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North-East Faroe-Shetland Channel MPA Monitoring Report

Gallyot, J., McBreen, F. & Hope, V.

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#### For further information please contact:

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

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#### **Please Note:**

This report contains references to EU legislation – the survey was undertaken prior to the UKs exit from the EU.

## **Executive summary**

North-East Faroe-Shetland Channel Nature Conservation Marine Protected Area (MPA), hereafter referred to as "NEFSC", is located to the north-eastern reaches of the Faroe-Shetland Channel, approximately 93 km from Shetland. The protected features of the site include deep-sea sponge aggregations, offshore deep-sea muds, offshore subtidal sands and gravels, and large-scale geodiversity features (the Continental slope, the North Sea Fan, the Miller Slide, the West-Shetland Margin Paleo-Depositional System and the Pilot Whale Diapirs). In 2017, the Joint Nature Conservation Committee (JNCC) and Marine Scotland Science (MSS) completed a survey to NEFSC to collect sediment samples (Hamon grab) and seabed images (drop-down camera and chariot tows) to characterise the site and provide the first point in a monitoring time series.

The aim of this report is to explore and describe the protected habitat features within the NEFSC and test a number of hypotheses using the evidence collected during this survey. Sampling took place in four areas: box A, box, B, box C and box D (Figure 7). The specific objectives are described in the table below, alongside a summary of the outcomes. As the large-scale geodiversity features are unlikely to change in their range, extent or condition over the monitoring timescales under consideration, they are not considered further in this report.

Objectives	Outcomes
Describe the <b>extent</b> and <b>distribution</b> , <b>structure</b> and <b>supporting processes</b> of deep-sea sponge aggregations within NEFSC (box A) and outside the site (boxes B and C).	Deep-sea sponge aggregations were found in all three sampling boxes, both within and outside the MPA. Suspected records were verified using a confidence assessment scoring system (Henry & Roberts 2014) which resulted in 15 high confidence records in addition to several medium and low confidence records. The structure of the sponges was described using functional morphotypes (e.g. Encrusting, Simple Massive and Erect forms). The community results support previous findings on sponge communities seen in the Faroe-Shetland region, with similar species communities identified including Galatheoidea squat lobsters, <i>Cidaris cidaris</i> , starfish (Asteroidea) such as <i>Ceramaster</i> spp., brittlestars (Ophiuroidea), Sabellidae polychaetes and brachiopods. The environmental variables that best explained the variation in the biological data were found to include depth, proportion of pebbles and mud, and bed shear stress, however this only accounted for 47% of the biological variance.

Objectives	Outcomes
Compare deep-sea sponge aggregations within the site (box A) to those outside the site (boxes B and C).	Within NEFSC, 10 high confidence records and eight medium confidence records were assigned. Outside the boundaries, a total of five high confidence deep-sea sponge aggregations were observed and a further seven medium confidence records. A significant difference with a weak effect was found between the overall epifaunal communities within and those outside the MPA ( $p = 0.03 R = 0.07$ ). There were no significant differences found between Encrusting or Simple Massive morphotype sponges (percentage cover colonial species) within or outside the MPA. The differences could be the result of three highly differentiated outlier stations seen outside the MPA where no evidence of aggregations was found, or the higher number of aggregations within the site. However, between the sampling boxes both the sponge morphotypes and the overall epifaunal communities display high levels of similarity.
Describe the <b>extent and distribution</b> and <b>structure</b> of offshore subtidal sands and gravels and offshore deep-sea muds (box D).	Only nine grab samples were obtained during the survey therefore results are limited. Offshore deep-sea muds extend further south of the site in shallower habitats (around the 500–600 m contour) than previously predicted. Offshore subtidal sands and gravels were most frequently recorded at the site and occur within box D as previously predicted. Further investigation is required to be able to conclude robustly on the extent and distribution of sedimentary Priority Marine Features (PMFs) at NEFSC.
Note the presence of any PMFs and Vulnerable Marine Ecosystems (VMEs) observed which are not designated features of the site.	No undesignated benthic PMFs were detected during the analysis of data from NEFSC. Some mobile species of interest were observed during the analysis which includes the PMF species <i>Molva dypterygia</i> , and rays identified at high taxonomic level (Rajiformes). Epifaunal species of interest such as soft corals, sea pens and megafauna burrows were recorded in low numbers.
Present any evidence of non-indigenous species (Marine Strategy Framework Directive (MSFD) Descriptor 2) and marine litter (MSFD Descriptor 10).	Several instances of litter were identified within the site. No non-indigenous species were identified.

The new evidence and the analysis detailed within this report have updated our understanding of the protected habitat features of NEFSC. In particular, the deep-sea sponge aggregations were found within and outside the MPA, on both sides of the boundary.

Further information on the observations made during the survey and the subsequent analysis of the data for each of the protected habitat features of the site are provided below.

a) Deep-sea sponge aggregations

For the deep-sea sponge aggregations, the analysis of the data suggests a greater extent and distribution then previously observed with high confidence records distributed within and outside the MPA. The new records at NEFSC improve the current known distribution of VMEs and will be provided to the International Council for the Exploration of the Sea (ICES) and Oslo Paris Convention (OSPAR) databases. The structure of the aggregations was explored using functional morphotypes which show sponge morphologies are dominated by Encrusting, Simple Massive and Erect growth forms. We have added to the understanding of the biological structure of the epifaunal communities. Our findings agree with previous studies in the area observing communities that are associated with high sponge density. This included Galatheoidea squat lobsters, long-spine slate pen sea urchins (Cidaris cidaris), Sabellidae polychaetes, starfish (Asteroidea), brittlestars (Ophiuroidea) and lamp shells (Brachiopoda). The supporting processes were investigated using functional morphotypes, environmental parameters from the conductivity, temperature and depth (CTD) data, and shear stress values from energy datasets. Depth, pebbles, mud and shear stress explain around half of the variation seen in the biological data. Links are discussed between sediment type, different levels of shear stress and morphotype as potential key factors determining the communities.

b) Offshore deep-sea muds and offshore subtidal sands and gravels

For the offshore deep-sea muds and offshore subtidal sands and gravels we can only report limited results from box D due to low sampling. Despite this, we have improved our understanding of the **extent and distribution** of the sedimentary PMFs. Offshore subtidal sands and gravels were found to agree with predictive models whilst offshore deep-sea muds show slight deviation from previously predicted habitats. We have also improved our understanding of the **biological structure** of these habitats. Communities were characterised by the presence of *Paramphionome jeffreysii*, nematodes, copepods, Cirratulid polychaetes and *Spiophanes* spp.. The grabs were obtained within a narrow depth range (520–600 m) during the survey. Sampling in depths greater than 800 m would improve our understanding of the sand and gravel habitats for which the MPA has been designated.

Recommendations for future monitoring of NEFSC	Recommendations for future monitoring of the wider MPA network	
<ul> <li>It is recommended that the chariot tows are reanalysed to obtain sponge density values and community metrics to enable subsequent verification of the suspected sponge aggregations.</li> </ul>	<ul> <li>More, regularly spaced images would decrease the need for heavy standardisation treatments prior to multivariate analysis.</li> </ul>	
<ul> <li>Camera stations should focus on depth gradients along the length of NEFSC (400 m, 450 m, 500 m, 550 m) to further evaluate extent and distribution of deep- sea sponge aggregations.</li> </ul>	<ul> <li>It is recommended that future surveys attempt to obtain images with an ideal range of field of view (FOV) around 1– 1.5 m.</li> </ul>	

	commendations for future monitoring NEFSC		ommendations for future monitoring ne wider MPA network
•	Additional environmental parameters should aim to be collected such as silicate concentration, particulate organic carbon levels, hydrodynamics and sedimentation values in order to assist our understanding of sponge distribution, sponge morphotypes and their prevailing environmental conditions (Howell <i>et al.</i> 2016; Schönberg 2021).	r r	Future surveys could evaluate whether nore suitable equipment is available to educe issues with image quality and sampling units.
•	It is recommended that the metrics originally obtained in this report are collected so that comparisons can be directly made, however more reliable metrics such as frequency of occurrence should also be recorded during analysis. A10 X 10 frequency of occurrence grid could increase the consistency and accuracy of abundance estimates for future comparisons (Moore <i>et al.</i> 2019; Hinchen <i>et al.</i> 2021).	i: s c ł	t is recommended that future analysis s conducted via image annotation software such as BIIGLE. Frequency of occurrence metrics have been shown to have better precision, power and consistency (Moore <i>et al.</i> 2019).
•	The use of operational taxonomic units (OTUs) or a global standardised marine taxon reference image database (e.g., SMarTaR-ID) would greatly improve the overall epifaunal dataset at NEFSC, significantly reducing the inconsistencies and uncertainties between analysts (Howell <i>et al.</i> 2019).	s c c v r	The use of a shared classification schemes would provide a standardised approach to analysis, increasing consistency, comparability and decreasing variation. Future analyses would benefit from the adoption of the nost widely used current classification scheme.
•	Future surveys should aim to repeat sampling either side of the limits of the current MPA designation particularly focusing on box C.	a f lı r	Determining a suitable sampling unit and sample size needs to be a priority or subsequent analysis. A pooled ength of tow or random mosaic would need to be determined in relation to the sampling population of focus.
•	To understand the extent of VMEs in the wider environment and further understand the distribution of the sponge belt in the Faroe-Shetland region, it is recommended that surveys could investigate between the Faroe- Shetland Sponge Belt MPA and NEFSC between the 400–600 m depth contours.		
•	Direct sampling via Remotely Operated Vehicle (ROV) may be considered in the future, targeting key sponge morphotypes precisely without causing high levels of damage.		

Recommendations for future monitoring of NEFSC	Recommendations for future monitoring of the wider MPA network
<ul> <li>Further sampling is required within NEFSC to investigate the extent and distribution of the PMFs offshore subtidal sands and gravels and offshore deep-sea muds. Different faunal compositions are expected below 800 m, where species can tolerate the cooler Arctic-influenced waters (Chamberlain &amp; Barnich 2018). Sampling in depths greater than 800 m would improve our understanding of the sand and gravel habitats for which the MPA has been designated.</li> </ul>	

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## **Abbreviations**

Term	Definition
ANOSIM	Analysis of Similarities
AUV	Autonomous Underwater Vehicle
BEST	Biota and Environmental Matching Analysis
СТД	Conductivity, Temperature and Depth sensor
DEM	Digital Elevation Model
DistLM	Distance-based Linear Modelling
DSSA	Deep-sea sponge aggregations
EUNIS	The European Nature Information System
FAO	Food and Agriculture Organization of the United Nations
FOV	Field of View
JNCC	Joint Nature Conservation Committee
NMBAQC	North-East Atlantic Marine Biological Analytical Quality Control Scheme
nMDS	Non-metric multidimensional scaling
MPA	Marine Protected Area
MSFD	Marine Strategy Framework Directive
NEFSC	North-East Faroe-Shetland Channel
NIS	Non-indigenous species
NMBAQC	NE Atlantic Marine Biological Analytical Quality Control Scheme
OSPAR	The Convention for the Protection of the Marine Environment of the North-East Atlantic
ODSM	Offshore deep-sea muds
OSSG	Offshore subtidal sands and gravels
ΟΤυ	Operational Taxonomic Unit
PMF	Priority Marine Features

Term	Definition
PSA	Particle Size Analysis
ROV	Remotely Operated Vehicle
SIMPER	Similarity Percentage Procedure
SIMPROF	Similarity Profile Analysis
SNCB	Statutory Nature Conservation Body
VME	Vulnerable Marine Ecosystem

## Glossary

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

Term	Definition
Activity	A human action which may have an effect on the marine environment (e.g. fishing, energy production) (Robinson <i>et al.</i> 2008).
Anthropogenic	Caused by humans or human activities; usually used in reference to environmental degradation.
Assemblage	A collection of plants and/or animals characteristically associated with a particular environment that can be used as an indicator of that environment. The term has a neutral connotation and does not imply any specific relationship between the component organisms, whereas terms such as 'community' imply interactions (Allaby 2015).
Benthic	A description for animals, plants and habitats associated with the seabed. All plants and animals that live in, on or near the seabed are benthos (e.g. sponges, crabs, seagrass beds).
Biotope	The physical habitat with its associated, distinctive biological communities. A biotope is the smallest unit of a habitat that can be delineated conveniently and is characterised by the community of plants and animals living there.
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). *
Deep-sea sponge aggregations	Deep-sea sponge aggregations are defined by OSPAR as occurring in the deep sea (typically > 250 m water depth), primarily characterised by the presence of structure-forming (usually megabenthic) glass sponges (Class Hexactinellida) or demosponges (Class Demospongiae) in relatively high densities typically ranging from 0.5–24 sponges/m <sup>2</sup> (OSPAR 2010).
Epifauna	Fauna living on the seabed surface.
Favourable Condition	When the ecological condition of a species or habitat is in line with the Conservation Objectives for that feature. The term 'favourable' encompasses a range of ecological conditions depending on the objectives for individual features. *

Term	Definition
Feature	A species, habitat, geological or geomorphological entity for which an MPA is identified and managed. *
Feature Attributes	Ecological characteristics defined for each feature within site- specific Supplementary Advice on Conservation Objectives (SACO). Feature Attributes are monitored to determine whether condition is favourable.
Impact	The consequence of pressures (e.g. habitat degradation) where a change occurs that is different to that expected under natural conditions (Robinson <i>et al.</i> 2008). *
Infauna	Fauna living within the seabed sediment.
Investigative	Objective: to investigate the cause of change.
monitoring to determine management needs and effectiveness (Type 3 monitoring)	This monitoring type provides evidence of causality. It complements the above types by testing specific hypotheses through targeted manipulative studies (i.e. excluding an impact or causing an impact for experimental purposes). The design and statistical approach that can be used in these cases gives confidence in identifying cause and effect. It is best suited to test state/pressure relationships and the efficacy of management measures (Kröger & Johnston 2016).
Joint Nature Conservation Committee (JNCC)	JNCC is the public body that advises the UK Government and devolved administrations on UK-wide and international nature conservation. JNCC has responsibility for nature conservation in the offshore marine environment, which begins at the edge of territorial waters and extends to the UK Continental Shelf (UKCS).
Marine Scotland Science (MSS)	The scientific division of Marine Scotland providing expert scientific, economic and technical advice and services on issues relating to marine and freshwater fisheries, aquaculture, marine renewable energy, and the aquatic environment and its flora and fauna. MSS provide evidence to support the policies and regulatory activities of the Scottish Government through a programme of monitoring and research.
Marine Strategy Framework Directive (MSFD)	Environmental Status (GES) of EU marine waters and to protect the resource base upon which marine-related economic and social activities depend.
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). *

Term	Definition
Nature Conservation Marine Protected Area (MPA)	Marine protected areas in Scottish sea areas which were designated by Scottish Ministers through powers granted by the Marine (Scotland) Act and UK Marine and Coastal Access Act.
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 historically, and which is separate from and lies outside the area where natural range extension could be expected (Eno <i>et al.</i> 1997).
Operational monitoring of	Objective: to measure state and relate observed change to possible causes.
pressure-state relationships (Type 2 monitoring)	This objective complements the monitoring of long-term trends and is best suited to exploring the likely impacts of anthropogenic pressures on habitats and species and identify emerging problems. It leads to the setting of hypotheses about processes underlying observed patterns and is generally best applied in areas where a gradient of pressure is present (e.g. no pressure increasing gradually to 'high' pressure) (Kröger & Johnston 2016).
	It relies on finding relationships between observed changes in biodiversity and observed variability in pressures and environmental factors. It provides inference, but it is not proof of cause and effect. The spatial and temporal scale for this type of monitoring will require careful consideration of the reality on the ground to ensure inference will be reliable; for example, inference will be poor in situations where the presence of a pressure is consistently correlated to the presence of an environmental driver (e.g., a specific depth stratum) (Kröger & Johnston 2016).
Pressure	The mechanism through which an activity has an effect on any part of the ecosystem (e.g. physical abrasion caused by trawling). Pressures can be physical, chemical or biological, and the same pressure can be caused by a number of different activities (Robinson <i>et al.</i> 2008).
Priority Marine Features (PMFs)	Priority Marine Features are habitats and species that are considered to be marine nature conservation priorities in Scottish waters.
Sentinel monitoring	Objective: to measure rate and direction of long-term change.
of long-term trends (Type 1 monitoring)	This type of monitoring provides the context for distinguishing directional trends from short-scale variability in space and time. To achieve this objective efficiently, a long-term commitment to regular and consistent data collection is necessary; this means time series must be established as their power in identifying trends is far superior to any combination of independent studies (Kröger & Johnston 2016).

Term	Definition
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.
Marine and Coastal Access Act 2009	The Marine Act provides the legal mechanism to help ensure clean, healthy, safe, productive and biologically diverse oceans and seas by putting in place a new system for improved management and protection of the marine and coastal environment. The Act provides executive devolution to Scottish Ministers of marine planning and nature conservation powers in the offshore region (12–200 nautical miles).
Vulnerable Marine Ecosystem	The term Vulnerable Marine Ecosystems refers to paragraphs 42 and 43 of the Food and Agriculture Organization of the United Nations (FAO) International Guidelines for the Management of Deep-Sea Fisheries in the High Seas (FC Doc 2008; CEM 2009- present) (FAO 2020).

## 1. Introduction

The North-East Faroe-Shetland Channel Nature Conservation Marine Protected Area (MPA), hereafter referred to as "NEFSC", is part of a network of nationally designated sites designed to meet conservation objectives under and the Marine and Coastal Access Act 2009. These sites contribute to an ecologically coherent network of Marine Protected Areas (MPAs) across the North-east Atlantic, as agreed under the Oslo Paris (OSPAR) Convention and other international commitments to which the UK is a signatory.

Under the Marine and Coastal Access Act 2009, Scottish Ministers have devolved responsibility to designate MPAs within Scottish Waters and must assess whether they are meeting their conservation objectives. Marine Scotland, in partnership with Marine Scotland Science (MSS), Scottish Natural Heritage (SNH) (now known as NatureScot) and the Joint Nature Conservation Committee (JNCC), has developed a Scottish MPA monitoring strategy (Marine Scotland 2017). The strategy aims to provide direction for monitoring, assessment and reporting on the MPA network and guidance on standardisation of monitoring objectives, sampling design and methodologies. JNCC is the Statutory Nature Conservation Body (SNCB) responsible for nature conservation in the UK offshore environment (from the territorial limit to 200 nm from the mean low-water mark of the shore). The aim of this monitoring programme is to collect the necessary information from the Scottish MPA network to underpin assessment and reporting obligations. Where possible, this monitoring should also inform assessment of the status of the wider UK marine environment; for example, assessment of whether Good Environmental Status has been achieved, as required under Article 11 of the Marine Strategy Framework Directive (MSFD).

This monitoring report explores data acquired from the first dedicated monitoring survey of NEFSC, which will aim to form the first point in a monitoring time series of the Priority Marine Feature (PMF) deep-sea sponge aggregations against which changes and trends in feature condition can be assessed in the future. The data will also inform the development of an effective site and feature-specific monitoring approach for the site. The specific aims of the report are detailed under Report aims and objectives (section 1.4.3).

## 1.1 Site overview

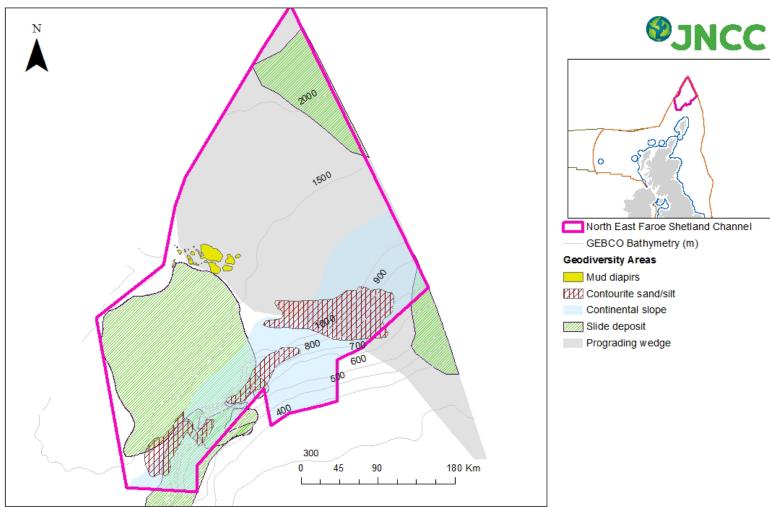
NEFSC spans 23,682 km<sup>2</sup> of the north-eastern reaches of the Faroe-Shetland Channel, approximately 93 km from Shetland. It lies within Charting Progress 2 Biogeographic Region: Atlantic North-West Approaches, Rockall Trough and Faroe-Shetland Channel.

The arrangement of habitats within the site are a result of discrete environmental conditions at different depth bands. Depths range from 330 m to 2,420 m. Between the 400-600 m contour is a highly unusual deep-sea habitat which is considered globally rare (Bett 2012). This habitat is formed by aggregations of deep-sea sponges. The combination of iceberg plough marks, seabed type and plentiful supply of nutrients, created by the mixing of temperate Atlantic waters and cold Arctic waters, makes it an ideal habitat for the establishment of deep-sea sponge aggregations. A narrow 'sponge belt' has previously been observed around the 500 m contour of the Faroe-Shetland Channel (Henry & Roberts 2014). Offshore deep-sea muds have been recorded across the site, predominantly below 800 m in the unique Greenland-Iceland-Scotland ridge system, where the sediment is more stable as reduced wave action allows for finer grains to settle, creating deep-sea muds. The muddy seabed is home to species that can tolerate cooler arctic-influenced waters, such as deepsea polychaetes (Bett 2012). In offshore waters, subtidal sand and gravel sediments are the most common seabed habitat in UK waters. The continental slope plays an important role in funnelling ocean currents that bring food and nutrients to the region, which in turn support a wide diversity of life. The NEFSC also includes several different features of geological

importance including the North Sea Fan, Miller Slide and the West Shetland Margin (Table 1; Figure 1).

Designated feature	Feature type
Deep-sea sponge aggregations	Low or limited mobility species
Offshore deep-sea muds	Habitat
Offshore subtidal sands and gravels	Habitat
Continental slope	Large-scale feature
The North Sea Fan	Geological and geomorphological feature
The Miller Slide	
The West-Shetland Margin Paleo-	
Depositional System	
The Pilot Whale Diapirs	

The EU deep-sea fishing regulation (EU) 2016/2336 (as amended by S.I. 2019/739, S.I. 2019/753 and S.I. 2020/1542) prohibits bottom trawling below 800 m across all European Union and UK waters. In the context of NEFSC, the legislation protects offshore-deep sea muds below 800 m from bottom trawls. Article 9 of (EU) 2016/2336 provides specific requirements for the protection of Vulnerable Marine Ecosystems (VMEs). A list where VMEs are known to occur, or are likely to occur, is due to be published by the European Commission. Where the listed VMEs occur at depths greater than 400 m, fishing with bottom gears will be prohibited within EU waters. Similar measures are expected to be put in place for UK waters. Between 400–800 m the 'move on' rules also apply for VMEs, i.e. if VMEs are found at or above the encounter thresholds, then fishing must cease, and the vessel must move at least 5 nm from the known VME. At NEFSC this is likely to provide a level of protection to the deep-sea sponge aggregations between 400 m and 600 m in the future. In relation to the rest of NEFSC and surrounding areas, under (EU) 2016/2336 (as amended by S.I. 2019/739, S.I. 2019/753 and S.I. 2020/1542), fishing activity is only to be permitted where deep-sea fishing activity has previously occurred between 2009 and 2011.



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Figure 1. Location of the NEFSC with key geodiversity areas.

## **1.2 Feature description**

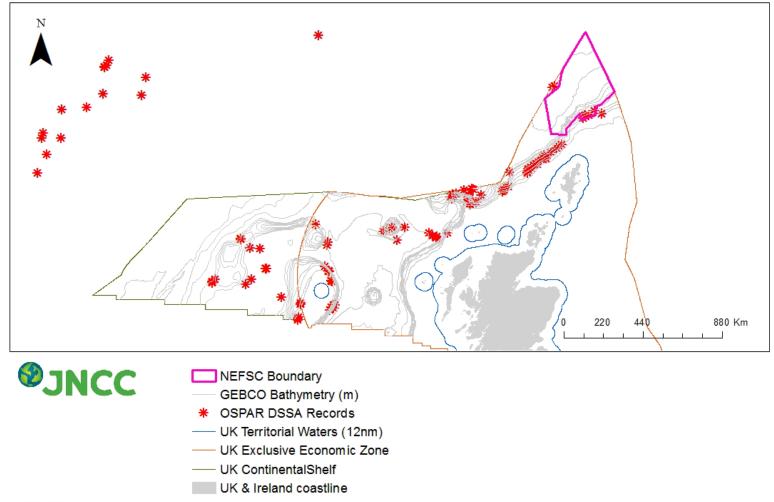
NEFSC has been designated to protect three PMFs: deep-sea sponge aggregations, offshore deep-sea muds and offshore subtidal sands and gravels. As the large-scale geodiversity features are unlikely to change in their range, extent or condition over the monitoring timescales under consideration, they are outside the scope of offshore MPA monitoring programme and are not included in this report. Table 2 provides correlating monitoring habitats that are included under PMFs that occur in NEFSC (Doggett *et al.* 2018).

Priority Marine Feature (PMF)	NEFSC corresponding Marine Habitat Classification of Britain and Ireland (v15.03)
Deep-sea sponge aggregations	<b>M.AAUB.Co.DeeSpo:</b> Deep sponge aggregation on Atlanto- Arctic upper bathyal coarse sediment
Offshore subtidal sands	M.AAUB.Sa: Atlanto-Arctic upper bathyal sand
and gravels	M.AAUB.Co; Atlanto-Arctic upper bathyal coarse sediment
	<b>M.AAUB.Mx:</b> Atlanto-Arctic upper bathyal mixed sediments
Offshore deep-sea muds	M.AAUB.Mu: Atlanto-Arctic upper bathyal mud

Table 2. NEFSC PMFs correlated to relevant monitoring habitats (Doggett et al. 2018).

#### 1.2.1 Deep-sea sponge aggregations

Deep-sea sponge aggregations are listed in the OSPAR list of Threatened and/or Declining species and habitats (OSPAR agreement 2008-07) and are defined by OSPAR as occurring in the deep-sea (typically at depths between 250 m and 1,300 m), primarily characterised by the presence of structure-forming glass sponges (Hexactinellida) or giant demosponges (Demospongiae) (OSPAR 2010). Sponge aggregation densities are defined by OSPAR as ranging from 0.5–24 sponges per m<sup>2</sup> (OSPAR 2010; Henry & Roberts 2014). Figure 2 shows confirmed records of deep-sea sponge aggregations within the UK Continental Shelf limits. Such aggregations are biodiversity hotspots, supporting a range of species that are unique to the surrounding seafloor communities. Deep-sea sponge aggregations are found in a wide range of habitats from muddy sediments to rock. In the Faroe-Shetland region, aggregations are often related to iceberg plough marks, attaching to the hard and coarse substrates associated with the scoured seabed (Marine Scotland 2016c). Sponge tissue is composed of small, spine-like, silicone spicules. Spicules from dead sponges form dense mats and can alter seabed characteristics, which in turn provide shelter for a wide range of small animals and elevated habitats for filter feeders (Tyler-Walters et al. 2016). Deep-sea sponges are thought to be slow-growing, and therefore damaged sponge communities are likely to take many years or even decades to recover. Physical disturbance is the greatest anthropogenic threat to sponge communities in the deep sea, and it is probable that bottom trawling and increased amounts of sediment in the water cause damage to aggregations. Resource exploitation (oil and gas operations) and future bioprospecting also pose potential threats to the survival of sponges (Tyler-Walters et al. 2016).



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Figure 2. Deep-sea sponge aggregations (DSSA) within the UK Continental Shelf limits (OSPAR 2018).

### 1.2.2 Offshore subtidal sands and gravels

Offshore subtidal sands and gravels are the most common habitat found in the subtidal waters of Scotland. Offshore subtidal sands and gravels encompass a wide range of broad-scale habitats and occur down to depths of 3,000 m (Tyler-Walters *et al.* 2016). This PMF spans a wide range of substrate types including sand and muddy sand, mixed sediment and coarse sediment. In offshore waters, sands and muddy sands support polychaetes (including tube building polychaetes), brittlestars and bivalve molluscs. Medium sands support communities dominated by the pea urchin *Echinocyamus pusillus*, whilst finer sands are characterised by burrowing crustaceans such as amphipods and cumaceans (Marine Scotland 2016a).

#### 1.2.3 Offshore deep-sea muds

Offshore deep-sea muds occur at depths from 200 m to 2,500 m and are widespread in the north and west offshore waters of Scotland, supporting diverse biological communities. The most common mobile benthic species are echinoderms; primarily sea urchins, brittlestars and sea cucumbers (Tyler-Walters *et al.* 2016). The relatively stable conditions associated with deep mud habitats often lead to the establishment of communities of burrowing megafaunal species where bathyal species may co-occur with coastal species. The burrowing megafaunal species include burrowing crustaceans such as the Norway Lobster, *Nephrops norvegicus,* and *Callianassa subterranea*. The mud habitats in deep water can also support sea pen populations and communities with brittlestars, including *Amphiura* spp. (UK BAP 2008).

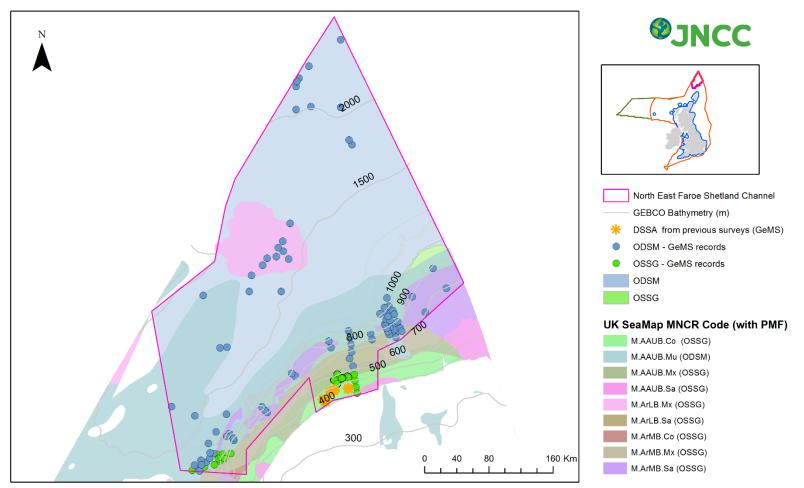
## **1.3** Existing data and habitat maps

Previous records of deep-sea sponge aggregations, offshore subtidal sands and gravels and offshore deep-sea muds exist within the site from a suite of dedicated surveys and are presented with predicted habitat models from UKSeaMap 2018 in Figure 3 (JNCC 2018b). Predictive habitat models for NEFSC are also available in the Geodatabase for Marine Habitats and Species in Scotland (GeMS) (SNH 2019).

NEFSC has been subject to a suite of dedicated environmental surveys between 1996 and 2006:

- Atlantic Frontier Environmental Surveys (AFEN) (1996, 1998);
- Strategic Environmental Assessment (SEA4) program (1999, 2000, 2002) (Holmes et al. 2003);
- Strategic Environmental Assessment (SEA7) program 2006 (DTI 2007; Bett 2012).

These surveys produced detailed mapping products including two multibeam datasets; one covering a large proportion of the deep waters of NEFSC (Figure 4) and one focusing on the shallower south of the site where aggregations have been previously recorded (Figure 5). Side scan sonar was also collected for the deep waters but is not included within the maps of this report. A marine Digital Elevation Model (DEM) exists for the site at six arc second resolution and is illustrated in Figure 6 (Environment Agency Geomatics 2020).



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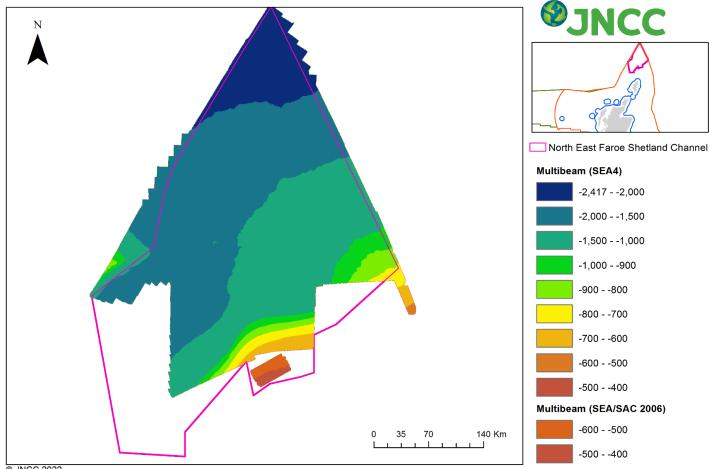
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**Figure 3.** PMF point data from GeMS records and predicted UKSeaMap 2018 habitats labelled with associated PMF (deep-sea sponge aggregations (DSSA), offshore subtidal sands and gravels (OSSG), offshore deep-sea muds (ODSM)).

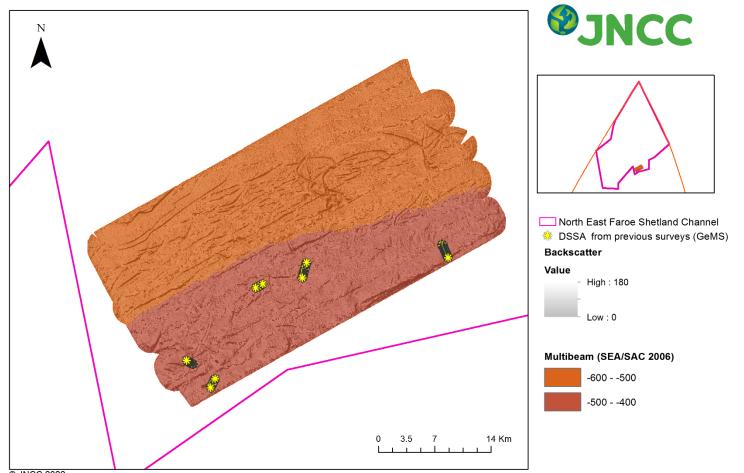


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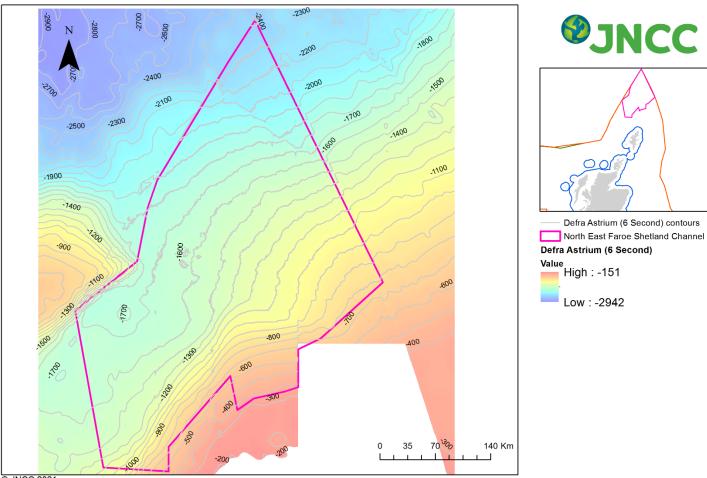
Figure 4. Existing multibeam data from Strategic Environmental Assessments; the large dataset covering the majority of NEFSC was collected during the SEA4 survey and the small box located in the south of the site was obtained during the SEA/SAC 2006 surveys.



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**Figure 5.** Multibeam data and accompanying backscatter from SEA/SAC 2006 survey with verified high confidence records of deep-sea sponge aggregations (DSSA) from GeMS records (2014).



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Figure 6. A mosaic of the 2020 Marine Digital Elevation Model (DEM) at six arc second resolution labelled with depth contours (Environment Agency Geomatics 2020).

## 1.4 Aims and objectives

#### 1.4.1 High-level conservation objectives

High-level, site-specific conservation objectives serve as a benchmark against which to monitor and assess the efficacy of management measures in protecting designated features within MPAs.

The high-level conservation objectives for NEFSC (JNCC 2018a, 2018c) 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.

In relation to the PMFs deep-sea sponge aggregations, offshore subtidal sands and gravels and offshore deep-sea muds, this means that:

- (a) habitat extent is stable or increasing; and
- (b) structures and functions, quality, and the composition of the habitat's characteristic biological communities are such as to ensure that it is in a condition which is healthy and not deteriorating.

#### **1.4.2** Definition of favourable condition

Specific attributes of the features will be monitored and assessed to determine whether conservation objectives have been achieved at the site level, as detailed in the high-level conservation objectives. Conservation objectives are to restore/maintain attributes to favourable condition. Supplementary conservation advice for this MPA (JNCC 2018b) lists several feature attributes for designated features. These attributes fall into broad attribute themes, which align with the terminology used in the Designation Order and are described below.

**Extent** refers to the total area within the site occupied by the feature while **distribution** refers to how a feature is spread out within the site. A reduction in extent has the potential to alter the biological and physical functioning of sedimentary habitat types (Elliott *et al.* 1998). The distribution of a habitat influences the component communities present and can contribute to the health and resilience of the feature (JNCC 2004).

**Structure** refers to the physical structure of a subtidal sedimentary habitat and its biological structure. Physical structure refers to: 1 - finer scale topography; and 2 - sediment composition. Biological structure refers to: 1 - key and influential species; and 2 - characteristic communities.

**Functions** are ecological processes (e.g. sediment processing, secondary production, habitat modification, supply of recruits, bioengineering and biodeposition). Functions are reliant on the growth and reproduction of characterising biological communities and provide a variety of functional roles within it (Norling *et al.* 2007). These can occur at a number of temporal and spatial scales and help to maintain the provision of ecosystem services (ETC 2011), both locally and to the wider marine environment.

**Supporting processes** refers to a range of natural processes to support and help any recovery from adverse impacts. For the site to fully deliver the conservation benefits (JNCC

2018b), these processes, namely hydrodynamic regime, water quality and sediment quality, must remain largely unimpeded. While this is a monitorable feature attribute, it is outside the scope of this report as no data on supporting processes were collected during the 1016S survey.

#### 1.4.3 Report aims and objectives

The primary aim of this monitoring report is to explore and describe the attributes of the designated features within NEFSC, to enable future assessment and monitoring of feature condition, including understanding of any change and trends. 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 specific objectives of this monitoring report are as follows (broad attribute themes, as defined in the site Designation Order are in bold text):

- 1. Describe the **extent and distribution**, **structure** and **supporting processes** of deep-sea sponge aggregations within NEFSC (box A) and outside the site (boxes B and C).
- 2. Compare deep-sea sponge aggregations within the site (box A) to those outside the site (boxes B and C).
- 3. Describe the **extent and distribution and structure** of offshore subtidal sands and gravels and offshore deep-sea muds (box D).
- 4. Note the presence of any PMFs and VMEs observed which are not designated features of the site.
- 5. Present any evidence of non-indigenous species (NIS) (MSFD Descriptor 2) and marine litter (MSFD Descriptor 10).
- 6. Recommend future monitoring approaches for NEFSC and the wider MPA network.

#### 1.4.4 Reporting sub-objectives

To achieve report objective 1 and objective 3, several reporting sub-objectives will be addressed to provide evidence on feature attributes and supporting processes (as defined in Supplementary Advice on Conservation Objectives (SACOs) developed by JNCC for NEFSC (JNCC 2018a). It should be noted that it was not possible to address all feature attributes as part of this monitoring survey given their comprehensive nature, the size of the site and the time available for survey. The feature attributes were therefore rationalised, prioritised and are presented below (Table 3).

 Table 3. Reporting sub-objectives addressed to achieve report objectives 1, 2 and 3 for feature attributes of NEFSC.

Feature attributes	Sub-attributes	Outputs	
Extent and distribution	Extent and distribution	Qualitative and semi-quantitative observations of deep-sea sponges.	
Structure	Sponge composition	Qualitative and semi-quantitative observations of deep-sea sponge	
	Sponge abundance	functional morphotypes. Univariate analysis of species of interest (Porifera morphotypes).	
	Characteristic communities	Multivariate analysis of epifaunal communities.	
Supporting processes	Water parameters	Conductivity, temperature and depth (CTD) data, bed shear stress energy layer outputs (West <i>et al.</i> 2010).	

<b>Objectives 1 and</b>	I 2: Deep-sea sponge	aggregations
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#### Objective 3: Offshore subtidal sands and gravels and offshore deep-sea muds

Feature attributes	Sub-attributes	Outputs	
Extent and distribution	Extent and distribution	Particle Size Analysis (PSA) point sample sediment distribution and qualitative evidence from imagery analysis.	
Structure	Physical structure: fine scale topography	PSA data and qualitative observations of seabed character.	
	Sediment composition		
	Biological structure: key and influential species	Multivariate analysis of infaunal communities.	
	Biological structure: characteristic communities		

#### **1.4.5** Outside the scope of this report

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

The focus of the offshore survey was to gather data for the first point in a monitoring time series of the biodiversity features of the site. The survey was designed to target and focus on the deep-sea sponge aggregations.

## 2. Methods

#### 2.1 Survey design

During October and November 2017, a dedicated monitoring survey (1517S) was conducted at NEFSC onboard MRV *Scotia* (Taylor *et al.* 2019). The aim of the survey was to acquire a robust initial (T0) sentinel monitoring (Type 1 monitoring) dataset to establish a monitoring time series for NEFSC, against which the rate and direction of change in the condition of the MPA features can be inferred over time. A detailed breakdown of the survey methodology is available in the cruise report (Taylor *et al.* 2019).

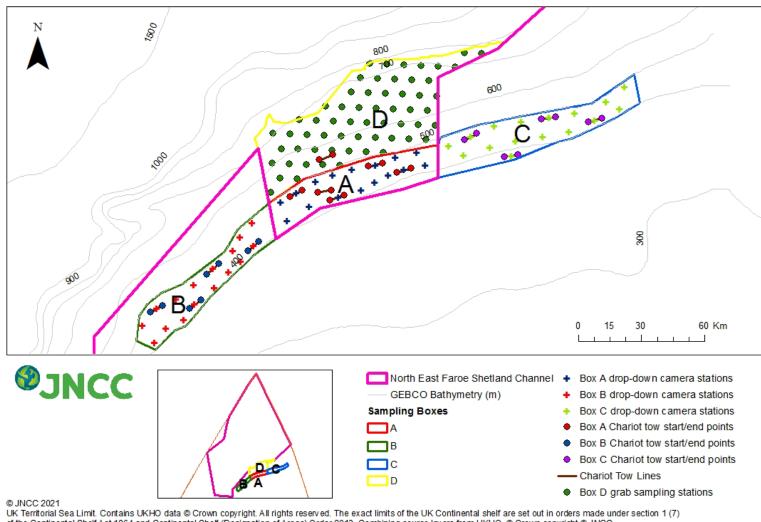
The three monitoring survey objectives are detailed in Table 4. To achieve survey objectives 1 and 2, three stratified boxes were assigned to the south of the site within the 400–600 m contour bands. Box A was located within the site and boxes B and C were allocated to the east and west of box A respectively, outside the current MPA boundaries. An additional box D was located north of box A to target offshore subtidal sands and gravels and offshore deep-sea muds within the site (Figure 7).

 Table 4. Survey objectives for the 1517S survey of NEFSC (features and attributes used to assess feature condition are in bold text).

Survey objective	Sub-objectives	Sampling box	
1. Collect evidence to inform monitoring of the <b>deep-sea sponge</b> <b>aggregations</b> within and near the	<b>1.1.</b> Visually sample within the area proposed as a full closure to demersal fisheries.	Box A	
<ul><li>site, specifically in relation to:</li><li>extent and distribution</li></ul>	<b>1.2.</b> Visually sample within the two boxes either side of the	Boxes B and C	
<ul> <li>biological structure.</li> </ul>	proposed closure investigated in sub-objective 1.1.		
2. Collect environmental data to improve understanding of environmental conditions and natural supporting processes within and near the site.	<b>2.1.</b> Acquire quantitative data on temperature and salinity within the areas surveyed for objective 1.	Boxes A, B and C	
<b>3.</b> Collect physical evidence to characterise the sediment composition and biological communities in areas of offshore subtidal sands and gravels and offshore deep-sea muds.	<b>3.1.</b> Acquire physical samples by means of grab sampler.	Box D	

To achieve objectives 1 and 2, the survey planned to obtain 16 short drop-frame camera transects (200 m) per box (48 stations in total), collecting high-definition video and still images and four long chariot tows (~3 km) to investigate the extent of deep-sea sponge aggregations within boxes A, B and C (Figure 7). Chariot station placement was based on a grid set along 425 m and 475 m depth contours with the chariot to be flown parallel to these depth contours. Drop-frame stations were proposed between chariot transect start and end points. Conductivity, temperature and depth (CTD) data would be collected for every drop-frame station to obtain environmental data.

To achieve objective 3, 60 sediment grab stations were assigned using a triangular systematic grid within box D located within the predicted offshore subtidal sands and gravels and offshore deep-sea muds close to boxes A, B and C.



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Figure 7. 1517S proposed sampling locations.

## 2.2 Completed sampling, data acquisition and processing

The 1517S survey was restricted by adverse weather conditions and time constraints. As deep-sea sponge aggregations were the focus of the survey, box D was low priority and in the face of unsuitable weather conditions the sampling effort was subsequently very low. This resulted in only eight infauna samples and a total of nine PSA samples. A significantly reduced number of drop-camera stations were obtained in box C (four stations) compared to box A (17 stations) and box B (16 stations) due to the operational constraints encountered (Taylor *et al.* 2019) (Figure 8).

#### 2.2.1 Drop-down camera

A total of 37 drop-down camera tows (approximately 200 m in length) were successfully collected during the 1517S survey (Figure 8). Drop camera transects were conducted to capture video data to describe the extent of the habitats, the presence of deep-sea sponge aggregations and to capture photographic stills for epibenthic analysis. Imagery data were collected in accordance with MESH (Mapping European Seabed Habitats) guidelines (Coggan *et al.* 2007), analysed by Envision Mapping Ltd. and subjected to external quality assurance (Benson & Sotheran 2018).

#### 2.2.2 Chariot tows

The chariot is a towed camera system that collects continuous video data and was used in the 1517S survey to delineate the extent of habitats. Fifteen chariot transects were completed during the survey (each of approximately 1 hour duration) (Figure 8).

### 2.2.3 Conductivity, temperature and depth (CTD) data

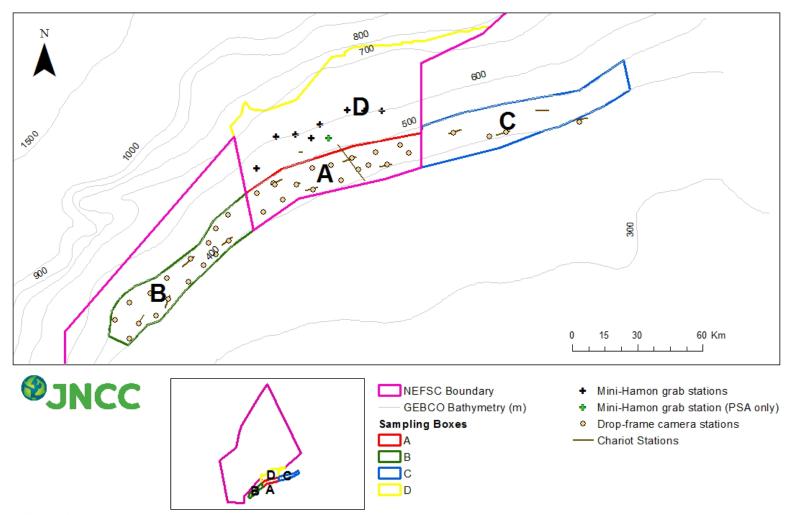
Multiparameter CTD profilers were attached to the drop-down camera frame to collect characteristics of the sea water including temperature and conductivity. CTD data were collected for 35 out of 37 drop down camera stations; two stations were unsuccessful due to CTD failure (A08 and B05). The data were subsequently used as environmental parameters during multivariate statistical analysis.

### 2.2.4 Sediment sampling

Within box D a total of nine seabed sediment samples were collected for particle size analysis (PSA) and benthic infauna analyses using a 0.1 m<sup>2</sup> Hamon Grab (also known as a 'mini' Hamon Grab) (Figure 8) (Taylor *et al.* 2019).

A 500 ml sub-sample was taken from each grab sample and stored at -20°C prior to determining the particle size distribution. Sediment samples were processed following the recommended methodology of the North-East Atlantic Marine Biological Analytical Quality Control (NMBAQC) scheme (Mason 2011) (section 2.3.1). The faunal fraction was sieved over a 0.5 mm and 0.25 mm mesh, photographed, then fixed in buffered 4% formaldehyde. The purpose of obtaining two fractions per sample was to compare the efficacy of different mesh sizes in deep-sea habitats. Although two faunal fractions were obtained it was decided that the results of such a comparison would not be statistically robust. Consequently, the two fractions taken from the same grab were added together and considered as one sample.

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Figure 8. Map of completed sampling during 1517S.

## 2.3 Data preparation and analysis

#### 2.3.1 Epifaunal data: drop-frame camera

Video and still imagery from the drop-down camera operations were analysed for several parameters:

- To identify habitat.
- To identify and quantify epifauna.
- To provide semi-quantitative data on seabed characteristics.
- To note transitions between substrata.
- To record any visually detectable human impacts.

#### 2.3.2 Field of view (FOV) threshold and the sum of relative density

Due to the variable total area covered by individual tows ( $13 \text{ m}^2$  to  $37 \text{ m}^2$ ), the sum of relative species density was calculated for all images within a tow (by station) and divided by total area covered to produce comparable figures for each tow, i.e. each species was represented by  $1 \text{ m}^2$ . A total of 37 drop camera tows and 672 images were included for the analysis (Appendix 2).

A large range in Field of View (FOV) was observed across the still imagery dataset (0.1 m<sup>2</sup> to 4.6 m<sup>2</sup>). To account for the variation, maintain a consistent degree of taxonomic detail and to reduce the potential for under/over estimation of abundance, a threshold for an ideal FOV was determined. FOV was plotted for each habitat with its assigned image quality (Appendix 3, Figure 36). As expected, images with large FOVs are associated with *Poor* quality. Further plots illustrating number of images per habitat against FOV show the majority of images fall within 0.5 m<sup>2</sup> and 2 m<sup>2</sup> (Appendix 3; Figure 37; Figure 38). To encapsulate as many images as possible within each habitat, particularly for deep-sea sponge aggregations, and to retain a consistent level of taxonomic detail, the threshold for ideal FOV was increased to 2.5 m<sup>2</sup>, therefore the FOV range used for analysis was 0.5 - 2.5 m<sup>2</sup>. Images assigned with *Zero* and *Very Poor* quality were removed from analysis as per the NMBAQC guidelines (Turner *et al.* 2016). Duplicate images were removed from the dataset.

#### 2.3.3 Deep-sea sponge aggregation identification

Suspected deep-sea sponge aggregations were assigned where the coverage of Erect, structure-forming sponges was approximately 3% or more of the still image (Benson & Sotheran 2018). Sponges were identified to morphotype during all imagery analyses based on descriptions by Berman *et al.* (2015) (Appendix 6) due to the difficulties identifying sponges to species level without physical sampling.

Drop-down video tows were analysed to identify whether deep-sea sponge aggregations formed the main habitat. OSPAR (OSPAR 2010) defines deep-sea sponge aggregations as occurring in the deep sea (typically > 250 m water depth), primarily characterised by the presence of structure-forming (usually megabenthic) glass sponges (Class Hexactinellida) or demosponges (Class Demospongiae) in relatively high densities typically ranging from 0.5–24 sponges per m<sup>2</sup> (Henry & Roberts 2014). During the analysis, video segments were recorded as suspected deep-sea sponge aggregations where "Erect, structure-forming sponges were observed at 1% or more of the percentage cover of the entire video tow" (Benson & Sotheran 2018). Changes in habitat are defined as a change in substrate type where tow length is greater than 5 m covering an area equal to or greater than 25 m<sup>2</sup>. One video tow (A06) was split into two segments where a significant change in habitat was

observed. This information was used to delineate the aggregations and used alongside the still images in subsequent analyses.

### 2.3.4 Categorising sponges into functional morphotypes

Eight sponge morphotypes were identified during the analysis as per Berman *et al.* (2015). These were further categorised into 'functional morphologies' for the purpose of this report. Schönberg (2021) identifies functional morphologies by relating morphotypes to their traits, function, environmental conditions and ecology. This helps to standardise subsequent comparisons and could increase our ability to monitor change over time. Categorising functional morphologies can additionally inform on the prevailing environmental conditions, further discussed in section 4. Morphotypes were allocated their corresponding category and were further grouped into the broader, overarching category. Five categories result from the eight morphotypes originally identified and are detailed in Table 5. This includes category 1 Encrusting; category 2 Creeping or Repent; category 3 Simple Massive; and category 4 Balls and Globular. Categories 11 and 12 were grouped together as they both represent Erect forms.

Original Porifera morphotype from imagery analysis as per Berman et al. (2015)	Functional morphologies as per Hanna and Shönberg (2021)	Group category for this report
Encrusting	Category 1.1 Encrusting	Catagony 1 Encrusting
Papillate	Category 1.2 Encrusting Papillate	Category 1 Encrusting
Repent	Category 2 Creeping or Repent	Category 2 Creeping or Repent
Massive	Category 3 Simple Massive	Category 3 Simple Massive
Globular	Category 4 Balls or Globular	
Pedunculate	Category 4 Balls or Globular	Category 4 Balls and Globular
Flabellate	Category 11.1 Erect Laminar	
Arborescent	Category 12 Erect Branching	Category 11 & 12 Erect Forms

 Table 5. Sponge functional morphology categories.

### 2.3.5 Epifaunal data: chariot video tows

In addition to the drop-frame analysis detailed above, the chariot video tows were analysed in the open-source image annotation software BIIGLE (Langenkämper *et al.* 2017) and segmented with the purpose of mapping the presence and absence of sponges. Habitats were segmented into three broad categories including sediment, patchy sponges and sponge aggregations. The mapping of the aggregations is a conservative estimate of the extent of aggregations and does not strictly apply the OSPAR (2010) density criteria for deep-sea sponge aggregations.

### 2.3.6 Sediment particle size distribution

Sediment samples collected at NEFSC were analysed by Cefas for half phi intervals using a combination of laser diffraction (<1 mm fraction) and dry sieving techniques (>1 mm) as

described in National Marine Biological Analytical Quality Control Scheme (NMBAQC) PSA guidance (Mason 2011). Mean particle size, sorting coefficient, skewness and kurtosis were also calculated for all samples and each sample was classified according to one of four European Nature Information system (EUNIS) sediment classes as defined by Long (2006). GRADISTAT software (Blott & Pye 2001) was then used to produce particle size distribution statistics.

### 2.3.7 Infaunal data preparation

Faunal samples were processed by Thomson Unicomarine and were identified to the lowest taxonomic level practicable, enumerated and weighed (blotted wet weight) to the nearest 0.0001 g. This was carried out following the recommendations of the NMBAQC scheme (Worsfold *et al.* 2010). Full details are available in the analysis report (Chamberlain *et al.* 2017).

## 2.4 Numerical and statistical analyses

### 2.4.1 Data truncation

The datasets were examined and truncated to ensure subsequent analyses were robust and any erroneous entries were removed. For example, records of juveniles and mobile species were removed. Many sponge morphotypes were provided with qualifiers on suspected species and colour, however it was decided that they would be merged by morphotype due to uncertainties and potential inconsistencies between analysts. Details of the truncation protocol for infauna and epifauna are available in Appendix 1.

### 2.4.2 Epifaunal multivariate analysis

Univariate indices of community structure were calculated for the communities to get an initial insight into the dataset. This included total number of species, total individuals, Margalef's species richness index, Shannon diversity index, Simpson diversity index and Pielou's evenness. The Margalef index reflects the total number of species relative to the natural log of total abundance, the Shannon index reflects both the total number of species and the evenness with which total abundance is distributed across species, and Pielou's evenness reflects the abundance of each species, scaled between 0 and 1 where 1 is perfect evenness.

The following null hypotheses were used to inform epibenthic analysis and investigated using multivariate analysis:

- H<sub>0</sub> There is no difference in **epifaunal** metric/trait or community composition within the sampling boxes.
- H<sub>0</sub> There is no difference in **epifaunal** metric/trait or community composition between sampling boxes within and outside NEFSC.

The first null hypothesis will be used to address reporting for objective 1 (section1.4.3) informing on the extent, distribution, structure and supporting processes of the deep-sea sponge aggregations in the sampling boxes. The second null hypothesis aims to inform reporting for objective 2, comparing the deep-sea sponge aggregations in box A to boxes B and C. Prior to analysis, an ideal FOV threshold was applied to account for variation in total area covered (per video tow) as per section 2.3.7. Images were pooled by station throughout the analyses.

The diversity indices were analysed for any correlation with the 15 suspected records of deep-sea sponge aggregations from the original drop-down camera video results. The correlation analyses were conducted using the statistical package PRIMER (v7: Clarke & Gorley 2015) using Pearson's correlation coefficient. Stations that had aggregations recorded were factored with a value of two and the rest of the stations were factored with a value of one.

### 2.4.3 Solitary and colonial epifaunal community

Measurements of epifaunal abundances for all habitats sampled in boxes A, B and C were provided in the form of individual counts for solitary species and percentage cover for colonial/Encrusting species. As each of these methodologies target highly divergent communities that have different biological traits and are enumerated on different scales, initial statistical investigations were carried out separately. Encrusting and Massive Porifera morphotypes were included in the colonial dataset and the remaining sponge morphologies were included in the solitary dataset. The datasets were visually examined using shade plots before being fourth root transformed to downweigh numerically dominant taxa. A resemblance matrix was generated using Bray-Curtis similarity.

### 2.4.4 Overall epifaunal community

To get an overall picture of the epifaunal community, the percentage cover and abundance count datasets were combined. When considering the analysis of the combined data, several applications were explored to factor in differences between measurements of abundance and the two different metrics they produce. Transformation can be applied to a mixed matrix of counts and percentage cover to downweigh the contributions of quantitatively dominant species to the similarities calculated between samples (Clarke & Gorley 2015). Presence-absence transformation can combine two metrics whilst providing a view into the community composition, however the severe transformation omits any detail in abundance and therefore cannot give a detailed view into the assemblage or community structure. Fourth root transformation is less severe and gives an insight into community structure, however it combines two metrics that are on different scales (i.e. percentage cover <<1-100% counts 0–400).

Standardisation was applied before transformation to overcome issues regarding the combination of two metrics. Standardisation ensured that the total number of individuals for each recorded taxon was equally weighted and on a relative scale of 100 within each image (Clarke & Gorley 2015). Shade plots were again created for the standardised data and stress values were observed. Fourth root transformation gave more weight to more taxa in the analysis, this included a greater representation across Porifera morphotypes. A resemblance matrix was created using Bray-Curtis similarity analyses and applied to the suite of multivariate analyses listed in Table 6. Whilst standardisation provides insight into the relative structure and composition, it must be noted that it is not a true representation of the data as it gives rare/low abundance species as much weight as common and abundant ones.

Environmental variables were visually examined with the aid of draftsman plots to assess for possible skewness in the data and the need for any subsequent transformation. The variables were investigated for collinearity using Pearson's correlation. The variables were normalised, and a resemblance matrix was generated using Euclidean distance. Additionally, univariate analysis of Porifera morphotypes and qualitative observations of deep-sea sponges were investigated.

 Table 6. Epifaunal multivariate analysis conducted using the statistical package PRIMER (v7: Clarke & Gorley 2015).

	Multivariate routine stages	Application
1.	Non-metric multidimensional scaling (nMDS)	To explore the relationships between samples.
2.	Similarity profiles (SIMPROF)	To determine if the dataset has a structure distinct from that derived by random permutation.
3.	Hierarchical agglomerative clustering	Used in conjunction with SIMPROF to look for divisions in the dataset and to determine where divisions could no longer be made appropriately (i.e. any sub cluster could be randomly permuted).
4.	Analysis of similarity (ANOSIM)	To investigate differences between boxes within NEFSC and outside NEFSC both globally and pairwise.
5.	Similarity percentages (SIMPER)	To further investigate the results from ANOSIM and inform which taxa characterised each group, and which taxa explained the dissimilarity between stations.
6.	RELATE	A non-parametric Mantel test to determine if there was a relationship between the biological and environmental resemblance matrices.
7.	Biota and/or environment matching (BEST)	To relate measured environmental factors (depth, sediment type, temperature and conductivity) to biological patterns and examine how well these factors (or a combination of) explain biological variability.
8.	DistLM	To determine how much of the biological variance was explained by the environmental drivers.

### 2.4.5 Confidence assessment of deep-sea sponge aggregations

The suspected deep-sea sponge aggregations from the drop-down camera stations were further assessed for confidence with supporting information from both the video and still analyses using criteria detailed in Henry and Roberts (2014). The following questions were asked of the data to characterise and verify suspected deep-sea sponge aggregations at the site:

 Density – Do the records conform to densities provided by OSPAR? This was calculated for NEFSC from the still imagery community data, dividing the sum of sponge counts or percentage cover over a whole station/segment by the total viewable area covered (calculated from the field of view per image). Any stations with counts greater than 0.5 per m<sup>2</sup> or 1% per m<sup>2</sup> were considered to satisfy this criterion. Where SIMPER identified sponges as truly characteristic of a community this would also satisfy this criterion.

- 2. Habitat Do suspected records conform most closely to a deep-sea sponge aggregation or would they be better characterised as another habitat type based on the key species present? In the case of NEFSC, this criterion is met when the analyst identified deep-sea sponge aggregations during the analysis of the drop-down video data using the threshold detailed above i.e., where sponges were observed at 1% or more over a station/segment.
- 3. **Ecological function** Do suspected records support a biological assemblage considered typical of a deep-sea sponge aggregation? This criterion focused on the SIMPER community results (the methodology for which is discussed in section 2.4.4), comparing them to the typical associated sponge species detailed from observed during investigations within the Faroe-Shetland region by Henry and Roberts (2014).

For this assessment a tick scoring system was used. A suspected record received a tick if the density criterion was met, two ticks if the density and one other criterion were met, and three ticks if all three criteria were met. The number of ticks correlated to the confidence score assigned to each suspected record, with one tick equating to low confidence, two to medium confidence, and three to high confidence (Henry & Roberts 2014).

### 2.4.6 Infaunal multivariate analysis

Due to the low number of successful sampling events a robust dataset was not obtained for infaunal communities meaning it was not feasible to produce a first point in a time series. The aim of the infaunal analysis was to describe the sampled communities to inform reporting for objective 3 (section 1.4.3) on the extent, distribution, structure and function of the PMFs offshore subtidal sands and gravel and offshore deep-sea muds. This meant that multivariate analysis was limited, so these results must be viewed with some caution. Prior to multivariate analysis, the dataset was visually examined using shade plots before being fourth root transformed to down weigh numerically dominant taxa. Abundance data was analysed to examine community structure. A resemblance matrix was generated using Bray-Curtis similarity and subject to the multivariate techniques listed below (Table 7).

Summary statistics and univariate indices of community structure were calculated for each grab sample to get an insight into the dataset. This included total abundance, total species, Margalef index and Pielou's evenness.

Multiv	ariate routine process	Application		
1.	Non-metric multidimensional scaling (nMDS)	To explore the relationships between samples.		
2.	Similarity profiles (SIMPROF)	To determine if the dataset has a structure distinct from that derived by random permutation.		
3.	Hierarchical agglomerative clustering	Used in conjunction with SIMPROF to look for divisions in the dataset and to determine where divisions could no longer be made appropriately (i.e. any sub-cluster could be randomly permuted).		

 Table 7. Infaunal multivariate analysis conducted using the statistical package PRIMER (v7: Clarke & Gorley 2015).

### 2.4.7 Non-indigenous species (NIS)

The infaunal and epifaunal taxon lists generated from the infaunal samples and seabed imagery data were cross-referenced against lists of non-indigenous target species. These lists have been selected for assessment of Good Environmental Status in UK waters under MSFD Descriptor 2 and identified as significant by the Great Britain Non-Native Species Secretariat. These taxa are listed in Appendix 5.

# 3. Results

# 3.1 Benthic habitat extent and distribution

### 3.1.1 Particle size analysis (PSA)

From the limited physical sampling within box D, nine PSA samples were obtained to investigate the presence of offshore deep-sea muds and offshore subtidal sands and gravels (objective 3). Samples were collected in depths ranging from 520–600 m. Seven samples were classified as M.AAUB.Mx (Atlanto-Arctic upper bathyal mixed sediments) which correspond to the PMF offshore subtidal sands and gravels. Two samples were classified as M.AAUB.Mu (Atlanto-Arctic upper bathyal mud) correlated to the PMF offshore deep-sea muds (Table 8).

The offshore subtidal sands and gravel samples were composed of gravelly muddy sand (four samples), muddy gravel (two samples) and gravelly mud (one sample). The offshore deep-sea mud samples were composed of (gravelly) sandy mud (two samples). Mud and sandy mud substrates are characterised by higher proportions of fines than the classified mixed sediment (Table 9; Figure 9; Figure 10). Overall, these results confirm presence of the PMF offshore subtidal sands and gravels from UKSeaMap 2018 (SNH 2019) (Figure 11). Offshore deep-sea muds extend further south of the site in shallower habitats (around the 500–600 m contour) than previously predicted by UKSeaMap 2018 (JNCC 2018b).

Priority Marine Feature (PMF)	Marine Habitat Classification Level 3	No. of Particle Size Analysis (PSA) samples
Offshore subtidal sands and gravels	M.AAUB.Mx	7
Offshore deep-sea muds	M.AAUB.Mu	2

Table 8. Priority Marine Features (PMFs) from Particle Size Analysis (PSA) results.

 Table 9. PSA results and corresponding classification.

Station	Gravel	Sand	Fines	Folk symbol (Long, 2006)	Depth	Broad description	Deep-sea JNCC Marine Habitat Classification Code	Associated PMF
D02	3.19	33.93	62.88	(g)sM	543	mud and sandy mud	M.AAUB.Mu Atlanto-Arctic upper bathyal mud	Offshore deep-sea mud
D10	13.09	60.46	26.45	gmS	585	mixed sediments	M.AAUB.Mx Atlanto-Arctic upper bathyal mixed sediments	Offshore subtidal sands and gravels
D11	11.74	67.78	20.48	gmS	572	mixed sediments	M.AAUB.Mx Atlanto-Arctic upper bathyal mixed sediments	Offshore subtidal sands and gravels
D12	49.35	15.32	35.33	mG	520	mixed sediments	M.AAUB.Mx Atlanto-Arctic upper bathyal mixed sediments	Offshore subtidal sands and gravels
D13 (PSA ONLY)	32.23	21.49	46.28	mG	545	mixed sediments	M.AAUB.Mx Atlanto-Arctic upper bathyal mixed sediments	Offshore subtidal sands and gravels
D20	3.41	36.85	59.74	(g)sM	583	mud and sandy mud	M.AAUB.Mu Atlanto-Arctic upper bathyal mud	Offshore deep-sea mud
D30	6.13	37.94	55.93	gM	600	mixed sediments	M.AAUB.Mx Atlanto-Arctic upper bathyal mixed sediments	Offshore subtidal sands and gravels
D31	5.07	52.18	42.76	gmS	596	mixed sediments	M.AAUB.Mx Atlanto-Arctic upper bathyal mixed sediments	Offshore subtidal sands and gravels
D32	8.72	49	42.28	gmS	589	mixed sediments	M.AAUB.Mx Atlanto-Arctic upper bathyal mixed sediments	Offshore subtidal sands and gravels

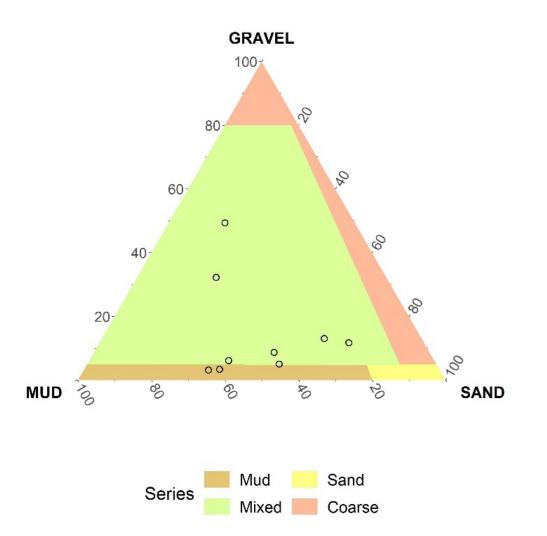
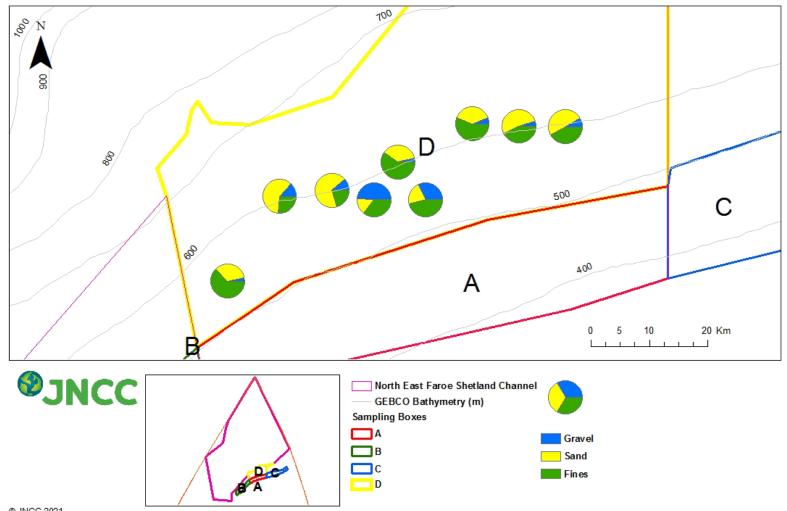


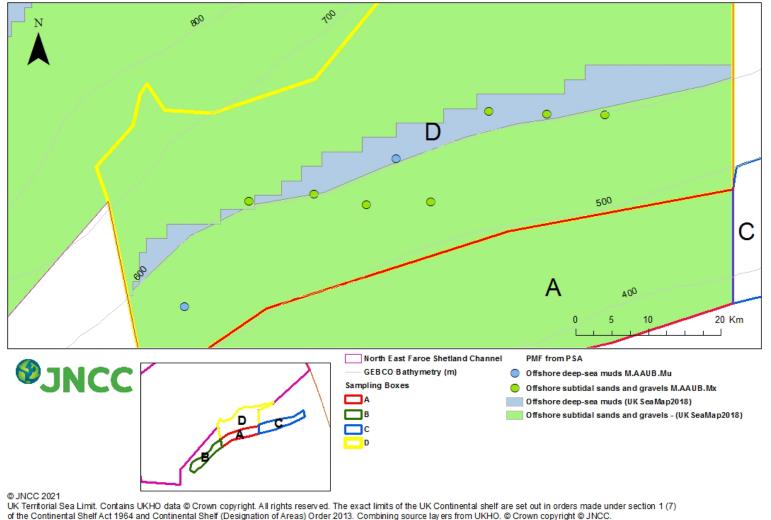
Figure 9. Classification of particle size distribution (half phi) information for each sampling point (black circles) into one of the broad-scale habitats (coloured areas) plotted on a true scale subdivision of the Folk triangle (Long 2006).



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Figure 10. Pie charts illustrating proportions of gravel sand and fines from grab sampling in box D.



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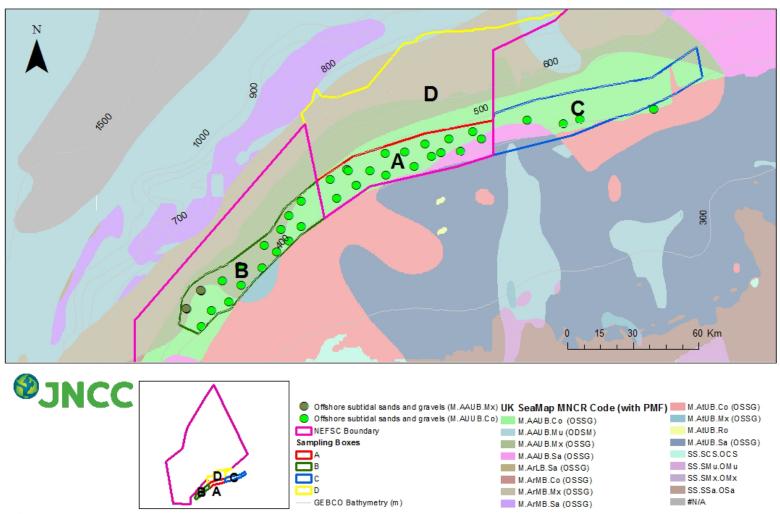
Figure 11. PMFs offshore subtidal sands and gravels and offshore deep-sea muds from PSA results and UKSeaMap 2018 habitats classed according to sedimentary PMFs.

### 3.1.2 Imagery sediment description

Further observations on the sedimentary PMFs were made from the imagery analysis. From the 37 drop-down video tows, one station was split into two segments (as per Coggan *et al.* 2007), making a total of 38 segments. Video and stills analysis identified all stations as different variants of the PMF offshore subtidal sands and gravel (Table 10). Most video segments were allocated as M.AAUB.Co (Atlanto-Arctic upper bathyal coarse sediment – 36 video segments) and two were assigned as M.AAUB.Mx (Atlanto-Arctic upper bathyal mixed sediment). Coarse and mixed sediment results are broadly consistent with the predicted UKSeaMap 2018 (Figure 12) with slight increase in coarse observations (as opposed to predicted mixed sediment) in box B. In addition, UKSeaMap predicts sand habitats towards the east of box A, however coarse sediment was assigned during analysis. Nevertheless, all the classified habitats coincide with the PMF offshore subtidal sands and gravels which is consistent with previous records from UKSeaMap records and the GeMS database (SNH 2019) (Figure 12).

 Table 10. PMFs from video habitat analysis.

Priority Marine Feature (PMF)	Marine Habitat Classification Level 3	No. of video segments
Offshore subtidal sands and gravels	M.AAUB.Co	36
gravels	M.AAUB.Mx	2



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Figure 12. Sedimentary PMFs from video analysis with predicted habitats from UKSeaMap 2018 and associated PMFs.

# 3.2 Deep-sea sponge aggregations PMF

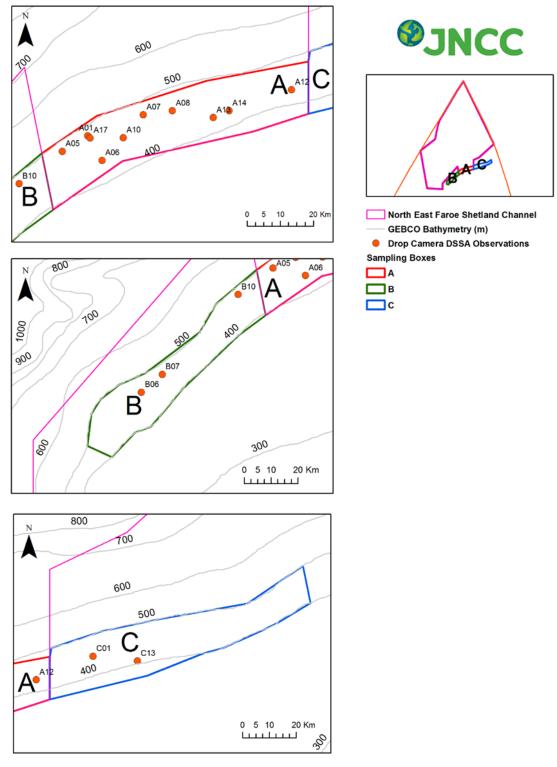
### 3.2.1 Drop-down camera video and stills

Suspected deep-sea sponge aggregations were allocated for 15 drop-down camera segments collected during the habitat analysis of the video footage (Table 11). Ten suspected records were observed within NEFSC boundaries (box A) (Figure 13). Outside the NEFSC boundary, three suspected records were identified within box B and two in box C. It is noted that reduced sampling effort in box C means that direct comparisons between all three boxes cannot be made with confidence as low numbers of aggregations may only reflect low sampling events.

Depth, temperature and conductivity were recorded during the drop-down camera video. The average values per video tow for each suspected deep-sea sponge aggregation are provided in Table 11 (drop-down camera results) and Table 12 (chariot tow). Average drop-down camera depth over a station ranged from 427 m to 495 m. Average temperatures ranged from 2.9 to 8°C. Conductivity at aggregations ranged from 31.7 to 36.5 S/m. A suspected deep-sea sponge aggregation was assigned at A08 however, due to a technical failure of the CTD temperature and conductivity were not collected.

Station	Camera type	Average depth	Average temperature	Average conductivity
A01	Drop-down	482	3.31	32.00
A05	Drop-down	484	3.13	31.86
A06b	Drop-down	427	4.44	33.01
A07	Drop-down	481	4.28	32.89
A08	Drop-down	471	NA	NA
A10	Drop-down	448	4.27	32.85
A12	Drop-down	457	7.31	35.78
A13	Drop-down	440	6.85	35.31
A14	Drop-down	443	6.75	35.22
A17	Drop-down	478	3.12	31.84
B06	Drop-down	495	3.47	32.17
B07	Drop-down	473	3.66	32.34
B10	Drop-down	488	2.95	31.71
C01	Drop-down	473	6.67	35.17
C13	Drop-down	430	8.10	36.54

 Table 11. Average environmental parameters for stations with suspected deep-sea sponge aggregations from drop-camera video analysis.



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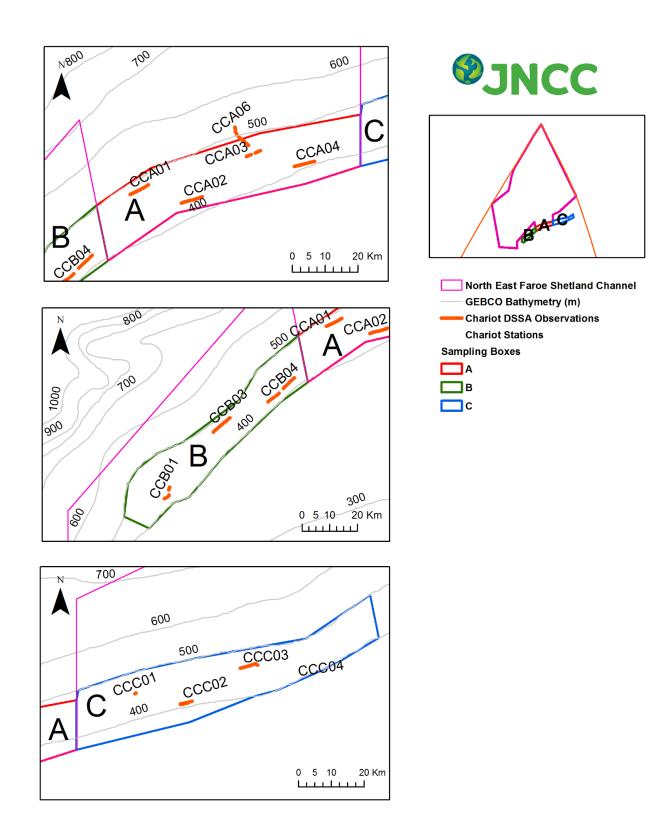
Figure 13. Suspected deep-sea sponge aggregation (DSSA) records from drop-down camera imagery results.

### 3.2.2 Chariot video tows

During the evaluation of chariot tow data, 11 stations included segments assigned as potential deep-sea sponge aggregations. This included five stations in box A, three stations in box B and three stations in box C (Figure 14). Depths ranged from 374 to 484 m, temperatures ranged from 3.6 to 9°C and conductivity ranged from 32.3 to 37.5 S/m. A technical failure at CC\_C02 meant that temperature and conductivity were not obtained at this station. Although average depths are provided as a value for each station, it is noted that chariot tow A06 intersected the 500 m depth contour and observed suspected aggregations in depths of approximately 495–525 m.

Station	Camera type	Average depth	Average temperature	Average conductivity
	Chariot			
CC_A01	tow	424.70	5.94	34.45
	Chariot			
CC_A02	tow	374.26	7.87	36.28
	Chariot			
CC_A03	tow	466.20	8.04	36.52
	Chariot			
CC_A04	tow	392.26	9.08	37.50
	Chariot			
CC_A06	tow	484.43	3.58	32.30
	Chariot			
CC_B01	tow	456.05	4.28	32.89
	Chariot			
CC_B03	tow	427.03	4.96	33.52
	Chariot			
CC_B04	tow	424.93	6.81	35.30
	Chariot			
CC_C01	tow	478.50	NA	NA
	Chariot			
CC_C02	tow	426.00	NA	NA
	Chariot			
CC_C03	tow	477.31	6.44	34.95

 Table 12. Average environmental parameters for chariot tow segments with suspected deep-sea sponge aggregations.



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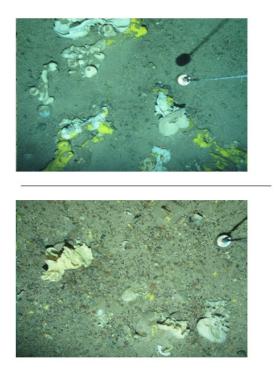
**Figure 14.** Suspected deep-sea sponge aggregation (DSSA) observations from chariot tow video analysis.

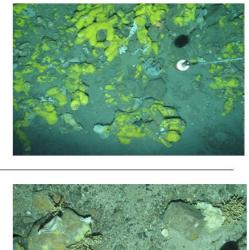
# 3.3 Epifaunal community analysis

### 3.3.1 Porifera morphotypes and structure

Eight sponge morphotypes were originally recorded within and outside NEFSC which were subsequently grouped into five categories as referred to in section 2.3.4. The most frequently occurring morphologies included Encrusting, Erect (Flabellate and Arborescent) and Simple Massive. Examples of the most frequently recorded morphotypes can be seen in Figure 15. The structure of the sponge community was explored using a series of analyses. Figure 16 and Figure 17 explore the contribution of morphotype abundances across each station for cover and count metrices. The highest percentage cover was recorded for Simple Massive growth forms at station A05 (Figure 16). Stations A06a, A06b and A08 had the highest recorded percentage cover for Encrusting morphotypes. Erect forms appear to occur similarly throughout the surveyed sites with the highest abundance of these growth forms seen at station A17 (Figure 17). The highest number of creeping or repent sponges was observed over station A06b. Creeping or Repent sponges had the lowest observations across the sampling boxes compared to the rest of the morphotypes.

Figure 18 illustrates the morphotypes present at a station and their contribution to the overall sponge structure. The pie charts broadly show a north-south divide in boxes A and B, with two groups dominated by either a) Massive and Encrusting and Erect growth forms (red-yellow-blue pie charts) or b) Erect and Encrusting morphologies (blue-yellow pie charts). Simple Massive growth forms are more abundant towards the northern 500 m contour of boxes A and B whilst Erect-Encrusting growth forms appear to be distributed toward the 400 m contour. In box C, Massive, Encrusting and Erect forms appear to occur in similar frequencies for most of the stations.

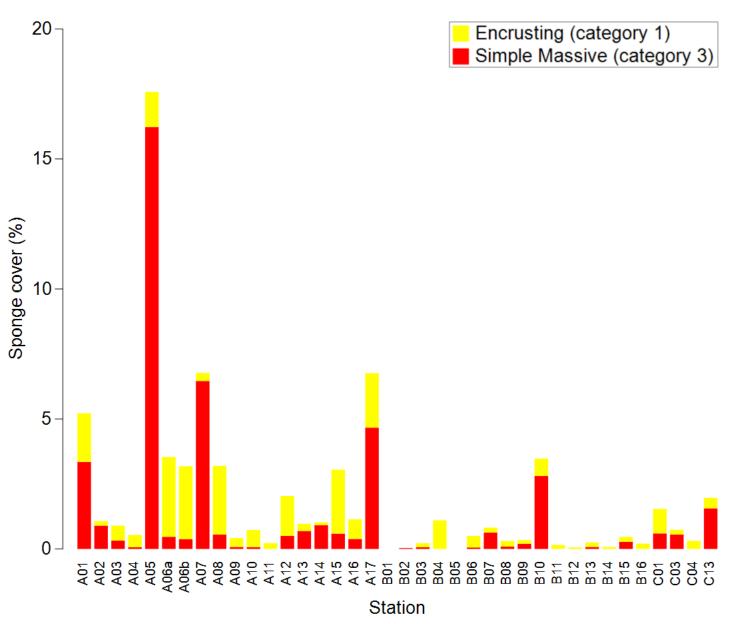




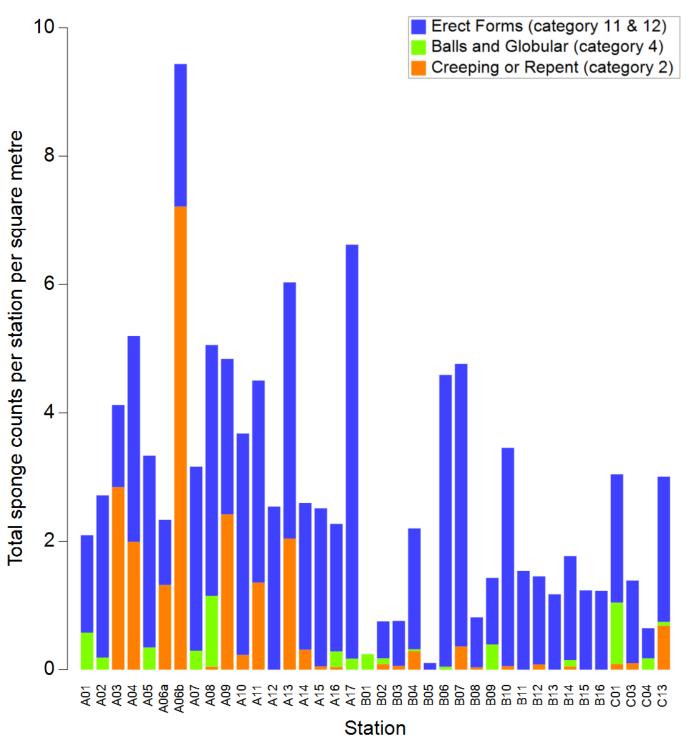


**Figure 15.** Examples of sponge aggregations observed within NEFSC. Clockwise from top left: Massive, Encrusting, Globular and Flabellate (Erect) (A12); Massive and Encrusting (A05); Arborescent (Erect) (B14); Erect Flabellate (A17).

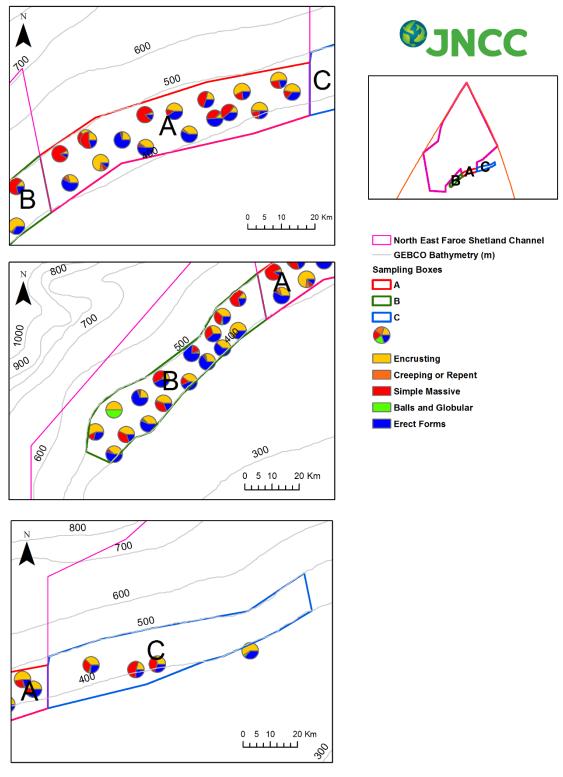
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**Figure 16.** Total percentage cover across the total viewable area of each station for Encrusting and Simple Massive morphotypes (category 1 and category 3) as described in Table 5.



**Figure 17.** Total sponge counts per station per m<sup>2</sup> for Erect growth forms (categories 11 & 12), Balls and Globular (category 4), and Creeping or Repent (category 2) morphotypes as described in Table 5.

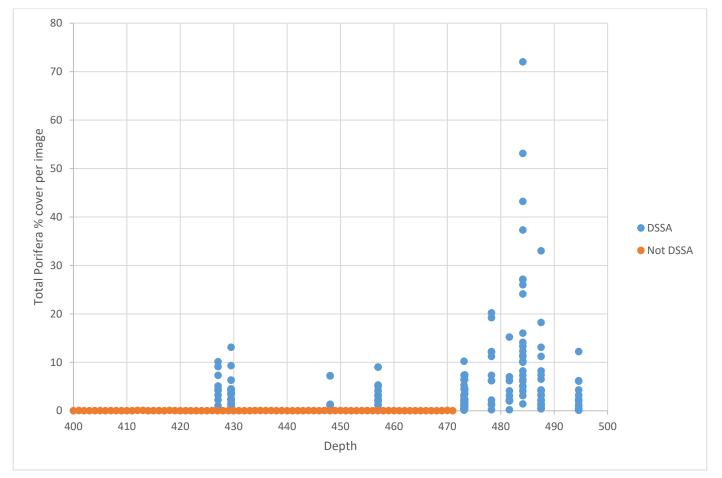


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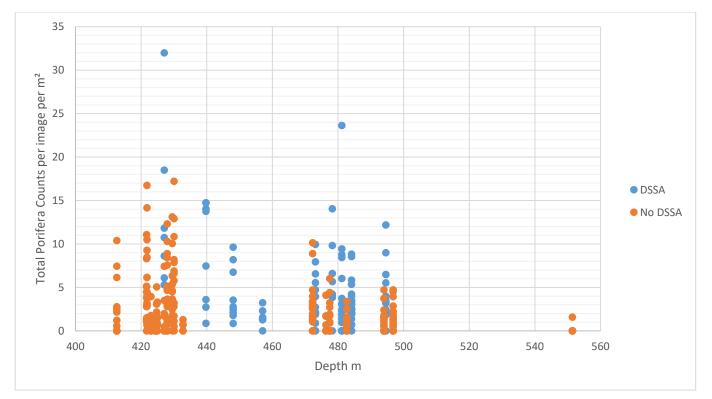
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Figure 18. Pie charts illustrating total sponge counts or cover at each station for each sponge category. Encrusting (category 1), Creeping or Repent (category 2), Simple Massive (category 3), Balls and Globular (category 4), Erect forms (categories 11 & 12).

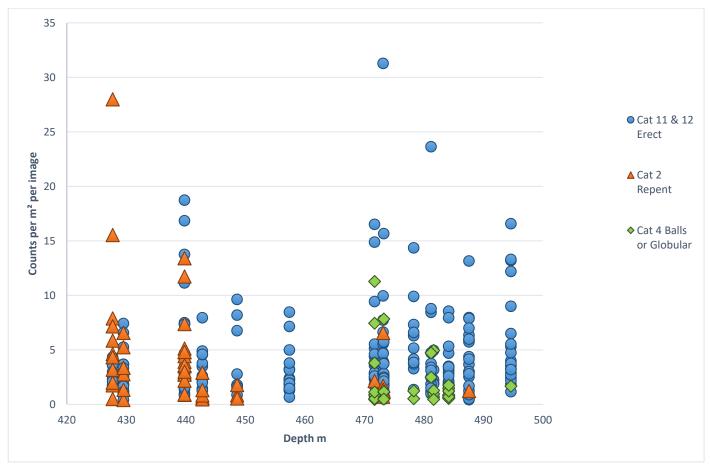
Scatterplots were produced to investigate sponge distribution between the 400–500 m depth contours (sponge individuals per image per category/m<sup>2</sup>). Figure 19 shows an association of tows with suspected deep-sea sponge aggregations with higher percentage cover (per image) from stills analysis and greater numbers of aggregations in deeper locations, mostly between 470–500 m. The same cannot be said for the count data (Figure 20), where high total sponge counts (per m<sup>2</sup>) do not necessarily correlate with stations allocated with suspected aggregations from the video analyses. Suspected aggregation records are also present throughout the depth ranges. Figure 21 focuses on the count data for suspected deep-sea sponge aggregation stations only. Erect morphotypes are present across the depth range. Repent growth forms are more frequently observed in shallower locations (420–450 m) whilst Ball and Globular morphotypes are more abundant in the deeper stations (470–500 m).



**Figure 19.** Scatterplot to explore the relationship between depth, suspected deep-sea sponge aggregations (DSSA) and total percent cover for colonial morphotypes (category 1 Encrusting & category 3 Simple Massive) per image.



**Figure 20.** Scatterplot to explore the relationship between depth, suspected deep-sea sponge aggregations (DSSA) and total Porifera counts per image per square metre.



**Figure 21.** Scatterplot showing counts for each solitary sponge morphotype for suspected deep-sea sponge aggregation records.

# 3.4 Univariate and multivariate analysis

### 3.4.1 Solitary and colonial communities

Univariate analysis was conducted on the solitary (count) community data from the dropdown camera stations. The count data accounted for most of the taxa at NEFSC and provided practical metrics for illustrative purposes and comparability across the site. The summary of the diversity indices is provided in Table 13. Margalef species richness index (d) shows an average of 2.04 across all the stations, with a maximum of 3.18 indicating that the communities do not have a large range in richness. The Shannon diversity results show a similar picture with an average of 1.50, a maximum of 2.20 and a range of 1.69 suggesting some variation between the stations but not a large range in diversity, which is also supported by similar Simpson diversity results. Pielou's evenness is averaged at 0.64 with a range of 0.76 suggesting that the distribution of individuals among taxa between the stations is quite varied with some showing a higher degree of evenness (maximum value of 0.96) compared to others (minimum of 0.19).

The univariate diversity metrics were used in subsequent correlation analyses to test whether the metrics had any correlation with stations that had suspected deep-sea sponge aggregations from the drop-down video results (Table 14). The only significant correlation was found between suspected aggregations and the sum of total individuals. The positive correlation shows that the total number of individuals increases for records of deep-sea sponge aggregations. This is a moderately low correlation (0.32) suggesting a weak relationship exists between deep-sea sponge aggregations and the total number of individuals. This is further reflected in the rest of the diversity metrics which do not show any significant correlation to stations that have suspected deep-sea sponge aggregations.

	Minimum	Maximum	Average	Sum	Standard deviation	Variance	Range
Total species (S)	4	19	10.97	384	2.90	8.38	15
Total individuals (N)	12	1,206	272.29	9,530	330.36	109,136.39	1,194
Species richness (d)	0.96	3.18	2.04	n/a	0.52	0.27	2.22
Pielou's evenness (J')	0.19	0.96	0.64	n/a	0.19	0.04	0.76
Shannon diversity (H'(loge))	0.51	2.20	1.50	n/a	0.45	0.20	1.69
Simpson's diversity (Lambda')	0.20	0.93	0.67	n/a	0.20	0.04	0.73

 Table 13. Summary of epifaunal diversity indices conducted on the count data for all the drop-frame camera stations.

**Table 14.** Pearson's correlation coefficient values for univariate metrics tested for correlation with stations with and without suspected deep-sea sponge aggregations (DSSA) from drop-down camera video results.

Correlation test	p value	Pearson's correlation coefficient	95% confidence interval
DSSA and number of species	0.06	0.31956	-0.01531507, 0.58998017
DSSA and total individuals	0.04	0.34375	0.01187076, 0.60742035
DSSA and Margalef's species richness index	0.88	-0.02553	-0.3557511, 0.3103557
DSSA and Shannon diversity index	0.66	-0.07594	-0.3990869, 0.2639872
DSSA and Simpson diversity index	0.55	-0.07594	-0.4221262, 0.2380213
DSSA and Pielou's eveness	0.40	-0.14803	-0.4586474, 0.1948319

### 3.4.2 Overall community (counts and percentage cover data)

To get a holistic view of both the solitary and colonial communities and understand the overall composition, percentage cover and counts were combined. The dataset was standardised ensuring the total number of individuals for each taxon was equally weighted on a relative scale of 100 across each station. This method enabled the combination of two enumeration techniques to understand the relative structure and composition of both communities. The dataset was subsequently transformed by fourth root prior to analysis.

Global patterns and structure in the data were investigated using hierarchical agglomerative clustering aided by the SIMPROF routine which determined nine clusters at the 5% significance level (Figure 22). Groups that can be considered statistically distinct from one another at the 95% confidence level are denoted by black lines on the cluster dendrogram, whilst red lines indicate sub-structure with no statistical support. The three significantly differentiated groups (groups **b**, **c**, and **g**) all contained only one station each (B02, B16 and B05 respectively). The supporting nMDS (Figure 23) resulted in a stress value of 0.15 indicating the plot is a reasonably good representation of the data. It illustrates that there are six main clusters: four overlapping and two distinct clusters. SIMPER identified the most important contributing species to the six main SIMPROF cluster groups (Table 15) and is aided by the shade plot in Figure 24 which displays abundance data alongside SIMPROF clusters and their associated SIMPER community. Each SIMPROF group is explained alongside the supporting SIMPER community results.

Results from the shade plot, dendrogram and nMDS show that the cluster groups and their associated communities all show a degree of similarity to one another. The overlapping of clusters is likely explained by the SIMPER results. Composition of the communities is dominated by the presence (in varying degrees of contribution) of galatheoid squat lobsters, long-spine slate pen sea urchin *Cidaris cidaris* and high-level taxonomic observations of starfish (Asteroidea), brittlestars (Ophiuroidea) and lamp shells (Brachiopoda). Average

similarity within the groups ranges from 77.5 (group **d**) to 38.9% (group **i**). The differences between the SIMPROF groups and their communities is further explained below.

Group **i** showed the greatest difference to the other cluster groups, separating them at approximately 25% similarity. The group consisted of two stations B01 and C04 that both had overall low abundance and coarse taxonomic detail. SIMPER community results showed 77% within-group similarity was due to the presence of Crustacea and Echinoidea.

The subsequent highest difference is seen for group **h** which separates from the rest of the overlapping clusters at 44.5% similarity. This group consisted of seven stations (A01, B09, A02, B10, A17, A05 and A07) and does not overlap with any of the other cluster groups in the nMDS. SIMPER results showed that this group was characterised by Sabellidae polychaetes which contributed 29% to the total 73% within-group similarity.

Group **a** separates from the other clusters at around 49.5% similarity and contains two stations (B14 and B11). Group **f** separates at around 52.5% and consists of seven stations (B03, B13, C03, A15, C01, B04 and C13). For both these groups the top three characterising species include Galathoid squat lobsters, the long-spine slate pen sea urchin *Cidaris cidaris* and star fish (Asteroidea). Group **f** however is more diverse and has crinoids, brachiopods, Encrusting (category 1 Encrusting) and Massive (category 3 Simple Massive) sponge morphotypes and cushion stars (*Ceramaster* spp.) within the characterising species.

Group **e** splits from the remaining groups at around 58% similarity and is the largest group containing 12 stations (B08, A11, B06, A08, A16, A06a, A06b, A09, A03, A04, A12 and A13). It has similar characterising species to many of the groups (Galatheoidea, <u>Cidaris</u> <u>cidaris</u>, Brachiopoda, Asteroidea) but differs in the presence of brittlestars (Ophiuroidea) and the sponge morphotype Flabellate (category 11.1 Erect laminar).

Group **d** separates from the last few stations at 65% similarity and contains 5 stations (B12, B07, A10, A14 and A15). It also has similar contributing species to the rest of the groups, brachiopods, galatheoid squat lobsters and the long-spine slate pen sea urchin *Cidaris cidaris*.

Groups **b**, **c** and **g** all represent one station each therefore the characterising species cannot be defined by SIMPER (B02, B16, B05) but the dissimilarities in the community structure can inform on the differences between these significantly differentiated stations. Group **g** (B05) differs from most of the stations due to a higher abundance of *Ceramaster* spp., the presence of *Crossaster* spp. and the lack of Galatheoidea and *Cidaris cidaris*. The shade plot with accompanying SIMPROF clusters illustrates that group **b** and **c** (B02 and B16) differ from the other stations due to overall low total species abundance and diversity (Figure 24).

Figure 25 shows SIMPER community distribution across the site. Box A is dominated by communities **h** and **e**. Community **h** stations are generally located toward the 500 m depth contour and community **e** stations broadly representing stations toward the 400 m contour. Two further communities **d** and **f** (representing two and one stations respectively) are observed in the middle of box A. Box B also has two stations as community **h** toward the 500 m contour but the rest of the box B is highly varied with a mix of all the communities found at NEFSC. Box C is dominated by community **f** (three stations) and one community **i** station.

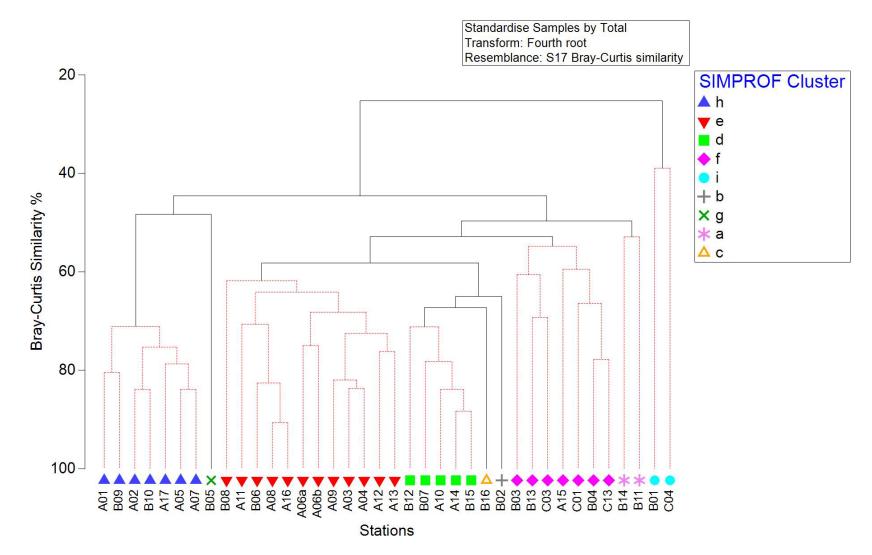


Figure 22. Hierarchical agglomerative clustering of FOV transformed, standardised, fourth root transformed overall community data (percentage cover and counts). Images pooled and labelled by station and symbolised by SIMPROF cluster. Cophenetic correlation = 0.79.

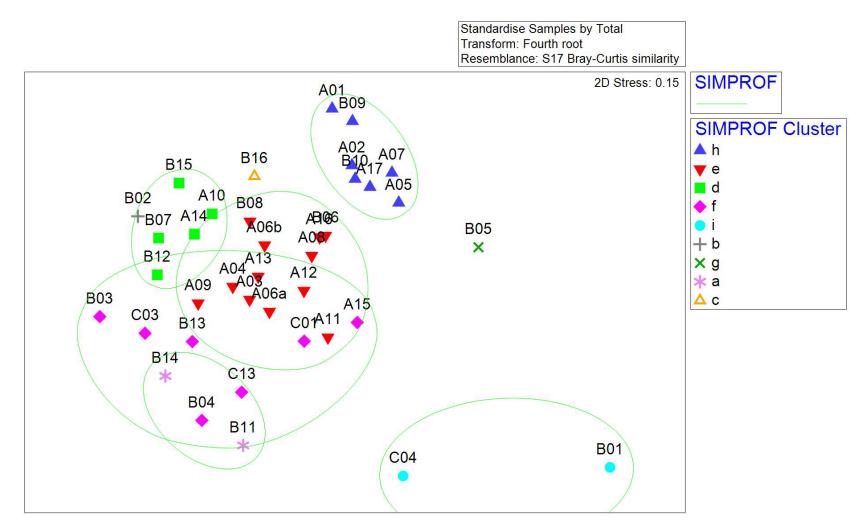


Figure 23. 2D nMDS plot of FOV transformed, standardised, fourth root transformed overall community data (percentage cover and counts). Images are pooled and labelled by station, green lines and symbols represent SIMPROF clusters.

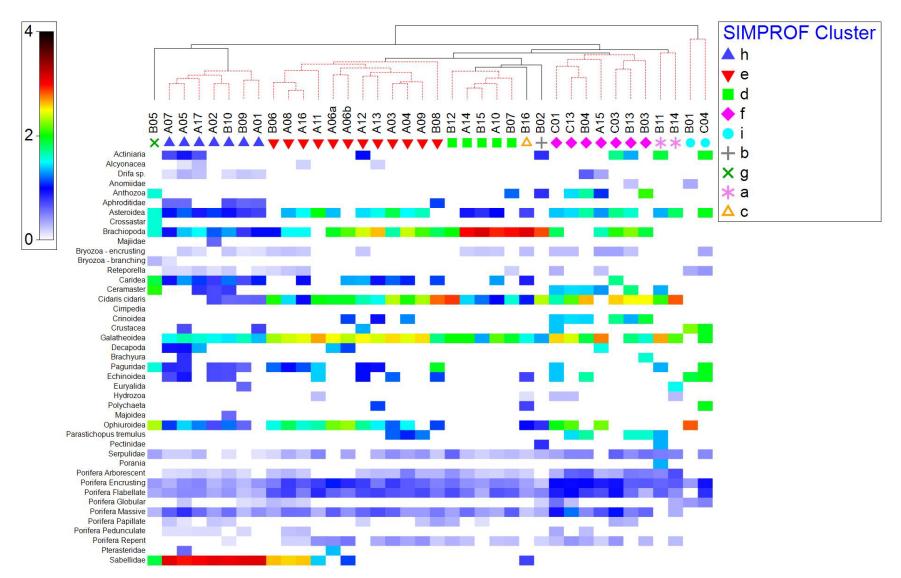


Figure 24. Shade plot for relative density, standardised, fourth root transformed community data with hierarchical agglomerative cluster dendrogram aided by the SIMPROF cluster routine.

**Table 15.** Results of the SIMPER test for overall community data. The clustering identifies a total of seven community assemblages, detailing the top five species that contribute most to the average community similarity. Includes values for average abundance (av.abund), average similarity (av.sim), standard deviation of the similarity (sim/SD) and its percentage contribution (contrib%) to the group's overall similarity and cumulative percentage (cum%) similarity of the taxa. Group c contained only one sample and was removed. Sim/SD of "n/a" indicates insufficient samples to calculate statistic.

Group <b>h</b> Average community similarity: 74.68					
Species	Av.abund	Av.sim	Sim/SD	Contrib%	Cum%
Sabellidae	3.05	21.37	7.54	28.61	28.61
Galatheoidea	1.52	10.27	9.32	13.76	42.37
Brachiopoda	1.14	6.86	6.94	9.19	51.56
Caridea	1	6.07	6.52	8.13	59.69
Asteroidea	0.94	5.92	10.29	7.92	67.61
Ophiuroidea	0.89	4	1.41	5.35	72.96

Group <b>e</b> Average community similarity: 67.57					
Species	Av.abund	Av.sim	Sim/SD	Contrib%	Cum%
Galatheoidea	2.4	14.76	8.25	21.84	21.84
Cidaris cidaris	1.91	10.57	3.76	15.65	37.49
Brachiopoda	1.75	8.71	1.95	12.89	50.38
Ophiuroidea	1.5	7.54	2.05	11.16	61.53
Asteroidea	1.17	5	1.08	7.39	68.93
Porifera Erect (Flabellate)	0.66	3.87	7.09	5.72	74.65

Group <b>d</b> Average community similarity: 77.56					
Species	Av.abund	Av.sim	Sim/SD	Contrib%	Cum%
Brachiopoda	2.8	26.29	4.67	33.9	33.9
Galatheoidea	1.87	17.14	6.76	22.1	56
Cidaris cidaris	1.61	12.22	5.39	15.76	71.76

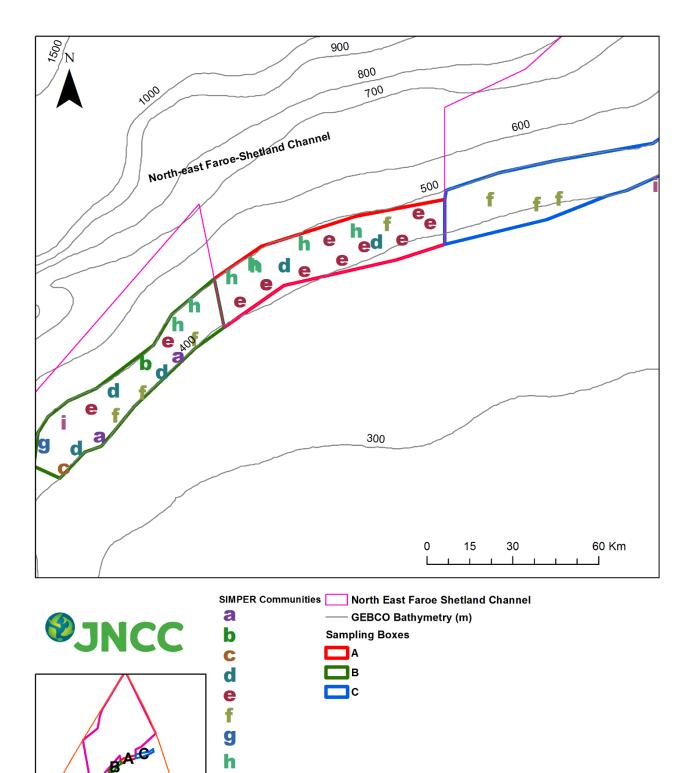
Group <b>f</b> Average community similarity: 58.92					
Species	Av.abund	Av.sim	Sim/SD	Contrib%	Cum%
Cidaris cidaris	1.99	8.43	1.42	14.31	14.31
Galatheoidea	1.84	7.37	1.48	12.51	26.81
Asteroidea	1.3	5.58	1.51	9.47	36.28
Crinoidea	1.31	5.53	1.51	9.39	45.67
Brachiopoda	1.37	4.9	0.92	8.31	53.98
Porifera Encrusting	0.85	4.3	5.78	7.3	61.28
Ceramaster	1.04	3.51	0.93	5.96	67.24
Porifera Massive	0.77	3.38	3.85	5.74	72.98

Group I Average community similarity: 38.96				
Species	Av.abund	Av.sim	Contrib%	Cum%
Crustacea	2.09	15.26	39.17	39.17
Echinoidea	1.91	14.81	38	77.17

Group <b>a</b> : Average community similarity: 52.91				
Species	Av.abund	Av.sim	Contrib%	Cum.%
Galatheoidea	2.4	15.03	28.4	28.4
Cidaris cidaris	2.45	14.6	27.6	56
Asteroidea	1.56	9.33	17.63	73.63

Groups **b**, **c** and **g** 

<2 samples in each group



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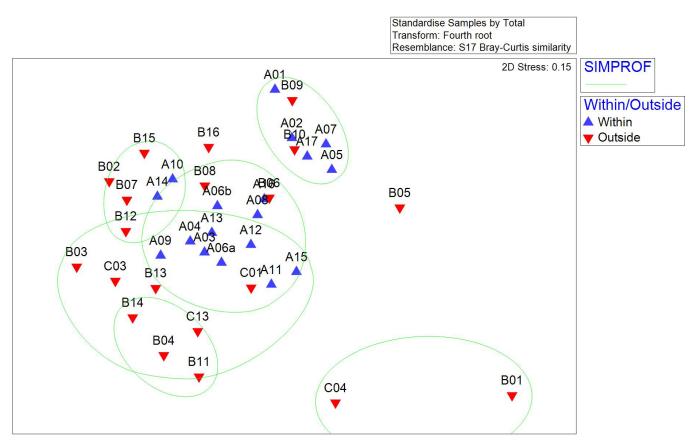
Figure 25. SIMPER community results from overall epifaunal cluster analysis at NEFSC.

i

### 3.4.3 Differences within and outside NEFSC

To understand whether we should accept or reject the null hypothesis that "there is no difference (in overall epifaunal communities) within or outside NEFSC", we can symbolise the nMDS to show if there are any clear differences between the site locations (within/outside NEFSC). Figure 26 illustrates that there is no clear distinction between the two factors, evident in the observed overlap. One-way analysis of similarities (ANOSIM) further investigates whether a significant difference between the two locations exists. The ANOSIM results in a significance level of 2.8% (p = 0.03) indicating that the locations are in fact significantly different. However, the Global R statistic is very low (0.078) which indicates that the effect of separation is weak and the differences between the two factors is subtle.

To further explore this difference, the count and cover data were individually subject to ANOSIM to test for differences between stations within and outside the site. The cover community result was not significantly different (p = 0.11) whereas the count community result was highly significant (p = 0.002) with a weak effect (R = 0.188). To see if there is a relationship between the solitary communities and the presence of deep-sea sponge aggregations another ANOSIM was carried out using suspected deep-sea sponge aggregations (from drop-down camera video analysis) as a factor. The result was significant (p = 2.7%), with a low effect (R = 0.1). We could then assume that a small but significant difference, for the count communities, exists between the communities that inhabit suspected deep-sea sponge aggregations compared to those that do not.



**Figure 26**. nMDS plot of standardised by variable, fourth root transformed overall community data (percentage cover and counts). Images are pooled and labelled by station, symbolised by location (within/outside NEFSC) and overlain by SIMPROF clusters.

# 3.5 BEST (biota and/or environment matching) analysis

To link biological data to environmental data, a biota and/or environment matching (BEST) analysis was employed to identify the optimal combination of environmental variables explaining the observed patterns in epifaunal community composition. For the BEST analysis, the biological data were pooled by station as per the previous analysis. The BEST analysis was conducted with parameters of location and tidal shear stress and values that were averaged over a station including depth, conductivity, temperature, and sediment data recorded during imagery analysis (Table 16). Prior to BEST the environmental variables were visually examined for any evidence of skewness within the distributions using draftsman plots, none of the variables needed any further treatment. The variables were investigated for collinearity using Pearson's correlation which found temperature and conductivity to have a perfect positive correlation (correlation coefficient = 1.0). Temperature was retained for use in BEST analyses as it is known to be an important factor affecting sponge growth and extent.

A RELATE test was carried out to see if a correlation exists between the environmental and the biological resemblance matrices. The RELATE resulted in a significant (p = 0.001) with a reasonably good correlation (Spearman rank Rho = 0.509).

The BEST global sample statistic was also significant (p = 0.01) with a moderately strong effect (Rho = 0.636). The environmental factors can explain over half of the patterns seen in the biological data, but they cannot fully explain all the variation observed. Pebbles appear to have the most influence as a singular variable with a value of 0.436, however it does not have the highest correlation value (Table 17). Depth, pebbles, mud and shear stress as a combination have the largest correlation of 0.636 and, from the variables recorded, this combination best match the patterns observed in the biological data.

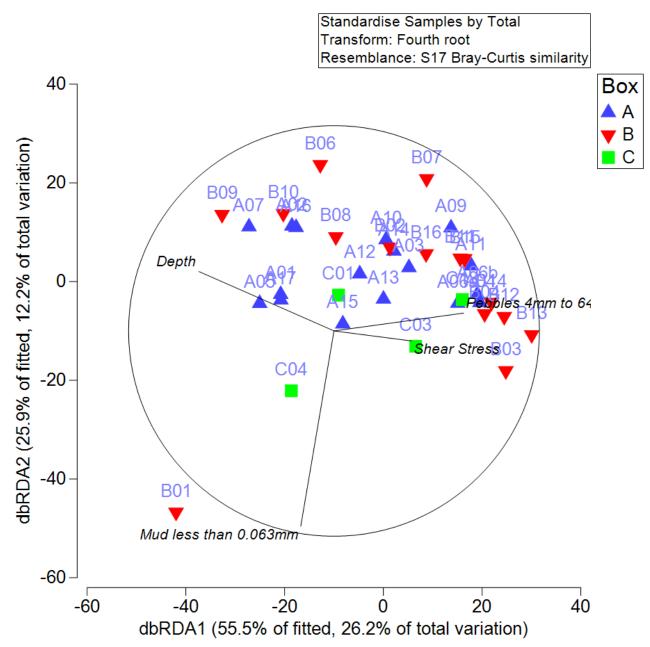
A DistLM analysis was undertaken to see how much of the biological variance could be explained by the environmental variables (Figure 27). It included the variables with the highest correlation from BEST results (depth, pebbles, mud and shear stress), using stepwise Akaike information criterion (AICc) selection. We can see here that nearly half of the total variation in the biological data can be explained by the BEST environmental variables (47%). The proportion of mud explains about 12% of the variation. The two outlier stations B01 and C04 are distributed towards the mud axis, suggesting the biological variance could be explained by higher percentage of finer, mud sediments compared to the rest of the stations. The rest of the variance is mostly explained by a combination of depth, proportion of pebbles and shear stress.

Environmental variable	Source
Depth (m)	Recorded from the vessel Davis system
Temperature (°C)	Recorded from CTD
Conductivity	Recorded from CTD
Tidal bed shear stress	Values extracted from MB0102 Bio- physical contract – Task 2E Energy Layers (accessing and developing the required biophysical datasets and data layers for MPA network planning and wider marine spatial planning purposes (West <i>et al.</i> 2010)
Location	Latitude
	Longitude
Sediment from imagery observations (per image)	Boulders (256 mm to 512 mm) Cobbles (64 mm to 256 mm) Pebbles (4 mm to 64mm) Empty shells Granule (2 mm to 4 mm) Shell (2 mm to 16 mm) Sand (0.063 mm to 2 mm) Mud (< 0.063 mm)

Table 16. Environmental variables obtained at NEFSC.

Table 17. BEST result for each number of environmental variables.

Number of variables	Correlation	Selections
1	0.436	Pebbles
2	0.614	Depth, pebbles
3	0.629	Depth, pebbles, mud
4	0.636	Depth, pebbles, mud, shear stress
5	0.632	Depth, cobbles, pebbles, mud, shear stress



**Figure 27.** DistLM RDA plot AICc step-wise selection of environmental variables with the highest correlation from BEST results.

# 3.6 Confidence assessment for suspected records of deep-sea sponge aggregations

Using the criteria provided in Henry and Roberts (2014) each station was assigned a confidence score to assess the likelihood that suspected records are representations of deep-sea sponge aggregations according to OSPAR (2010) (Table 18). All stations were assessed for density, ecological function, and habitat criteria. This enabled a more holistic assessment of the sponges rather than solely relying on the delineation of sponge aggregations from video analyses.

## 3.6.1 Density

Density was determined from the drop-down camera stills community data. The density criterion was defined by the raw measurements of abundance that equal or exceed densities defined in the OSPAR definition. Suspected records passed the density criterion if sponge counts were equal to or greater than 0.5 per m<sup>2</sup>, or sponge cover was equal to or greater than 1% per m<sup>2</sup>. The density value was calculated from the total counts/percentage cover for all sponge morphotypes within a station divided by the total viewable area of a station (determined from the total viewable area of each image). Where sponges were characteristic of the SIMPER community, but the density threshold was not met, the suspected record passed the density criterion. This was only the case for one station (B05) at NEFSC. Thirty-five stations met the density criteria for the count abundance data and 15 for the percentage cover.

## 3.6.2 Ecological function

Ecological function was also established using the drop-down camera stills community data, determined from multivariate SIMPER community results (Table 15). Two SIMPER communities had sponge morphotypes within their characteristic species (**e** and **f**); community **e** had category 11 Erect forms and community **f** had category 3 Simple Massive forms, both alongside species typical of aggregations. Extensive sponge growth in the Faroe-Shetland region has been previously associated with the presence of Galatheoidea squat lobsters, *Cidaris cidaris*, Asteroidea including cushion stars *Ceramaster* spp., brittlestars Ophiuroidea, Sabellidae polychaetes and brachiopods (Bett & Axelsson 2000; Axelsson 2003; Bett & Jacobs 2007; Henry & Roberts 2014). The majority of the NEFSC SIMPER communities had biological assemblages which included these typical species in varying contributions, except for communities **i**, **b**, **c**, and **g** (only representing five of 38 stations).

## 3.6.3 Habitat

The habitat criterion was identified during the drop-down camera video analysis. Henry and Roberts (2014) applied this criterion where assemblages could not be defined as anything other than a potential deep-sea sponge habitat. For the purpose of this assessment the habitat criterion was passed where stations were identified as "deep sponge aggregation on Atlanto-Arctic upper bathyal mixed sediment" (M.AAUB.Co.DeeSpo), totalling 15 stations.

## 3.6.4 Verification

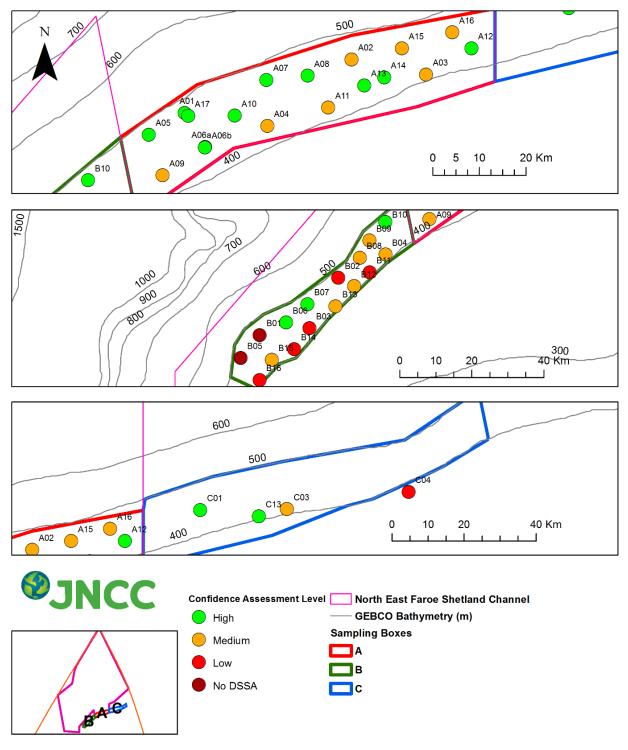
The confidence assessment identified 15 high confidence deep-sea sponge aggregations; a summary of the assessment is provided in Table 18 with the full detailed results provided in Appendix 7. The distribution for each record is mapped in Figure 28. The high-level confidence records support the 15 suspected aggregations identified during the drop-camera video analysis (Table 11; Figure 13). The assessment also identified 15 notable medium

confidence records, 11 of which had sponges within their characterising species. Furthermore another 11 of the 15 were identified as "sparse Encrusting community on Atlantic mid-bathyal coarse sediment". Six records were identified as having low confidence, five of which received one tick each for count density. Two stations did not pass any of the criteria: B01 and B04 (Table 18; Figure 28). **Table 18.** Summary table of the verification of suspected deep-sea sponge aggregations at NEFSC applying the criteria and confidence assessment from Henry and Roberts (2014). Double ticks under *Ecological function* symbolise SIMPER communities with sponges within the characterising species. The number of ticks under *Criteria result* indicate the number of criteria met.

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Station	Density (counts)	Density (cover)	Ecological function	Habitat	Criteria result	Confidence result
A10	✓	×	✓	✓	$\checkmark\checkmark\checkmark$	High
A14	✓	×	✓	✓	$\checkmark\checkmark\checkmark$	High
B06	✓	×	$\checkmark\checkmark$	✓	$\checkmark\checkmark\checkmark$	High
A01	✓	✓	✓	✓	$\checkmark\checkmark\checkmark$	High
A05	✓	✓	✓	✓	$\checkmark\checkmark\checkmark$	High
A06b	✓	✓	$\checkmark\checkmark$	✓	$\checkmark\checkmark\checkmark$	High
A07	✓	✓	✓	✓	$\checkmark\checkmark\checkmark$	High
A08	$\checkmark$	✓	$\checkmark\checkmark$	✓	$\checkmark \checkmark \checkmark$	High
A12	$\checkmark$	✓	$\checkmark\checkmark$	✓	$\checkmark \checkmark \checkmark$	High
A13	$\checkmark$	✓	$\checkmark\checkmark$	✓	$\checkmark \checkmark \checkmark$	High
A17	$\checkmark$	✓	$\checkmark$	✓	$\checkmark \checkmark \checkmark$	High
B07	$\checkmark$	✓	$\checkmark$	✓	$\checkmark\checkmark\checkmark$	High
B10	$\checkmark$	✓	$\checkmark$	✓	$\checkmark\checkmark\checkmark$	High
C01	$\checkmark$	✓	$\checkmark\checkmark$	✓	$\checkmark\checkmark\checkmark$	High
C13	$\checkmark$	$\checkmark$	$\checkmark\checkmark$	$\checkmark$	$\checkmark\checkmark\checkmark$	High
A02	$\checkmark$	×	$\checkmark$	×	$\checkmark\checkmark$	Medium
A03	$\checkmark$	×	$\checkmark\checkmark$	×	$\checkmark\checkmark$	Medium
A04	$\checkmark$	×	$\checkmark\checkmark$	×	$\checkmark\checkmark$	Medium
A09	$\checkmark$	×	$\checkmark\checkmark$	×	$\checkmark\checkmark$	Medium
A11	$\checkmark$	×	$\checkmark\checkmark$	×	$\checkmark\checkmark$	Medium
B04	$\checkmark$	×	$\checkmark\checkmark$	×	$\checkmark\checkmark$	Medium
B08	✓	×	$\checkmark\checkmark$	×	$\checkmark\checkmark$	Medium
B09	$\checkmark$	×	$\checkmark$	×	$\checkmark\checkmark$	Medium
B12	$\checkmark$	×	$\checkmark$	×	$\checkmark\checkmark$	Medium
B13	$\checkmark$	×	$\checkmark\checkmark$	×	$\checkmark\checkmark$	Medium
B15	$\checkmark$	×	$\checkmark$	×	$\checkmark\checkmark$	Medium
C03	✓	×	$\checkmark\checkmark$	×	$\checkmark\checkmark$	Medium
A06a	✓	✓	$\checkmark\checkmark$	×	$\checkmark\checkmark$	Medium
A15	✓	✓	$\checkmark\checkmark$	×	$\checkmark\checkmark$	Medium
A16	✓	✓	$\checkmark\checkmark$	×	$\checkmark\checkmark$	Medium
B02	✓	×	×	×	✓	Low
B03	×	×	$\checkmark\checkmark$	×	✓	Low
B11	✓	×	×	×	✓	Low
B14	✓	×	×	×	✓	Low
B16	✓	×	×	×	✓	Low
C04	✓	×	×	×	✓	Low
B01	×	×	×	×	×	No DSSA
B05	×	×	×	×	×	No DSSA



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**Figure 28.** Confidence assessment results for suspected deep-sea sponge aggregations (DSSA) at NEFSC.

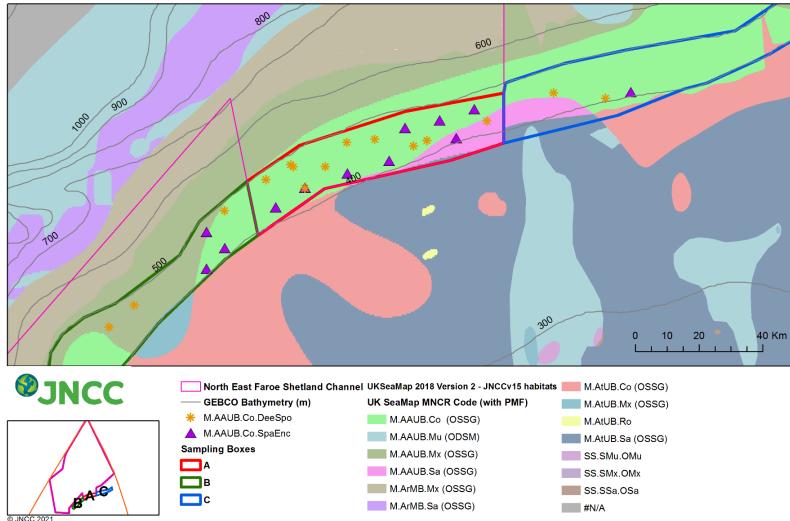
## 3.7 Epifaunal biotopes

Two biotopes were assigned to 27 of the video segments, all of which fall within the coarse habitat M.AAUB.Co (Table 10; Figure 12). M.AAUB.Co.DeeSpo (Deep sponge aggregations on Atlanto-Arctic upper bathyal coarse sediment) was allocated for 11 segments and M.AAUB.Co.SpaEnc (Sparse Encrusting community on Atlanto-Arctic upper bathyal coarse sediment) for 16 segments (Table 19; Figure 29). It is noted that the biotope M.AAUB.Co.DeeSpo was assigned at every station where the PMF deep-sea sponge aggregations were allocated during the video analysis.

Biotope	MNCR classification	Video segments
M.AAUB.Co.DeeSpo	Deep sponge aggregation on Atlanto-Arctic upper bathyal coarse sediment	15
M.AAUB.Co.SpaEnc	Sparse Encrusting community on Atlanto- Arctic upper bathyal coarse sediment	16

Table 19. Video analysis and assigned epifaunal biotopes.

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Figure 29. Biotopes assigned from video analysis.

## 3.8 Infaunal community analysis

Limited grab sampling was completed at NEFSC. As such, the multivariate analysis can only provide an insight into the infaunal communities within box D. Infaunal samples were obtained for eight out of nine grab samples from box D; one grab was of insufficient volume to analyse for infauna and was only used for PSA. Infauna samples were collected to investigate the biological structure of offshore subtidal sands and gravels and offshore deepsea muds (objective 3). However, the low number of samples do not constitute a statistically robust sample size, therefore observations cannot be inferred to overall populations and the dataset can only be subject to limited multivariate analysis.

#### 3.8.1 Summary statistics

A total of 121 taxa and 1,815 individuals were collected from eight grab stations. Summary statistics (Table 20) provide an overview on the abundance and diversity indices for each grab sample. Grab sample D32 had the highest number of species (72) and total individuals (664). Species richness ranged from 5.7 to 10.9. Pielous's evenness ranged from 0.65 to 0.90.

Station	Total species	Total number of individuals	Species richness (Margalef index)	Pielous's eveness
D02	32	130	6.4	0.71
D10	27	57	6.4	0.90
D11	32	137	6.3	0.81
D12	55	251	9.8	0.77
D20	29	142	5.7	0.77
D30	36	169	6.8	0.76
D31	72	664	10.9	0.65
D32	48	265	8.4	0.73

 Table 20.
 Summary statistics from infaunal communities by station.

#### 3.8.2 Structure of infaunal community

Without a robust dataset it is difficult, if not impossible, to determine if there are any patterns within the data. nMDS ordination was conducted to test for any structure, however, these results should be viewed with caution (Figure 30). The nMDS plot shows that most stations show a degree of approximately 48% similarity to one another except for two stations (D02 and D10), which provided the only significantly distinct samples.



Figure 30. nMDS plot of fourth root transformed infaunal data labelled by station, symbolised by SIMPER communities, and overlain with SIMPROF cluster.

SIMPER identifies the most contributing species for the observed similarities and differences. The largest SIMPER community z was characterised by the presence of *Paramphionome jeffreysii*, nematodes, copepods, Cirratulid polychaetes and *Spiophanes* spp. (Table 21). The two significantly distinct stations (x and y) differ from the rest of the dataset due to the presence or absence of specific fauna (Table 22). Community y does not contain the Terrebellids *Pista* spp. and has low numbers of *P. jeffreysii*. Community x does not contain any nematodes and was the only station with the sea cucumber *Labidoplax buskii*. Stations D02 and D20 were the only stations allocated as deep-sea muds as opposed to the rest of the data which were classified as offshore subtidal sands and gravels. The significant difference of D02 (group x) could represent a move towards communities that prefer muddy habitats, such as *L. buskii*.

**Table 21.** Results of the SIMPER test for infaunal community data. The clustering identifies a total of three community assemblages, however communities x and y contained only one sample each and were removed. The five species contributing most to average community similarity are detailed including average abundance (av.abund), average similarity (av.sim), standard deviation of the similarity (sim/SD) and its percentage contribution (contrib%) to the group's overall similarity and cumulative percentage (cum%) similarity of the taxa.

<b>Group z</b> : Average similarity: 53.45					
Species	Av.abund	Av.sim	Sim/SD	Contrib%	Cum%
Paramphinome jeffreysii	2.87	4.58	5.04	8.56	8.56
Nematoda	2.42	3.77	5.53	7.06	15.62
Copepoda	1.87	3.03	4.86	5.68	21.3
Cirratulidae	1.75	2.75	3.29	5.15	26.45
Spiophanes spp.	1.77	2.58	5.53	4.82	31.27

**Table 22.** The dissimilarity between infaunal community groups x, y and z, from the SIMPER cluster analysis. The clustering identified the primary contributing species to the dissimilarity, the ratio of their average individual contribution to dissimilarity and standard deviation of this value (diss/SD) and their percentage contribution to the dissimilarity between groups.

Groups x & y Average dissimilarity = 70.67 Species	Group a Av.abund	Group b Av.abund	Av.diss	Diss/SD	Contrib%	Cum%
Pista (sensu Jirkov 2001)	1.86	0	2.66	n/a	3.76	3.76
Nematoda	0	1.68	2.4	n/a	3.4	7.16
Sipuncula	1.57	0	2.24	n/a	3.16	10.33
Paramphinome jeffreysii	2.72	1.19	2.19	n/a	3.1	13.43
Galathowenia	1.41	0	2.02	n/a	2.86	16.29
Labidoplax buskii	1.41	0	2.02	n/a	2.86	19.15

Groups x & z: Average dissimilarity = 57.77 Species	Group a Av.abund	Group c Av.abund	Av.diss	Diss/SD	Contrib%	Cum%
Nematoda	0	2.42	2.49	10.14	4.32	4.32
Labidoplax buskii	1.41	0	1.5	4.87	2.6	6.91
Chaetozone sp.	0	1.43	1.46	5.36	2.52	9.43
Urothoe elegans	1.19	0	1.26	4.87	2.18	11.62
<i>Pulsellum</i> sp.	1.19	0	1.26	4.87	2.18	13.8

#### 3.8.3 Infaunal biotopes

PSA results describe two habitats equivalent to Atlanto-Arctic upper bathyal mixed sediment (M.AAUB.Mx) and two Atlanto-Arctic upper bathyal mud habitats (M.AAUB.Mu) (Table 23).

Table 23. PSA	results and	l assigned	habitats.
---------------	-------------	------------	-----------

Habitat	MNCR Classification	Number of PSA samples
M.AAUB.Mx	Atlanto-Arctic upper bathyal mixed sediment	7
M.AAUB.Mu	Atlanto-Arctic upper bathyal mud habitats	2

## 4. Other Priority Marine Features (PMFs)

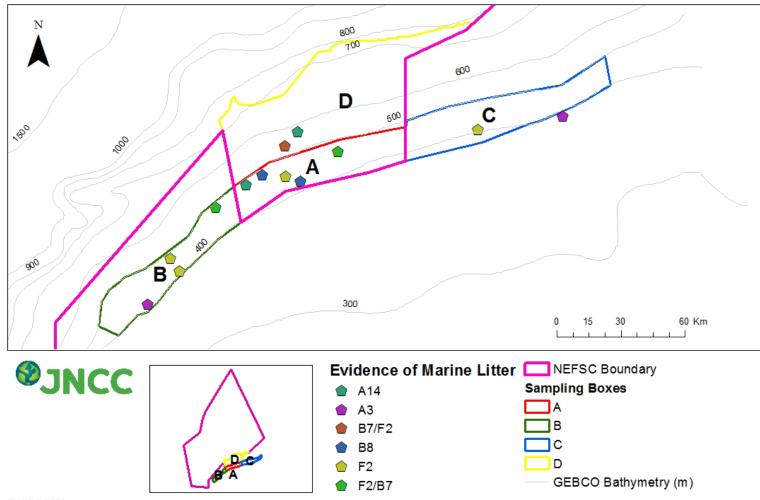
The PMF species *Molva dypterygia* (Blue Ling) could be seen throughout the footage. Several species were observed that could be considered 'features of interest' in terms of protection or conservation status. Rays were observed but only identified to 'Rajiformes'. Epifaunal species of interest such as soft corals, sea pens and megafauna burrows were recorded, albeit in low numbers (Benson & Sotheran 2018).

## 5. Marine litter

Non-natural materials were observed in several tows, including several possible discarded lengths of rope/cable/warp, bags or plastic material, pieces of metal or pipe/tube (Table 24; Figure 31) (Benson & Sotheran 2018). Further detail on the MSFD litter categories (MSFD Technical Subgroup on Marine Litter 2013) can be found in Appendix 4.

Box	Station	Equipment	Visual impacts or modifiers	
Box A	A01	Chariot camera	B8 Piece of metal	
	A02	Drop-down camera	F2/B7 Rope/cable	
		Chariot camera	F2/B7 Rope/cable	
	A04	Chariot camera	B8 Metal bar	
	A05	Drop-down camera	A14 Pipe/tube	
	A10	Drop-down camera	F2 Rope	
Box B	B03	Chariot camera	F2 Rope	
	B10	Drop-down camera	F2/B7 Rope/cable	
	B13	Drop-down camera	F2 Rope	
	B14	Drop-down camera	A3 Bag	
Box C	C02	Chariot camera	F2 Warp	
	C04	Drop-down camera	A3 Bag	
Box D	D01	Chariot camera	B7/F2 Cable/warp	
	D12	Hamon grab	A14 White plastic in sample	

 Table 24. Marine litter observed from camera and grab operations.



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Figure 31. Locations with evidence of marine litter.

## 6. Non-indigenous species (NIS)

Report objective 5 (section 1.4.3) calls for any evidence of non-indigenous species (NIS) at the site. The infaunal and epifaunal taxon lists generated from the infaunal samples and seabed imagery data were cross-referenced against lists of non-indigenous target species which have been selected for assessment of Good Environmental Status in UK waters under MSFD Descriptor 2 and identified as significant by the GB Non-Native Species Secretariat. These taxa are listed in Appendix 4. None of the identified taxa at NEFSC were listed on the NIS list (Stebbing *et al.* 2014) (Appendix 5).

## 7. Anthropogenic activities and pressures

Potential trawling evidence was observed at six stations (small patches of broken clumps of muddy sand), however upon further investigation it was difficult to discern the true origin or nature of these marks. It is possible that some are changes in sediment type and some may be very old trawl marks.

## 8. Discussion

## 8.1 Deep-sea sponge aggregations

#### 8.1.1 Extent and distribution

The principal objective of the survey was to describe the extent and distribution of deep-sea sponge aggregations and compare the extent and structure of the aggregations found within the MPA to those outside NEFSC. Verified high confidence and other notable suspected records were found in all three sampling boxes (Figure 28), both within and outside the MPA. High confidence aggregations were identified throughout the sampling boxes A, B and C occurring between depths of 427–495 m. Within NEFSC, 10 high confidence records and eight medium confidence records were assigned. Outside the boundaries a total of five high confidence deep-sea sponge aggregations were observed (three in box B and two in box C) and a further seven medium confidence records (six in box B and one in box C).

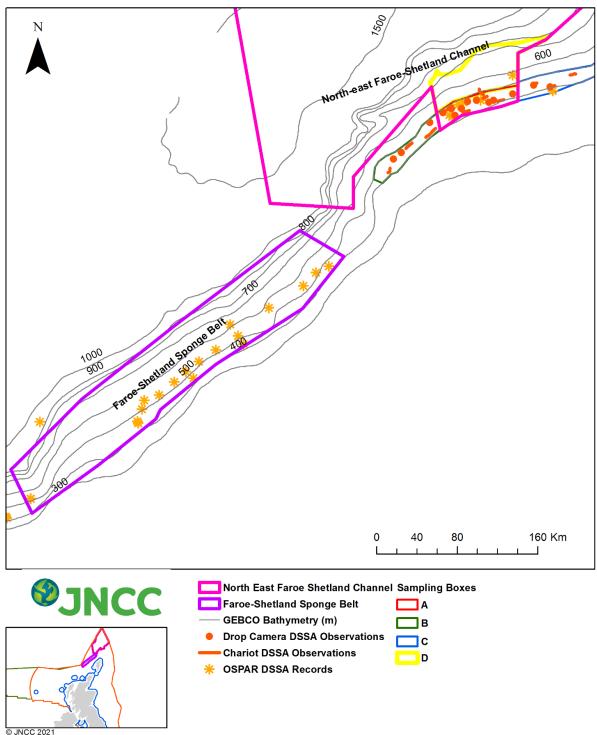
Suspected deep-sea sponge aggregations were also seen across 11 chariot tows in varying degrees. This included five tows within the MPA and six outside the site (three in both boxes). The chariot results show suspected aggregations at depths ranging from 374 to 525 m. These results see records in both shallower and deeper waters than previously noted in the area. The chariot results add value to our understanding of potential sponge distribution at the site but without accompanying density and community values we cannot confidently report that they represent a deep-sea sponge aggregation *sensu* OPSAR (2010). Further assessment of the chariot data would add to our understanding of the extent of the sponge belt in the area, filling some of the sampling gaps and improving on the current results and between box comparability, which in turn would contribute to more robust standing as a first point in a time series.

The results from box B indicate that potential aggregations (including verified and unverified records) extend at least 70 km southwest of the NEFSC boundary (box A). The results from box C show potential aggregations extend at least 60 km east of the boundary. The Faroe-Shetland Sponge Belt MPA lies to the south-west of NEFSC (Figure 32). Sponge aggregations are known to occur here around the 400–600 m contour. It is possible that the narrow sponge belt may continue between the two MPAs along the narrow contour belt. However, our results show stations that do not have aggregations, or support communities that are associated with high sponge density, at the sampling extremities of both boxes outside the MPA. The limit to the western extent of the sponge belt may be evident at stations B01, B04 and to the eastern extent at station C04. These three outlier stations had the highest levels of difference from clustering and nMDS results (Figure 22; Figure 23). They fell within communities i and g (Table 15) with high-level taxonomic assemblages (Crustacea-Echinoidea) and had either low or no confidence results following the verification assessment.

The stations all had relatively low species diversity and low sponge abundance. These outlier stations could be the driving the significant difference seen between the overall epifaunal communities outside NEFSC to those within it. B01 and B04 were also found to be the only two stations recorded as mixed sediments which could suggest that aggregations are limited to coarse sediments. Mixed sediments tend to have higher proportions of fine sediment which can create unsuitable conditions for sponge growth, due to the risk of smothering.

It cannot be said with certainty that these outlier stations denote the extent of the sponge belt as sponge aggregations are known to be patchy in their distribution; varying levels of confidence could infer this patchy nature rather than the limits. The results from the biotopes could also support this – the allocation of M.AAUB.Co.SpaEnc could show transition areas of reduced sponge density and not necessarily indicate the limits. Further investigations between the two MPAs would add to our understanding of the extent of VMEs within the Faroe-Shetland region. The verified records from the current study at NEFSC improve the current known distribution of this VME which feeds directly into updating ICES and OSPAR databases.

From the results of the ANOSIM we can see that there are slight (albeit significant) differences in the overall (combined) and the solitary communities within and outside the MPA. On the other hand, the colonial communities are the same throughout the boxes; no significant difference was found from ANOSIM results. This reflects that Encrusting-Massive sponge communities are present throughout the surveyed area. Higher abundances correlate with more deep-sea sponge aggregation allocation from video analyses and deeper sampled areas (approximately 470–500 m) (Figure 19). It could be assumed that the Encrusting-Simple Massive deep-sea sponge aggregations are present throughout all three sampling boxes most commonly between depths of 470-500 m. Conclusions on the overall or solitary communities are more complex. A subtle, significant difference exists within and outside the MPA and between the solitary communities where deep-sea sponge aggregations are identified from video analyses. The difference between the sites could be linked to the higher number of aggregations found within the site as compared to outside the site. Furthermore, the differences could be linked to lower numbers of stations obtained outside the MPA therefore fewer deep-sea sponge aggregations and associated communities.



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**Figure 32.** Location of North-East Faroe Shetland Channel MPA and the Faroe-Shetland Sponge Belt MPA with deep-sea sponge aggregation (DSSA) records from OSPAR (2010) and results from the 1517S survey.

#### 8.1.2 Structure

This report highlights the complexities defining deep-sea sponge aggregations and the usefulness of a confidence scoring system (using the criteria from Henry and Roberts (2014)) to verify suspected records. The results at NEFSC could have been improved if sponge density and community data were recorded from the video tow analyses as opposed to using data from the still imagery. We do not have the same supporting verification for the chariot tow analysis so we can only record these as suspected deep-sea sponge aggregations. Our assessment is limited to still imagery therefore the sponge and community data can only represent a proportion of the true values. Nevertheless, the assessment verified the 15 suspected records that were identified during the initial video analysis with high confidence, fulfilling all three criteria. The assessment verified that densities conform to the OSPAR definition and support species that are associated with enhanced biodiversity. The confidence assessment also identified a further 15 medium confidence records. This suggests that aggregations could have been underrepresented during the initial analyses or that a more cautious application of the definition was applied in the first instance. Many of these records qualified the density and ecological function criteria but failed the habitat criterion as they were not identified as deep-sea sponge aggregations during the original habitat analysis of the video data. This could be due to difficulties identifying deep-sea sponge aggregations from video analysis without supporting density values. Univariate data exploration shows a better link between aggregation identification and higher sponge percentage cover (Figure 19). It is likely that Encrusting and Massive deep-sea sponge aggregations are easier to identify whereas solitary sponge forms are harder for analysts to visualise whether densities have been met during video tows.

Deep-sea sponge aggregations were seen to correlate positively with the total number of individuals. SIMPER communities where aggregations were allocated included groups with sponges within the characterising species; communities **e** (five stations) and **f** (two stations) and those without; communities d (three stations) and h (five stations). Our results do not show a clear link between the allocation of suspected deep-sea sponge aggregations and sponges characterising the SIMPER communities, nor do we see any links between other species diversity indices. However, many of the communities are characterised by species that are known to inhabit sponges including galatheoid squat lobsters, sabellid polychaetes, brachiopods, and urchins, Cidaris cidaris, supporting other findings on deep-sea sponge aggregations in the Faroe-Shetland region (Henry & Roberts 2014; Kazadinis 2019). The high abundance of squat lobsters of the Munida genus throughout the SIMPER communities could reflect extensive sponge density and growth (Henry & Roberts 2014; Kazadinis 2019). The over-arching presence of typical sponge supporting species in varying degrees across the SIMPER communities shows little variation exists between them and could imply that all the stations are part of the wider sponge belt community. It is noteworthy that although community h was absent of sponges within the characterising species, it was commonly associated with stations where aggregations were identified as the main habitat from video analyses. Sabellidae worms were characteristic of community h, a class of polychaetes associated with sponges and observed links to populations of Erect (stalked) sponges (Bett & Jacobs 2007; Henry & Roberts 2014). There are, however, limitations to using the SIMPER routine to characterise a relatively homogenous site. The outputs of the SIMPER routine can be difficult to interpret as the routine does not account for the fact that taxa with smaller variances are often not identified as important contributors by the SIMPER routine (Warton et al. 2012). This could explain why sponges were not present within the characterising species where they were identified as forming the main habitat during video analyses. Henry and Roberts (2014) note that using it to define the ecological function criteria is not perfect. Characteristic species are dependent on the spatial distribution of fauna, different metrics of abundance, the type of data transformation and similarity metric employed (Henry & Roberts 2014).

Imagery surveys are restricted by morphotype identification methods. Identifying sponges to species is complex and relies on physical sampling for spicule and skeleton analysis; a costly and lengthy process (Schönberg 2021). Previous studies in the Faroe-Shetland area identify aggregations as Boreal-Ostur or otherwise known as "cheesy-bottoms" due to their yellow-white carpet like nature. Past investigations recorded massive morphotypes as *Geodia* spp. and Erect laminar sponges as *Phakellia ventilabrum* and it is likely that this is the case for these morphotypes at NEFSC. However, without direct sampling, identification cannot be confirmed (Klitgaard & Tendal 2004; Henry & Roberts 2014; Kazanidis 2019). Some of the large aggregations observed are indicative of the biotope *Geodia* and other massive sponges on Atlanto-Arctic upper bathyal sediment (M.AAUB.Co.DeeSpo.GeoSpo) (Last *et al.* 2019).

Identifying sponge growth forms rather than species has been considered sufficient for the purpose of identifying deep-sea sponge aggregations. Where the result identifies sponges as the main feature of the habitat it is argued that this is sufficient for this type of report (Berman et al. 2013; Henry & Roberts 2014). Spatial and temporal comparisons can be made from data obtained in this way and will aid future monitoring. However, it is noted that imagery analysis can lack detail, reducing insights into the potential diversity of sponge communities. Morphotype identification is also limited by analyst subjectivity, the use of different morphotype classifications and image quality. Even with consistent morphotype identification there are things to consider; different species can share the same morphologies and in other instances there can be high levels of morphological plasticity within a singular species (Schönberg 2021). The results of the current study were hampered by inconsistencies between analysts and subsequent reduction in identification confidence; our results were therefore restricted to high taxonomic level as a result. The use of a global standardised marine taxon reference image database such as SMarTaR-ID (Howell et al. 2019) would significantly reduce this problem and enable more consistent and accurate metrics to be obtained.

#### 8.1.3 Supporting processes

Dominant sponge morphologies can act as surrogates for prevailing environmental conditions. Schönberg (2021) identifies 'functional morphologies' relating morphotypes to function, environmental conditions and sponge ecology. Categorising sponge morphotypes in this way has provided a different insight into the sponge community and could aid future comparisons for monitoring. In Figure 18 we can see in boxes A and B that stations located toward the 400 m contour are characterised by higher abundances of Erect and Encrusting growth forms (category 1, categories 11 & 12). Towards the 500 m contour there are higher abundances of Massive growth forms (category 3). Dominant growth forms can be used as a proxy for the prevailing environmental conditions (Schönberg 2021). Crusts and Massive forms dominate towards the 500 m contour indicating water movement is strong/turbulent and substrates are harder in nature. SIMPER community f stations have Encrusting and Simple Massive sponges within the characterising species and could be indicative of the dynamic mixing zone known to occur in the channel. Five different water masses converge in the channel which creates turbulent water mixing in the area. This dynamic mixing zone occurs where warmer Atlantic waters flow over cooler Arctic waters (Bett 2012). Where Erect sponges dominate towards the 400 m contour, this indicates potentially strong to moderate laminar flow with finer sediments. Erect morphotypes commonly occur in areas with more predictable flow regimes therefore SIMPER community e stations, which have Erect growth forms within the characterising species, could indicate more stable conditions. Balls and Globular growth forms appear to be distributed in the deeper areas of the site at depth ranging 470–485 m (Figure 21). These morphotypes can tolerate a wider range of environmental conditions including high sedimentation rates and burial and are often indicative of sediment substrates (Schönberg 2021 and references therein). The occurrence

of these growth forms could be related to the settlement of finer sediments from other areas that experience turbulent hydrodynamic conditions.

Depth, pebbles, mud and shear stress explain 47% of the variation seen in the biological data. The inclusion of pebbles and mud could be related to different levels of shear stress experienced throughout the site. Areas of increased shear stress may be linked to harder substrates, such as pebbles, and areas that experience lower levels of shear stress could be linked with muddier habitats. This in turn will affect morphotype distribution; areas of higher mud content would have sponges that can tolerate smothering (e.g. Erect and Globular), and higher numbers of Encrusting and Simple Massive morphotypes would favour harder substrates (e.g. pebbles). Pebbles (classed as 4-64 mm in size) were found to be the variable with the highest correlation from BEST, and as a singular variable explained 20% of the variation (from DistLM results). As a habitat, coarse sediments dominated the site and characterised stations with suspected deep-sea sponge aggregations (Figure 12; Figure 29). It is likely that pebbles are important at NEFSC providing suitable substrate for sponge settlement and growth. However, further work is required, and additional parameters need to be obtained to inform on the driving forces behind sponge communities, morphotype and distribution. It would be beneficial to obtain further hydrodynamic and sedimentation data during future surveys. Howell et al. (2016) found that, in addition to depth and temperature, silicate concentration and particulate organic carbon levels are the most important drivers of sponge distribution. Other environmental parameters affecting predominant growth forms are thought to include nutrients, physical damage (natural or anthropogenic), biological interaction (predation, competition, symbiosis), illumination, pH, bathymetry and oxygen levels (Schönberg 2021).

Although temperature was not found to influence the biological variation in this analysis, it is known to be key variable affecting sponge distribution. Water temperature is also a consequence of the mixing of cold and temperate waters within the channel (Bett 2012). Water temperatures at the fifteen high confidence deep-sea sponge aggregation records ranged from 2.9 to 8°C. Other studies in the Faroe-Shetland region reported on Boreal-Ostur (Geodiid) species occurring at water temperatures ranging from -2 to 9°C (Henry & Roberts 2014; Davison *et al.* 2019).

# 8.2 Offshore subtidal sands and gravels and offshore deep-sea muds

#### 8.2.1 Extent, distribution and structure

The sediment sampling results agree with previously recorded locations of the PMF offshore subtidal sands and gravels from GeMS records and the UKSeaMap 2018 predictive habitat map (SNH 2019) (Figure 11). Further investigation is required to be able to conclude robustly on extent and distribution of sedimentary PMFs at NEFSC.

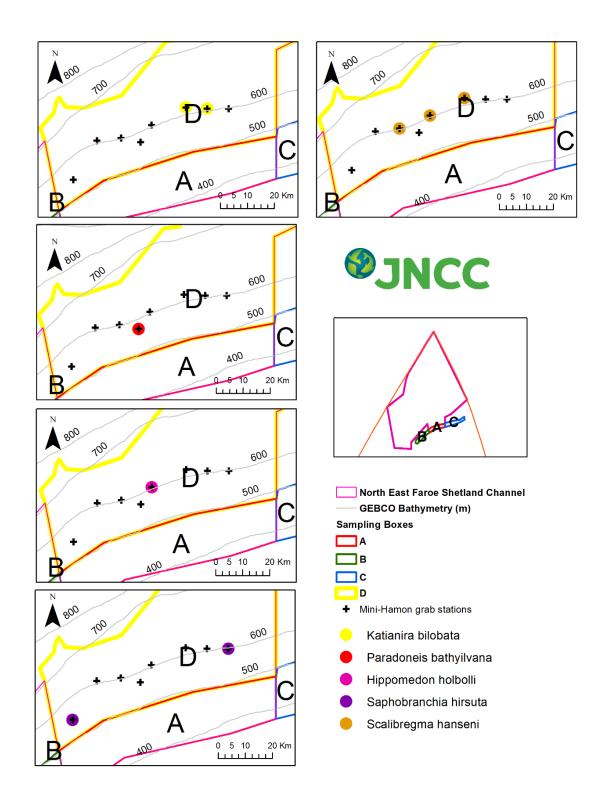
## 8.2.2 Biological structure

It is interesting to note that many deep-water species and species that have only been previously recorded in Nordic waters (hereafter referred to Nordic species) were recorded from samples in box D (Table 25; Figure 33; Figure 34) (Chamberlain & Barnich 2018). The deep-water polychaete *Paradoneis bathyilvana* was found in grab sample D12. This species is originally described from the Capbreton Canyon in the Bay of Biscay (Aguirrezabalaga & Gil 2009) and its presence at the site may represent the northernmost record known to date. The polychaete *Scalibregma hanseni* is a deep-water Nordic species found on the continental slope from depths of 500 to 1,200 m in the Nordic Sea and Northern North Atlantic (Bakken *et al.* 2014), corroborating results from the present study. The deep-water

amphipod, *Haploops abyssorum*, was originally described from the Azores, confirmed for the Faroe region in 1996 by Dauvin and Bellan-Santini. The deep-water and Nordic isopod species *Katianira bilobata* was recorded in two samples and is typical for sponge habitats in depths of 70 m to 1,000 m (Svavarsson 1987). However, is hard to report on indigeneity or rarity of species without an understanding of current distributions to support it. These findings reflect the paucity of deep-sea data and the limited number of datasets from UK offshore waters means that supporting information on the distribution of species is unavailable.

Major group	Species/genus	Authority	Distribution
Polychaeta	Saphobranchia hirsuta (original ID now unaccepted Diplocirrus hirsutus)	Hansen 1978	Eurybathic Nordic species
	Nicomache quadrispinata	Arwidsson 1906	Eurybathic Nordic species
	Paradoneis bathyilvana	Aguirrezabalaga & Gil 2009	Deep-water species. Originally recorded in Capbreton Canyon (Bay of Biscay)
	Scalibregma hanseni	Bakken <i>et al.</i> 2014	Deep-water Nordic species
Pcynogonidae (sea spider)	Nymphon tenellum	Bamber 2010	Nordic species
Amphipoda	Byblis crassicornis	Sars 1890	Nordic species
	Harpinia mucronata	Sars 1890	Nordic species
	Harpinia propinqua	Sars 1890	Nordic species
	Hippomedon holbolli	Sars 1890	Nordic species
	Haploops abyssorum	Dauvin & Bellan-Santini 1996	Deep-water species. Originally described from the Azores and confirmed in Faroe region by Dauvin <i>et al.</i> (1996)
Isopoda	Austroniscus sp.	Brix & Svavarsson 2009	Deep-water Nordic species
	Katianira bilobate	Svavarsson 1987	Deep-water and Nordic isopod typical for sponge habitats in depths of 70–1,000 m
	Typhlotanais sp	Sars 1899	Deep-water Nordic species
	Pseudosphyrapus anomalus	Sars 1899	Deep-water Nordic species
Bivalvia	Yoldiella sp.	Oliver <i>et al.</i> 2016	Deep-water species
	Dacrydium ockelmanni	Oliver <i>et al.</i> 2016	Deep-water species
Bryozoa	Tervia irregularis	Hayward & Ryland 1985	Deep-water species
Echinodermata	Elasipodida	Mortensen 1977	Deep-water species
Ophiuroidea (brittlestars)	Ophiactis abyssicola	Paterson 1985	Deep-water species

 Table 25. Nordic and deep-water species identified from grab samples obtained in box D.

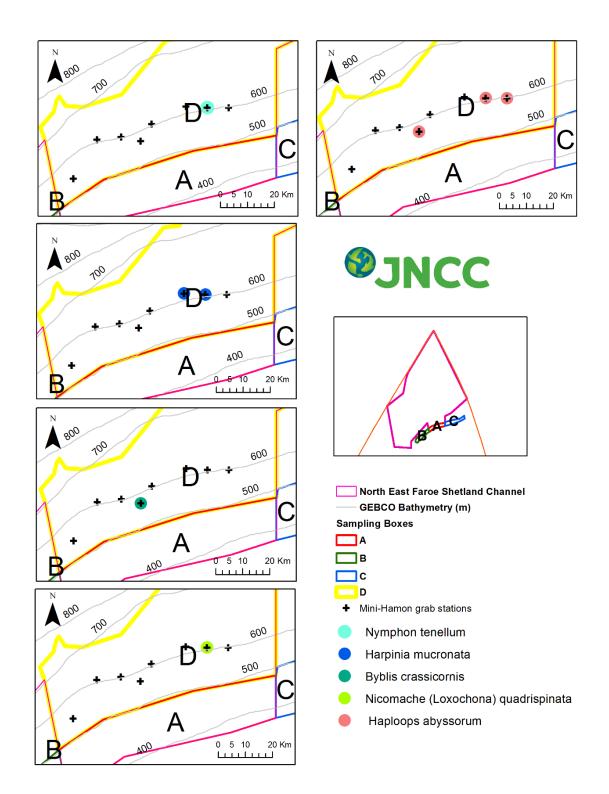


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**Figure 33.** Nordic deep-sea infauna at NEFSC (*Katianira bilobata*, *Paradoneis bathyilvana*, *Hippomedon holbolli*, *Saphobranchia hirsuta*, *Scalibregma hanseni*).



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World Vector Shoreline © US Defence Mapping Agency. Projection: Web Mercator (EPSG: 3857). Not to be used for navigation.

**Figure 34**. Nordic deep-sea infauna at NEFSC (*Nymphon tenellum*, *Harpinia mucronate*, *Byblis crassicornis*, *Nicomache* (*Loxochona*) *quadrispinata*, *Haploops abyssorum*).

#### 8.2.3 Biotope classification

Little is currently known about deep-sea sedimentary biotopes and their infaunal communities including the Atlanto-Arctic upper bathyal sediments found at NEFSC due to the paucity of deep-sea sampling. Low taxonomic resolution from video analysis means designation of biotopes is limited to identifying broad community type based on taxa present (Level 4) of the Marine Habitat Classification (Parry *et al.* 2015). It was not possible to identify specific species assemblages (Level 5). Improvements to epifaunal imagery analysis and data collection standards to enable more accurate and consistent results across analysts and methods would improve data quality and understanding of biotopes and communities. There is a need for higher resolution deep-sea imagery data to aid development of the classification.

## 9. Recommendations for future monitoring

## 9.1 Data collection

- Suspected deep-sea sponge aggregations were recorded in box A in the northern, deeper extents of the sampling box during chariot tow analysis (525 m). Future camera stations should focus on depth gradients along the length of NEFSC (400 m, 450 m, 500 m, 550 m) to further evaluate extent and distribution of deep-sea sponge aggregations.
- Deep-sea sponge aggregations were observed within and outside the site but due to lower numbers of stations taken in box C (outside the site) compared to the other boxes, our understanding of the extent of the aggregations in this box is incomplete. Future surveys should repeat sampling either side of the limits of the current MPA designation particularly focusing on box C.
- Previous work by Howell *et al.* (2016) and Schönberg (2021) suggest additional environmental parameters such as silicate concentration, particulate organic carbon levels, hydrodynamics and sedimentation values can assist our understanding of sponge distribution, sponge morphotypes and their prevailing environmental conditions. It is recommended that these variables are obtained during future surveys where possible.
- It is recommended that more, regularly spaced images would decrease the need for heavy standardisation treatments prior to multivariate analysis.
- To maintain a consistent degree of taxonomic detail and to reduce the potential for over/under estimation of species it is recommended that future surveys attempt to obtain images with an ideal range in field of view (FOV) of 1–1.5 m.
- To understand the extent of VMEs in the wider environment and further understand the distribution of the sponge belt in the Faroe-Shetland region, it is recommended that surveys could investigate between the Faroe-Shetland Sponge Belt MPA and NEFSC between the 400–600 m depth contours.
- Future surveys could evaluate whether more suitable equipment is available to reduce issues with image quality and sampling units. Whilst drop-down camera systems provide one of the cheapest, easiest systems to operate, they have accuracy and guality limitations. Autonomous Underwater Vehicles (AUVs) and Remotely Operated Vehicles (ROVs) can collect high resolution imagery and environmental data. AUVs and ROVs can maintain slow speeds for more precise seabed image collection, enabling more suitable images and the ability focus on the fine-scale spatial extent of deep-sea sponge aggregations. They have the advantage of maintaining an ideal distance from the seabed, increasing consistency in FOV and obtain more comparable metrics. Both systems can be equipped with a suite of sensors collecting environmental metrics in situ. ROVs have the added ability to collect direct physical samples which would enable sponge identification to species. ROVs or AUVs could utilise acoustic tools to detect aggregations at wider spatial scales (Przeslawski et al. 2018). The advantages of using such equipment would need to outweigh the disadvantages, namely the high costs associated with both equipment hire/purchase and additional pilot and technician costs (Przeslawski et al. 2018; Hinchen et al. 2019).
- Direct sampling via ROV may be considered in the future, targeting key sponge morphotypes precisely without causing high levels of damage. The cost of sampling

such slow-growing species would need to be outweighed by the benefit that would be gained from increased taxonomic resolution in the dataset.

• Further sampling is required within NEFSC to investigate the extent and distribution of the PMFs offshore subtidal sands and gravels and offshore deep-sea muds. The boundaries of NEFSC cover a depth range of 330 m to 2,420 m. Different faunal compositions are expected below 800 m, where species can tolerate the cooler Arctic-influenced waters (Chamberlain & Barnich 2018). During 1517S a limited number of samples were collected in a narrow depth range of 520 m to 600 m, so the range of different assemblages expected have not been sampled. Sampling in depths greater than 800 m would improve our understanding of the sand and gravel habitats for which the MPA has been designated. Furthermore, the low number of samples does not constitute a sufficient sample size to achieve adequate statistical power to reliably detect change.

## 9.2 Data analysis

- It is recommended that the chariot tows are reanalysed to obtain sponge density values and community metrics to enable subsequent verification of the suspected sponge aggregations. This would contribute to the current number of verified aggregations at the site enabling the current study to have a more robust standing as a first point in a time series against which the rate and direction of change in condition of the features could be inferred in the long term.
- It is recommended that future analysis is conducted via image annotation software such as BIIGLE. Advantages of BIIGLE include the ability to obtain standard metrics, use shared classification schemes via label trees and share examples of previously applied identifications.
- It is recommended that the metrics originally obtained in this report are collected in the future so that comparisons can be directly made, however more reliable metrics such as frequency of occurrence should also be recorded during analysis. Frequency of occurrence metrics have been shown to have better precision, power, and consistency (Moore *et al.* 2019). A10 X 10 frequency of occurrence grid could increase the consistency and accuracy of abundance estimates for future comparisons (Moore *et al.* 2019; Hinchen *et al.* 2021).
- Porifera enumeration/estimation from seabed imagery is known to be problematic. Global standardisation for recording sponge abundances will enable future comparability of findings and could increase our power to detect change. The use of a shared classification schemes would provide a standardised approach to analysis, increasing consistency, comparability and decreasing variation. Sponges are more reliably classified to morphological growth forms from imagery as opposed to species, therefore such classification schemes would suit the long-term monitoring of deep-sea sponge aggregations. Future analyses would benefit from the most used classification scheme at the time, but it is recommended that the sponges are categorised into functional morphotypes as per Hanna and Shönberg (2019), and that the overall community analysis uses SMarTaR-ID, a global standardised marine taxon reference image database that is currently under development (Howell et al. 2019). The use of OTUs (Howell et al. 2019) would have greatly improved the overall epifaunal dataset at NEFSC, significantly reducing the inconsistencies and uncertainties between analysts that restricted the results to such to high taxonomic levels.

During analysis of still imagery, multiple images are combined by the stations or split by habitat if there was a significant change. Within the literature it is evident that determining a suitable sampling unit and sample size needs to be a priority for subsequent analysis (Durden *et al.* 2016). A pooled length of tow or random mosaic would need to be determined in relation to our sampling population of focus. Sponge aggregation densities are defined by OSPAR as ranging from 0.5–24 sponges per m<sup>2</sup> (OSPAR 2010; Henry & Roberts 2014) and biotopes are assigned to areas of at least 25 m<sup>2</sup> (e.g. a 5 m tow maintained at 1 m from the subsurface), two factors that could be considered here. Durden *et al.* (2016) suggest that segments should have at least 100 total individuals. Once a sample unit is decided upon, the statistical power should be investigated, and sample size can then be considered. Trade-offs between the number of photographs pooled (sampling unit) and the number of replicates per habitat (sample size) should also be considered.

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## 11. Appendix 1: Infauna data truncation

Raw taxon abundance and biomass matrices can often contain entries that include the same taxa recorded differently, erroneously or differentiated according to unorthodox, subjective criteria. Therefore, ahead of analysis, data should be checked and truncated to ensure that each row represents a legitimate taxon and that 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 must be merged to a level in the taxonomic hierarchy that is higher than the level at which they were identified. In such situations, a compromise must be reached between the level of information lost by discarding recorded detail on a taxon's identity and the potential for error in analyses, results and interpretation if that detail is retained.

Details of the data preparation and truncation protocols applied to the infaunal datasets acquired at NEFSC ahead of the analyses reported here are provided below.

- Where there are records of one named species together with records of members of the same genus (but the latter not identified to species level), the entries are merged, and the resulting entry retains only the name of the genus.
- Taxa are often assigned as 'juveniles' during the identification stage with little evidence for their actual reproductive natural history (with the exception of some well-studied molluscs and commercial species). Many truncation methods involve the removal of all 'juveniles'. However, a decision must be made on whether removal of all juveniles from the dataset is appropriate or whether they should be combined with the adults of the same species where present. For the infaunal data collected at NEFSC, where a species level identification was labelled 'juvenile', the record was combined with the associated adult species level identification when present; or the 'juvenile' label removed where no adults of the same species had been recorded.
- Records of fish species were removed.

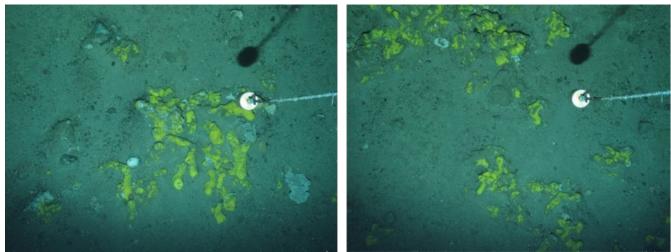
### 12. Appendix 2: Epifauna data standardisation for dropdown camera images

Images assigned with *Zero* and *Very Poor* quality were removed from analysis as per the NMBAQC guidelines (Turner *et al.* 2016). Images assigned as *Poor* were included for analysis as they provided sufficient clarity to identify the PMF deep-sea sponge aggregations (Figure 33). However, following application of the FOV threshold (discussed below) many images of *Poor* quality were excluded from multivariate community analysis.

Reason for removal from dataset	Number of images
Metadata issues or duplicate images	44
35 images with missing CTD data	35
Image quality = <i>Very Poor</i>	22
Image quality = <i>Zero</i>	7
No Field of View (FOV)	7
FOV > 2.5 m <sup>2</sup>	51

 Table 26. Selection of images for quality and consistency.

Original number of images	Number of images included for analysis
838	672



**Figure 35.** Examples of images deemed as *Poor* quality with deep-sea sponge aggregations (A01\_04, A01\_10, A01\_11).

### 13. Appendix 3: Field of View (FOV) threshold

A large range in FOV was observed within the dataset (0.1 m<sup>2</sup> to 4.6 m<sup>2</sup>). To maintain a consistent degree of taxonomic detail and to reduce the potential for under/over estimation of abundance, a threshold for an ideal FOV was determined. FOV was plotted for each habitat with its assigned image quality (Figure 36). As expected, images with large FOVs are associated with *Poor* quality. Further plots illustrating number of images per habitat against FOV show the majority of images fall within 0.5 m<sup>2</sup> and 2 m<sup>2</sup> (Figure 37; Figure 38). To encapsulate as many images as possible within each habitat, particularly for deep-sea sponge aggregations, and to retain a consistent level of taxonomic detail the threshold for ideal FOV was set to 2.5 m<sup>2</sup>.

Due to the variable total area covered by individual tows ( $13 \text{ m}^2$  to  $37 \text{ m}^2$ ), the sum of species was calculated for all images within a tow (by station) and divided by total area covered to produce sums of relative densities for each tow (i.e. each species represented by  $1 \text{ m}^2$ ).

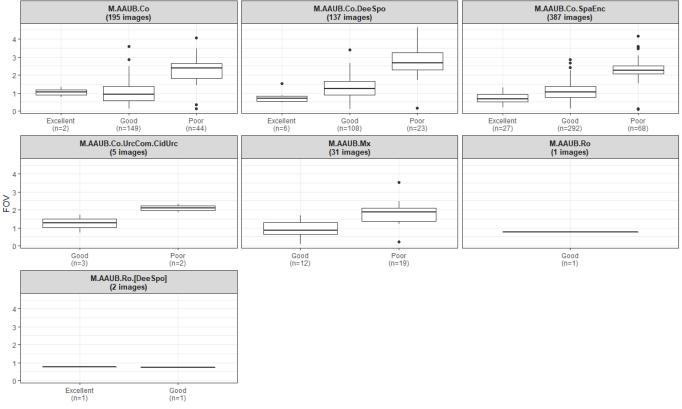


Image Quality

**Figure 36.** Range of image FOV per  $m^2$  across each habitat (Marine Habitat Classification of Britain and Ireland (v15.03)) for each image quality, n = number of images.

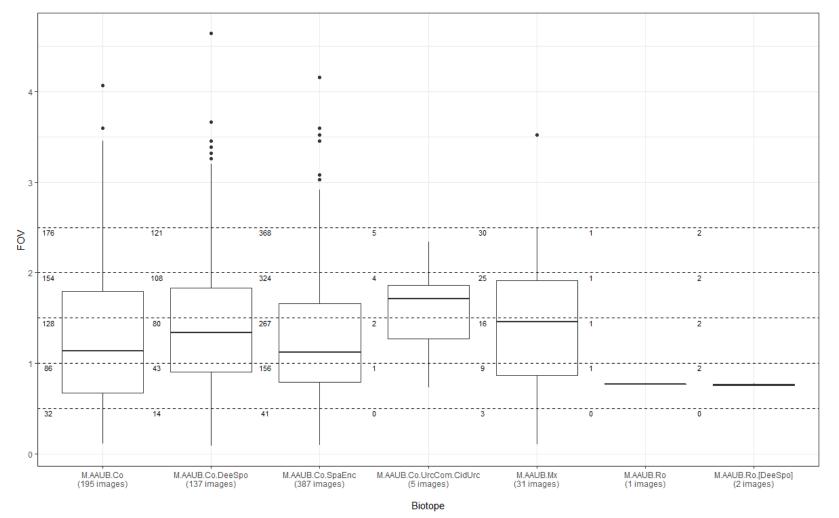
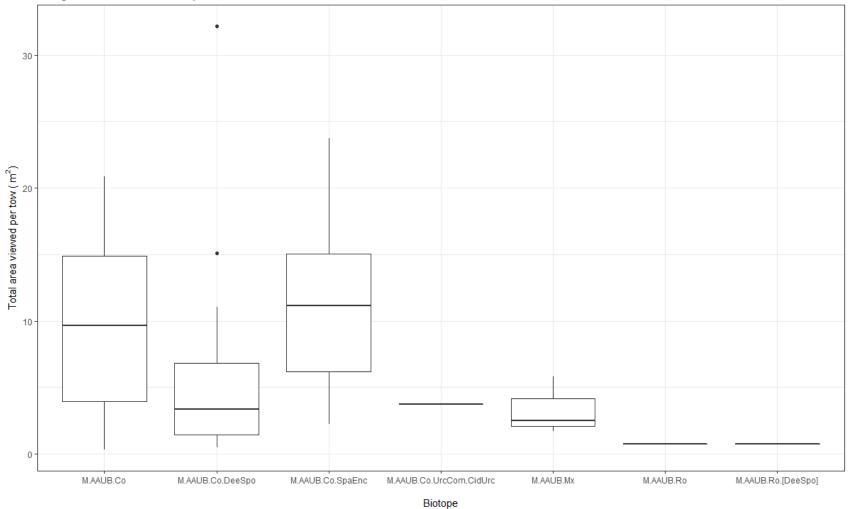


Figure 37. FOV per habitat (Marine Habitat Classification of Britain and Ireland (v15.03)) with number of images illustrated on the dotted axis.



Range of total area viewed per tow for each MNCR habitat.

Figure 38. Range of total area viewed per tow (m<sup>2</sup>) for each habitat (Marine Habitat Classification of Britain and Ireland (v15.03)).

### 14. Appendix 4: Marine litter categories

Categories and sub-categories of litter items for seafloor from the OSPAR/ICES/IBTS (International Bottom Trawl Survey) for North-East Atlantic and Baltic.

A: Plastic	B: Metals	C: Rubber	D: Glass/ Ceramics	E: Natural products/ Clothes	F: Miscellaneous	
A1. Bottle	B1. Cans (food)	C1. Boots	D1. Jar	E1. Clothing/ rags	F1. Wood (processed)	
A2. Sheet	B2. Cans (beverage)	<mark>C2</mark> . Balloons	D2. Bottle	E2. Shoes	F2. Rope	
A3. Bag	B3. Fishing related	<mark>C3</mark> . Bobbins (fishing)	D3. Piece	E3. Other	F3. Paper/ cardboard	
A4. Caps/ lids A5. Fishing line (monofilament)	B4. Drums B5. Appliances	C4. Tyre C5. Other	D4. Other		F4. Pallets F5. Other	
A6. Fishing line (entangled)	B6. Car parts					
A7. Synthetic rope	B7. Cables			Related size categories A: $\leq 5*5 \text{ cm} = 25 \text{ cm}^2$ B: $\leq 10*10 \text{ cm} = 100 \text{ cm}^2$ C: $\leq 20*20 \text{ cm} = 400 \text{ cm}^2$ D: $\leq 50*50 \text{ cm} = 2500 \text{ cm}^2$ E: $\leq 100*100 \text{ cm} = 10000 \text{ cm}^2$ F: $\geq 100*100 \text{ cm} = 10000 \text{ cm}^2$		
A8. Fishing net	B8. Other					
A9. Cable ties A10. Strapping band A11. Crates and containers						
A12. Plastic diapers A13. Sanitary towels/ tampons A14. Other						

Source: MSFD Technical Subgroup on Marine Litter (2013).

### 15. Appendix 5: Non-indigenous species (NIS) lists

**Table 27.** Taxa listed as NIS (present and horizon) which have been selected for assessment of Good Environmental Status in GB waters under MSFD Descriptor 2 (Stebbing *et al.* 2014).

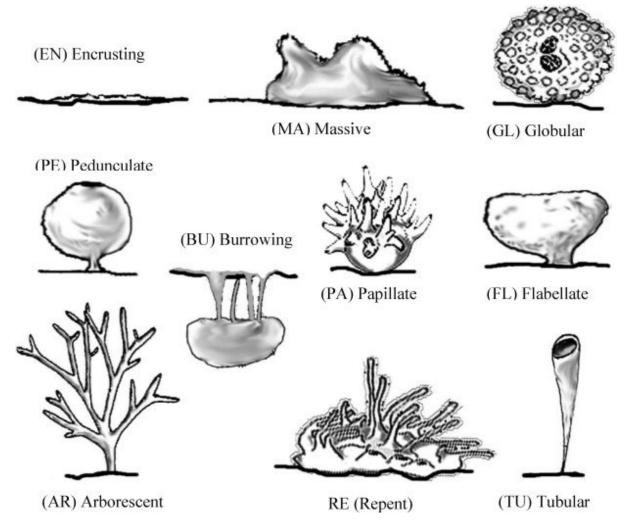
Species name	List	Species name	List
Acartia (Acanthacartia) tonsa	Present	Alexandrium catenella	Horizon
Amphibalanus amphitrite	Present	Amphibalanus reticulatus	Horizon
Asterocarpa humilis	Present	Asterias amurensis	Horizon
Bonnemaisonia hamifera	Present	Caulerpa racemosa	Horizon
Caprella mutica	Present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

**Table 28.** Additional taxa listed as NIS in the JNCC 'Non-native marine species in British waters: a review and directory' report by Eno *et al.* (1997) which have not been selected for assessment of Good Environmental Status in GB waters under MSFD.

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	

Species name (1997)	Updated name (2017)
Tiostrea lutaria	Tiostrea chilensis
Mercenaria mercenaria	
Petricola pholadiformis	
Mya arenaria	

# 16. Appendix 6: Sponge morphotypes for imagery analysis



**Figure 39.** Sponge morphologies as described by Berman *et al.* (2013) used during the identification of sponges from video and still imagery.

### **17.** Appendix 7: Confidence assessment – full results

**Table 29.** Verification of suspected deep-sea sponge aggregations (DSSA) at NEFSC applying the criteria and confidence assessment from Henry and Roberts (2014) – complete results. Ticks indicate number of Criteria met.

Station	Density (counts >0.5 per m <sup>2</sup> )	Density (% cover >1% per m²)	Ecological function	Habitat (Marine Habitat Classification)	Criteria	Confidence result
A10	yes	no	Brachiopoda-Galatheoidea-Cidaris cidaris (d)	M.AAUB.Co.DeeSpo	$\checkmark \checkmark \checkmark$	High
A14	yes	no	Brachiopoda-Galatheoidea-Cidaris cidaris (d)	M.AAUB.Co.DeeSpo	$\checkmark \checkmark \checkmark$	High
B06	yes	no	Galatheoidea-Cidaris cidaris-Brachiopoda- Ophiuroidea-Asteroidea- category 11 & 12 Erect forms (Porifera Flabellate) (e)	M.AAUB.Co.DeeSpo	<b>√√√</b>	High
A01	yes	yes	Sabellidae-Galatheoidea-Brachiopoda-Caridea- Asteroidea-Ophiuroidea (h)	M.AAUB.Co.DeeSpo	<b>~ ~ ~</b>	High
A05	yes	yes	Sabellidae-Galatheoidea-Brachiopoda-Caridea- Asteroidea-Ophiuroidea (h)	M.AAUB.Co.DeeSpo	<b>~ ~ ~</b>	High
A06b	yes	yes	Galatheoidea-Cidaris cidaris-Brachiopoda- Ophiuroidea-Asteroidea- Porifera category 11 & 12 Erect forms (e)	M.AAUB.Co.DeeSpo	<b>√√√</b>	High
A07	yes	yes	Sabellidae-Galatheoidea-Brachiopoda-Caridea- Asteroidea-Ophiuroidea (h)	M.AAUB.Co.DeeSpo	<b>~ ~ ~</b>	High
A08	yes	yes	Galatheoidea-Cidaris cidaris-Brachiopoda- Ophiuroidea-Asteroidea- Porifera category 11 & 12 Erect forms (e)	M.AAUB.Co.DeeSpo	<b>√</b> √ √	High
A12	yes	yes	Galatheoidea-Cidaris cidaris-Brachiopoda- Ophiuroidea-Asteroidea- Porifera category 11 & 12 Erect forms (e)	M.AAUB.Co.DeeSpo	<b>√√√</b>	High

Station	Density (counts >0.5 per m <sup>2</sup> )	Density (% cover >1% per m²)	Ecological function	Habitat (Marine Habitat Classification)	Criteria	Confidence result
A13	yes	yes	Galatheoidea-Cidaris cidaris-Brachiopoda- Ophiuroidea-Asteroidea- Porifera category 11 & 12 Erect forms (e)	M.AAUB.Co.DeeSpo	<b>√√√</b>	High
A17	yes	yes	Sabellidae-Galatheoidea-Brachiopoda-Caridea- Asteroidea-Ophiuroidea (h)	M.AAUB.Co.DeeSpo	<b>~ ~ ~</b>	High
B07	yes	yes	Brachiopoda-Galatheoidea-Cidaris cidaris (d)	M.AAUB.Co.DeeSpo	$\checkmark\checkmark\checkmark$	High
B10	yes	yes	Sabellidae-Galatheoidea-Brachiopoda-Caridea- Asteroidea-Ophiuroidea (h)	M.AAUB.Co.DeeSpo	<b>~ ~ ~</b>	High
C01	yes	yes	Cidaris cidaris-Galatheoidea-Asteroidea-Crinoidea- Brachiopoda-category 1 Encrusting-Ceramaster- category 3 Simple Massive (f)	M.AAUB.Co.DeeSpo	<b>V V V</b>	High
C13	yes	yes	Cidaris cidaris-Galatheoidea-Asteroidea-Crinoidea- Brachiopoda-category 1 Encrusting-Ceramaster- category 3 Simple Massive (f)	M.AAUB.Co.DeeSpo	<b>√√√</b>	High
A02	yes	no	Sabellidae-Galatheoidea-Brachiopoda-Caridea- Asteroidea-Ophiuroidea (h)	M.AAUB.Co.SpaEnc	$\checkmark\checkmark$	Medium
A03	yes	no	Galatheoidea-Cidaris cidaris-Brachiopoda- Ophiuroidea-Asteroidea- Porifera category 11 & 12 Erect forms (e)	M.AAUB.Co.SpaEnc	<b>√ √</b>	Medium
A04	yes	no	Galatheoidea-Cidaris cidaris-Brachiopoda- Ophiuroidea-Asteroidea- Porifera category 11 & 12 Erect forms (e)	M.AAUB.Co.SpaEnc	<b>√ √</b>	Medium
A09	yes	no	Galatheoidea-Cidaris cidaris-Brachiopoda- Ophiuroidea-Asteroidea- Porifera category 11 & 12 Erect forms (e)	M.AAUB.Co.SpaEnc	<b>√ √</b>	Medium

Station	Density (counts >0.5 per m <sup>2</sup> )	Density (% cover >1% per m²)	Ecological function	Habitat (Marine Habitat Classification)	Criteria	Confidence result
A11	yes	no	Galatheoidea-Cidaris cidaris-Brachiopoda- Ophiuroidea-Asteroidea- Porifera category 11 & 12 Erect forms (e)	M.AAUB.Co.SpaEnc	<b>√</b> √	Medium
B04	yes	no	Cidaris cidaris-Galatheoidea-Asteroidea-Crinoidea- Brachiopoda-category 1 Encrusting-Ceramaster- category 3 Simple Massive (f)	M.AAUB.Co.SpaEnc	<b>√ √</b>	Medium
B08	yes	no	Galatheoidea-Cidaris cidaris-Brachiopoda- Ophiuroidea-Asteroidea- category 11 & 12 Erect forms (Porifera Flabellate) (e)	M.AAUB.Co	<b>√</b> √	Medium
B09	yes	no	Sabellidae-Galatheoidea-Brachiopoda-Caridea- Asteroidea-Ophiuroidea (h)	M.AAUB.Co.SpaEnc	$\checkmark\checkmark$	Medium
B12	yes	no	Brachiopoda-Galatheoidea-Cidaris cidaris (d)	M.AAUB.Co	<b>√</b> √	Medium
B13	yes	no	Cidaris cidaris-Galatheoidea-Asteroidea-Crinoidea- Brachiopoda-category 1 Encrusting-Ceramaster- category 3 Simple Massive (f)	M.AAUB.Co	<b>√</b> √	Medium
B15	yes	no	Brachiopoda-Galatheoidea-Cidaris cidaris (d)	M.AAUB.Co	$\checkmark\checkmark$	Medium
C03	yes	no	Cidaris cidaris-Galatheoidea-Asteroidea-Crinoidea- Brachiopoda-category 1 Encrusting-Ceramaster- category 3 Simple Massive (f)	M.AAUB.Co.SpaEnc	<b>√</b> √	Medium
A06a	yes	yes	Galatheoidea-Cidaris cidaris-Brachiopoda- Ophiuroidea-Asteroidea- Porifera category 11 & 12 Erect forms (e)	M.AAUB.Co.SpaEnc	<b>√ √</b>	Medium
A15	yes	yes	Cidaris cidaris-Galatheoidea-Asteroidea-Crinoidea- Brachiopoda-category 1 Encrusting-Ceramaster- category 3 Simple Massive (f)	M.AAUB.Co.SpaEnc	<b>√ √</b>	Medium

Station	Density (counts >0.5 per m <sup>2</sup> )	Density (% cover >1% per m²)	Ecological function	Habitat (Marine Habitat Classification)	Criteria	Confidence result
A16	yes	yes	Galatheoidea-Cidaris cidaris-Brachiopoda- Ophiuroidea-Asteroidea- category 11 & 12 Erect forms (e)	M.AAUB.Co.SpaEnc	<b>√</b> √	Medium
B02	yes	no	Less than two samples in group	M.AAUB.Co	✓	Low
B03	no	no	Cidaris cidaris-Galatheoidea-Asteroidea-Crinoidea- Brachiopoda-category 1 Encrusting-Ceramaster- category 3 Simple Massive (f)	M.AAUB.Co	✓	Low
B11	yes	no	Less than two samples in group	M.AAUB.Co.SpaEnc	✓	Low
B14	yes	no	Less than two samples in group	M.AAUB.Co	$\checkmark$	Low
B16	yes	no	Less than two samples in group	M.AAUB.Co	$\checkmark$	Low
C04	yes	no	Crustacea-Echinoidea (i)	M.AAUB.Co	$\checkmark$	Low
B01	no	no	Crustacea-Echinoidea (i)	M.AAUB.Mx		No DSSA
B05	no	no	Less than two samples in group	M.AAUB.Mx		No DSSA

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# marinescotland





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