The brittle star *Ophiura* spp. and hermit crabs of the family Paguridae were also commonly observed.

Epifauna observed in ‘A5.2 Subtidal sand’ were statistically distinguishable ($p < 0.05$) from those observed in association with ‘A5.1 Subtidal coarse sediment’ and ‘A5.4 Subtidal mixed sediments’, but there was considerable overlap in communities (R < 0.25). Still images of ‘A5.2 Subtidal sand’ were assigned to two sand biotopes: ‘Sublittoral sands and muddy sands’ (SS.SSa) and its sub-biotope ‘Circalittoral muddy sand’ (SS.SSa.CMuSa). Very few epifaunal taxa were observed in still images of this BSH. The only characterising taxa for sand were small tube-dwelling polychaetes. Hydrozoan turf was observed where shell or pebbles were present on the sand (Figure 14). All sediment BSHs were substantially different from ‘A4.2 Moderate energy circalittoral rock’ (R > 0.6; $p < 0.01$; Table 4).

Table 4. R values from pairwise ANOSIM tests of differences in epifaunal community composition (on the SACFOR scale of abundance) in relation to Broadscale Habitat (BSH) (‘A5.1 Subtidal coarse sediment’, ‘A5.2 Subtidal sand’, ‘A5.4 Subtidal mud’ and ‘A4.2 Moderate energy circalittoral rock’) within the East of Haig Fras MCZ in 2015. The R value shown in grey was not statistically significant; all other pairwise comparisons were significant (* = $p < 0.05$, ** = $p < 0.01$). R values that indicate low overlap in communities (R > 0.5) are shown in bold.

	A5.1	**A5.2**	**A5.4**
A5.2	0.108 *		
A5.4	0.089	0.223 *	
A4.2	**0.639 ****	**0.862 ****	**0.637 ****

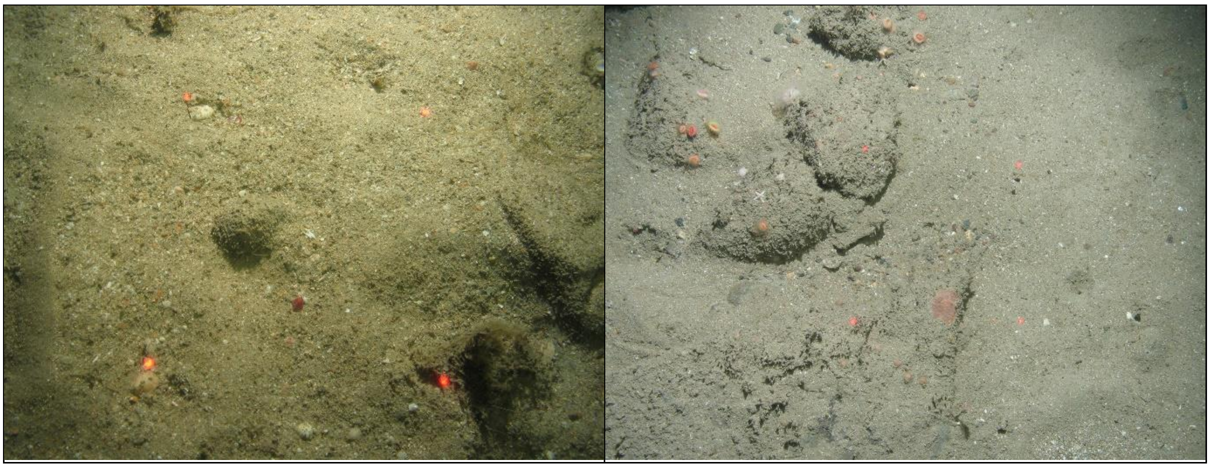


Figure 12. Example images of epifauna observed in association with ‘A5.1 Subtidal coarse sediment’ at the East of Haig Fras MCZ in 2015.

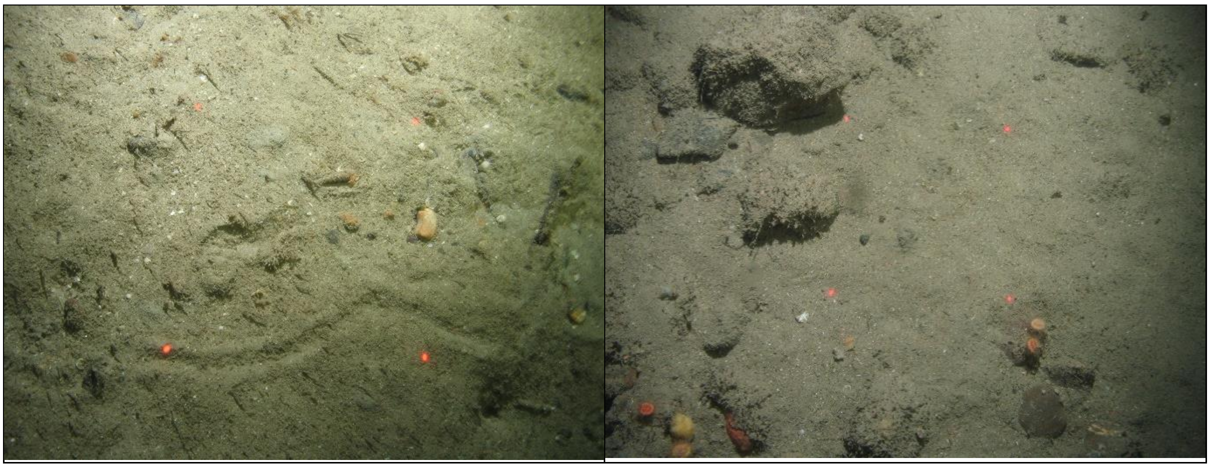


Figure 13. Example images of epifauna observed in association with 'A5.4 Subtidal mixed sediments' at the East of Haig Fras MCZ in 2015.

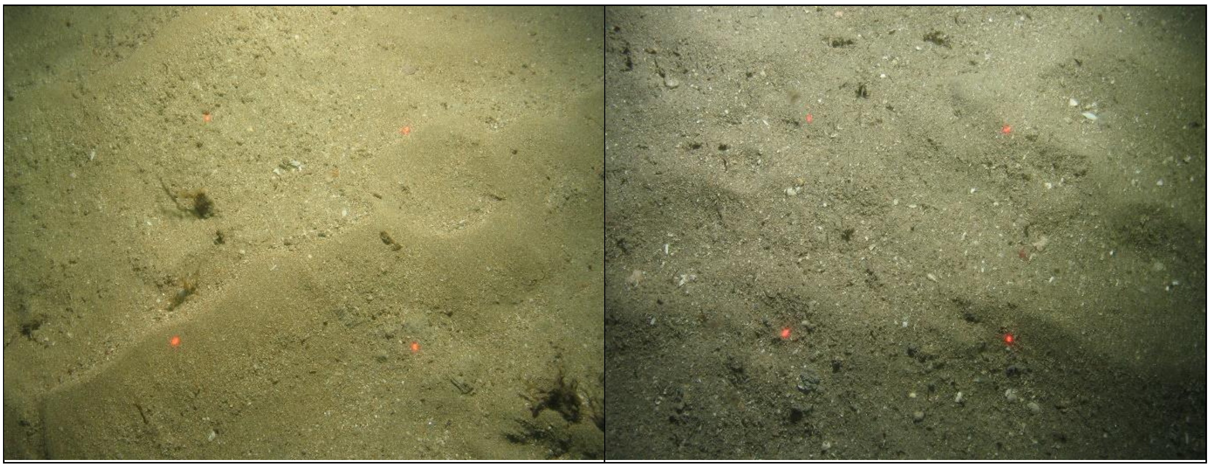


Figure 14. Example images of epifauna observed in association with 'A5.2 Subtidal sand' at the East of Haig Fras MCZ in 2015.

SIMPROF separated epifaunal communities into three sediment groups (and one rock group) at 55% similarity. A single station was also separated from all others at this level of similarity. The three sediment cluster groups did not correspond to specific BSHs, instead consisting of mixtures of several BSHs (Figure 15). 'A5.1 Subtidal coarse sediment' and 'A5.4 Subtidal mixed sediments' each span a wide range of sediment compositions, from clean to gravelly sand to a matrix of sand, gravel, pebbles and cobbles. Community composition in sediment BSHs was largely driven by the presence of hard substrata suitable for attached sessile fauna (i.e. cobbles and boulders) and the relative proportions of sand vs. gravel (including shell gravel) (Figure 15). These communities ranged a single unidentified tube-dwelling polychaete in clean sand to diverse assemblages similar to those found in 'A4.2 Moderate energy circalittoral rock', consisting of hydrozoan turf, encrusting bryozoans, serpulid worms and caryophyllid corals as pebble and cobble content increased. BEST analysis indicated that 49% of variation in community structure was explained by the percentage of gravel, shell gravel and sand. Percentages of pebbles and cobbles decrease proportionally as percentages of sand and gravel increase.

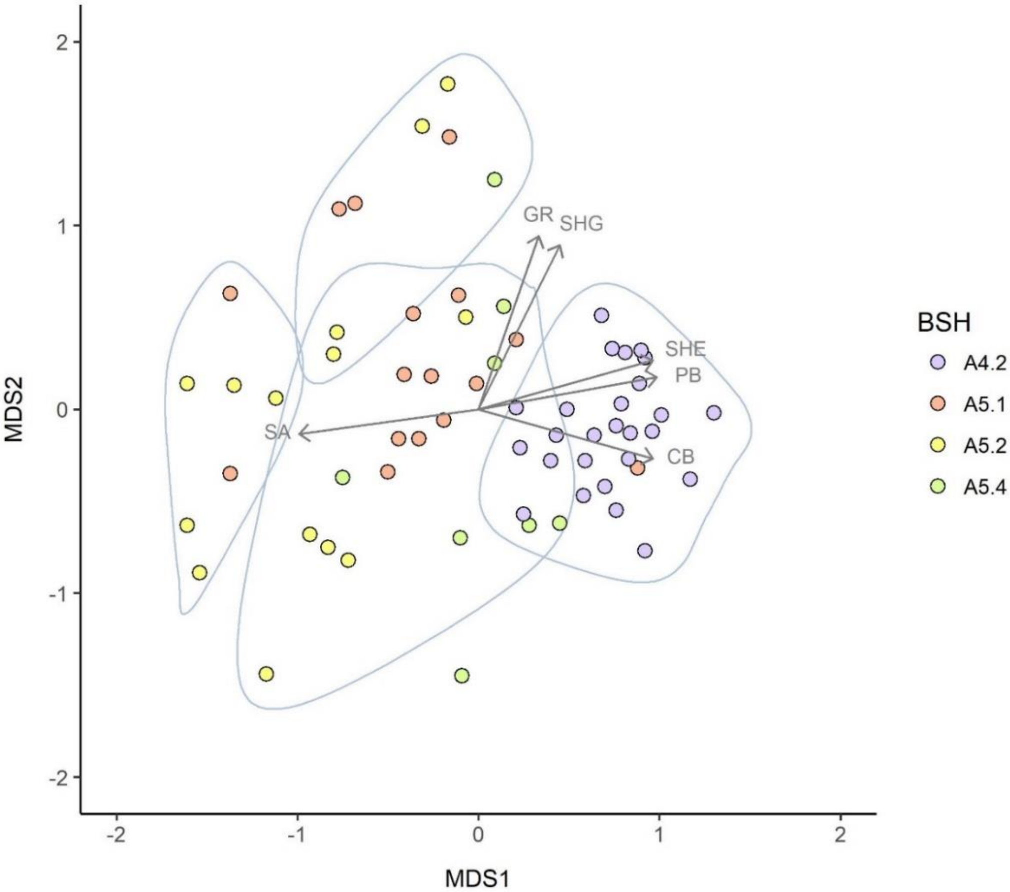


Figure 15. Non-metric multidimensional scaling ordination of epifaunal communities in relation to Broadscale Habitat (BSH) (‘A4.2 Moderate energy circalittoral rock’, ‘A5.1 Subtidal coarse sediment’, ‘A5.2 Subtidal sand’ and ‘A5.4 Subtidal mixed sediments’) and cluster groups (< 55% similarity; blue lines) and the contents of sediment components (arrows) within the East of Haig Fras MCZ in 2015. CB = cobbles, PB = pebbles, SHE = Shell, SHG = shell gravel, GR = gravel, SA = sand.

A similar trend was seen in species richness across BSHs. ‘A5.2 Subtidal sand’ had the lowest mean number of taxa (5.3 ± 1.8; standard deviation) in the 1-2m^2 sample area. As with community composition, ‘A5.1 Subtidal coarse sediment’ and ‘A5.4 Subtidal mixed sediments’ did not differ significantly in species richness (9.8 ± 5.3 and 11.7 ± 5.5 taxa in the sample area, respectively). All sediment BSHs had significantly fewer taxa than ‘A4.2 Moderate energy circalittoral rock’.

Within-BSH variability in species richness was highest in ‘A5.1 Subtidal coarse sediment’ and ‘A5.4 Subtidal mixed sediments’, ranging from 4 to 24 taxa per sample. This variability is likely to reflect both BSHs being formed of a heterogenous mixture of sand, gravel, pebbles, and cobbles, with the diversity of sessile epifauna dependent on the amount of ‘hard’ surface available for attachment. A linear model of species richness based on samples from all BSHs indicates that 86% of variability is explained by the percent cover of boulders and cobbles, although the relationship is not linear (best fit is achieved using a third-degree polynomial model; Figure 16). The increase in epifaunal diversity with increasing boulder and cobble contents was observed only for sediment habitats (< 30% boulder and cobble contents) and not rock habitats (> 30% boulder and cobble contents) (Figure 16).

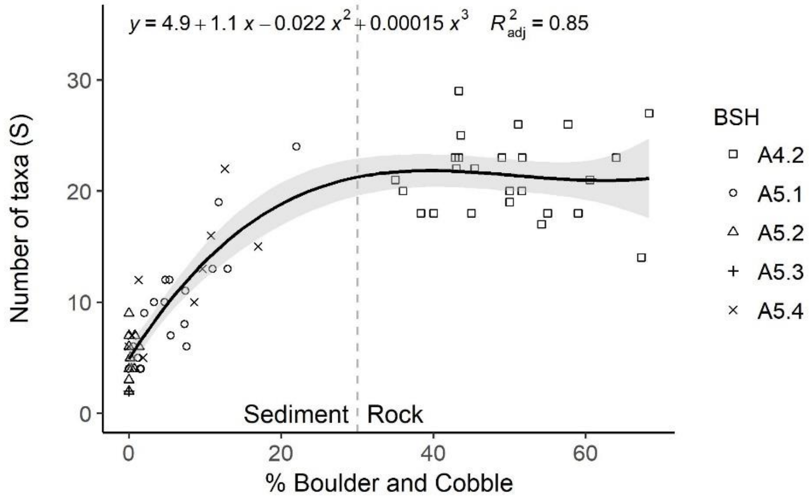


Figure 16. Relationship between epifaunal species richness and the combined percent cover of boulders and cobbles in the East of Haig Fras MCZ in 2015, showing the fit of a third-degree polynomial model with standard error. R^2_{adj} = Adjusted R^2.

3.3.3 Biological structure of infauna

Infauna in sediment BSHs were statistically distinguishable ($p < 0.05$) in terms of community composition (ANOSIM: Global R = 0.220; $p < 0.001$; Table 5). However, there was a high level of overlap in communities observed in each BSH (Figure 17) and, as with epifauna, the differences between infaunal communities in sediment BSHs were generally small (R < 0.25). The exception was the difference between ‘A5.1 Subtidal coarse sediment’ and ‘A5.3 Subtidal mud’ (R = 0.65) and, to a lesser degree, between ‘A5.1 Subtidal coarse sediment’ and ‘A5.2 Subtidal sand’ (R = 0.26). The four sediment BSHs shared characterising infaunal taxa, including the Pea Urchin (*Echinocyamus pusillus*), Nemerteans and the polychaete *Spiophanes* spp. Various other taxa were characteristic of two or more sediment BSHs. The taxa that characterised each BSH are shown in Table 6.

Table 5. R values from pairwise ANOSIM tests of differences in infaunal community composition (based on $\log_e$ (x+1)-transformed taxa abundances) in relation to sediment Broadscale Habitat (‘A5.1 Subtidal coarse sediment’, ‘A5.2 Subtidal sand’, ‘A5.3 Subtidal mud’, and ‘A5.4 Subtidal mixed sediments’) within the East of Haig Fras MCZ in 2015. R values were statistically significant (p < 0.05) in all pairwise comparisons. R values that indicate low overlap in communities (R > 0.5) are shown in bold.

	A5.1	**A5.2**	**A5.3**
A5.2	0.261		
A5.3	**0.649**	0.191	
A5.4	0.175	0.183	0.182

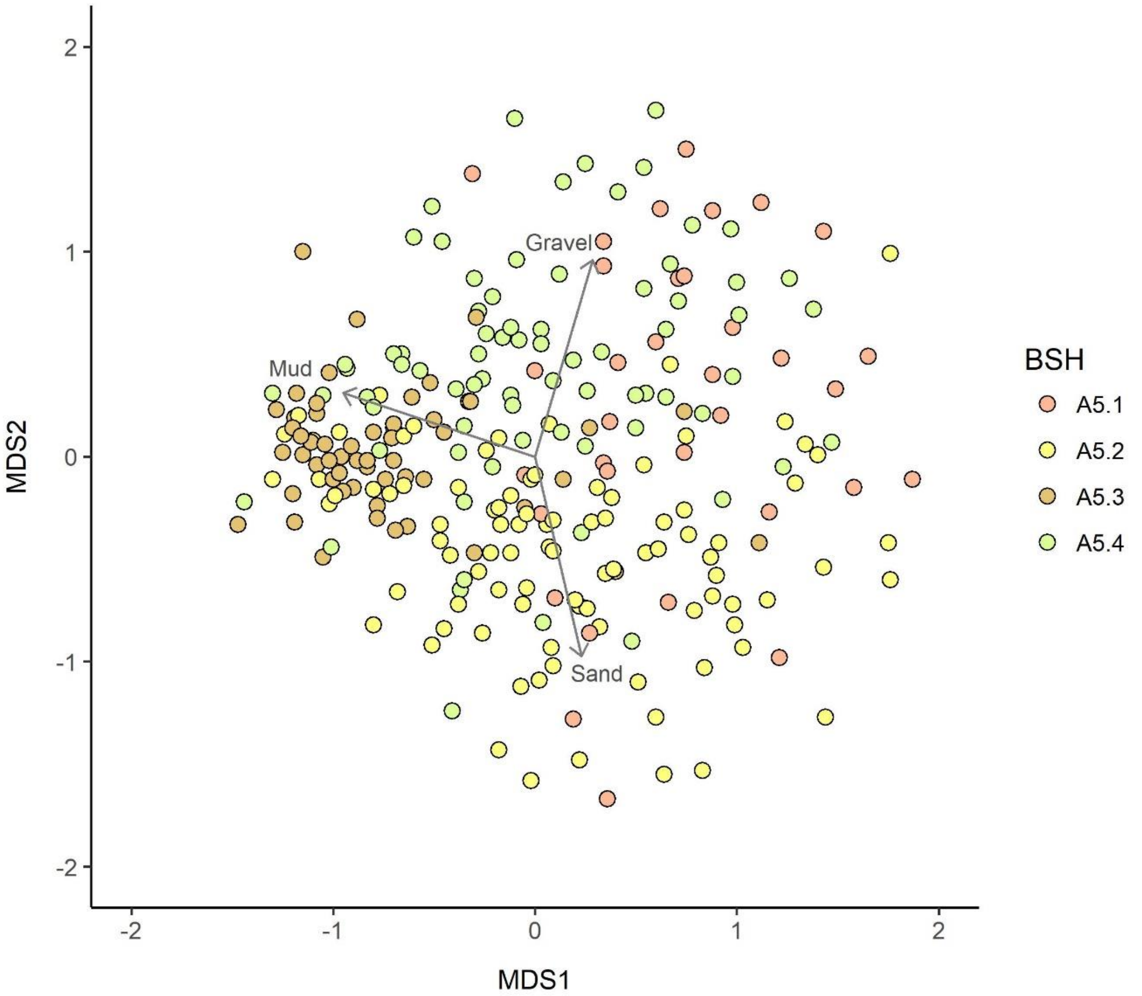


Figure 17. Non-metric multidimensional scaling ordination of infaunal community composition (based on $\log_e(x+1)$-transformed taxa abundances) in relation to sediment Broadscale Habitat (○ = 'A5.1 Subtidal coarse sediment', ○ = 'A5.2 Subtidal sand', ○ = 'A5.3 Subtidal mud', ○ = 'A5.4 Subtidal mixed sediment') and sediment composition vectors (arrows) within the East of Haig Fras MCZ in 2015. Two-dimensional stress = 0.25.

Table 6. Taxa that made the greatest % contributions to the internal similarity of infaunal samples collected from sediment Broadscale Habitats (BSHs) ('A5.1 Subtidal coarse sediment', 'A5.2 Subtidal sand', 'A5.3. Subtidal mud', and 'A5.4 Subtidal mixed sediments') in the East of Haig Fras MCZ in 2015.

BSH	Internal similarity	Main Taxa	% contribution
A5.1	28.94	*Echinocyamus pusillus*	11.28
		Glycera spp.	7.41
		Chaetozone spp.	7.29
		Nemertea	6.47
		Spiophanes spp.	6.14
		Aponuphis bilineata	4.88
		Unciola planipes	4.53
		Glycinde nordmanni	3.76
A5.2	31.76	*Echinocyamus pusillus*	21.30
		Spiophanes spp.	8.19
		Nemertea	7.52
		Glycera spp.	5.99
		Amphiura filiformis	5.83
		Phaxas pellucidus	4.31
		Chaetozone spp.	4.05
		Lumbrineris spp.	3.79
A5.3	40.81	*Echinocyamus pusillus*	9.34
		Phaxas pellucidus	7.73
		Spiophanes spp.	6.52
		Nemertea	6.09
		Ampharete falcata	5.45
		Magelona minuta	4.77
		Terebellides spp.	4.34
		Amphiura filiformis	4.25
A5.4	30.58	*Echinocyamus pusillus*	9.28
		Nemertea	8.40
		Spiophanes spp.	7.28
		Lumbrineris spp.	6.02
		Glycera spp.	5.98
		Terebellides spp.	5.02
		Phaxas pellucidus	4.85
		Chaetozone spp.	3.78

There was significant variation ($p < 0.05$) across sediment BSHs in univariate indices of infaunal density and diversity (Figure 18; Annex 7). Mean total abundance and species richness were higher in 'A5.3 Subtidal mud' than in other sediment BSHs and were higher in 'A5.4 Subtidal mixed sediments' than in 'A5.1 Subtidal coarse sediment' and 'A5.2 Subtidal sand' (Figure 18 a, b). The Margalef Index was higher in 'A5.3 Subtidal mud' and 'A5.4 Subtidal mixed sediments' than in the other two sediment BSHs on average (Figure 18 c), whereas mean Shannon Index was relatively low in 'A5.2 Subtidal sand' (Figure 18 d) but reasonably high (2.8–3.0) across all sediment BSHs in absolute terms (Shannon Index typically lies between 1.5–3.5 in ecological studies; May 1975).

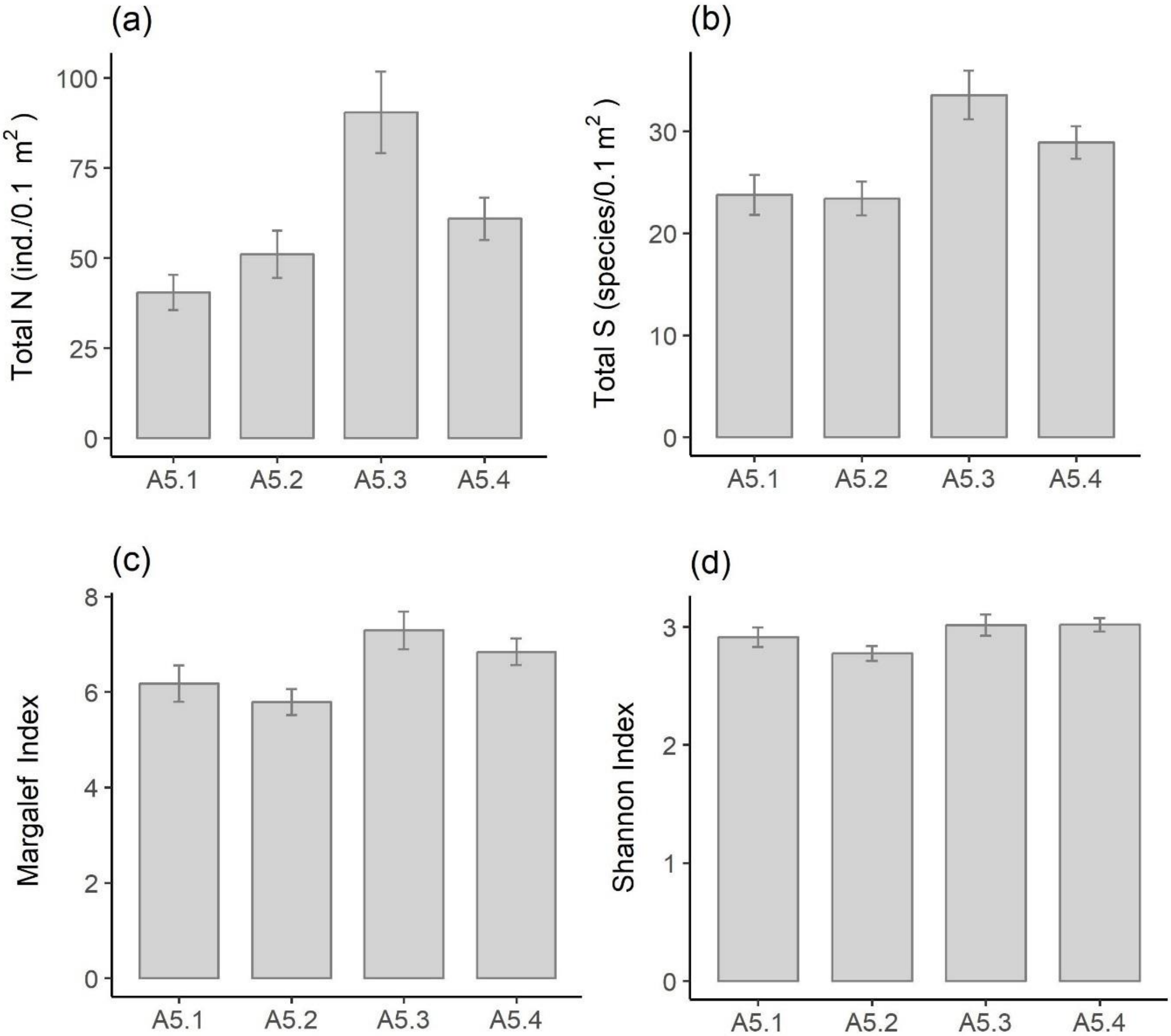


Figure 18. Mean and 95% confidence intervals of (a) total abundance, (b) total number of species (species richness), (c) Margalef Index, and (d) Shannon Index of infauna in 'A5.1 Subtidal coarse sediment' (n = 34), 'A5.2 Subtidal sand' (n = 93), 'A5.3 Subtidal mud' (n = 53), and 'A5.4 Subtidal mixed sediments' (n = 77) within the East of Haig Fras MCZ in 2015.

Further exploration of variation in the benthos within and across sediment BSHs within the MCZ indicated that infaunal assemblages were represented by 17 significantly different clusters (SIMPROF; $p < 0.05$). These clusters were somewhat spatially dispersed throughout the MCZ and most were not associated with a single BSH (Figure 19; Figure 20). Some clusters did, however, show a degree of spatial pattern that appeared to relate to habitat type. For example, clusters f, h, j, k, and l were found mainly in the southwest of the site, where muddy sediments are relatively common, and cluster n was found mainly in the southeast, where coarser sediments (i.e. 'A5.1 Subtidal coarse sediment' and 'A5.4 Subtidal mixed sediments') are relatively common (Figure 20). It should also be noted that while communities in clusters f, h, j, k, and l are statistically distinguishable ($p < 0.05$), they are more similar to each other than they are to communities from other clusters (indicated by their similarity in colour in Figure 20). The infaunal taxa that characterised each cluster, the BSHs over which the clusters were distributed and, where possible, the biotopes to which these clusters were matched are shown in Table 7.

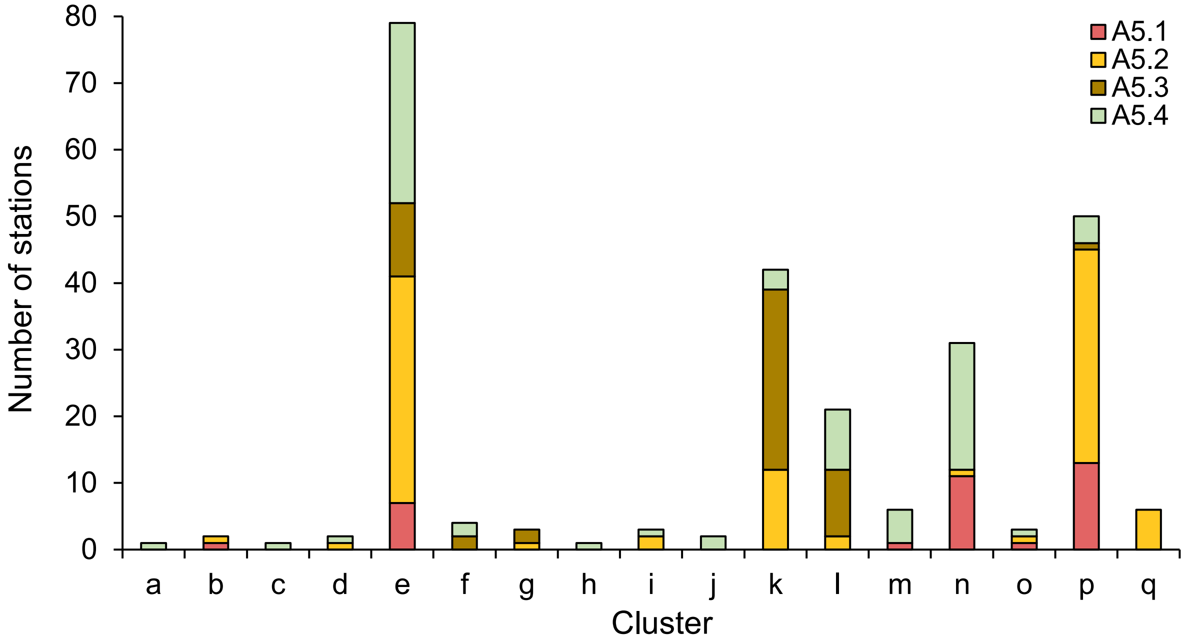


Figure 19. The number of stations at which each infaunal community cluster was observed and the Broadscale Habitats (BSHs) of these stations (■ = 'A5.1 Subtidal coarse sediment', ■ = 'A5.2 Subtidal sand', ■ = 'A5.3 Subtidal mud', and ■ = 'A5.4 Subtidal mixed sediments') based on grab samples collected from the East of Haig Fras MCZ in 2015. The taxa that characterise each cluster are shown in Table 7.

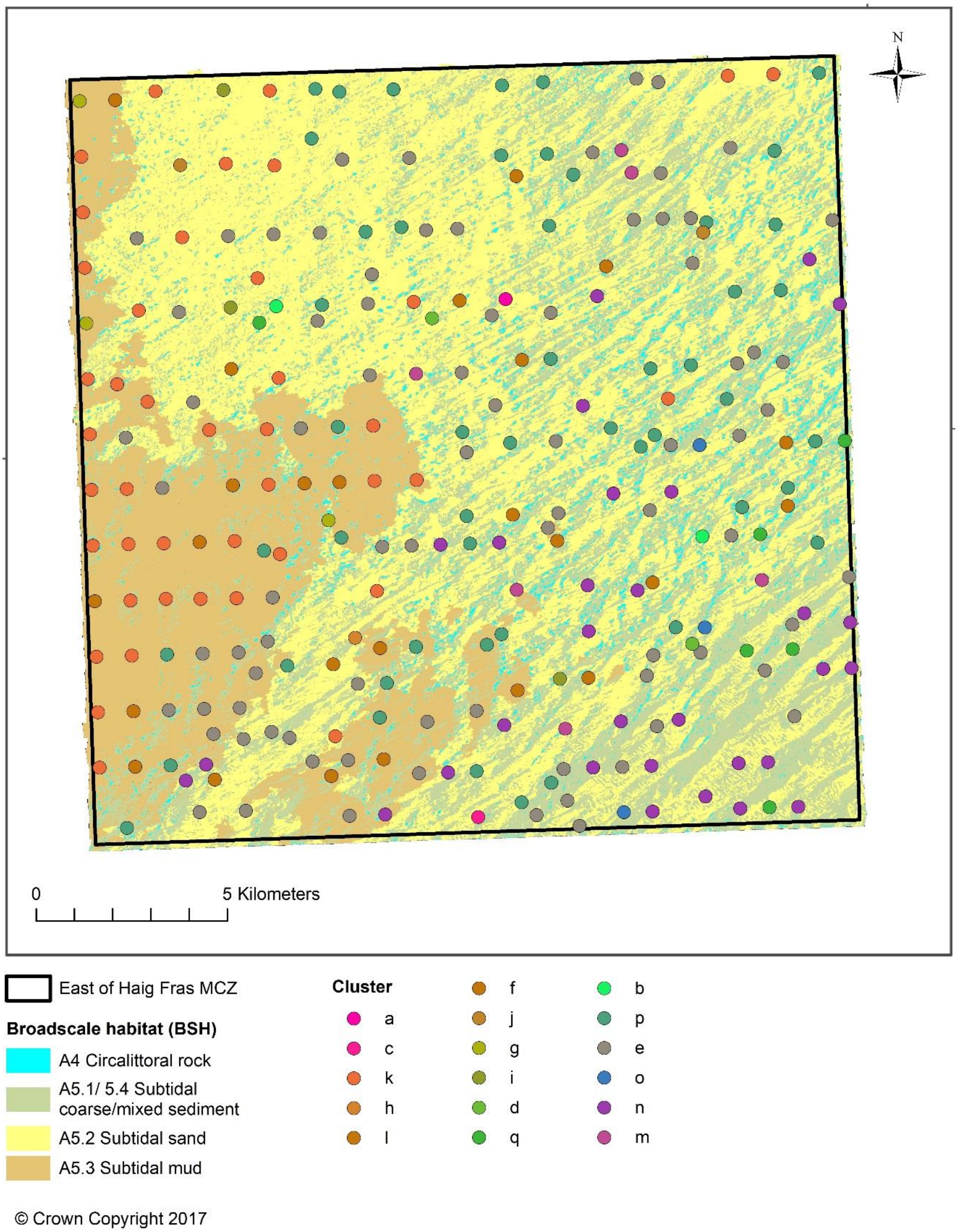


Figure 20. The spatial distribution of clusters in infaunal community composition within the East of Haig Fras MCZ in 2015. Communities within the same cluster are not significantly different from each other ($p > 0.05$), whereas those in different clusters are significantly different ($p < 0.05$). The similarity of communities from different clusters is indicated by their colour; those that are more similar in composition are more similar in colour, whereas those that are more dissimilar in composition are more dissimilar in colour. The Broadscale Habitat map is from Eggleton and Downie (2017).

Table 7. Taxa that made the greatest % contributions to the internal similarity of infaunal clusters observed in the East of Haig Fras MCZ in 2015. When a cluster consisted of just one sample, the numerically dominant taxa are listed as the main taxa. The Broadscale Habitats (BSHs) ('A5.1 Subtidal coarse sediment', 'A5.2 Subtidal sand', 'A5.3 Subtidal mud', and 'A5.4 Subtidal mixed sediments') over which the clusters were distributed and, where possible, the inferred biotopes are reported.

Cluster	Internal similarity	Main Taxa	% contribution
a	-	*Goniadella gracilis*	-
		Laonice bahusiensis	-
		Cerianthus lloydii	-
		Edwardsia claparedii	-
		Owenia borealis	-
BSH: A5.4 (1 sample)			
Biotope: A5.441 *Cerianthus lloydii* and other burrowing anemones in circalittoral muddy mixed sediment (SS.SMx.CMx.ClloMx)			
b	35.2	*Amphiura filiformis*	28.38
		Echinocyamus pusillus	17.91
		Goniada maculata	17.91
		Lumbrineris spp.	17.91
		Nemertea	17.91
BSH: A5.1 (1 sample), A5.2 (1 sample)			
Biotope: N/A			
c	-	*Aspidosiphon muelleri*	-
		Eumida sanguinea	-
		Glycera spp.	-
		Terebellides spp.	-
		Lumbrineris spp.	-
BSH: A5.4 (1 sample)			
Biotope: N/A			
d	38.75	*Lumbrineris* spp.	18.84
		Amphiura filiformis	16.23
		Aricidea wassi	8.12
		Dyopedos monacanthus	8.12
		Magelona minuta	8.12
BSH: A5.2 (1 sample), A5.4 (1 sample)			
Biotope: N/A			
e	36.61	*Echinocyamus pusillus*	12.57
		Lumbrineris spp.	8.30
		Nemertea	7.16
		Phaxas pellucidus	6.90
		Amphiura filiformis	6.46
BSH: A5.1 (7 samples), A5.2 (34 samples), A5.3 (11 samples), A5.4 (27 samples)			
Biotope: N/A			

f	39.6	*Spiophanes* spp.	16.36
		Diastylis spp.	15.21
		Ampharete falcata	10.74
		Nemertea	10.74
		Phaxas pellucidus	8.66
BSH: A5.3 (2 samples), A5.4 (2 samples)			
Biotope: N/A			
g	44.12	*Ampharete falcata*	16.03
		Phaxas pellucidus	14.69
		Amphiura filiformis	13.45
		Echinocyamus pusillus	11.38
		Spiophanes spp.	10.18
BSH: A5.2 (1 sample), A5.3 (2 samples)			
Biotope: N/A			
h	-	*Ampharete falcata*	-
		Magelona minuta	-
		Phaxas pellucidus	-
		Phoronis spp.	-
		Nemertea	-
BSH: A5.4 (1 sample)			
Biotope: N/A			
i	47.7	*Echinocyamus pusillus*	19.15
		Phaxas pellucidus	9.82
		Nemertea	9.14
		Euclymene oerstedii	7.07
		Magelona minuta	6.86
BSH: A5.2 (2 samples), A5.4 (1 sample)			
Biotope: N/A			
j	48.21	*Phaxas pellucidus*	9.67
		Abyssoninoe spp.	7.66
		Amphiura filiformis	7.66
		Euclymene oerstedii	7.66
		Lumbrineris spp.	7.66
BSH: A5.4 (2 samples)			
Biotope: N/A			
k	49.83	*Ampharete falcata*	7.62
		Magelona minuta	7.49
		Echinocyamus pusillus	7.44
		Nemertea	5.74
		Spiophanes spp.	5.63
BSH: A5.2 (12 samples), A5.3 (27 samples), A5.4 (3 samples)			
Biotope: N/A			

l	46.74	*Echinocyamus pusillus*	8.63
		Spiophanes spp.	8.10
		Phaxas pellucidus	7.89
		Nemertea	4.55
		Iphinoe serrata	4.54
BSH: A5.2 (2 samples), A5.3 (10 samples), A5.4 (9 samples)			
Biotope: N/A			
m	30.11	*Spiophanes* spp.	14.31
		Terebellides spp.	13.48
		Lumbrineris spp.	9.92
		Nemertea	8.29
		Diastylis spp.	5.64
BSH: A5.1 (1 sample), A5.4 (5 samples)			
Biotope: N/A			
n	34.53	*Spiophanes* spp.	9.33
		Echinocyamus pusillus	8.75
		Chaetozone spp.	8.61
		Goniadella gracilis	6.98
		Nemertea	6.53
BSH: A5.1 (11 samples), A5.2 (1 sample), A5.4 (19 samples)			
Biotope: N/A			
o	38.51	*Unciola planipes*	24.06
		Abra spp.	15.18
		Aricidea cerrutii	15.18
		Echinocyamus pusillus	15.18
		Glycera spp.	6.07
BSH: A5.1 (1 sample), A5.2 (1 sample), A5.4 (1 sample)			
Biotope: N/A			
p	33.74	*Echinocyamus pusillus*	21.05
		Ophelia borealis	8.97
		Glycera spp.	6.42
		Nemertea	6.14
		Amphiura filiformis	5.60
BSH: A5.1 (13 samples), A5.2 (32 samples), A5.3 (1 sample), A5.4 (4 samples)			
Biotope: N/A			
q	46.32	*Echinocyamus pusillus*	35.19
		Spiophanes spp.	14.71
		Nemertea	8.59
		Ophelia borealis	8.21
		Galathowenia spp.	7.86
BSH: A5.2 (6 samples)			
Biotope: A5.251 *Echinocyamus pusillus*, *Ophelia borealis* and *Abra prismatica* in circalittoral fine sand (SS.SSa.CFiSa.EpusOborApri)			

Analysis of variation in univariate biotic indices in relation to sediment components that determine BSH type (% content of gravel, sand and mud) revealed that each index varied significantly in relation to an interaction between sand and mud contents (linear model output is presented in the second table of Annex 5). The density and diversity of infauna declined gradually as the dominant sediment component shifted from gravel (i.e. when % sand and mud contents were both close to 0%) toward either sand or mud, but peaked when sediment consisted of a sand and mud mixture (Figure 21). The Shannon Index varied in relation to an interaction between sand and gravel contents as well as between sand and mud (second table of Annex 5). Variation in infaunal communities in sediment habitats may therefore be shaped by interdependent effects of sediment components that vary in their relative proportions within and between BSHs (i.e. the effect of changing the content of one sediment component on infauna depends on the content of another component). The amount of variation in univariate biotic indices explained by sediment composition (R^2) ranged from 18% for the Shannon Index to 41% for total abundance.

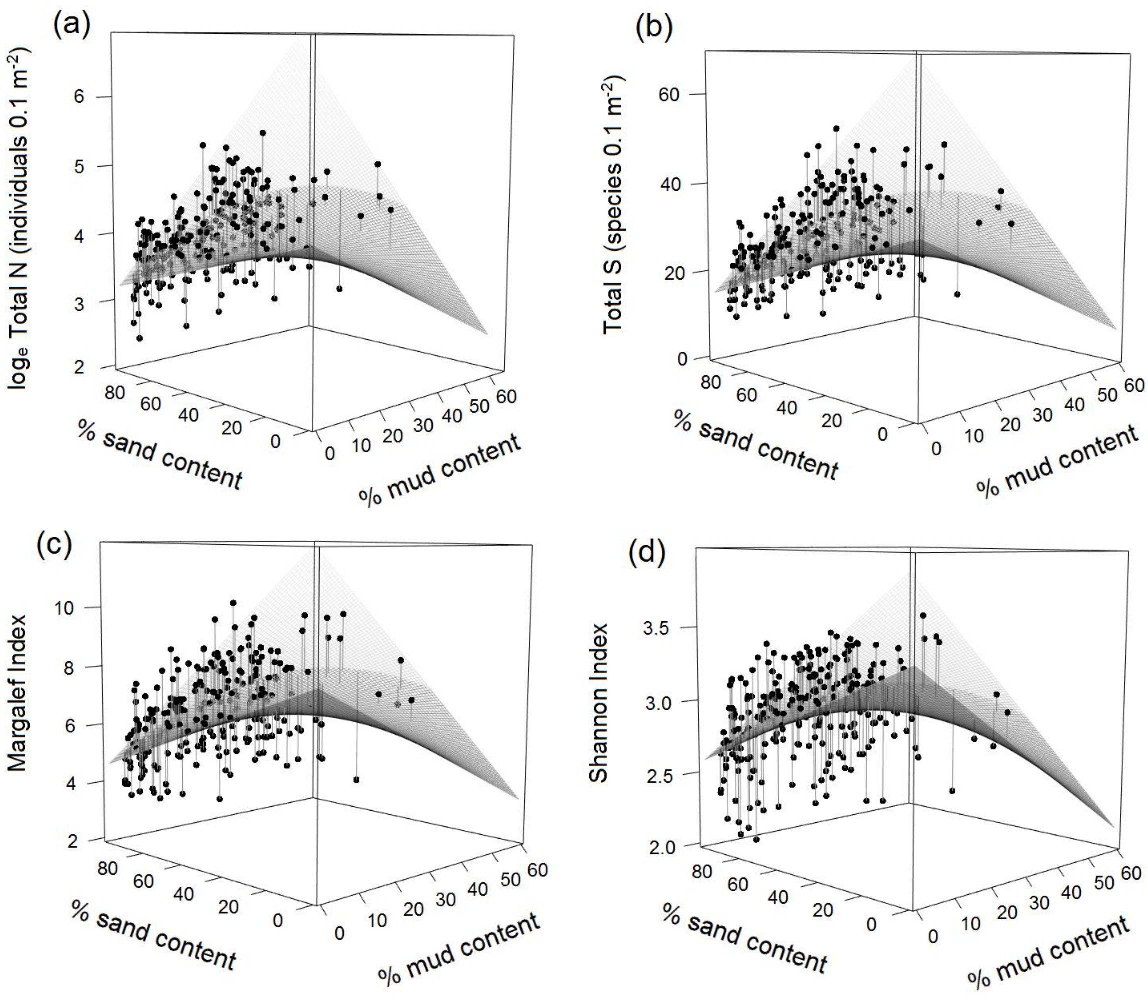


Figure 21. Variation in (a) total abundance (N), (b) total number of species (S; species richness), (c) Margalef Index, and (d) Shannon Index of infauna in relation to % sand content and % mud content of the sediment within the East of Haig Fras MCZ in 2015. The points represent the actual observations and the lines connecting the points to the 3D surface represent the size of the residuals. The 3D surface is coloured white where sand + mud contents theoretically exceed 100%.

The subset of taxa whose abundances are described by the first principal component (PC 1) varied mainly in relation to an interaction between sand and mud contents (R^2 = 63%), thus mirroring the relationships observed for total abundance and diversity (third table of Annex

5). Taxa that increased (positively-loaded taxa) and decreased (negatively-loaded taxa) in abundance as sediment composition tended toward sand and mud mixtures are shown in Table 8. The subset of taxa whose abundances are described by the second principal component (PC 2) varied mainly in relation to an interaction between sand and gravel contents (R^2 = 36%). Some of these taxa increased in numbers when sediment consisted of mainly sand or gravel (positively-loaded taxa), whereas others showed the opposite pattern (negatively-loaded taxa) (Table 8). PCs 1 and 2 together captured 18% of total spatial variation in community composition (11% for PC1, 7% for PC 2). Analysis of the correlation between the full community (based on Bray-Curtis similarity of $\log_e$ (x+1) transformed taxa abundance data) and sediment composition also indicated a statistically significant relationship ($p < 0.05$), which was best explained by the same two interactions highlighted in the analysis of principal components, i.e. sand x mud and sand x gravel (R = 0.39). These results reaffirm that variation in infaunal communities within and across sediment BSH is attributable, in part, to interdependent effects of sediment components (i.e. gravel, sand, and mud contents).

Table 8. Taxa positively-loaded (above dashed line) and negatively-loaded (below dashed line) on the first and second principal components of Hellinger-transformed abundance data for infauna in the East of Haig Fras MCZ in 2015.

Taxa PC 1	Eigenvector	Taxa PC 2	Eigenvector
Ampharete falcata	0.375	*Amphiura filiformis*	0.644
Magelona minuta	0.352	*Echinocyamus pusillus*	0.381
Terebellides spp.	0.24	*Ophelia borealis*	0.156
Phaxas pellucidus	0.23	*Scoloplos (Scoloplos) armiger*	0.111
Iphinoe serrata	0.223	*Phaxas pellucidus*	0.101
Diplocirrus glaucus	0.198	*Echinocardium* spp.	0.097
Euclymene oerstedii	0.144	*Astrorhiza* spp.	0.095
Nephtys spp.	0.131	*Goniada maculata*	0.08
Ampelisca spp.	0.125	*Sthenelais limicola*	0.079
Falcidens crossotus	0.125	*Lumbrineris* spp.	0.077
Cerianthus lloydii	-0.091	*Cerianthus lloydii*	-0.088
Harmothoe glabra	-0.097	*Grania* spp.	-0.09
Goniadella gracilis	-0.119	*Eulalia mustela*	-0.123
Nototropis vedlomensis	-0.122	*Chaetozone* spp.	-0.125
Aricidea (Acmira) cerrutii	-0.132	*Notomastus* spp.	-0.133
Echinocyamus pusillus	-0.141	*Nototropis vedlomensis*	-0.145
Chaetozone spp.	-0.176	*Aponuphis bilineata*	-0.146
Ophelia borealis	-0.186	*Spiophanes* spp.	-0.148
Unciola planipes	-0.195	*Goniadella gracilis*	-0.183
Aponuphis bilineata	-0.25	*Ampharete falcata*	-0.257

3.3.4 Grab gear comparison

Analysis of mud content data derived from Hamon Grab and Day Grab samples collected at fifteen stations where the BSH appeared, based on visual inspection of samples, to be ‘A5.3 Subtidal mud’ revealed a significant difference ($p < 0.05$) between gears. Average mud content was 27% (± 4; standard deviation) for the Day Grab and 23% (± 4) for the Hamon Grab. At two stations, PSA led to the BSH being classified as ‘A5.3 Subtidal mud’ based on the Day Grab sample and ‘A5.2 Subtidal sand’ based on the Hamon Grab sample. Moreover, at one of these stations, mud content of the Day Grab sample was 31% whereas in the Hamon Grab sample mud content was just 17%. On the other hand, the two gears did not differ significantly in terms of any of the univariate indices used to describe the density and diversity of infauna (i.e. total abundance, species richness, the Margalef Index and the Shannon Index).

3.4 Features of Conservation Importance (FOCI)

3.4.1 Habitat Features of Conservation Importance (FOCI)

Several taxa considered indicative of the habitat FOCI 'Sea-Pen and Burrowing Megafauna Communities' were observed in the MCZ (Figure 22). The taxa that make up this FOCI are key species that influence biogeochemical cycling and add three-dimensional structure to the seabed (see report objective 1 and sub-objectives; Table 2). *Callianassa subterranea* and *Goneplax rhomboides* were found in grab samples collected at two and eleven stations, respectively, in the western half of the site. The sea-pen *Virgularia mirabilis* was observed in a grab sample at one station and in four still images taken across three stations. However, these records were largely associated with 'A5.1 Subtidal coarse sediment' and 'A5.2 Subtidal sand'. Only *G. rhomboides* was found in 'A5.3 Subtidal mud' (seven occurrences); the BSH that the 'Sea-Pen and Burrowing Megafauna Communities' feature is typically associated with.

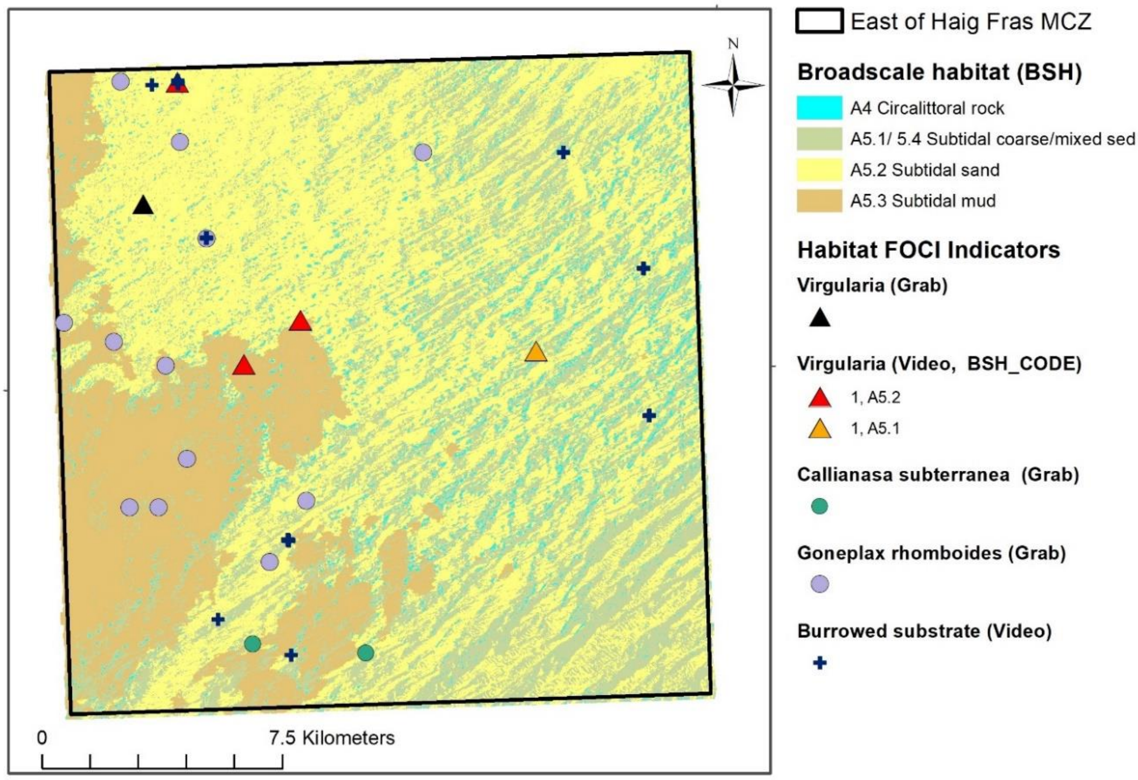


Figure 22. Locations where species indicative of 'Sea-Pen and Burrowing Megafauna Communities' were observed in grab samples and still images (drop camera) during a survey of the East of Haig Fras MCZ in 2015. Locations where burrowed substrate was observed in still images are also shown.

Active burrows were observed in 52 images across nine stations within the MCZ (Figure 22). At eight of these stations, burrows occurred at a sufficient density to meet classification criteria for this FOCI (i.e. one burrow per 10m^2; JNCC, 2014). Burrows were observed in association with 'A5.2 Subtidal sand' and 'A5.3 Subtidal mud' (Annex 7); however, it should be noted, that the latter BSH was not targeted with the drop camera. Representative images of burrows are shown in Figure 23.

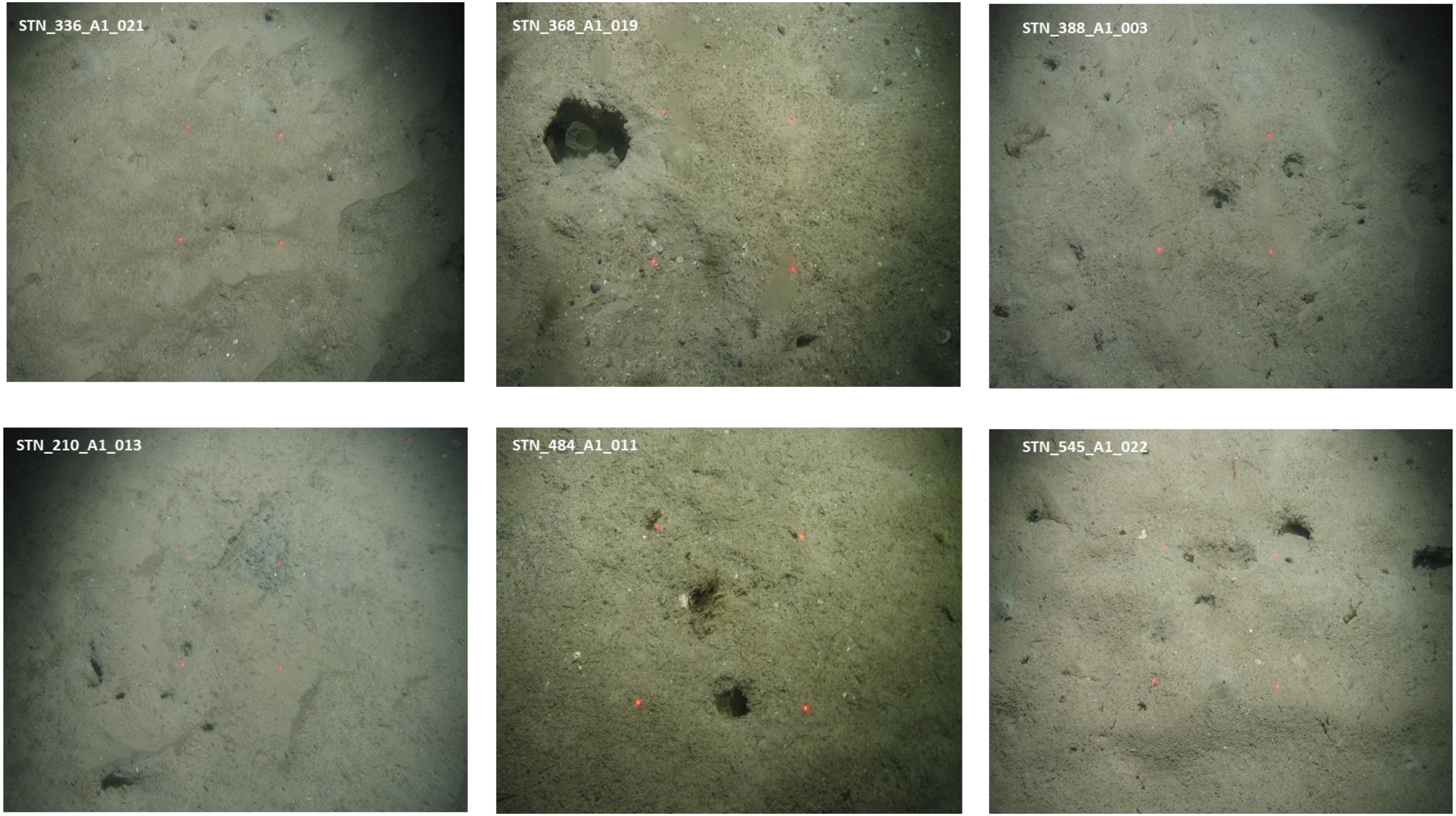


Figure 23. Still images representative of burrowed substrate observed at nine stations during a drop camera survey within the East of Haig Fras MCZ in 2015.

3.4.2 Species Features of Conservation Importance (FOCI)

Two taxa included in the MCZ list of species FOCI were recorded during the survey. The Fan Mussel (*Atrina fragilis*) was observed in 37 images from 29 stations (out of 162, 18%) within the site (Figure 24). The Pink Sea-Fan (*Eunicella verrucosa*) was observed in one image (Figure 24). No species FOCI were found in grab samples.

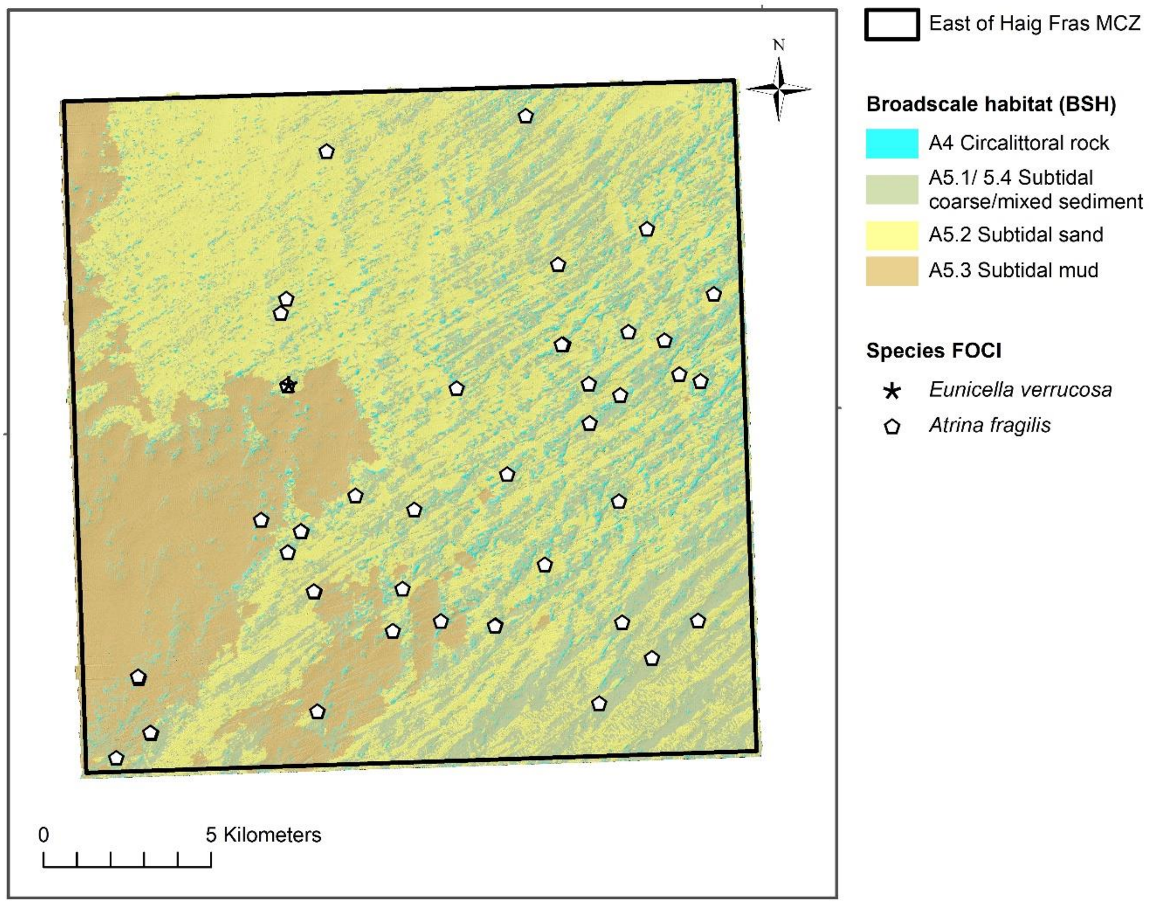


Figure 24. Locations where species FOCI were observed in still images within the East of Haig Fras MCZ in 2015.

3.5 Non-indigenous species (NIS)

No NIS were recorded in the epifaunal (seabed imagery) or infaunal (grab sample) community datasets.

4 Discussion

This monitoring report has achieved the broad objectives 1, 3 and 4, describing the distribution and structural attributes of designated features within the East of Haig Fras MCZ, noting the presence of any potential habitat or species FOCI not covered by the site designation order and non-indigenous species. The following sections discuss the evidence pertaining to these objectives and provide monitoring recommendations for the designated features in order to meet broad objective 5. Aside of the production of the tidal model no further data have been presented or discussed in order to inform on the supporting processes of the designated features (report objective 2; see section 1.3.3 and Table 2). No targeted sampling was undertaken in 2015 to inform on wider supporting processes and none is expected to be planned in the future therefore data to support this objective must be sought from wider, existing monitoring programmes in the future. This is reflected in the recommendations in section 5.

4.1 Benthic and environmental overview

The extent and distribution of sediment BSHs based on data acquired in 2015 is in broad agreement with predictions of the habitat map (Eggleton & Downie 2017). However, there is some evidence for a possible migration eastward of the northern section of ‘A5.3 Subtidal mud’, with a notable series of stations (on a north–south axis) assigned this BSH where ‘A5.2 Subtidal sand’ was predicted. The hydrodynamic model shows that, although low in predicted maximum and mean velocity, the dominant current direction is west–east, thus providing a possible mechanism to explain any eastward migration of fine sediment fractions. Data collected in 2015 indicate that ‘A5.3 Subtidal mud’ continues to be associated with relatively deep areas within the MCZ (> 98m).

The observed rock habitats, predicted in the habitat map as ‘A4 Circalittoral rock’ (Eggleton & Downie 2017), were shown to consist of ‘A4.2 Moderate energy circalittoral rock’ and the distribution of this BSH, according to seabed imagery data collected in 2015, is consistent with predictions of the 2013 habitat map. This BSH was found to comprise a high density of cobbles with low relief and was associated with the crests of large coarse substrate ridges observed in the central and eastern sections of the MCZ.

4.2 Subtidal rock BSH: Physical and biological structure

Subtidal rock within the East of Haig Fras MCZ is formed of boulders and cobbles, interspersed with variable amounts of pebbles, gravel and sand. The only rock BSH identified at the site from the 2015 data was ‘A4.2 Moderate energy circalittoral rock’. The matrix of grain sizes present was reflected in the faunal communities associated with the ‘A4.2 Moderate energy circalittoral rock’ BSH and included some burrowing taxa alongside a range of sessile taxa that typically characterise such subtidal rock habitats. These fauna included: encrusting bryozoans and sponges, hydrozoan turf, tubiculous polychaetes belonging to the family Serpulidae, the cup coral *Caryophyllia* spp. and the branching colonies of the bryozoan *Porella* sp.

Epifaunal diversity and community composition were observed to be relatively stable in rock habitats compared to sediment habitats. While the diversity of epifauna increased with increasing cobble/boulder content in sediment habitats (i.e. where cobble/boulder content was low; < 30%), there was no additional increase in epifaunal diversity with increasing cobble/boulder content in habitats classified as rock (i.e. when cobble/boulder content was > 30%) (Figure 16). The differences in the spatial variability of communities between rock and sediment habitats have implications for future monitoring of designated BSHs at the East of Haig Fras MCZ (discussed in section 4.5).

Only one rock biotope, 'Echinoderms and crustose communities' (CR.MCR.EcCr), was identified using the 2015 seabed imagery data. Consequently, all rock habitat observed in the 2015 survey was classified as 'A4.2 Moderate energy circalittoral rock'. Analysis of images from the earlier habitat verification survey in 2013 included a second biotope 'Mixed faunal turf communities' (CR.HCR.XFa), and the presence of 'A4.1 High energy circalittoral rock' was reported at the site verification stage (Eggleton & Downie 2017). The communities associated with these biotopes are very similar and it was a comparable taxa list that characterised the 'A4.2 Moderate energy circalittoral rock' communities in 2015 that was split across the two rock BSHs in 2013. Both biotopes assigned bear some resemblance to the collection of taxa observed in the images; hydrozoan and bryozoan turf with associated sponges, tubiculous worms (Serpulidae) and cup corals (*Caryophyllia* spp.), but neither are a particularly good fit.

Due to the topography of the site, it is observed that taxa spanning both biotopes are observed in close proximity to one another, with the species characteristic of the 'A4.1 High energy circalittoral rock' present at the tops of the cobbles and boulders where they may be subject to increased energy compared to the lower-lying rocky areas. It is for this reason that distinction between 'A4.1 High energy circalittoral rock' and 'A4.2 Moderate energy circalittoral rock' cannot be easily made for mapping purposes, as it is an intricate mosaic of moderate and high energy species.

Multivariate analysis of the 2013 epifaunal community data carried out by Allen *et al*. (2016) confirmed that there was not enough evidence to support the split into two rock biotopes. Allen *et al*. (2016) highlighted the presence of two sponge taxa (*Axinella* spp. and *Phakellia ventilabrum*) that are characteristic of another biotope, '*Phakellia ventilabrum* and Axinellid sponges on deep, wave-exposed circalittoral rock' (CR.HCR.DpSp.PhaAxi) and assigned all rock habitat in the 2013 data to this biotope. Whilst flabellate sponges (*Axinella infundibuliformis* / *P. ventilabrum*) were observed in three still images in the 2015 dataset, they were not identified as a characterising species in 2015. Consequently, there was not sufficient evidence to warrant a change from the CR.MCR.EcCr biotope given for the 2015 data to the CR.HCR.DpSp.PhaAxi biotope suggested by Allen *et al*. (2016).

Additionally, there is uncertainty around the identification of *Henricia* sp., which was prominent in the 2013 dataset and a biotope defining species for Allen *et al*. (2016). No records of *Henricia* sp. occur in the 2015 data however a very similar, if less common species, *Stichastrella rosea*, is regularly recorded. On comparison of the images from each year, many of identifications of *Henricia* sp. in 2013 are questionable and some are considered the same species as identified in 2015 as *Stichastrella rosea.* On balance, upon review of the images, the 2015 identification of *Stichastrella rosea* is considered overall more accurate.

The different opinions of analysts looking at the raw images and those assigning biotopes based on community analysis all contributes the subjective nature of using biotope classifications. It is difficult to match a community to a description which it may only partially resemble and there is a tendency for analysts' identification of specific taxa to be highly coloured by previous experience. As demonstrated here this variability can have a big effect on the biotopes recorded. In this case it may be that the rocky community observed at East of Haig Fras is a subtype of one of the biotopes suggested above, or it may be a mixture of coarse sediment and rock community components that is not currently recognised in the classification. Regardless based on these data using biotopes to describe rock habitats for the purposes of repeat monitoring at East of Haig Fras is not recommended.

Rock-associated epifauna were not matched to biotopes using the 2012 survey, and taxa were identified to a much lower taxonomic resolution, but epifaunal communities were characterised by the same broad taxon groups as observed in 2013 and 2015 (e.g. hydroids, bryozoans and sponges).

4.3 Subtidal sediment BSH: Sediment composition and biological structure

Grab samples indicated that the Pea Urchin (*Echinocyamus pusillus*) characterises sediment BSHs in the East of Haig Fras MCZ (Table 6), reaffirming the findings of a previous habitat verification survey (Allen *et al.* 2016). Polychaetes (e.g. *Spiophanes* spp. and *Glycera* spp.), Nemerteans and the razor shell *Phaxas pellucidus* were also commonly observed among the infauna in sediment habitats. Of these taxa, *Spiophanes* and Nemerteans were commonly observed in grab samples during the 2012 survey; however, *Glycera* and *Phaxas* were typically not among the characterising taxa (Allen *et al.* 2016). This suggests that there may have been some temporal change in faunal community composition of sediment habitats in the East of Haig Fras MCZ in the years between the two surveys (2012-15). However, the grab surveys were conducted at different times of the year (February in 2012 and May in 2015) and therefore differences in infaunal composition could be, in part, due to seasonal cycles. The dataset from the more recent survey (i.e. the dataset analysed in this report) is also much larger than the previous survey (257 vs 48 grab stations), which could also contribute to the apparent differences in infaunal composition between the two surveys. No grab samples were collected during the habitat verification survey in April 2013 and, therefore, no comparison can be made to infaunal communities at the site in this year. In both years when grab samples were collected from the East of Haig Fras MCZ, infaunal communities were generally characterised by taxa considered to be indicative of low to intermediate levels of physical disturbance and chemical stress, such as *Amphiura* and *Terebellides* (low levels) and *Chaetozone* and *Glycera* (intermediate levels) (Gray & Elliott 2009; Chapter 9). The common occurrence of sensitive taxa suggests that sedimentary habitats within the East of Haig Fras MCZ may be in a generally good ecological condition.

Regarding variability in infauna within the East of Haig Fras MCZ in 2015, all sediment BSHs were statistically distinguishable in terms of community composition ($p < 0.05$). However, there was also a high level of variability in the infauna within all sediment BSHs, with communities from the same BSH clustering into different groups and communities from different BSHs clustering together (Figure 19). The latter of these observations, along with the makeup of taxa that characterised each cluster, prevented biotopes from being identified using the grab data in most cases. Likewise, biotopes could rarely be unambiguously identified using data collected during the 2012 habitat verification survey (Allen *et al.* 2016). The lack of a clear association between infauna and BSH appears, at least in part, to be due to the range of sediment compositions that occur within the same BSH (see Figure 10). Indeed, some of the infaunal taxa that characterised a BSH showed opposing relationships with the same sediment components (i.e. sand, mud or gravel contents). For example, a subset of taxa that characterised ‘A5.1 Subtidal coarse sediment’ were relatively abundant when the sediment consisted mainly of either gravel or sand, but not gravel and sand mixtures, while others that characterised the same BSH were relatively abundant in gravel and sand mixtures. Such an unintuitive occurrence is possible because ‘A5.1 Subtidal coarse sediment’ covers sediment compositions ranging from mainly sand through to mainly gravel (Figure 10). The likelihood that infauna respond to changes in the proportions of sediment components, rather than BSH *per se*, was proposed to explain the findings of the earlier survey of this site (Allen *et al*. 2016), and the analyses conducted for this report support this proposition.

Sediment BSHs were generally also distinguishable in terms of epifaunal community composition, the only exception being ‘A5.1 Subtidal coarse sediment’ and ‘A5.4 Subtidal mixed sediments’. With larger sessile species most likely to be observed in seabed imagery, the diversity and composition of epifauna was linked to the amount of substrate available for attached fauna (i.e. % cover of pebbles and cobbles). For example, the number of epifaunal species increased with increasing prevalence of cobble and boulders, regardless of whether they inhabited ‘A5.1 Subtidal coarse sediment’ or ‘A5.4 Subtidal mixed sediments’ (Figure 16). As with infauna, results suggest that faunal communities are not strongly linked to the BSH in which they occur, but rather are influenced primarily by fine-scale differences in substrate composition.

Biotopes in sediment BSHs could only be classified to a very coarse level using seabed imagery and could not be classified with respect to differences in epifaunal community composition. The biotopes identified were ‘Circalittoral coarse sediment’ (SS.SCS.CCS), ‘Circalittoral mixed sediments’ (SS.SMx.CMx), and ‘Sublittoral sands and muddy sands’ (SS.SSa) or its sub-biotope ‘Circalittoral muddy sand’ (SS.SSa.CMuSa). This matches the resolution achieved when identifying sediment biotopes using data collected during the 2012 and 2013 habitat verification surveys (Allen *et al.* 2016). In all years, epifaunal taxa were sparse and consisted mainly of hydroids and bryozoans, along with some sponges, tubiculous worms of the family Serpulidae and hermit crabs of the family Paguridae. It therefore appears that there has been little temporal change in epifaunal communities associated with sediment BSHs in the East of Haig Fras MCZ between 2012 and 2015. A new biotope denoting circalittoral sediment with sparse fauna may be warranted from these observations.

4.4 Implications for future monitoring

4.4.1 Seabed imagery acquisition

The nature of the seabed within the East of Haig Fras MCZ necessitated the use of a drop camera to collect imagery. Using a drop camera, as opposed to a camera sledge or flying array, introduces uncertainty into the data derived from video and still imagery due to the variability in height above the seabed during video segments and between still images. The variability within video segments makes it very difficult to estimate the area sampled and to select an appropriate level of taxonomic identification. Hence, this report only utilised data derived from the still images. The image sub-setting process (described in Annex 1) enabled the use of quantitative measures, such as species richness, but also greatly reduced the number of stations with sufficient stills to be included in the dataset. The poor coverage of rock habitat in images resulted from the patchy nature of the seabed, where changes in BSH type occur at the scale of tens of metres. Consequently, most video tows covered more than one BSH, yielding a lower number of still images per BSH per tow. This patchiness in BSH will have to be accounted for in the execution of drop camera transects and in defining what constitutes a sample during future monitoring (see recommendations in Section 5).

The height of the camera above seabed influences image resolution and consequently affects the size of individuals that can be observed in images, as well as the level of taxonomic identification possible. A FOV of < 0.4m^2 was found to correspond to ‘Good’ and a FOV of < 0.25m^2 to ‘Excellent’ image quality as specified by the NMBAQC digital imagery interpretation guidelines (Turner *et. al* 2016). Keeping the image quality and FOV more consistent across the entire dataset made taxonomic truncation of the community dataset easier and reduced arbitrary variability in the resulting community matrix. Species accumulation curves, calculated as part of the image data sub-setting procedure, revealed that the appropriate area of seabed to sample the rock community with still imagery on the scale of a 100-150 metre drop camera transect was ~ 4m^2 (Figure 25). This corresponds to ten 0.4m^2 images, or 20 images at a FOV of 0.2m^2. Far fewer images of sufficient quality were collected for the majority of transects at East of Haig Fras in the 2015 survey on which this report is based.

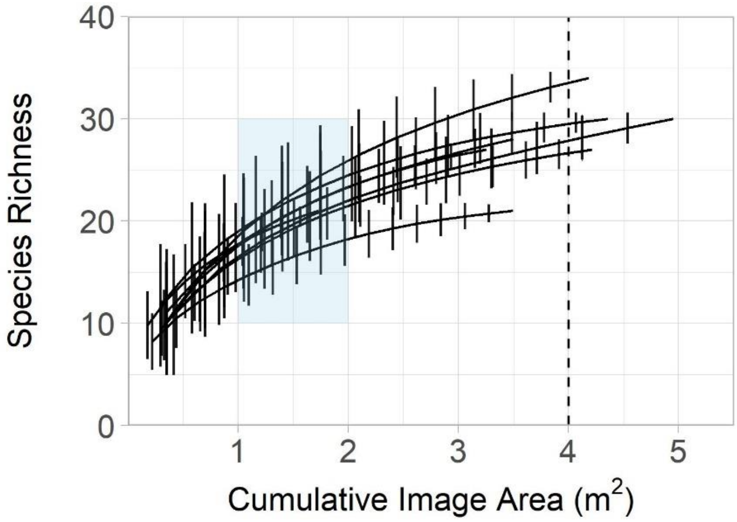


Figure 25. Species accumulation curves for transects on rock habitat, with estimated confidence intervals (2 standard deviations). The curves are derived from transects with a minimum of ten images. The standard sample area used for this report is highlighted in blue. Vertical dashed line shows the minimum area recommended for sampling.

Imagery collected closer to the seabed has a superior quality compared to that taken further from the bed, which results in greater taxonomic accuracy. However, this approach requires more images to sufficiently sample the rock community. Increasing the length of the transect will provide a more complete assessment of associated epifauna but will also increase the likelihood of variability in BSH (i.e. between ‘A4.2 moderate energy circalittoral rock’ and sediment BSHs). In view of the fine-scale spatial variability of the seabed throughout most of the site and the limitations of drop camera systems, future monitoring of ‘A4.2 Moderate energy circalittoral rock’ at the East of Haig Fras MCZ may benefit from targeted sampling of the largest known or likely patches of rock and using transects of shorter lengths. A reliable and repeatable representation of the rock habitat can be achieved by collecting images within a specified spatial neighbourhood at a uniform FOV, analysed to an appropriate level of taxonomic identification and consistently truncated.

4.4.2 Observed physical and biological structure of BSHs

As epifaunal communities associated with ‘A4.2 Moderate energy circalittoral rock’ showed little spatial variability, it seems appropriate to treat this BSH as a single unit for monitoring. The primary issue to consider when monitoring ‘A4.2 Moderate energy circalittoral rock’ is therefore locating areas where this BSH is of sufficient extent to give a reliable representation of its associated taxa (see Section 4.4.1 and 5). In contrast, the variability in infaunal assemblages within sediment BSHs, which is associated with fine-scale variability in sediment composition, may mean that it is inappropriate to treat all grab samples from the same sediment BSH as comparable replicates during monitoring. By extension, a grouping together of the BSHs ‘A5.1 Subtidal coarse sediment’ and ‘A5.4 Subtidal mixed sediments’ for analysis (as is required for survey design; see section 2.3.4) also seems inappropriate. It may instead be necessary to divide sediment BSHs into sub-habitats with narrower, more ecologically relevant sediment compositions to provide an improved basis for monitoring. However, if this approach is adopted, targeting areas of the seabed that are sufficiently similar in sediment composition to be considered as replicates could be challenging. While the survey reported on here has provided information on sediment composition throughout the MCZ, the fine-scale spatial heterogeneity of the seabed may make it difficult to locate stations within the narrow range of particle size distributions targeted. It is also possible that sediments will be spatially displaced by currents over time, which, given the level of heterogeneity at the site, could lead to temporal changes in sediment composition at localities throughout the MCZ. If such temporal variability in sediment composition occurs, then even returning to stations and sampling the same areas of the seabed in consecutive surveys may not provide the data required to assess temporal ecological change under the same habitat conditions. It is also unlikely that acoustic techniques could be used to target specific sediment compositions, as they are unsuitable for resolving fine-scale gradations in particle size distribution, particularly in areas where substrate is spatially heterogenous, as is the case in the East of Haig Fras MCZ.

A possible approach for addressing the issue of seabed heterogeneity in future surveys of the East of Haig Fras MCZ could be to continue to collect large numbers of samples across BSHs so that the range of sediment compositions within each BSH is likely to be covered. Data could then be retrospectively divided into ecologically meaningful sub-habitats for monitoring of biological structure. However, this may not fully resolve the problem, as the level of fine-scale spatial heterogeneity means that patches of the seabed with the same (or similar) sediment compositions could have different community compositions as a result of being neighboured by very different habitat types. Patches of similar sediment may also be separated by large distances and thus be exposed to differences in environmental conditions that also influence benthic community composition. These suggestions are supported by the observation that some faunal clusters were spatially aggregated and made up of communities from every sediment BSH (Figure 19). A possible solution may be to use Figure 11 to identify areas where sediment composition appears to be the least heterogeneous and, from these areas, attempt to select locations where each BSH or BSH sub-category can be targeted. If replicate samples are collected from each sampling station, then this would allow the level of small-scale seabed heterogeneity to be confirmed and increase the ability to accurately describe the biological structure of the targeted habitat. A consequence of this approach, however, would be a reduced spatial coverage of samples (compared to taking a single sample at many stations) and therefore a reduced ability to monitor changes to the extent and distribution of designated features, which is another monitoring objective (see report objective 1 and Table 2). Rather than attempting to control for seabed heterogeneity through survey design, another option is to include sediment composition (e.g. gravel, sand and/or mud contents) and spatial parameters (e.g. latitude and longitude) as covariates in models used to assess temporal change in biological structure. This would help to statistically separate temporal changes that occur under the same habitat conditions (e.g. changes caused by human activities) from any effects of spatiotemporal variation in sediment composition (either due to sediment redistribution or subtle differences in station location between surveys).

A further consideration for future surveys of sediment habitats is that the potential benefits of using video imagery, as opposed (or as a supplement) to grab sampling need to be weighed against the limitations. Imagery is the more appropriate tool for observing conspicuous epifaunal taxa with low densities and sporadic distributions, such as Sea-Pens, and for assessing the quality of their associated habitats. Very little epifaunal diversity was observed in ‘A5.2 Subtidal sand’ and therefore the value of using video imagery for future monitoring of this feature at the site would likely be low. However, as a range of epifaunal communities were observed in both ‘A5.1 Subtidal coarse sediment’ and ‘A5.4 Subtidal mixed sediments’ (driven mainly by the area available for attached fauna, i.e. pebble and cobble content), video imagery will likely be useful for sampling these BSHs, particularly sediments with a large cobble component, as this sediment component is not sufficiently sampled using grabs. Therefore, while direct sampling (i.e. grabs) should be the main monitoring tool used for sediment BSHs at the East of Haig Fras MCZ, imagery can add useful information on the epifaunal component of ‘A5.1 Subtidal coarse sediment’ and ‘A5.4 Subtidal mixed sediments’. The inability to separate these two BSHs appropriately in imagery means that data acquired from them should be combined for analysis, with consideration given mainly to variation in the hard substrata component that apparently drives variation in the species observed in still images. Imagery would also be the appropriate tool to verify and monitor the potential ‘Sea-Pen and Burrowing Megafauna Communities’ habitat FOCI, should it be considered for inclusion as a designated feature of the site.

4.4.3 Grab gear comparison

As analysis of sediment samples that appeared, based on visual inspection, to be ‘A5.3 Subtidal mud’ revealed significant differences in mud content between the Hamon Grab and Day Grab, the choice of gear clearly has implications for monitoring. This is emphasised by the fact that at two stations, samples collected using different gears implied different BSHs (‘A5.2 Subtidal sand’ vs ‘A5.3 Subtidal mud’), which in one case was associated with a substantial difference in mud content (17% vs 34%). The tendency for the Hamon Grab to indicate relatively low mud content relative to the Day Grab might be explained by differences in how sediment sub-samples are extracted using the two gears, with samples from the former gear requiring homogenisation by hand prior to sediment sub-sampling, thus making the process subject to human biases, whereas samples from the latter gear have the full, intact vertical profile of the sediment extracted from them using a corer. However, other differences between the gears, such as sediment penetration depth (11cm for the Hamon Grab, 15cm for the Day Grab), might also partly or fully explain the differences in mud content. While no significant differences in biotic indices were observed for infaunal communities sampled using the different grabs, the fact that the same community can be inferred to inhabit a different BSH depending on whether it was sampled using a Hamon Grab or Day Grab could clearly have implications for the characterisation and monitoring of biological structure. Specifically, if it is assumed that the Day Grab provides representative sediment samples, then the Hamon Grab will occasionally attribute taxa and communities associated with ‘A5.3 Subtidal mud’ to ‘A5.2 Subtidal sand’, or *vice versa* for the Day Grab if it is assumed that the Hamon Grab provides representative samples. The degree to which this affects results will depend on the distinctness of communities that inhabit the two BSHs; the more distinct the communities, the more misleading the results will be. It should be noted, however, that in most cases (thirteen out of fifteen stations) samples collected using the different gears gave the same indication of BSH. Therefore, if many samples are collected from a site, then site-level assessments of biological structure associated with different BSHs may not be severely affected.

4.5 Habitat Features of Conservation Importance (FOCI)

Taxa indicative of the habitat FOCI ‘Sea-Pen and Burrowing Megafauna Communities’ were observed in both grab samples and still images (Figure 22). However, Sea-Pens were associated with sand and coarse sediment, rather than mud, while burrowing megafauna sampled by grabs were mainly associated with sand or mixed sediments. This could be partly because much of the BSH ‘A5.2 Subtidal sand’ at East of Haig Fras MCZ is muddy sand, which may be sufficient to meet the habitat requirements of some burrowing taxa typically associated with ‘A5.3 Subtidal mud’. Similarly, it is likely to be the mud component of ‘A5.4 Subtidal mixed sediments’ that makes this BSH capable of supporting burrowing megafauna. Nevertheless, the observations made during the survey of the East of Haig Fras MCZ suggest that the substrate requirements of taxa that characterise this FOCI are wider than is typically assumed.

The JNCC advice on identifying ‘Sea-pen and Burrowing Megafauna Communities’ available at the time of analysis (JNCC 2014) indicated between one and nine burrows should be recorded per 10m^2, in conjunction with the collection of infaunal samples confirming the presence of relevant taxa, and PSD data confirming a fine mud habitat[7]. Burrows were observed in 52 still images, across 9 stations, in sufficient numbers per image for the

[7] Note that updated advice is now available (see Hawes 2020; JNCC 2020).

classification of ‘Sea-pen and Burrowing Megafauna Communities’ to be assigned. Moreover, there were two cases in which a FOCI-indicative species was directly observed at a station where burrowed substrate was evident (stations EHGF002 and EHGF112). However, the BSH at both stations was ‘A5.2 Subtidal sand’. While this FOCI is typically associated with fine mud, its presence has been recorded in substrates consisting of a mixture of sand and mud (JNCC 2014); a substrate commonly found in the East of Haig Fras MCZ. The findings of the present report could therefore be interpreted as evidence for the presence of ‘Sea-Pen and Burrowing Megafauna Communities’ within the East of Haig Fras MCZ; however, the presence of this habitat FOCI within the MCZ could not be demonstrated if classification criteria pertaining to substrate type and the direct sampling of identifying taxa are strictly adhered to (JNCC 2014). The dearth of evidence for burrowing megafauna in their typical habitat may simply be a consequence of ‘A5.3 Subtidal mud’ not being targeted for the collection of seabed imagery.

4.6 Species Features of Conservation Importance (FOCI)

Previous surveys during the verification stage have indicated that the species FOCI *Atrina fragilis* (Fan Mussel) is present at the site (Eggleton & Downie 2017). The 2015 monitoring survey has confirmed the presence of *A. fragilis* and indicates a wide distribution throughout the site. This species is highly sensitive to physical disturbance (Tyler-Walters & Wilding 2017). Therefore, an increase to physical disturbance within the East of Haig Fras MCZ could have a negative impact on this feature. The species FOCI *Eunicella verrucosa* (Pink Sea-Fan) was also observed in 2015 (although in just one image) and is similarly sensitive to disturbance (Readman & Hiscock 2017).

4.7 Non-indigenous species (NIS)

No NIS were recorded within the East of Haig Fras MCZ during the 2015 survey.

5 Recommendations for future monitoring

The East of Haig Fras MCZ is spatially variable in BSH type and has high within-BSH variability in sediment composition and, consequently, is also variable in faunal community structure. Where 'A4.2 Moderate energy circalittoral rock' is present, it occurs in patches surrounded by sediment BSHs. The variable nature of the seabed at the site leads to the following recommendations:

- Monitoring of rock features by drop camera transects must ensure sufficient volume and quality of imagery is collected for quantitative analysis in future monitoring. With a drop camera system, still images are the most appropriate tool for achieving good visual representation of the habitat.
    - Each camera tow should be considered as a single sample, with a set of still images describing a specified area of seabed (minimum of $4m^2$).
    - Camera tows should target the largest known patches of rock to ensure consistency of habitat along a set length transect.
    - Multiple shorter camera tows may be more appropriate to sample the patchy habitats.
    - Still images should be collected with a uniform FOV to ensure they can be analysed to an appropriate level of taxonomic identification and consistently truncated.
    - Oversampling with images is preferred so enough adequate quality images are collected to enable random selection of images covering an equal area of seafloor over the same distance travelled for each transect.

- Monitoring the biological structure of designated sediment BSHs might be best achieved by selecting a set of fixed monitoring stations and targeting with grab samples.
    - If this approach is adopted, stations should be selected to represent points along the range of sediment compositions (or sub-habitats) covered by each BSH using data on sediment composition collected during the 2015 survey. By targeting a smaller number of stations for temporal sampling, the noise introduced when all stations from a BSH are treated as comparable replicates will be reduced. This approach would therefore allow more consistent comparisons across time intervals while still capturing the full range of sediment compositions across BSHs and the associated range of biodiversity within the MCZ.
    - Each fixed monitoring station would ideally have an initial characterisation of small-scale spatial variability. Multiple replicates could subsequently be collected from each station (with the exact number of replicates based on the observed level of variability) and these replicates could be used to accurately describe benthic communities at each station and assess changes to these communities over time.
    - Possible temporal changes in sediment composition within stations, through the movement of sediments across the site, must be accounted for. It may be possible to place sampling stations in areas of the site where sediment composition is relatively homogeneous, therefore minimising the possibility that sediment composition at a station will change substantially over time.
    - Focusing on a smaller number of stations with increased replication will limit the ability to monitor changes to BSH extent and distribution. The collection of sediment samples from stations throughout the MCZ, in addition to replicated samples collected at fixed stations to assess BSH condition, would allow this monitoring objective to also be achieved.

- Alternatively, samples could continue to be collected from many stations throughout the site and variation in fine-scale sediment composition and other relevant variables (e.g.

spatial coordinates) could be incorporated into statistical models that assess ecological change over time in BSHs.

- With this approach, ecological changes associated with variation in sediment composition could be separated from those related to other drivers (e.g. direct physical disturbance or chemical stress).
- This approach would be better suited to monitoring changes to BSH extent and distribution than using a relatively small set of fixed monitoring stations.

- Indices that respond predictably to environmental stress may be the most useful for assessing changes in condition.

  - There is some evidence that the Margalef Index might be useful as a general indicator physical, organic, and chemical disturbance (van Loon *et al.* 2018).
  - Indices based on specific suites of life-history traits may reveal more specific anthropogenic effects on the ecosystem, e.g. reductions in large and long-lived taxa in response to trawling (Tillin *et al.* 2006; van Denderen *et al.* 2015; Rijnsdorp *et al.* 2018).
  - Similar trait-based indices may also be useful for monitoring likely changes to ecological processes (e.g. sediment reworking and aeration) and associated ecosystem functions (e.g. Solan *et al.* 2004; Morys *et al.* 2017; Wrede *et al.* 2018).
  - Once indices have been selected, power analyses should be performed to determine how many samples are needed to be able to detect temporal changes in these indices in the habitats (or sub-habitats) being monitored.

- Consistency should be maintained in the type of grab used to sample sediments and infaunal communities.

  - Hamon Grabs and Day Grabs provided different indications of sediment composition (percent mud content) and occasionally BSH type but did not differ in their efficacy at sampling infaunal communities of ‘A5.3 Subtidal mud’. On this basis, either gear type could theoretically be used to effectively sample the infauna of this BSH.
  - It is unclear why the gears provide different indications of sediment composition. Given that they do, once a gear has been selected it should continue to be used to monitor changes at the site. For the East of Haig Fras MCZ, this means using the Hamon Grab if the full set of samples targeting ‘A5.3 Subtidal mud’ in 2015 are to be used as a baseline for future monitoring.
  - When selecting a gear, it should be considered that the Day Grab keeps sediment intact. This gear should therefore be used if information on the vertical profile of the sediment or sediment contaminant concentration is sought.

- The species FOCI *Atrina fragilis* (Fan Mussel) was recorded throughout the site and is highly sensitive to several pressures (Tyler-Walters & Wilding 2017). The density and distribution of *Atrina fragilis* should therefore be quantitatively monitored in the future.

- Additional surveys would be required to better understand the extent and distribution of the habitat FOCI ‘Sea-Pen and burrowing megafauna communities’, as this feature was not targeted as part of the imagery survey in the 2015 survey.

  - A camera survey should be conducted throughout the BSH ‘A5.3 Subtidal mud’ to identify areas where there are active burrows.
  - Infaunal samples should be collected from any stations where burrows are evident, to verify the presence of identifying taxa. A NIOZ Corer would be preferable to a Day

Grab or Hamon Grab, as it penetrates deeper into the seabed and is therefore more likely to capture burrowing megafauna.

- Recommendations for monitoring of supporting processes include:
  - Making optimal use of wider monitoring data (e.g., acquired as part of existing integrated marine monitoring programmes) to provide context in relation to wider ecosystem processes operating at a landscape or regional scale.
- Future surveys should monitor marine litter (Descriptor 10), to satisfy the requirements of the MSFD.

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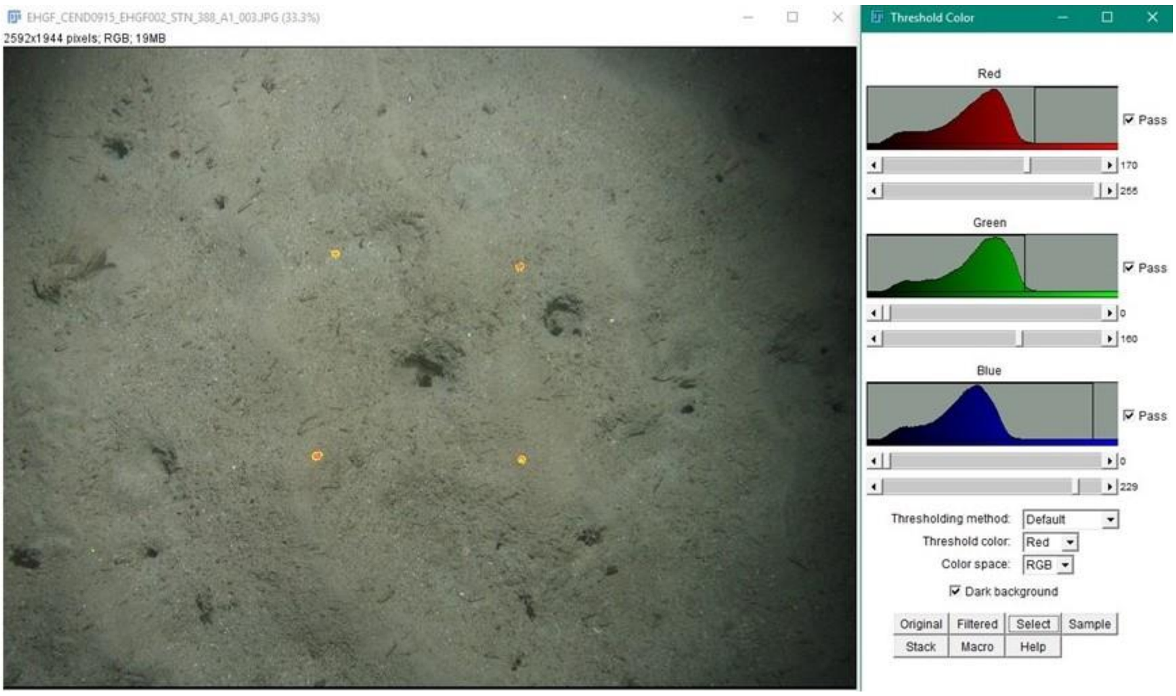
Annex 1. Selection of still images for quantitative analysis

Video and still images acquired using cameras towed on the sea floor have a consistent field of view across the tow and are hence readily applicable to quantitative analysis. Imagery acquired using a drop-frame camera, however, often consists of video segments and images taken at a wide range of heights above the seabed, leading to variability both in the field of view and the pixel ground resolution in images. Imagery from drop cameras is consequently not readily comparable across the tow, and consequently not suitable for quantitative analysis. The still images were chosen for analysis of species richness and multivariate community statistics, due to the relative ease of evaluating sampled area, in comparison to the video across the tow and, hence, create a quantitative dataset. To subset still images to comparable sampled area, first the area of each image was calculated, and consequently a representative and comparable subset of images was selected for each habitat for each tow.

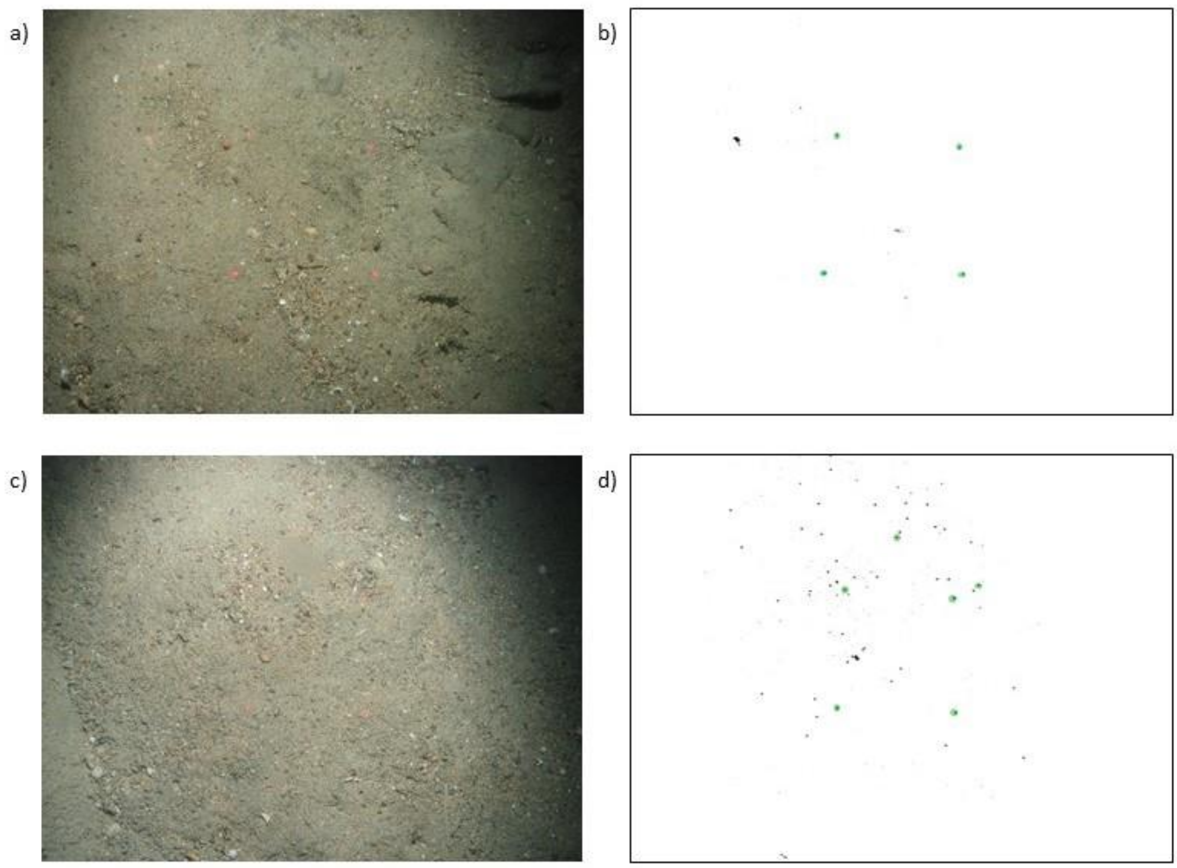
Automated Image Field of View Calculation

Field of view (FOV), measured in m^2, was calculated for each still image by relating the horizontally measured number of pixels between points projected by each laser pair (set at 170mm apart) in a four-spot laser-scaling device to the pixel dimensions of the images (2592 x 1944px). The measurements were taken using a batch processing macro in ImageJ v1.51n (Rasband 1999-2016) to automatically identify the laser points. The ‘Colour Threshold’ tool was used to create a selection of pixels, within threshold values (see figure below).



Selection of laser pixels through colour thresholds.

The ‘Analyze Particles’ tool was used to select ‘particles’ formed by contiguous pixels in the selection by both size and circularity. The particle selection step was included to exclude other objects with similar colour values picked up in the mask (see panels a & b in figure below). The particle selection was not always successful, resulting in too few or too many ‘spots’ identified in the image (see panels c & d in figure below). Those images with less or more than four spots identified were not measured and were copied into a separate folder for further action.



Examples of the 'Particle Analysis' stage of the automated FOV procedure. The mask of pixels selected by the ‘Colour Threshold’ step is shown to the right of its respective image. Contiguous pixel aggregations selected in the ‘Particle Analysis’ are highlighted in green in the masks. Objects in an image (a) with similar colour values to the laser spots were excluded from the particle selection (b). In some images (c) several objects had the same size and shape as laser points, leading to selection of more than four points (d).

As an additional check, percentage difference in the pixel distance between each laser pair was also calculated and any measured pair that differed by more than 30% was flagged up as unmeasured. Different images had different optimal thresholds for picking up the laser spots. Several runs of the macro were repeated with slightly altered threshold and particle attribute settings, to maximise the number of images measured (see table below). Each new run included the images that were left unmeasured by the previous run. Images still left unmeasured by the end of the final run, were either of very poor quality and ignored, or had biota with very similar reflectance to the laser points and were measured manually.

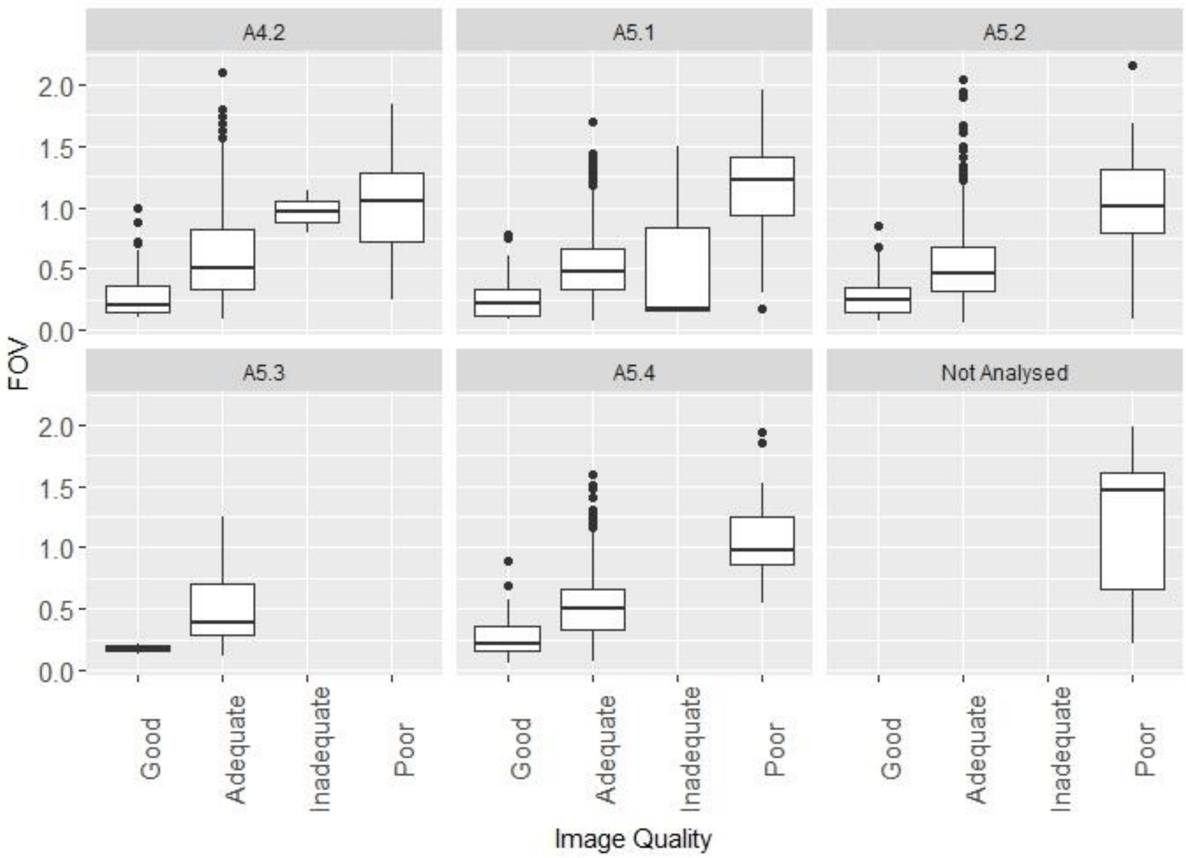
Thresholds used in ImageJ for each batch run to automatically select and horizontally measure the number of pixels between both laser-scaling device pairs.

	Red	Green	Blue	Particle size	Circularity	No. images measured
Run1	200-256	0-180	0-180	10-Inf	0.35-Inf	1680
Run2	200-256	0-180	0-180	35-Inf	0.35-Inf	423
Run3	170-256	0-135	0-170	35-Inf	0.35-Inf	81
Run4	240-256	0-230	0-230	50-Inf	0.5-Inf	228
Run5	240-256	0-230	0-230	30-Inf	0.3-Inf	158
Run6	240-256	0-230	0-230	5-Inf	0.3-Inf	28
Manual	/	/	/	/	/	231

Not measured	375
Total	3101

Selection of images for quality and consistency

The range of FOV in images was plotted for each habitat type, with the analyst defined Quality Score to gauge the appropriate FOV range for quantitative analysis (see figure below). Good quality images were mainly below a FOV of 0.25m^2, whereas images with a FOV above 0.75m^2 tend to be of inadequate or poor quality. Better quality images with a small FOV number contain more taxonomic diversity due to the smaller number of uncertain identifications in well-lit high-resolution images. The majority of images, however, were in the 0.5–1m^2 FOV range. The final image quality parameter threshold was chosen to optimise both the number of sampling station with a sufficient number of images (Table below) and taxonomic detail retained. A FOV of 0.6 m^2 was chosen as the threshold for the East of Haig Fras dataset. Any images below 'Adequate' quality were further filtered out of the dataset.



Range of image FOV (m^2) across Broadscale Habitats for each image quality class (assigned by the analyst during image processing).

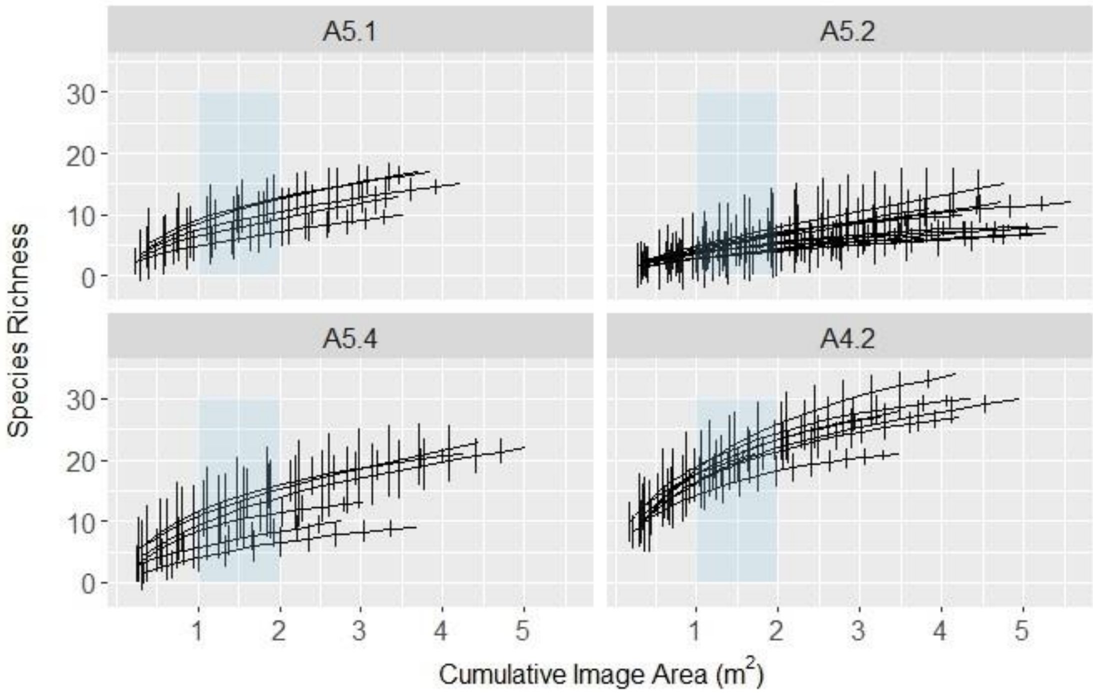
The number of stations with a set number of images retained after applying various field of view thresholds by Broadscale Habitat type.

	FOV <= 0.7m^2	FOV <= 0.6m^2	FOV <= 0.5m^2

No. Img.	A4.2	A5.1	A5.2	A5.3	A5.4	A4.2	A5.1	A5.2	A5.3	A5.4	A4.2	A5.1	A5.2	A5.3	A5.4
>=1	101	115	101	6	68	100	112	98	6	66	98	104	93	5	60
>=5	51	44	56	3	29	44	38	49	2	26	38	29	44	1	21
>=6	44	32	53	2	24	33	27	46	1	22	27	21	36	1	14
>=7	29	26	47	2	20	21	19	40	1	18	16	13	27	1	11
>=8	21	17	35	1	17	16	13	30	1	13	13	9	24	1	9
>=10	10	9	25	1	11	8	8	20	1	8	6	4	17	1	4

Quantitative data subset

Species accumulation curves per tow were computed across the Broadscale Habitat types using the filtered dataset. Plots of species accumulation with increasing area covered by images were used to determine the standard sample area per transect per habitat type to include in the final dataset (see figure below). The species accumulation curves indicated that a sampled area between 3-5m^2, depending on the Broadscale Habitat type, was required to sufficiently describe diversity along a transect. Very few transects had enough images to achieve such a large area. As a compromise, a standard area range of 1-2m^2 was selected, to minimise area dependence in quantitative estimates. Images for each transect were randomly sub-sampled within Broadscale Habitat until the maximum area of 2m^2 was achieved for a station – habitat combination. All station – habitat combinations that did not reach an area of 1m^2 were rejected. A total of 68 station – habitat combinations, from 62 stations were included in the final dataset.



Species accumulation curves for transects with a minimum of 10 images, with estimated confidence intervals (2 x standard deviations) for each Broadscale Habitat. The selected standard sample cumulative area range is highlighted in blue.

The final taxon matrix was truncated according to the protocols laid out in Annex 2. SACFOR abundance from individual images in each transect were pooled into one abundance value per taxon by taking the median numeric SACFOR value across all images.

Annex 2. Epifauna data truncation protocol applied to seabed imagery data

Still image data were all from one drop camera survey carried out in 2015. Initially, all assigned taxon names were collated with accompanying counts of occurrences in each data set. All taxon names were linked to an entry in an aggregation matrix forming a truncation matrix that was used as a basis for decisions. The table below shows an extract of the truncation matrix used to reassign taxon labels.

The taxonomic entries in the data were compared to the taxonomic reference collection of example stills to examine which taxon entries were exclusive of others. Taxa were recorded over many taxonomic levels between species and phyla. In some cases, especially for Arthropods, Cnidarians, Echinoderms and Molluscs, the taxonomic level used for uncertain identifications was prohibitively high (Class or Order level) to allow for truncation to the lowest common denominator. The coarser taxonomic categories were used for individuals that were small or partially obscured. They could not be ruled mutually exclusive from other taxa in the dataset overall, but generally were different from other taxa identified in the same image. Instead of aggregating taxa up to the coarsest level and losing all of the taxonomic detail below, those entries were dealt with in two ways depending on the intended use of the output dataset:

1) The very general taxonomic categories were removed entirely from the community matrix used for multivariate statistics. Inclusion of the overlapping high-level taxa would introduce too much noise into an analysis which relies on taxa being exclusive.
2) The entries were kept alongside the lower taxonomic categories in the community matrix used for calculating diversity statistics and species accumulation curves. The likelihood of overestimating diversity by adding the taxa is much less than underestimating diversity by removing them.

Otherwise, epifauna data preparation and truncation in both datasets followed the steps detailed below:

i. All fish, cephalopods and eggs were removed. Other taxa were combined to the highest common taxonomic level with some exceptions detailed below (see table below for examples).

ii. Porifera were reduced to morphotypes. Generally, each morphotype was represented by one dominant species, with very few observations of a secondary species and almost all observations were not made at the species or genus levels.

iii. Large and easily distinguishable taxa identified to species or genus were kept separate even where others were truncated to a higher taxonomic category above them, where there was no chance of overlap.

iv. Where a Class/Order level was used for a taxon that was clearly different from taxa identified to a more detailed level below it, the higher-level taxon was kept separate, instead of truncating all taxa to highest common denominator.

v. Where an uncertain species identification overlapped with a morphotype, the species was truncated to morphotype (e.g. *Palmiskenea skenei* was included in Erect bryozoa, which always referred to a small orange bryozoan visually similar to *P. Skenei*).

vi. *Swiftia pallida* was renamed *Eunicella verrucosa* due to physical samples of similar small white coloured Sea-Fans collected at Haig Fras being identified as *E. verrucosa* and the unlikely extension of the range of *S. pallida* to East of Haig Fras (Downie *et al.* 2018).

Extract from the epifauna truncation matrix. Original taxon is the identification made by the still image analyst. N = Number of still images the taxon is recorded in. Assigned taxon shows the taxon name after truncation. F = Filter category, indicating whether the taxon should be included in diversity dataset only (D) or both datasets (c).

Class	Order	Family	Genus	Species	Original taxon	N	Assigned taxon	F	Notes
Polychaeta	Eunicida	Onuphidae	Hyalinoecia		Hyalinoecia sp	33	Hyalinoecia sp	C	Large easily identifiable taxon
Polychaeta	Phyllodocida	Aphroditidae	Aphrodita	aculeata	Aphrodita aculeata	3	Aphrodita aculeata	C	Large easily identifiable taxon
Polychaeta	Phyllodocida	Hesionidae	Oxydromus	flexuosus	Oxydromus flexuosus	9	Oxydromus flexuosus	C	Large easily identifiable taxon
Polychaeta	Sabellida	Sabellidae	Myxicola		Myxicola sp	3	Myxicola sp	C	Sabellids not really separately identifiable
Polychaeta	Sabellida	Sabellidae	Sabella	pavonina	Sabella pavonina	3	Sabellidae	C	
Polychaeta	Sabellida	Sabellidae	Sabella	pavonina	Sabella pavonina tube	4	Sabellidae	C	
Polychaeta	Sabellida	Sabellidae			Sabellidae sp	17	Sabellidae	C	
Polychaeta	Sabellida	Serpulidae	Salmacina	dysteri	Salmacina dysteri ?or Filograna sp	2	Salmacina	C	Large easily identifiable taxon
Polychaeta	Sabellida	Serpulidae	Spirobranchus		Spirobranchus sp; tube	6	Serpulidae	C	Not really separately identifiable
Polychaeta	Sabellida	Serpulidae			Serpulidae	418	Serpulidae	C	
Polychaeta	Terebellida	Terebellidae	Lanice	conchilega	Lanice conchilega	7	Terebellidae	C	Not really separately identifiable
Polychaeta	Terebellida	Terebellidae			Terebellidae	7	Terebellidae	C	
Polychaeta					Polychaeta ?Ditrupa sp	2	Polychaeta (Ditrupa)	C	Specific different type of polychaete, not positively identified.
Polychaeta					Polychaeta tube	1026	Polychaeta	C	Smaller tubes than those identified as Sabellidae
Hexanauplia					Thoracica	0	Hexanauplia	C	
Malacostraca	Decapoda				Decapoda	56	Decapoda	D	Decapoda and Brahyura are both used to denote very small crabs that have not been possible to identify to a specific taxon. Decapoda often occurrs alongside a more detailed ID of other larger decapods, when referring to a small, different but unidentifiable Crab. Hence the category should be kept in for diversity, but not community analysis
Malacostraca	Decapoda				Brachyura	10	Decapoda	D	
Malacostraca	Decapoda				Majoidea	1	Decapoda	D	
Malacostraca	Decapoda	Inachidae	Inachus		Inachus sp	1	Inachidae	C	Truncated to lowest common denominator.
Malacostraca	Decapoda	Inachidae	Macropodia		Macropodia sp	5	Inachidae	C	
Malacostraca	Decapoda	Inachidae			Inachidae	7	Inachidae	C	
Malacostraca	Decapoda	Leucosiidae	Ebalia		Ebalia sp	21	Ebalia sp	C	
Malacostraca	Decapoda				Galatheoidea	10	Galatheoidea	C	Truncated to lowest common denominator.
Malacostraca	Decapoda	Munididae	Munida	rugosa	Munida rugosa	72	Galatheoidea	C	

Malacostraca	Decapoda	Paguridae			Paguridae	227	Paguridae	C	
Malacostraca	Decapoda				Caridea	37	Caridea	C	Truncated to lowest common denominator.
Malacostraca	Decapoda	Pandalidae			Pandalidae	0	Caridea	C	
Hydrozoa					Hydrozoa clumps / solitary	564	Hydrozoa A	C	Kept separate from other hydrozoa as, on inspection of a subset of images, seems to refer to a specific type of hydrozoa not covered by other identified taxa
Hydrozoa					Hydrozoa turf	826	Hydrozoa turf	C	Kept separate from other hydrozoa as, on inspection of a subset of images, seems to refer to a specific type of hydrozoa not covered by other identified taxa
Hydrozoa	Anthoathecata	Corymorphidae	Corymorpha		Corymorpha sp	2	Corymorpha sp	C	No overlapping higher taxonomic categories
Hydrozoa	Anthoathecata	Tubulariidae	Tubularia	indivisa	Tubularia indivisa	2	Tubularia indivisa	C	No overlapping higher taxonomic categories
Hydrozoa	Leptothecata	Aglaopheniidae	Aglaophenia		Aglaophenia sp	24	Aglaophenia sp	C	
Hydrozoa	Leptothecata	Haleciidae			Haleciidae	3	Haleciidae	C	
Hydrozoa	Leptothecata				Plumularioidea	6	Plumularioidea	C	Always other than Nemertesia (only other taxa in plumularioidea recorded in data)
Hydrozoa	Leptothecata	Plumulariidae	Nemertesia	antennina	Nemertesia antennina	28	Nemertesia sp	C	
Hydrozoa	Leptothecata	Plumulariidae	Nemertesia	ramosa	Nemertesia ramosa	9	Nemertesia sp	C	Truncated to lowest common denominator.
Hydrozoa	Leptothecata	Plumulariidae	Nemertesia		Nemertesia sp	87	Nemertesia sp	C	
Hydrozoa	Leptothecata	Sertulariidae			Sertulariidae	1	Sertulariidae	D	This can be kept for diversity analysis, as does not overlap with other more detailed ID of Sertulariidae in the same image - will be removed for community analysis to keep other more specific taxa in.
Hydrozoa	Leptothecata	Sertulariidae	Abietinaria	abietina	Abietinaria abietina	9	Abietinaria abietina	C	
Hydrozoa	Leptothecata	Sertulariidae	Diphasia	alata	Diphasia alata	2	Diphasia sp	C	Truncated to lowest common denominator.
Hydrozoa	Leptothecata	Sertulariidae	Diphasia		Diphasia sp	0	Diphasia sp	C	
Hydrozoa	Leptothecata	Sertulariidae	Sertularia		Sertularia sp	1	Sertularia sp	C	

Annex 3. Infauna data truncation protocol

Raw taxon abundance and biomass matrices can often contain entries that include the same taxa recorded differently, erroneously or differentiated according to unorthodox, subjective criteria. Therefore, ahead of analysis, data should be checked and truncated to ensure that each row represents a legitimate taxon and they are consistently recorded within the dataset. An artificially inflated taxon list (i.e. one that has not had spurious entries removed) risks distorting the interpretation of pattern contained within the sampled assemblage.
It is often the case that some taxa have to be merged to a level in the taxonomic hierarchy that is higher than the level at which they were identified. In such situations, a compromise must be reached between the level of information lost by discarding recorded detail on a taxon’s identity and the potential for error in analyses, results and interpretation if that detail is retained.

Details of the data preparation and truncation protocols applied to the infaunal datasets acquired at the East of Haig Fras MCZ ahead of the analyses reported here are provided below:

- Where there are records of one named species together with records of members of the same genus (but the latter not identified to species level) the entries are merged, and the resulting entry retains only the name of the genus.

- Taxa recorded above the genus level were removed from the dataset when lower taxonomic levels of the same group were recorded to avoid having to reduce the taxonomic resolution of records.

- Taxa are often assigned as ‘juveniles’ during the identification stage with little evidence for their actual reproductive natural history (with the exception of some well-studied molluscs and commercial species). Many truncation methods involve the removal of all ‘juveniles’. However, a decision must be made on whether removal of all juveniles from the dataset is appropriate or whether they should be combined with the adults of the same species where present. For the Infaunal data collected at the East of Haig Fras MCZ, if ‘juvenile’ records were recorded at the same taxonomic level as ‘adult’ records then the two records were combined, whereas if juveniles were recorded at a higher taxonomic level than adults then the ‘juvenile’ records were removed to avoid having to reduce the taxonomic resolution of the ‘adult’ records.

- Records of meiofauna (i.e., nematodes) were removed.

- Records of fish species were removed.

The full set of truncation steps applied to the infaunal community data are listed below.

Truncation steps for the infaunal community dataset

Records of 'P' changes to '1' in abundance dataset
All 3 ANIMALIA records removed
Abra, A. alba, A. nitida, and A. prismatica merged
Abyssoninoe and A. hibernica merged
Acanthocardia juveniles changed to Acanthocardia
ACTINOPTERYGII (a fish) eggs and larvae removed
Aglaophamus agilis adults and juveniles merged
Ammodytes (a fish) removed

ANOMURA zoea removed

Aphelochaeta species A changed to Aphelochaeta

Aspidosiphon (Aspidosiphon) muelleri muelleri and Aspidosiphon (Aspidosiphon) muelleri muelleri juveniles merged

Astacilla, A. dilatata, A. longicornis, and A. pusilla merged

Atelecyclus rotundatus adults and juveniles merged

BRACHYURA megalopa and zoea removed

Caulleri+A17:A56ella species B +A17:A66chnaged to Caulleriella

Cerianthus lloydii adults & juveniles merged

Chaetozone, C. setosa, C. sp 1., C. sp D, and C. zetlandica merged

Cheirocratus and Cheirocratus intermedius merged

Chone merged with Chone fauveli

Corystes cassivelaunus megalopa removed

Merge Cucumariidae adults and juveniles

Diastylis, D. bradyi, D. laevis, and D. lucifera merged

DIPTERA removed

Ebalia, E. cranchii, E. granulosa, and E. tuberosa merged, Echinocardium adults and juveniles, E. flavescens adults and juveniles, and E. pennatifidum merged

Eteone cf. longa changed to Eteone

Eunereis longissima adults and juveniles merged

Exogone naidina adults and epitokes merged

Galatheidae zoea removed

Gammaropsis, G. maculata, and G. nitida merged

Gari fervensis adults and juveniles merged

Glycera, G. alba adults and juveniles, G. celtica, G. fallax, G. lapidum, G. oxycephala adults and juveniles, and G. unicornis adults and juveniles merged

Gnathiidae juveniles changed to Gnathiidae

Golfingia, G. elongata, and G. vulgaris merged

Goniada maculata adults and juveniles merged

Harmothoe antilopes adults and juveniles merged

Harmothoe glabra adults and juveniles merged

Hyalinoecia tubicola adults and juveniles merged

Laetmonice juveniles changed to Laetmonice

Laevicardium crassum juveniles changed to Laevicardium crassum

Lanice conchilega adults and juveniles merged

Leptosynapta, L. decaria, L. inhaerens, and L. minuta merged

Liocarcinus juveniles changed to Liocarcinus

Lucinoma borealis adults and juveniles merged

Lumbrineris cf. cingulata changed to Lumbrineris

Lysidice unicornis juveniles changed to Lysidice unicornis

Malmgrenia, M. arenicolae, M. castanea, M. darbouxi, M. lunulata, M. mcintoshi, and Malmgrenia sp.1 (new species) merged

Marphysa bellii adults and juveniles merged

Myriochele, M. danielsseni, and M. olgae merged

Myrtea spinifera adults and juveniles merged

Nephrops norvegicus juveniles changed to Nephrops norvegicus

Nephtys adults and juveniles, N. cirrosa, N. hombergii, N. hystricis, N. incisa, and N. kersivalensis merged

Nothria britannica adults and juveniles merged
Oestergrenia, O. digitata, and O. thomsonii merged
Orbiniidae adults and juveniles merged
Paguridae megalopa removed
Panningia hyndmani adults and juveniles merged
Paradoneis, P. ilvana, and P. lyra merged
Phyllodoce cf. longipes changed to Phyllodoce
Phyllodoce adults and juveniles, P. groenlandica, P. lineata, and P. rosea merged
Pista, P. cristata, and P. mediterranea merged
Polycirrus and P. tenuisetis merged
Polynoidae adults and juveniles merged
Prionospio, P. cirrifera, P. dubia, P. fallax, and multibranchiata merged
Pseudopolydora cf. paucibranchiata changed to Pseudopolydora
Pseudothyone raphanus adults and juveniles merged
Scolelepis, S. bonnieri, S. korsuni, and S. tridentata
Scoloplos (Scoloplos) armiger adults and juveniles merged
Sphaerosyllis sp.1 / aff. Taylori changed to Sphaerosyllis taylori
Spiophanes, S. bombyx, and S. kroyeri merged
Sthenelais limicola adults and juveniles merged
Streblosoma and S. intestinalis merged
Syllides adults and epitokes, S. convoluta, and S. japonica merged
Terebellides, T. shetlandica, and T. stroemii merged
Thracia convexa juveniles changed to Thracia convexa
Thysanocardia procera adults and juveniles merged
Turritella communis adults and juveniles merged
Aphrodita juveniles and Aphroditidae removed
Dosnia juveniles removed
Euchone juveniles removed
Galathea juveniles removed
Nephtyidae juveniles removed
Nereididae juveniles removed
Opheliidae juveniles removed
Orbinia juveniles removed
Paguridae juveniles removed
Pectinaria juveniles removed
Sabellidae juveniles removed
ACTINARIA removed
Ampharetidae removed
AMPHIPODA removed
ANTHOATHECATA removed
Aoridae removed
ASCIDIACEA removed
Aricidea (Acmira) removed
ASTEROIDEA removed
BIVALVIA removed
Bopyridae removed

Campanulariidae removed
Capitellidae removed
CNIDARIA removed
COPEPODA removed
CRUSTACEA removed
Cucumariidae removed
CUMACEA removed
DECAPODA removed
DENDROCHIROTIDA removed
ECHINOIDEA removed
Edwardsiidae removed
GASTROPODA removed
Gnathiidae removed
Golfingiidae removed
HYDROZOA removed
Lumbrineridae removed
Maldanidae removed
MOLLUSCA removed
NEMATODA removed
Oedicerotidae removed
Onuphidae removed
Ophiuridae removed
OPHIUROIDEA removed
Orbiniidae removed
Oweniidae removed
Paraonidae removed
PECTINOIDEA removed
Photidae removed
Phoxocephalidae removed
Phyllodocidae removed
POLYCHAETA removed
Polynoidae removed
PORIFERA removed
PYCNOGONIDA removed
Serpulidae removed
SIPUNCULA removed
SPATANGOIDA removed
Spionidae removed
Synaptidae removed
Teribellidae removed
TEREBELOMORPHA removed

Annex 4. Non-indigenous species (NIS)

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

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

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

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

Tiostrea lutaria	*Tiostrea chilensis*
Mercenaria mercenaria	
Petricola pholadiformis	
Mya arenaria	

Annex 5. Output from analysis of univariate biotic indices for infauna

ANOVA output for tests of variation in univariate biotic indices across sediment Broadscale Habitats (‘A5.1 Subtidal coarse sediment’, ‘A5.2 Subtidal sand’, ‘A5.3. Subtidal mud’, and ‘A5.4 Subtidal mixed sediments’) in the East of Haig Fras MCZ in 2015. s.o.s. = sum of squares, d.f. = degrees of freedom. Total abundance was transformed by $\log_e$ (x+1) prior to analysis to meet test assumptions of normality and homogenous variance.

Index	s.o.s.	d.f.	F	*p*
Total abundance	16.4	3	26.072	<0.0001
Residuals	53.0	253		
Total number of species	4111.9	3	23.636	<0.0001
Residuals	14671.3	253		
Margalef Index	93.4	3	18.742	<0.0001
Residuals	420.1	253		
Shannon Index	3.1	3	12.584	<0.0001
Residuals	21.1	253		

General linear model output for tests of variation in univariate biotic indices in relation to the sediment components that determine Broadscale Habitat type (% gravel, sand, and mud contents) in the East of Haig Fras MCZ in 2015. s.o.s. = sum of squares, d.f. = degrees of freedom. Significant relationships ($p < 0.05$) are in bold. Total abundance was transformed by $\log_e$ (x+1) prior to analysis to meet test assumptions of normality and homogenous variance.

Index	Sediment component	s.o.s.	d.f.	F	*p*
Total abundance	Gravel	0.01	1	0.0567	0.8119
	Sand	0.01	1	0.0730	0.7872
	Mud	0.02	1	0.1168	0.7329
	Gravel x Sand	0.31	1	1.8464	0.1754
	Gravel x Mud	0.51	1	3.0786	0.0806
	Sand x Mud	**5.57**	**1**	**33.4930**	**<0.0001**
	Residuals	41.54	250		
Total number of species	Gravel	0.6	1	0.0130	0.9094
	Sand	5.5	1	0.1106	0.7397
	Mud	8.8	1	0.1776	0.6738
	Gravel x Sand	1.0	1	0.0196	0.8887
	Gravel x Mud	81.6	1	1.6521	0.1999
	Sand x Mud	**1250.8**	**1**	**25.3359**	**<0.0001**
	Residuals	12342.3	250		
Margalef Index	Gravel	0.05	1	0.0345	0.8527
	Sand	0.06	1	0.0411	0.8396
	Mud	0.13	1	0.0857	0.7699
	Gravel x Sand	2.19	1	1.4518	0.2294
	Gravel x Mud	1.92	1	1.2754	0.2598
	Sand x Mud	**29.27**	**1**	**19.4402**	**<0.0001**

	Residuals	376.43	250		
Shannon Index	Gravel	0.01	1	0.1027	0.7489
	Sand	0.00	1	0.0024	0.9613
	Mud	0.00	1	0.0186	0.8917
	Gravel x Sand	**0.38**	1	**4.8093**	**0.0292**
	Gravel x Mud	0.15	1	1.8575	0.1741
	Sand x Mud	**1.47**	**1**	**18.5053**	**<0.0001**
	Residuals	19.86	250		

General linear model output for tests of variation in the first and second principal components (PCs 1 & 2) of infauna abundances in relation to the sediment components that determine Broadscale Habitat type (% gravel, sand, and mud contents) in the East of Haig Fras MCZ in 2015. s.o.s. = sum of squares, d.f. = degrees of freedom. Significant relationships ($p < 0.05$) are in bold.

Index	Sediment component	s.o.s.	d.f.	F	*p*
PC 1	Gravel	0.00	1	0.0003	0.9865
	Sand	0.01	1	0.4269	0.5141
	Mud	0.02	1	0.6151	0.4336
	Gravel x Sand	**0.15**	**1**	**4.6618**	**0.0318**
	Gravel x Mud	0.06	1	1.7264	0.1901
	Sand x Mud	**1.73**	**1**	**53.7794**	**<0.0001**
	Residuals	8.05	250		
PC 2	Gravel	0.00	1	0.0282	0.8669
	Sand	0.00	1	0.0143	0.9050
	Mud	0.00	1	0.0609	0.8053
	Gravel x Sand	**0.50**	**1**	**17.9337**	**<0.0001**
	Gravel x Mud	**0.15**	**1**	**5.4209**	**0.0207**
	Sand x Mud	0.03	1	1.2262	0.2692
	Residuals	6.97	250		

Annex 6. Burrow density of burrowing megafauna

The clarification advice on identifying 'Sea-pen and Burrowing Megafauna Communities used in this report (JNCC 2014) indicated between one and nine burrows should be recorded per 10m^2, in conjunction with the collection of infaunal samples confirming the presence of relevant taxa and PSD data confirming a fine mud habitat. As it was not an objective of the survey to identify the presence of the habitat FOCI 'Sea-Pen and burrowing megafauna communities', the number of burrows per image was not recorded during image analysis. Likewise, the substrate type with which this FOCI is typically associated was not targeted during the collection of seabed imagery data.

The table below shows those stations (transects) where active megafaunal burrows were observed in still images. The sum-total field of view (FOV) from all stills acquired along the transect has been calculated, alongside the number of stills per transect where burrows were observed. As burrows were not enumerated, it is simply assumed that each image has at least one active burrow present. As such, the density criterion is considered met (for the transect as a whole) if there is at least one image containing at least one burrow per 10m^2 of summed FOV. Where the FOV is greater than 10m^2 and only one image along the transect contained burrows (i.e. stations EHGF091, EHGF206, and EHGF215), these stations were identified as not meeting the FOCI classification criteria with respect to burrow density. In these cases, the number of burrows in each image was enumerated by the authors of this report. If this number was large enough that more than one burrow was observed per 10m^2 along the transect, then density criteria was considered met for this station.

Stations where megafaunal burrows were observed, the total field of view for all images acquired from these stations, and the number of still images containing burrows within each station (allowing determination of whether a minimum burrow density of 1 per 10m^2 was met for each station). * if a minimum burrow density of < 1 per 10m^2 was calculated for a station, then burrows were counted in the relevant images to determine whether the actual burrow density was > 1 per 10m^2.

Station	Total FoV (m^2)	No. of stills containing burrows	No. of burrows per still*	Density criteria met?	Broadscale Habitat
EHGF002	5.81	20	-	Yes	A5.2 Subtidal sand
EHGF091	13.47	1	2	Yes	A5.2 Subtidal sand
EHGF112	7.46	5	-	Yes	A5.2 Subtidal sand
EHGF155	5.88	3	-	Yes	A5.2 Subtidal sand
EHGF183	6.21	10	-	Yes	A5.3 Subtidal mud
EHGF206	12.49	1	1	No	A5.4 Subtidal mixed sediments
EHGF215	10.99	1	3	Yes	A5.3 Subtidal mud
EHGF238	9.80	7	-	Yes	A5.3 Subtidal mud
EHGF249	11.57	4	-	Yes	A5.3 Subtidal mud

Annex 7. Acknowledgement

East of Haig Fras
Marine Conservation Zone (MCZ)
Monitoring Report 2015

MPA Monitoring Programme

Contract Reference: MB0129

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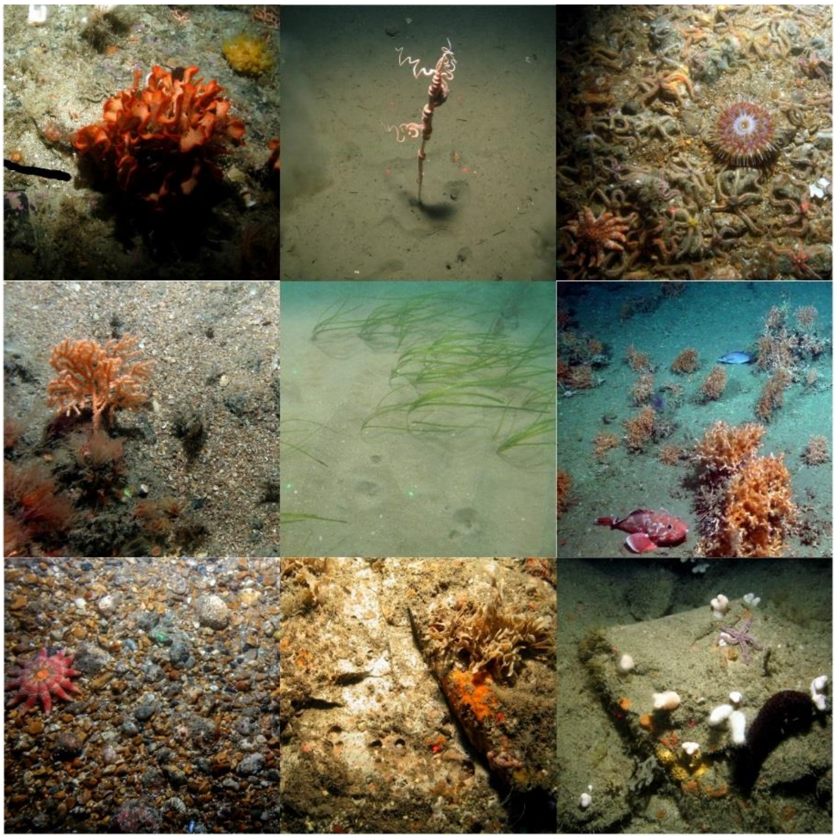

Association of IFCA · Cefas · Department for Environment Food & Rural Affairs · Environment Agency · JNCC Joint Nature Conservation Committee · Marine Management Organisation · Natural England

Marine Protected Areas Survey Coordination & Evidence Delivery Group

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

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

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