



**JNCC Report
No. 509**

**Biotope Assignment of Grab Samples from Four Surveys Undertaken in 2011
Across Scotland's Seas (2012)**

Pearce, B., Grubb, L., Earnshaw, S., Pitts, J. & Goodchild, R.

August 2014

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ISSN 0963 8901

For further information please contact:

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

This report should be cited as:

Pearce, B., Grubb, L., Earnshaw, S., Pitts, J. & Goodchild, R. 2014. Biotope Assignment of Grab Samples from Four Surveys Undertaken in 2011 Across Scotland's Seas (2012). *JNCC Report*, No. 509.

Acknowledgement

This report was prepared from analysis conducted in 2012.

Executive Summary

In 2012 the Joint Nature Conservation Committee (JNCC) commissioned Gardline Caledonia Ltd. to undertake analysis of benthic grab data collected during four surveys in order to assign biotope classifications and identify potential features of conservation interest. These surveys were commissioned to provide new data to enhance our understanding of the distribution of MPA search features across Scotland's seas. The four surveys were conducted between July and November 2011 in the Firth of Forth Banks Complex area, the northern North Sea, North East Atlantic shelf area and from within the Windsock fisheries restriction area and were designed to target habitats for which our understanding could be improved. These included areas that have been identified by the predictive habitat mapping project UKSeaMap 2010 (McBreen *et al* 2011) as sandy gravel habitats which are not well documented in Scottish waters.

Data Analysis

The biological and Particle Size Distribution (PSD) data (where available) from 291 benthic grab samples were analysed during the course of this study. Once the data had been organised and truncated to ensure taxonomic naming consistency, they were analysed using multivariate statistical techniques to identify natural groupings in the biological data, and to investigate the environmental niche requirements of the same.

Faunal Assemblages

A number of statistical trials were undertaken as a means of identifying the best method to identify faunal assemblages that are akin to existing biotopes of the EUNIS¹ classification scheme and the Marine Habitat Classification for Britain and Ireland (version 04.05)², also known as MNCR classification (Connor *et al* 2004). The biological data were subjected to a number of different transformations (untransformed, standardised, square root, fourth root, log and presence-absence) and faunal assemblages were identified using a 20% similarity slice and a SIMPROF test on clusters created from a series of Bray-Curtis similarity resemblance matrices.

The groupings identified using both the SIMPROF test and the similarity slice were found to be relatively faithful, regardless of the transformation used. The number of groups was also fairly consistent, with the exception of the untransformed data which gave rise to a higher number of groups using both methods. Examination of the SIMPER results revealed that this increase was primarily due to an increase in outlying samples with the core groups remaining consistent with groups identified using data that had been transformed. Using the SIMPROF test to identify faunal assemblages gave rise to many more groups than was useful in this context (between 71 and 106).

Ultimately, a 20% similarity slice through a cluster created using standardised benthic abundance data was used to identify the faunal assemblages that form the basis of the biotope assignments in this study. This method of transformation was chosen primarily because the data had been collected using different sampling gear of unspecified dimensions. Using this method, a total of 16 faunal assemblages were identified as shown overleaf in Table 1.

¹ EUNIS - <http://eunis.eea.europa.eu/>

² MNCR - <http://jncc.defra.gov.uk/page-1584>

Table 1. Summary of the faunal assemblages identified from benthic grab data collected from four surveys (Firth of Forth Banks, North Sea, North East Atlantic shelf and Windsack) carried out across Scotland's seas.

Cluster	Faunal assemblage
a	Sparse faunal assemblage including <i>Thelepus cincinnatus</i> and some hydroid species
b	A rich polychaete and epifaunal community including <i>Galathea intermedia</i>
c	<i>Spiophanes bombyx</i> aggregations
d	<i>Paramphinome jeffreysii</i> , <i>Amphiura filiformis</i> and other echinoderms
e	<i>Nephtys cirrosa</i> and <i>Bathyporeia</i> spp.
f	Dense <i>Owenia fusiformis</i> aggregations
g	<i>Diplocirrus glaucus</i> , <i>Paramphinome jeffreysii</i> and <i>Amphiura chiajei</i>
h	<i>Ophiactis balli</i> with encrusting bryozoans, saddle oysters and serpulids
i	<i>Owenia fusiformis</i> and <i>Galathea oculata</i> aggregations
j	<i>Aponuphis bilineata</i> and <i>Echinocyamus</i> and a rich polychaete community
k	Sparse fauna
l	<i>Angulus pygmaeus</i> (<i>Moerella pygmaea</i>) and sparse polychaetes
m	Sparse fauna including pagurids
n	<i>Placostegus tridentatus</i> and <i>Galathea intermedia</i> with encrusting fauna
o	Sipunculid community
p	<i>Aponuphis bilineata</i> and <i>Echinocyamus</i> with encrusting fauna and a rich polychaete community

Environmental Niche Requirements

The links between biological communities and the environmental conditions across Scotland's seas were investigated using a range of environmental variables extracted from UKSeaMap 2010, GEBCO and variables calculated from the PSD data.

Multivariate analysis revealed a strong relationship between the biological communities and the Particle Size Distribution of the sediments in which they occur ($p = 0.619$, 0.1% significance level) and a moderate relationship between the biological communities and a suite of derived environmental variables including sediment statistics, biological zone, depth, energy and geographical location ($p = 0.527$, 0.2% significance level). This indicates that the composition of the sediments has the strongest influence in shaping the biological communities in Scotland's seas. It should be noted however, that there are a number of environmental variables that were not available to include in this analysis, which are likely to have a strong influence on benthic communities including temperature at the seabed, sheer bed stress and the location of the oxygen departure zones. There was also an absence of information regarding the level of anthropogenic disturbance these communities are subjected to, which is likely to be another key factor influencing biological communities in these areas.

Biotope Assignment

A number of methods were employed to identify existing MNCR biotopes that correspond with the faunal assemblages identified in this study. The first method used multivariate statistical techniques to look for faunal assemblages and biotopes that were not statistically separable based on their faunal composition. A number of existing biotopes that were similar to the assemblages identified in this study were found using this method but none that were statistically inseparable from one another. The second method involved manually searching existing biotopes for the presence of the characterising species from each of the faunal assemblages. Both the JNCC website and the most recent comparative tables were searched in this way, resulting in just one confident match. Finally, expert moderation was employed to identify biotopes that had been missed by each of these methods, particularly those which were described on the basis of the ecological functioning rather than their

biological composition e.g. SS.SSa.IFiSa.TbAmPo - Semi-permanent tube-dwelling amphipods and polychaetes in sublittoral sand. This gave rise to a further three confident matches with existing MNCR biotopes.

The majority of assemblages identified in this study were found to have significantly different characterising fauna to those described for existing biotopes, and many also occurred in different environmental conditions. Where a faunal assemblage occurred in similar environmental conditions in more than five samples, from more than three locations, there was considered to be sufficient evidence to propose a new biotope. In total, ten new biotopes were proposed, four of which occurred in offshore coarse sediments, five in offshore sands and one in offshore mixed sediments. Where there was not sufficient information to propose a new biotope and the faunal assemblage was significantly different to all existing biotopes the sample was assigned to a habitat level biotope based on the environmental conditions recorded.

The existing and proposed biotopes that were ultimately assigned to the benthic grab samples included in this study are summarised below in Table 2.

Table 2. Summary of the biotopes assigned to samples taken from the Firth of Forth Banks, North Sea, North East Atlantic shelf and Windsock areas between July and November 2011. Brackets are used to highlight temporary codes assigned to illustrate indicative placing of these new biotopes within the current MNCR and EUNIS biotope classification schemes.

EUNIS Level	EUNIS Code	MNCR Code	MNCR Description
3	A4.2	CR.MCR	Moderate energy circalittoral rock
4	A4.21	CR.MCR.EcCr	Echinoderms and crustose communities
4	A5.13	SS.SCS.ICS	Infralittoral coarse sediment
4	A5.15	SS.SCS.OCS	Offshore circalittoral coarse sediments
5	A5.15[5]	SS.SCS.OCS.[PoGintBy]	Polychaete-rich <i>Galathea</i> community with encrusting bryozoans and other epifauna on offshore coarse sediment
5	A5.15[6]	SS.SCS.OCS.[Sbom]	<i>Spiophanes bombyx</i> aggregations in offshore coarse sands
5	A5.15[7]	SS.SCS.OCS.[PtriGintFaCr]	<i>Placostegus tridentatus</i> and <i>Galathea intermedia</i> on faunal encrusted gravelly sands and sandy gravels
5	A5.15[8]	SS.SCS.OCS.[AbilEpusFaCrPo]	<i>Aponuphis bilineata</i> and <i>Echinocyamus pusillus</i> in faunal encrusted polychaete-rich offshore circalittoral gravelly sands and sandy gravels
5	A5.233	SS.SSa.IFiSa.NcirBat	<i>Nephtys cirrosa</i> and <i>Bathyporeia</i> spp. in infralittoral sand
4	A5.27	SS.SSa.OSa	Offshore circalittoral sand
5	A5.272	SS.SSa.OSa.OfusAfil	<i>Owenia fusiformis</i> and <i>Amphiura filiformis</i> in offshore circalittoral sand or muddy sand
5	A5.27[4]	SS.SSa.OSa.[Sbom]	<i>Spiophanes bombyx</i> aggregations in offshore sands
5	A5.27[5]	SS.SSa.OSa.[AbilEpusPo]	<i>Aponuphis bilineata</i> and <i>Echinocyamus pusillus</i> in polychaete rich offshore circalittoral sands
5	A5.27[6]	SS.SSa.OSa.[MoePo]	<i>Moerella pygmaea</i> and sparse polychaetes in offshore circalittoral sands
5	A5.27[7]	SS.SSa.OSa.[PjefAfilEc]	<i>Paramphinome jeffreysii</i> , <i>Amphiura filiformis</i> and other echinoderms in stable offshore circalittoral sand
5	A5.27[8]	SS.SSa.OSa.[EpusFaCrPo]	<i>Echinocyamus pusillus</i> in polychaete-rich, deep circalittoral sands with some faunal encrusted gravel
4	A5.37	SS.SMu.OMu	Offshore circalittoral mud
4	A5.45	SS.SMx.OMx	Offshore circalittoral mixed sediments
5	A5.45[2]	SS.SMx.OMx.[PoGintBy]	Polychaete-rich <i>Galathea</i> community with encrusting bryozoans and other epifauna on offshore circalittoral mixed sediment
5	A5.611	SS.SBR.PoR.SspiMx	<i>Sabellaria spinulosa</i> on stable circalittoral mixed sediment

Once biotopes were assigned to each sample, these were cross-checked against the biotopes assigned to collocated video and still images. There was a relatively low level of correspondence between the biotopes assigned to the videos and stills and those assigned to the benthic grab samples, with just over a half matching at the habitat level and just over a third matching at the biotope level. The primary reason that the classifications differed was that a different substrate had been recorded, which is a reflection of the different scales of sampling as well as the local heterogeneity in sediment type. The remaining differences were as a result of the video and stills samples being assigned to biotopes of a different biological zone. For example, numerous samples collected from the deep-circalittoral had been assigned to circalittoral biotopes. This again is largely attributable to the gaps that are known to exist in the EUNIS and MNCR biotope classification schemes, but is also a reflection of the methods used to assign biotopes to these photographic imagery samples.

Features of Conservation Interest

MPA Search Features

The data examined as part of this study were screened for the presence of MPA search features including “*seabed habitats and their component biotopes and species*” and “*low or limited mobility species*” which highlighted a number of features that should be considered during the development of the Scottish MPA network:

- **Offshore Subtidal Sands and Gravels**

The majority of the samples analysed as part of this study fall under the MPA search feature of offshore subtidal sands and gravels of which SS.SCS.OCS and SS.SSa.OSa are all component biotopes. Given the gravel component, SS.SMx.OMx is also being considered under this search feature.

- **Offshore Deep Sea Muds**

Of the samples examined as part of this project there were five (NS002-A and NS008-A -D) that were identified as offshore muds (SS.SMu.OMu) which would fall under the MPA search feature of Offshore Deep Sea Muds.

- **Ocean Quahog Aggregations**

Seventy nine ocean quahog, *Arctica islandica*, were recorded in the grab samples analysed as part of this study, most of which were sampled from the Firth of Forth Banks and the North Sea. Of these, 22 samples contained more than one individual and may be considered the search feature “ocean quahog ‘aggregations’”.

- **Northern Sea Fan and Sponge Communities**

Whilst no sea fans were recorded in the benthic grab samples used for this study, a number of the species associated with this community were identified at six stations (FF005-B, WS009-D, WS027-B, WS038-C, WS042-D and WS045-C) and these would warrant further investigation in light of the fact that the main characterising species are unlikely to be adequately sampled using a sediment grab.

Annex I Reef Features

Annex I of the EC Habitats Directive lists a number of seabed communities that fall under the classification of “Reef”. These include biogenic reefs formed by mussels, tubicolous polychaetes and deep-sea corals, as well as rocky and stony reefs which are geological in origin but which often support a diverse epifaunal community including sponges and ascidians. Because of their high conservation status, Scotland has a commitment to protect these reef habitats through the MPA network, and hence information regarding the whereabouts of these features is very valuable.

Examination of the benthic grab data used in this study revealed evidence of both biogenic, and rocky and stony reef as summarised below:

Biogenic reef

High abundances (≥ 20 per grab sample) of the biogenic reef forming species, *Sabellaria spinulosa* were recorded in three samples collected from a single station in the Firth of Forth survey area (Station FF056). The abundance of *S. spinulosa* and the diversity of fauna present in these samples are indicative of *S. spinulosa* reef. However, information regarding the topographical height, the extent and ideally the longevity of the aggregation would be needed before it could be assessed against the EC habitats Directive reef criteria. No other biogenic reef forming species were identified in the course of this study.

Rocky and Stony Reef

One hundred samples were found to support fauna indicative of rocky or stony reef and a further 35 samples were found to support some fauna indicative of stony and rocky reefs. However, only 2 of these samples were taken in substrates indicative of stony and rocky reef ($> 10\%$ Cobbles (> 45 mm)). The vast majority of the samples that were found to support fauna indicative of rocky or stony reef were collected from the Windsock survey area, which is generally characterised by very stable coarse sediments.

The cup coral *Caryophyllia smithii* was also identified in a single sample collected from the Firth of Forth Banks (FF005-B). The grab sample in which *C. smithii* was identified was found to contain some fauna recorded in the biotopes CR.MCR.EcCr.CarSwi, CR.MCR.EcCr.CarSp and CR.MCR.EcCr.FaAlCr, although all of these biotopes are described on the basis of conspicuous epifauna present, so it is difficult to determine the degree of overlap. Since all of these biotopes fall under the EC Habitats Directive definition of reef, examination of the video footage and still images collected at this site would be advisable.

Rare and Alien Species

A number of rare and alien species were identified in grab samples taken across Scotland's seas, these are summarised below.

Table 3. Summary of the rare and alien species identified in samples taken from the Firth of Forth Banks, North Sea, North East Atlantic and Windsock between July and November 2011.

Species	Common Name	Status	Abundance				Total
			Firth of Forth	North East Atlantic	North Sea	Windsock	
<i>Arctica islandica</i>	Ocean Quahog	OSPAR Listed	61	~	17	1	79
<i>Okenia leachii</i>	A sea slug	Nationally Rare	1	~	~	~	1
<i>Paradulichia typica</i>	Amphipod	Nationally Rare	~	~	1	~	1
<i>Escharoides mamillata</i>	Bryozoan	Nationally Rare	~	~	~	2	2
<i>Tamarisca tamarisca</i>	Sea Tamarisk	Nationally Scarce	2	~	~	~	2
<i>Harpinia laevis</i>	Amphipod	Nationally Scarce	1	~	~	~	1
<i>Paracentrotus lividus</i>	Purple Sea Urchin	Nationally Scarce	1	~	~	~	1
<i>Eriopisa elongata</i>	Amphipod	Nationally Scarce	~	~	3	~	3
<i>Mendicula pygmaea</i>	Venerid Bivalve	Not found in UK	~	~	14	~	14

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Glossary of Acronyms

ANOSIM	Analysis of Similarities
EUNIS	European Nature Information System
GEBCO	General Bathymetric Chart of the Oceans
GIS	Geographical Information System
IBTS	International Bottom Trawl Survey
IUCN	International Union for the Conservation of Nature and Natural Resources
MDS	Multi-Dimensional Scaling Ordination
MNCR	Marine Nature Conservation Review
MPA	Marine Protected Area
NBN	National Biodiversity Network
OSPAR	Oslo-Paris Convention for the Protection of the Marine Environment of the North East Atlantic
PRIMER	Plymouth Routines in Multivariate Ecological Research
PSD	Particle Size Distribution
SAC	Special Area of Conservation
SIMPER	Similarity Percentage Routine
SIMPROF	Similarity Profile Permutation Test
SPA	Special Protection Area
TREx	Taxonomic Routines for Excel
WoRMS	World Register of Marine Species

1 Introduction

1.1 Scotland's Seas

Scotland has over 11,000km of coastline and its inshore and offshore areas account for approximately 13% of all European seas. The offshore environment in Scotland ranges from shelf sea areas which are generally shallower than 250m (average ~100m) to deep ocean regions with depths greater than 2,000m. The shelf seas are also marked by notable features including granite rises (e.g. Stanton Banks); large sand banks (e.g. Viking Bank) and deep channels (e.g. Beauforts Dyke). The marine environment in Scotland also encompasses a diverse range of tidal regimes including sheltered sea lochs, shallow bays and estuaries, and long straight stretches of coastline that have little shelter from waves and storms. The mixing of warm and cold-water currents further enhances the physical diversity of Scottish waters making them an important place for marine species and habitats.

The Marine (Scotland) Act 2010 provides a framework to help balance competing demands on our maritime environment, integrating the economic growth of industry with the need to protect Scotland's seas. Where new conservation measures are needed, these may be implemented at a national or regional level (e.g. through marine planning); targeted at specific species (e.g. improved protection for seals); or delivered within key locations (e.g. through the identification of Marine Protected Areas - MPAs). The powers in the Act complement the provisions of the UK Marine and Coastal Access Act 2009, giving Marine Scotland responsibility for marine planning and marine nature conservation in territorial and offshore waters.

1.2 Marine Protected Areas

The Marine (Scotland) Act 2010 and the UK Marine and Coastal Access Act 2009 include new powers for Scottish Ministers to designate Marine Protected Areas (MPAs) in the seas around Scotland as part of a range of measures to manage and protect Scotland's seas. The Scottish MPAs will be selected using a science-based approach through the collaborative Scottish MPA project.

The development of the MPA network must reflect the known biogeographic differences across Scotland's seas. Initially the Scottish waters were split into a useful framework of regions within which it was possible to assess whether or not the geographic range and known ecological variation of MPA search features had been captured. However, there is a need to consider ecological coherence within the broader context of OSPAR to help ensure the network delivers its objectives related to OSPAR. As such the Scottish MPA project adopted the OSPAR regions for this purpose and used them to support the assessment of adequacy and the contribution of proposals to the network in Scotland's seas and wider networks (Figure 1).

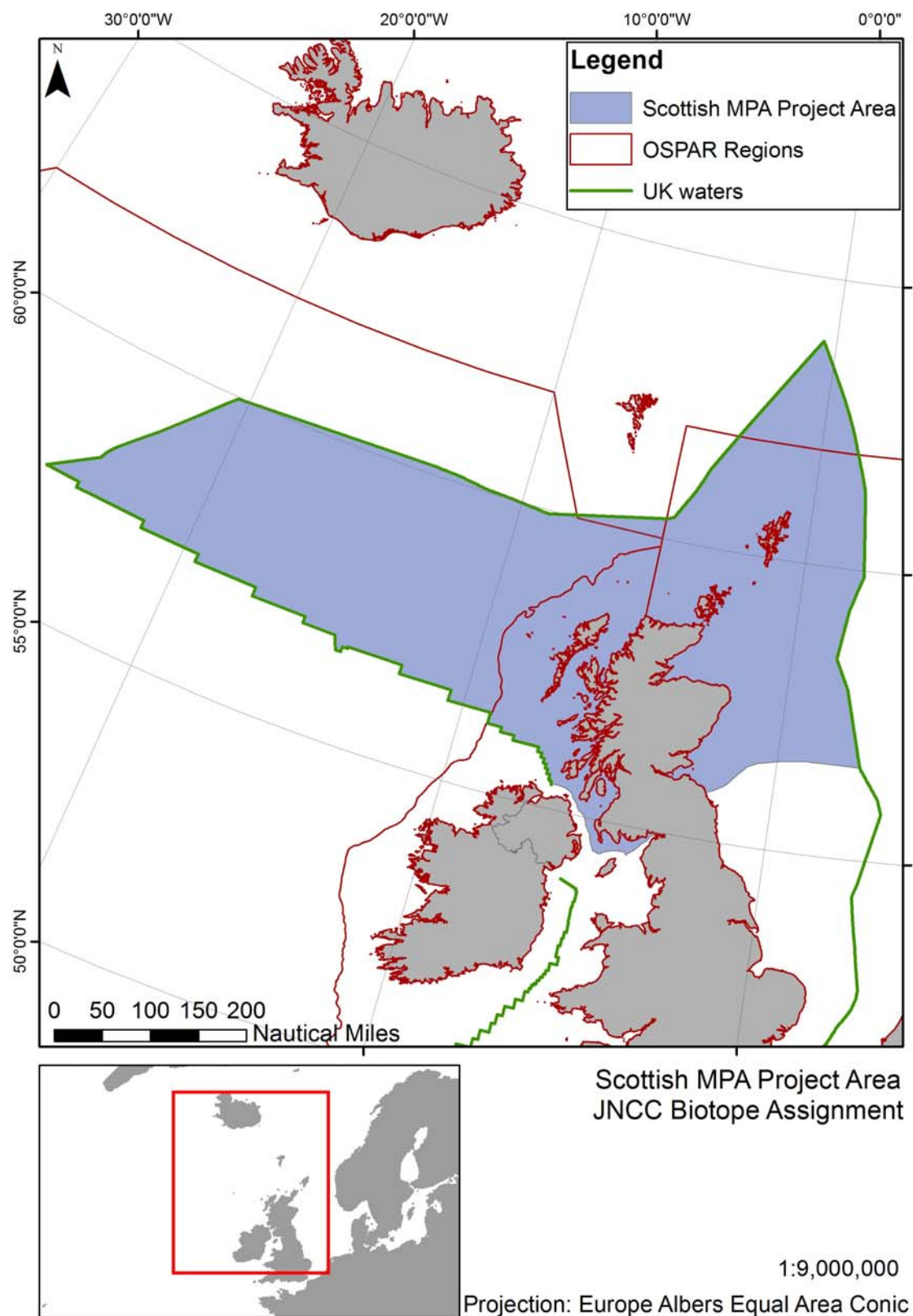


Figure 1. Map showing the extent of Scottish waters and the MPA regions and the OSPAR Regions.

The MPA network in Scottish waters will comprise existing protected areas, primarily European Marine Sites (marine areas designated as Special Areas of Conservation (SACs) under the EC Habitats Directive and Special Protection Areas (SPAs) under the EC Birds Directive), as well as those subject to other types of area-based management and MPAs designated under the new legislation.

A discrete list of habitats, species and large-scale features of functional importance to Scotland's seas (collectively termed MPA search features) will drive the identification of the Nature Conservation MPAs, these are listed below in Table 4.

Table 4. List of MPA search features being used to underpin the selection of Nature Conservation MPAs within Scottish waters (Marine Scotland, 2011).

Seabed Habitats	Low or Limited Mobility Species
Blue mussel beds	Burrowing sea anemone aggregations
Burrowed mud	Northern sea star aggregations on mixed substrata
Carbonate mound communities	Fan mussel aggregations
Coral gardens	Heart cockle aggregations
Deep sea sponge aggregations	Ocean quahog aggregations
Flame shell beds	Mobile Species
Horse mussel beds	European spiny lobster
Inshore deep mud with burrowing heart urchins	Blue ling
Kelp and seaweed communities on sublittoral sediment	Orange roughy
Low or variable salinity habitats	Sandeels
Maerl beds	Basking shark
Maerl or coarse shell gravel with burrowing sea cucumbers	Common skate
Native oysters	Minke whale
Northern sea fan and sponge communities	Risso's dolphin
Offshore deep sea muds	White-beaked dolphin
Offshore subtidal sands and gravels	Black guillemot
Seagrass beds	Large-scale Features
Sea loch egg wrack beds	Fronts
Seamount communities	Shelf banks and mounds
Shallow tide-swept coarse sands with burrowing bivalves	Shelf deeps
	Continental slope
Tide-swept algal communities	Seamounts

1.3 Seabed Classification

Over the past two decades there have been considerable advances in the way that the distribution of seabed resources are mapped, driven by the inherent value of such maps for marine planning and conservation. One of these advances has been to standardise the way in which marine habitats are classified. This work began in the UK with a national programme called the Marine Nature Conservation Review (MNCR) through which the Marine Habitat Classification for Britain and Ireland (Connor *et al* 2004) was developed. This has now been adopted and further developed at the European level to provide a single classification system that is applicable across the whole of Europe. The agreed classification is part of the European Nature Information System, commonly referred to as the “EUNIS” system (Davies & Moss 2004). Both of these classification systems are hierarchical in that they first define the physical habitat and then describe the biological communities that occur within these habitats, referred to hereafter as biotopes.

A “**biotope**” is an area of uniform environmental conditions providing a living place for a specific assemblage of plants and animals. Biotope is almost synonymous with the term habitat, which is more commonly used in English-speaking countries. However, in some countries these two terms are distinguished: the subject of a habitat is a species or a population, the subject of a biotope is a biological community (Moss *et al* 1994).

1.4 Aims and Objectives

A number of research projects have been commissioned to collate existing data on the distribution of MPA “search features” and a number of surveys have been carried out to provide new data in areas where there are gaps in our understanding. These include areas that have been identified by UKSeaMap 2010 (McBreen *et al* 2011) as being sandy gravel habitats which are not well documented in Scottish waters.

Data from four of these surveys have been used in this study:

- The Firth of Forth survey, took place across the Firth of Forth Banks Complex in the North Sea, aboard the NLV Pole Star, focussing on an area of sandy gravel habitat. This area was identified as being an important habitat for sandeels and was chosen based on the bank and mound features as well as its classification of ‘least damaged/more natural’.
- The North Sea survey was conducted as part of the Marine Scotland Science International Bottom Trawl survey (IBTS Q3) in July 2011. During the course of this survey subtidal sands and gravels, and burrowed mud were sampled.
- The North East Atlantic survey took place in November 2011 aboard the FRV Scotia during the Marine Scotland Science IBTS Q4. Only three grab samples were collected on this survey due to poor weather.
- The Windsock survey took place from 17 September to 2 October aboard the FRV Scotia in a fisheries closure area north of Rona which is one of very few areas around Scotland known to contain sublittoral sand biotopes in offshore shelf waters.

In 2012 JNCC commissioned Gardline Caledonia Ltd. to undertake analysis of benthic grab data collected during these four surveys in order to assign biotope classifications and identify potential features of conservation interest, more specifically to:

- Match sediment granulometry or Particle Size Distribution (PSD) data and benthic species abundance data collected from the North Sea, Windsock, the Firth of Forth and the North East Atlantic surveys, to recognised biotopes listed in the Marine Habitat Classification for Britain and Ireland (version 04.05).
- Propose new biotopes that describe the environmental conditions and faunal assemblages recorded in the grab samples, where recognised biotopes are found to be inadequate and sufficient evidence exists.
- Compare biotopes assigned to the grab samples with biotopes assigned to video footage and stills images taken in the same locations to provide an assessment of the accuracy of using these methods when used in isolation or in combination for the purpose of biotope assignments.
- Identify evidence of MPA search features as well as any other features of conservation significance (e.g. Annex I habitats, rare and alien species) that occur in the areas surveyed.

2 Methodology

2.1 Data Organisation and Cleaning

Since the data provided had been collected opportunistically as well as through targeted surveys, a degree of data organisation, truncation and formatting was required before the data could be used for biotope assignment purposes. The processes undertaken to organise and clean the data are clearly documented in audit trails associated with each of the raw data spreadsheets which are provided as appendices to this report (Electronic Appendices 5.1 and 5.2). Each stage in the data organisation and cleaning was quality checked and where necessary the data were revisited to address any issues identified.

2.1.1 Data Labelling

As the data were collected by a number of different organisations and for a number of different purposes the labelling conventions applied to the data were not consistent and there was some duplication in the sample labels. A new labelling convention was therefore applied to the data to ensure that it could be formatted for use in the PRIMER (Plymouth Routines in Multivariate Ecological Research) software and to provide a means of quickly identifying the survey of origin. The labelling convention applied, included a two letter code to identify the survey of origin (FF = Firth of Forth, NS = North Sea, NA = North East Atlantic and WS = Windsock) followed by the station number (e.g. 001) and the sample replicate (e.g. -A). Original labels were retained as a “factor” in the data matrices for ease of cross-referencing.

2.1.2 Truncation and Cleaning of Benthic Data

Juveniles were listed separately in the benthic data supplied for this study, which also contained non-specific identifications e.g. *Syllis* Type A, making it necessary to truncate and clean the data before it could be used for biotope assignment purposes. It was also necessary to standardise the species lists since several different laboratories were responsible for the sample analysis and a number of species occurred in the data under different synonyms and / or spellings. The species lists were combined and standardised in accordance with the World Register of Marine Species (WoRMS; <http://www.marinespecies.org/>). The full list of species names and their status in WoRMs is provided in Electronic Appendix 5.1.1. Full details of the subsequent data truncation are provided in the audit trail attached to each of the benthic datasets (Electronic Appendices 5.1.2 - 5.1.5).

2.1.3 Transformation of Sediment Data

The raw PSD data used in this project is provided in Electronic Appendix 5.2.1. PSD data had not been collected for all of the samples examined. Wherever possible these data gaps were filled by extrapolating PSD data belonging to the same sampling station. Where no PSD data had been collected for the sampling station, EUNIS sediments extracted from UKSeaMap 2010 (McBreen *et al* 2011) were used as a proxy. Sediment data were provided in Excel spreadsheets as raw PSD data and in some instances as sediment classes based on modified Folk (Long 2006). Raw PSD data were used to define the sediment classes where they had not been supplied and sediment classes supplied were transformed so that they conform to the classes proposed in UKSeaMap 2010 (McBreen *et al* 2011). “Sand and muddy sand” and “Mud and sandy mud” classes were identified using the sand to mud ratios, to allow the assignment of EUNIS Level 4 biotopes. These were then also transformed into EUNIS Level 3 biotopes and equivalent MNCR habitat classes. The project team developed a macro in Excel for the transformation of PSD data to these sediment classes which improved the efficiency of this process (Electronic Appendix 5.2.3).

2.1.4 Formatting of Video/Stills Data

The results of the video and stills image analysis were formatted during the data organisation and cleaning process to ensure consistent labelling throughout. Original sample labels were retained (as a factor) in all data matrices for quality assurance and cross-referencing purposes (Electronic Appendix 5.1.6).

2.1.5 Additional Environmental Data Extraction

Environmental data were extracted from UKSeaMap 2010 (McBreen *et al* 2011) layers and the General Bathymetric Chart of the Oceans (GEBCO) as well as being derived from the data supplied by JNCC, for each sampling station. These data were collated in an Excel spreadsheet for use in PRIMER analyses to identify the preferred environmental niche of the faunal assemblages identified. The nature and source of the environmental data used in this study is listed below in Table 5. All of the environmental data used are provided in Electronic Appendices 5.2.1 - 5.2.3.

Table 5. List of the environmental data sets used to define the environmental niche of faunal groups identified through multivariate analysis.

Environmental Data	Description	Source
Latitude	Geographical position of the sampling station	Supplied by JNCC as shape files and in spreadsheets
Longitude	Geographical position of the sampling station	Supplied by JNCC as shape files and in spreadsheets
Area	Survey area from which the samples were collected	Derived from the data provided
Depth	Water depth at which sample was taken (m)	Extracted from GEBCO (http://www.gebco.net/)
Biological Zone	Deep Circalittoral, Circalittoral or Infralittoral	UKSeaMap 2010 (McBreen <i>et al</i> 2011)
Energy	Combined wave and tidal energy (High, Moderate or Low)	UKSeaMap 2010 (McBreen <i>et al</i> 2011)
PSD	Granulometry data collected from grab samples	Supplied by JNCC as Excel spreadsheets
% Gravel, Sand and Mud	% Gravel, Sand and Mud recorded in collocated PSD samples	Calculated from the raw PSD data
Mean Grain Size	Mean (arithmetic average) particle size (mm) recorded from collocated PSD samples (Folk & Ward, 1957)	Calculated from the raw PSD data
Sorting	Sorting (standard deviation) of the particle size (mm) recorded from collocated PSD samples (Folk & Ward, 1957)	Calculated from the raw PSD data
Skewness	Skewness (asymmetry) of the particle size (mm) distribution recorded from collocated PSD samples (Folk & Ward, 1957)	Calculated from the raw PSD data
Kurtosis	Kurtosis (the degree of "bimodality") of the particle size (mm) distribution recorded from collocated PSD samples (Folk & Ward, 1957)	Calculated from the raw PSD data

2.2 Biotope Assignment

The primary objective of this study was to assign biotopes to the grab data collected across four surveys areas in Scottish waters. This was achieved by identifying faunal assemblages, investigating their relationship with the physical environment and then matching them to existing biotopes. Where it was not possible to identify a good match with existing biotopes, new ones were proposed.

2.2.1 Identification of Faunal Assemblages

Multivariate analyses were carried out using the PRIMER version 6 software package (Clarke & Gorley 2006; Clarke & Warwick 2001).

Data Transformation

Data used in this project were collected using a variety of different sampling gear namely a Day grab, a Hamon grab and a box grab. To correct for the differences in the resulting sample volume the benthic abundance data was standardised. However, a number of trials were also undertaken to test the applicability of different transformations for the purpose of biotope assignment, the results of which are presented in Electronic Appendices 5.3.1 - 5.3.8.

Hierarchical Cluster Analysis

Cluster analysis is employed to identify natural groupings in the data, such that samples within a group are more similar to each other than samples in different groups. The most commonly used clustering techniques (and those used in this study) are the hierarchical agglomerative methods. These start with a similarity matrix and “fuse” the samples into groups then groups into clusters, starting with the highest level of mutual similarity then gradually lowering the similarity level at which groups are formed until all of the samples are contained in a single cluster. The results of hierarchical clustering are represented by a tree diagram or dendrogram, with the x axis representing the full set of samples and the y axis representing the similarity level at which the groups are considered to have fused.

Delineation of Groups from the Cluster Analysis: The Similarity Profile Permutation (SIMPROF) Test & % Similarity Cut-Offs

Once cluster analysis has been carried out on a data set, it is necessary to delineate groups which are likely to represent ecologically meaningful faunal assemblages. This was once done by eye but is now carried out in a more systematic and scientifically robust way using % similarity cut-offs and the SIMPROF test. Cut-off's are relatively arbitrary although the chosen level of similarity can be defined according to the types of groups you are hoping to identify. For example, a lower level of similarity will give broader assemblages more akin to biotopes whilst a higher level of similarity would give very distinct groups which might reflect local-scale variability. The SIMPROF test looks for statistically significant evidence of genuine clusters in samples. Tests are performed at every node in the dendrogram, to see whether the group that has been divided has significant internal structure. The latter method is the most scientifically robust method but groups identified may not be at an appropriate level for management purposes. The critical piece of information to be gained from a SIMPROF test is that groups identified as having significant internal structure should not be split. Amalgamating those groups at a lower level of similarity is however acceptable and often required if broad grouping or trends are being investigated.

Both methods were utilised to define groups in the data used in this study and the results of these are provided in Electronic Appendices 5.3.1 – 5.3.8. A summary of the benthic species composition for each of the faunal assemblages identified is provided in Electronic Appendix 5.3.9.

Multi-Dimensional Scaling (MDS) Ordination

Multi-Dimensional Scaling ordinations are essentially a “map” or configuration of the samples in multidimensional space. The configuration attempts to position the samples as accurately as possible to reflect the similarity between the samples. For example, if sample 1 has a greater similarity to sample 2 than it does to sample 3, then sample 1 will be positioned closer to sample 2 than it is to sample 3. This “map” of the relative similarities between samples is then plotted in two dimensions. It is important to remember that this two-dimensional plot is a representation of a multi-dimensional picture. When large numbers of samples are analysed, or datasets include samples that are very different from one another, the accuracy of the two-dimensional plot may be reduced. A measure of the accuracy of the two-dimensional representation (stress) is given on the MDS plot. Stress values <0.1 correspond to a good ordination; values between 0.1 and 0.3 give a useful two-dimensional picture but one should not place too much reliance on the fine details of the plot; stress >0.3 indicates that the samples are close to being positioned in an arbitrary manner and should not be regarded as necessarily similar to one another, particularly in the upper half of this range.

The Similarity Percentage (SIMPER) Routine

The SIMPER routine allows a group of samples to be compared with other groups of samples. Species (or other variables) responsible for the dissimilarity between the two groups are then listed in decreasing order of importance in the discrimination of the groups of samples. This routine also provides information on which species are responsible for the within-group similarity and how much they contribute to this similarity.

Investigating the Environmental Niche Requirements of the Faunal Assemblages

A number of routines were employed within PRIMER to investigate the environmental niche requirements of the faunal groups identified through cluster analysis (Electronic Appendix 5.3.10).

The **RELATE** test, a non-parametric form of the Mantel test, was used to test for correlations between the multivariate patterns in the biological data and different combinations of environmental data. This test measures the level of agreement between the two datasets as a correlation coefficient, in this case Spearman correlation coefficient (denoted as rho or ρ). A Spearman correlation of 0 indicates that there is no correlation between the two datasets (or variables) being compared. A Spearman correlation of 1 indicates that the two datasets (or variables) being compared are monotonically related, although their relationship may not be linear.

The **BIO-ENV** routine was used as an exploratory tool to identify the combination of environmental variables (see Table 5) that correlated best (highest ρ value) with the biological community patterns.

Finally, a **LINKTREE** was created to identify the key differences in the environmental niche requirements of the different faunal assemblages. The LINKTREE algorithm creates a series of successive divisions in the biological data such that the degree of separation between each group is maximised. An absolute measure of the difference between groups is presented on the y-axis of the tree as B%. B% is generally largest for the first split in the data (A) and then declines as groups become more similar to one another. The LINKTREE results also provide the significance of each split measured as ANOSIM-R where large values (approaching 1) are indicative of complete separation between the groups and small values (close to zero) imply little or no separation. Note that the ANOSIM R values have no tendency to decrease or increase as you move down the tree as they are re-scaled for each subset of samples. The LINKTREE routine also identifies the environmental parameters that best correlate with the splits in the biological data making it a very useful tool to explore environmental niche requirements.

2.2.2 Biotope Matching

Multivariate Biotope Matching

The biological data for each of the faunal assemblages identified through multivariate analysis of the grab data was converted to % occurrence and was then re-analysed in PRIMER with the corresponding data for existing biotopes. A cluster analysis and SIMPROF test were then employed to identify existing biotopes that were not statistically separable from the faunal assemblages. The results of these analyses are summarised in subsequent sections of this report and are provided as an Electronic Appendix (5.3.11).

Manual Biotope Matching

In parallel to the multivariate biotope matching, faunal assemblages were cross-checked manually with existing biotopes listed on the JNCC website and in the biotope summary data provided by JNCC. To aid this process, spreadsheets were populated with a list of the characterising species, identified using the SIMPER routine. The characterising species list was limited to species contributing to 50% of the internal group similarity or, where the list was lengthy, the top five characterising species. Environmental parameters were also added to the summary including dominant sediment type, energy and derived EUNIS Level 3 habitat codes (Table 5).

Once the summary information for each faunal group had been collated, the genera that characterising species belong to were entered individually into the JNCC web-based biotope search tool. Where a family or higher level taxonomic group was included in the list of characterising species, all genera belonging to that family / higher level were used as search terms in addition to the family/higher level names as appropriate. Where species names did not occur in biotope data, known synonyms were also used as search terms. All biotopes identified in the web search were transposed to the summary spreadsheets including the number of matches with the search terms used.

The biotope summary data supplied by JNCC were transposed to allow for bespoke data sorting for each faunal group identified in the analysis. Using the transposed data a series of data sorting criteria were applied such that the data was organised by the % occurrence of the characterising species in their order of importance. In this way biotopes which match the identified faunal groups best, that is those which have the highest number of characterising taxa, appeared at the top of the biotope list. The biotopes which had the most taxa in common with the faunal groups were then examined using expert judgement to assess the degree to which the biotope matched the faunal group. The manual biotope matching has been summarised in a series of spreadsheets provided in Electronic Appendix 5.4.1.

Expert Moderation

Expert moderation was subsequently applied to the biotope matching process to identify biotopes that showed some correspondence to the faunal assemblages identified in this study, but were missed by the multivariate analysis and manual matching. This was considered necessary as many biotopes are described only qualitatively whilst others provide a good match based on ecological functioning rather than species composition.

Where no reasonable match was identified with existing biotopes using either multivariate or iterative species matching, new biotope assignments were proposed (Electronic Appendix 5.5.1) using the proforma and guidance notes supplied by JNCC. In some instances this process involved splitting faunal assemblages where they occurred under very different environmental conditions, for example in two or more different biological zones or substrates. Where this was necessary a second SIMPER analysis was carried out to identify any differences in the faunal composition associated with the different environmental conditions (Electronic Appendix 5.3.12).

2.2.2.1 Biotope Comparisons & Validation

Once all of the benthic grab data were assigned to biotope classifications, these were cross-checked against the biotopes assigned to corresponding video and stills images, selected on the basis of their proximity to the grab samples. Differences were then analysed to ascertain the primary causes and to make recommendations regarding the most appropriate biotope assignment for each station (Electronic Appendix 5.4.2). It should be noted that in some instances, the video and stills images that were closest to the benthic grab samples were over 1km away and as such we would anticipate differences in the biotopes observed.

A full summary of the biotopes ultimately assigned to each sample as well as a description of the observed assemblage and details of the environmental conditions are given in Electronic Appendix 5.5.2.

2.3 Features of Conservation Interest

2.3.1 Annex I Reef Assessment

Annex I of the EC Habitats Directive lists a number of seabed communities that fall under the classification of “Reef”. These include biogenic reefs formed by mussels, tubicolous polychaetes and deep-sea corals, as well as rocky and stony reefs which are geological in origin but which often support a diverse epifaunal community including sponges and ascidians (Irving 2009). Because of their high conservation status, Scotland has a commitment to protect these reef habitats through the MPA network and hence information regarding the whereabouts of these features is very valuable.

The benthic grab data analysed as part of this study were examined for the presence of Annex I features using the following reef indicators;

- High abundances (≥ 20 individuals per sample) of the following biogenic reef forming organisms:
 - Blue mussel, *Mytilus edulis*
 - Horse mussel, *Modiolus modiolus*
 - Ross worm, *Sabellaria spinulosa*
 - Honeycomb worm, *Sabellaria alveolata*
 - Fan worm, *Serpula vermicularis*
- The presence of coral species:
 - Cold water coral, *Lophelia pertusa*
 - Cup coral, *Caryophyllia smithii*
- High abundances (≥ 20 individuals per sample) of solitary encrusting species e.g. barnacles and solitary anemones or high diversity (≥ 10 species per sample) of colonial encrusting species e.g. sponges and bryozoans. As there is a level of uncertainty associated with the quantification of colonial species as well as the relationship between these species and the presence of rocky and stony reef, samples containing moderate abundances of solitary encrusting species (≥ 10 individuals per sample) or a moderate diversity of colonial species (≥ 5 species per sample) were also flagged as having “Some fauna indicative of Bedrock and Stony Reef”.

Encrusting and colonial species data were examined in combination with the corresponding PSD data to help identify rock and stony reef features. These species all require a hard surface for attachment and are therefore indicative of such habitats. A cobble (> 64mm) content of greater than 10% is generally considered necessary for samples to be representative of stony and rocky reef. The maximum sieve size used for the PSD analysis in this study however was 45mm and hence the sediment retained on this sieve was used as a proxy for cobble content. It should be noted however, that it is not standard practice to retain cobbles for PSD analysis as a very large volume of sediment, far exceeding the volume of a grab sample, would be required to accurately analyse the granulometry (British Standards Institution 1996; Passchier, S. 2007). It should therefore be noted that grab samples are unlikely to provide a reliable method of identifying rocky and stony reef features without corresponding video or stills images or the associated field notes.

The reef assessments for each area are supplied as Electronic Appendices to this report (5.6.1 - 5.6.4).

2.3.2 MPA Search Features

The benthic grab data were screened for the presence of habitats and species that are listed as MPA search features or species that are indicative of habitats listed as MPA search features (see Table 4).

2.3.3 Rare and Alien Species

The benthic grab data were screened for the presence of any rare, threatened or alien species using the Taxonomic Routines in Excel (TREx) software (available to download at: <http://www.thomsonecologysoftware.com/trex-2>). This programme identifies species that appear on the OSPAR list of Threatened and / or Declining Species and Habitats (OSPAR Commission, 2008) as well as those listed on the International Union for the Conservation of Nature and Natural Resources (IUCN) Red List of Threatened species (IUCN, 2012). In addition, species that are known to be nationally rare or scarce (Sanderson, 1996) were identified as well as those that are known to be non-native. The version of TREx (3d) used in this study does not recognise the WoRMs nomenclature and hence this analysis was carried out on the cleaned species names as supplied.

The results of the TREx screening are provided in Electronic Appendices 5.1.2 - 5.1.5.

3 Results

A total of 291 grab samples collected from four surveys across Scotland's seas were examined for their environmental and biological composition in order to assign a biotope classification to each and to identify features of potential conservation importance. The location from which each of these samples was collected is presented in Figure 2, along with the name of the survey. Subsequent sections will describe the sediments and faunal assemblages identified in each of these grab samples as well as the biotope that was ultimately assigned.

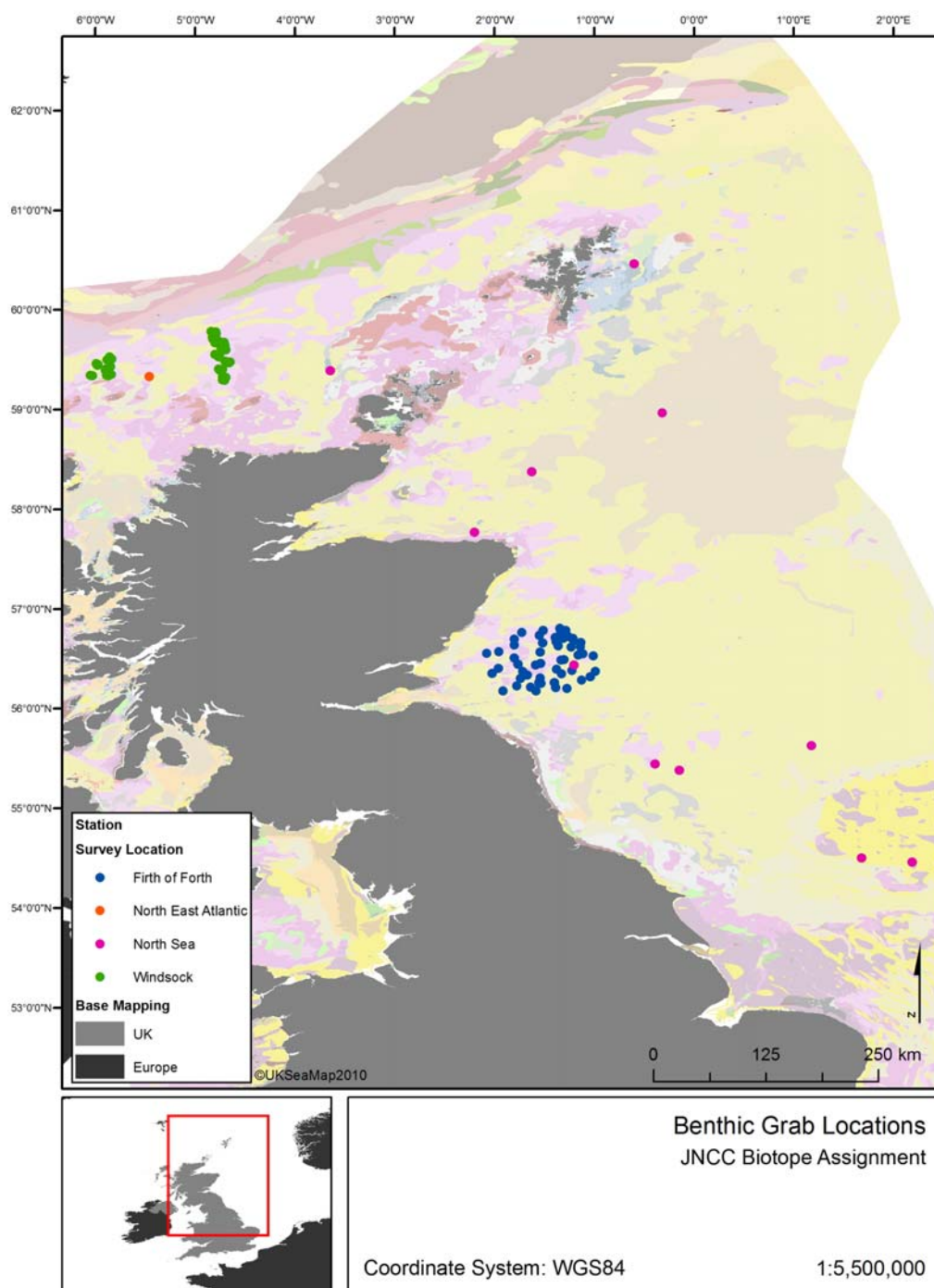


Figure 2. Chart showing the location of stations sampled during four surveys across Scotland's seas (Firth of Forth, North Sea, North East Atlantic and Windsock).

3.1 Sediment Deposits

Granulometry (PSD) data were collected at all stations with the exception of two (NS005 A-D and NS012 A-D). These stations were predicted to be sand by UKSeaMap 2010 and as the fauna recorded were typical of sand deposits they were treated as sand for the purposes of biotope assignment, but were excluded from multivariate analysis requiring environmental data. PSD data were not collected for all samples and hence were extrapolated from other samples taken at the same station, with the exception of five samples which were noted as being rock (WS009D, WS027B, WS038C, WS042D and WS045C). Although no information was provided regarding the nature of these rock samples the fauna again confirmed the presence of hard substrate.

3.1.1 Folk Sediment Classes

Figures 3 and 4 show the folk sediment classes assigned to each sample, overlaid on the UKSeaMap 2010 predicted EUNIS biotope distribution map (McBreen *et al* 2011). Sandy gravel and gravelly sand were the dominant sediment types sampled in all four surveys which broadly correspond with the predicted habitats, whilst also demonstrating the localised variability.

3.1.2 EUNIS Sediment Classes

Figures 5 and 6 show the EUNIS sediment classes derived from the raw PSD data, overlaid on the UKSeaMap 2010 predicted EUNIS biotope distribution map (McBreen *et al* 2011). The majority of stations were classified as either coarse sediment or sand with only two stations being classified as mud and four being classified as mixed sediments. The distribution of EUNIS sediment classes recorded from this study correlates well with the sediments predicted by UKSeaMap 2010 (McBreen *et al* 2011) with the exception of the mixed sediments identified in the Firth of Forth.

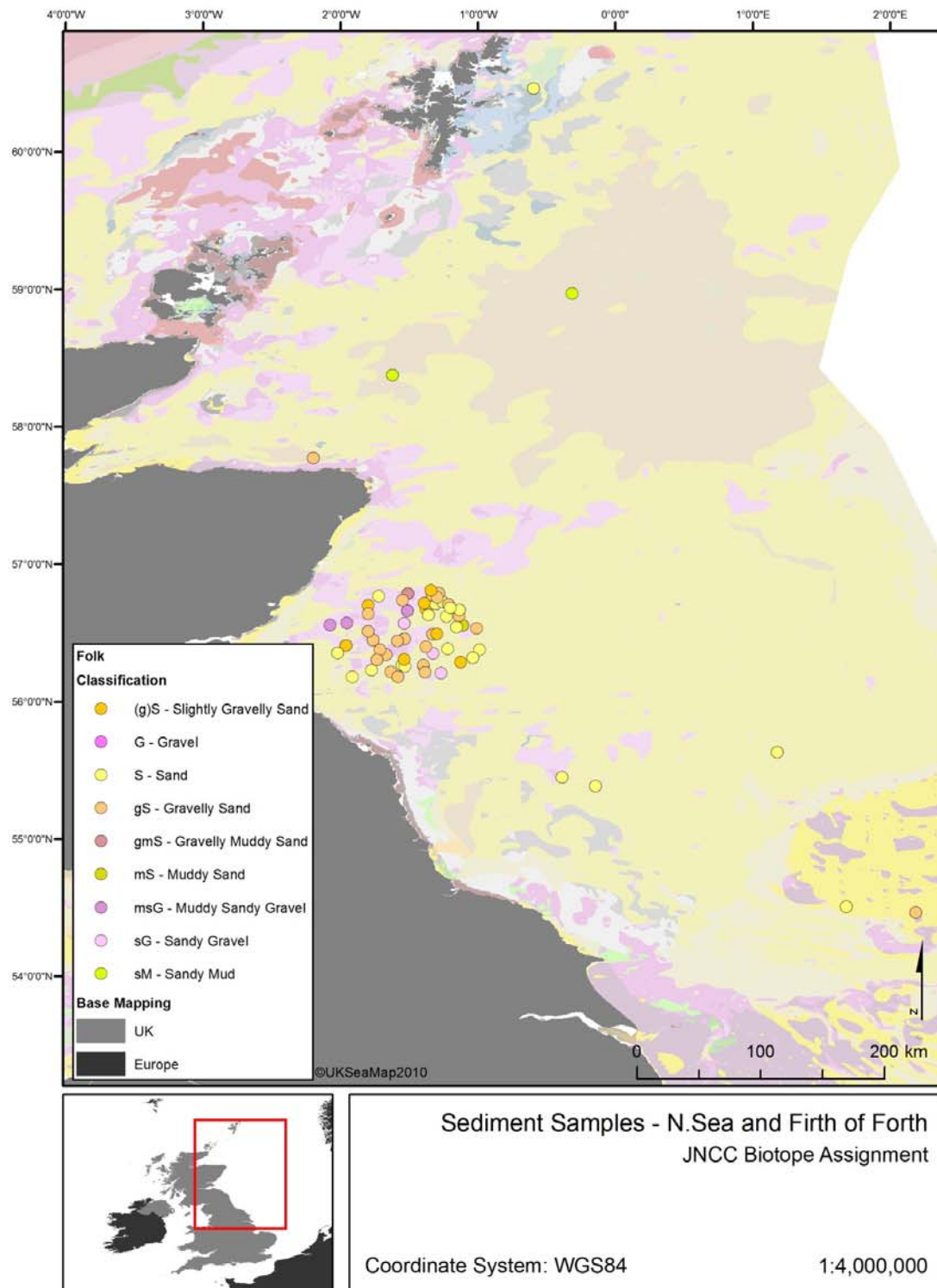


Figure 3. Chart showing the Folk sediment classes identified from each of the samples collected during the Firth of Forth and North Sea surveys.

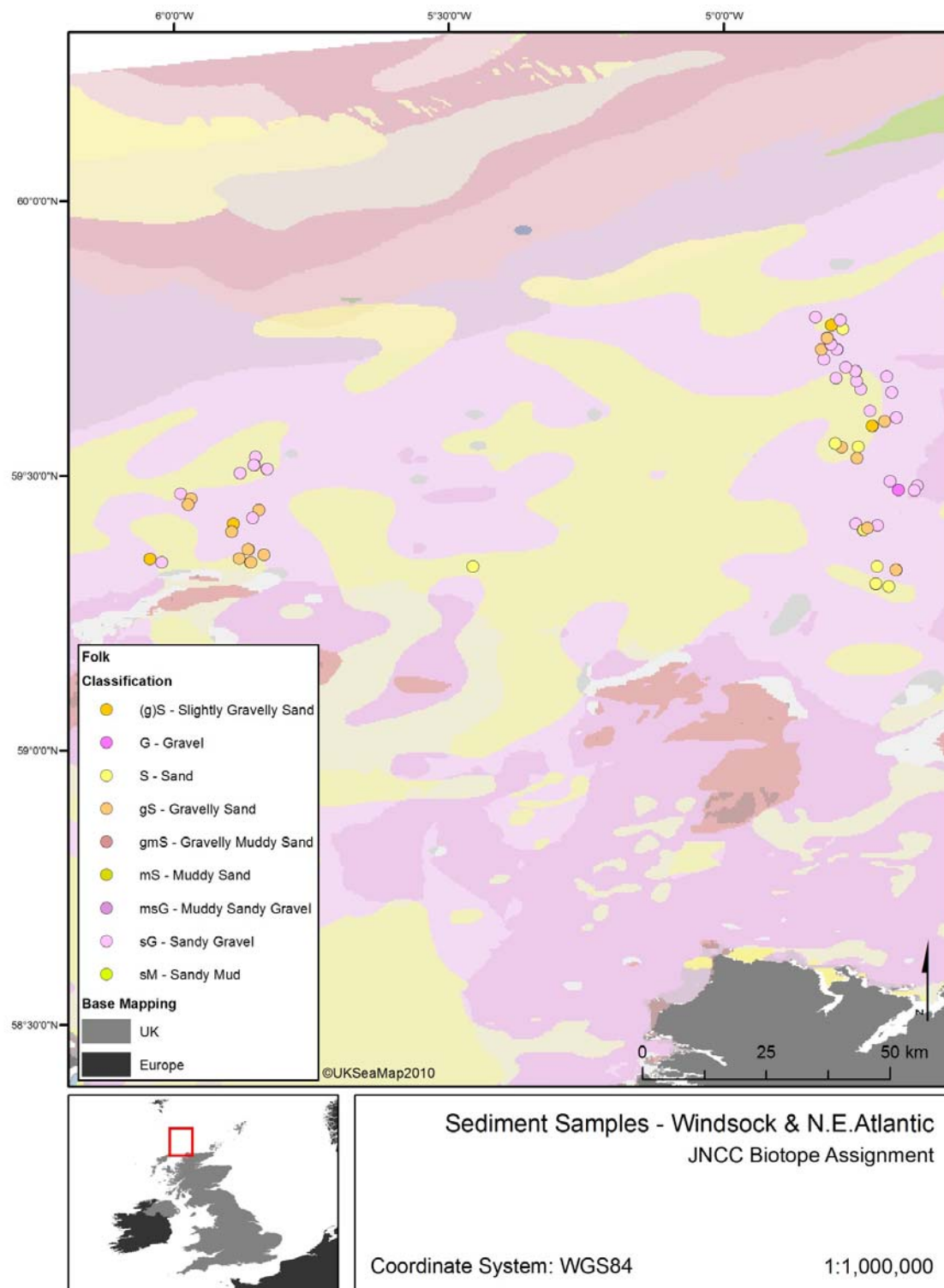


Figure 4. Chart showing the Folk sediment classes identified from each of the samples collected during the Windsock and North East Atlantic surveys.

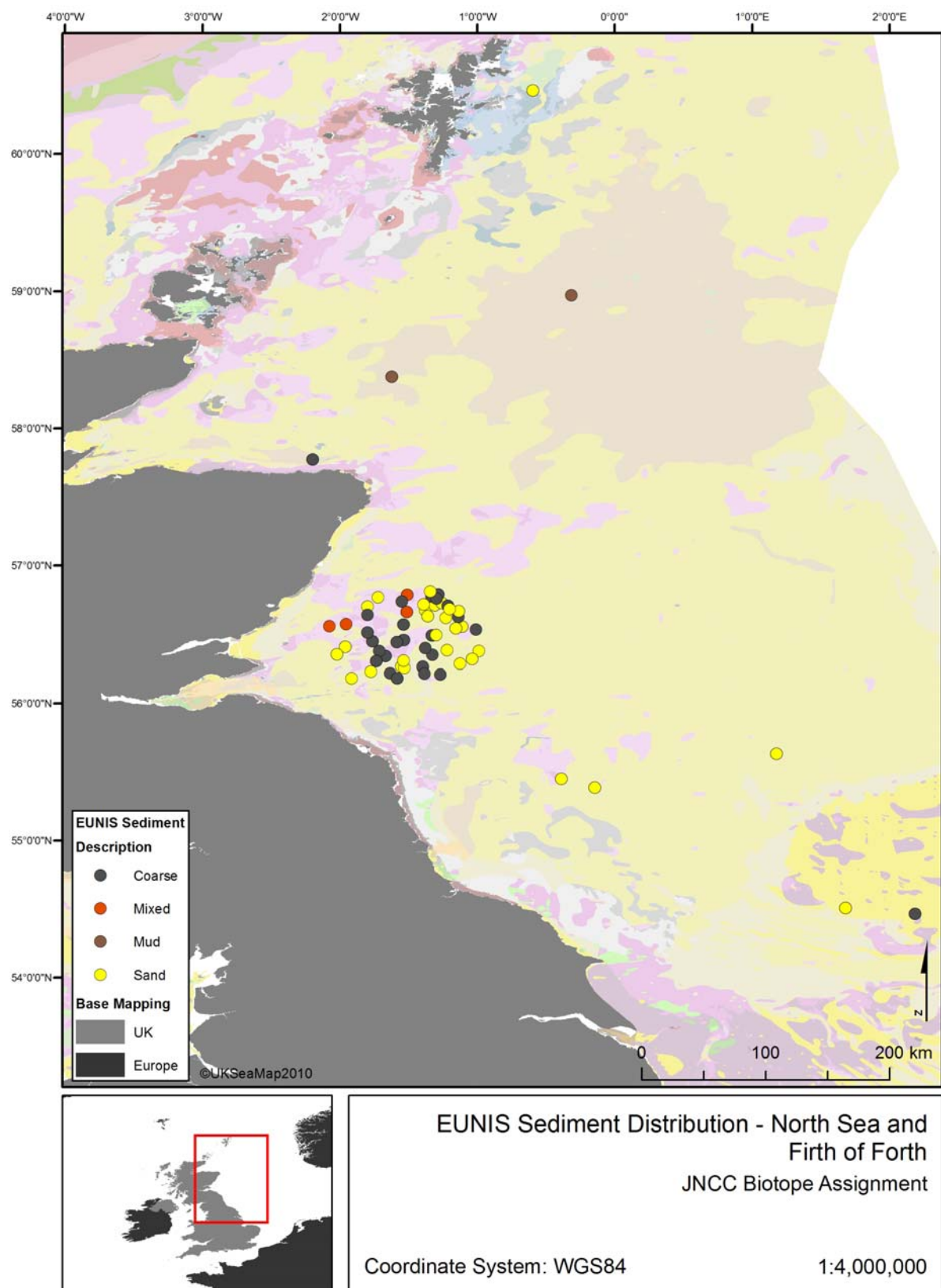


Figure 5. Chart showing the Folk sediment classes identified from each of the samples collected during the Firth of Forth and North Sea surveys.

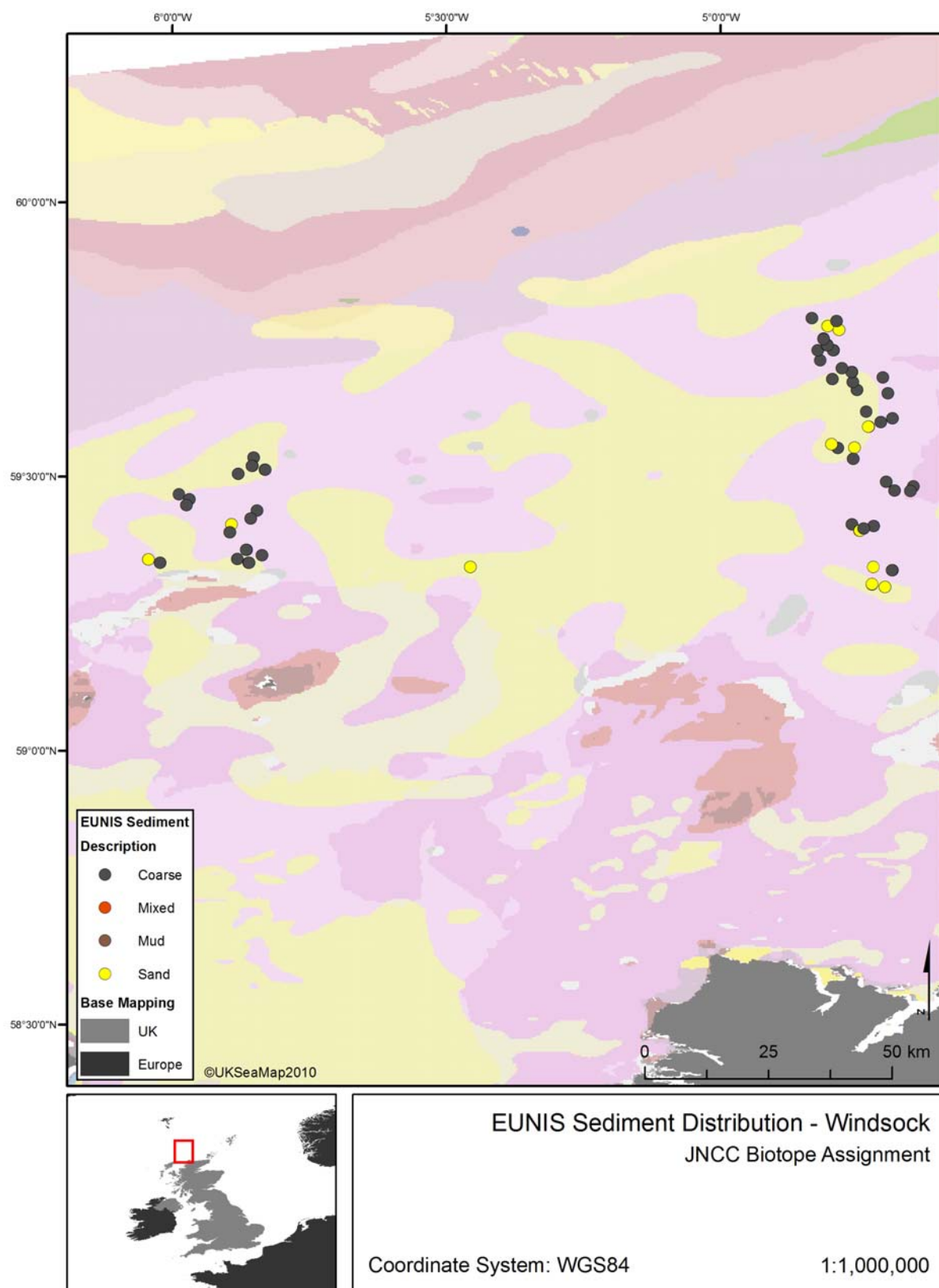


Figure 6. Chart showing the Folk sediment classes identified from each of the samples collected during the Windsock and North East Atlantic surveys.

3.2 Faunal Assemblages

Benthic abundance data collected during four surveys across Scotland's seas (Firth of Forth, North Sea, North East Atlantic and Windsock) were analysed using the multivariate statistical package PRIMER v6 (Clarke & Gorley 2006; Clarke & Warwick 2001). Different sampling gears (of unspecified dimensions) were used across the four surveys and the data were therefore standardised to reduce any influence of sample volume. The data were also subjected to a variety of transformations in order to investigate their applicability for the purpose of biotope assignment, the results of which are presented in Electronic Appendices 5.3.1 - 5.3.8.

The data analysis trials showed that the faunal groups identified through SIMPROF analysis and using a 20% similarity slice were relatively faithful with only minor differences observed as an effect of the different transformations. A slightly larger number of groups were identified using untransformed data indicating that relative abundance makes an important contribution to the structure of these benthic communities. Examination of the SIMPER results revealed that this increase was primarily due to an increase in outlying samples with the core groups remaining consistent with groups identified using transformed data.

The SIMPROF tests revealed a far greater number of groups in the data than the 20% similarity slice indicating that there is significant structure within these communities, even at a very local scale. The detailed groupings identified using the SIMPROF test were not considered useful for describing broad scale faunal assemblages or biotopes and so were used only to inform group divisions later in the process.

Although the SIMPROF test did not prove useful on this occasion it is possible that a better application of this tool could be developed for the purposes of biotope assignment to remove some of the subjectivity associated with other methods, including the similarity slice. The Humber REC for example developed a second stage SIMPROF test which was used successfully in the assignment of biotopes (Tappin *et al* 2011). This would require more testing though before it could be adopted as standard practice.

Following the data transformation and group delineation trials, a 20% similarity slice through a cluster based on Bray-Curtis-similarity of standardised benthic abundance was chosen to identify faunal assemblages occurring across the four surveys. A total of 16 faunal assemblages (a - p) were identified using these methods as illustrated in Figure 7.



Figure 7. MDS plot showing the faunal assemblages derived from a 20% similarity slice through a cluster based on Bray-Curtis-similarity of standardised benthic abundance data collected from four surveys across Scotland's seas.

The distribution of the faunal assemblages, illustrated in Figure 8, shows that there is a strong geographical separation in the assemblages, with only two assemblages (f and l) occurring in more than one survey area. The Firth of Forth survey area supports a relatively homogeneous benthic fauna with all samples collected there being assigned to faunal assemblage b or c with the exception of one sample which was afaunal (i.e. did not contain any species). In contrast the Windsock site supports a heterogeneous benthic fauna with ten distinct faunal assemblages being recorded.

SIMPER analysis was used to identify the species which characterise these faunal assemblages, the results of which are provided in Electronic Appendix 5.3.2.

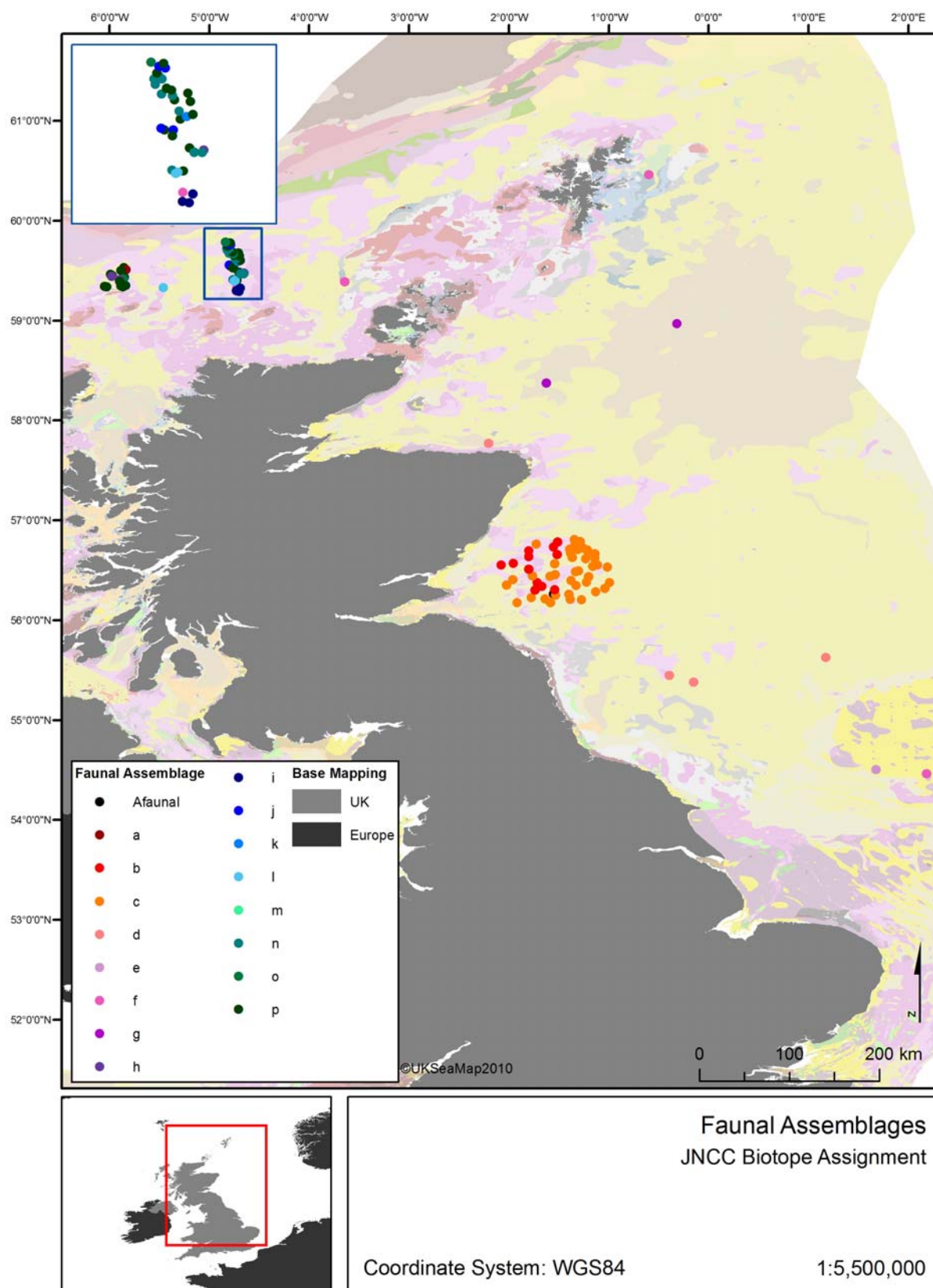


Figure 8. Chart showing the distribution of the sixteen faunal assemblages derived from a 20% similarity slice through a cluster based on Bray-Curtis-similarity of standardised benthic abundance data collected from four surveys across Scotland's seas (Figure 5).

3.3 Environmental Niche Requirements

The relationship between biological communities and the environment is a central theme of marine research (Seiderer and Newell 1999; Newell *et al* 2001; Robinson *et al* 2005; Rodil *et al* 2009). Understanding how environmental conditions influence marine fauna is of key importance to conservation since it helps us to predict the likely distribution of sensitive species and biodiversity hotspots as well as the impacts of natural and anthropogenic-induced change. In this context it helps us to understand the differences between the faunal assemblages identified and whether these are likely to be regional or temporal variants of one another or truly different communities occupying a different environmental niche.

The links between biological communities and the environmental conditions across Scotland's seas were investigated using a variety of environmental variables extracted from UKSeaMap 2010 and GEBCO as well as variables calculated from the associated PSD data (see Table 5).

3.3.1 Sediment Composition

The relationship between the benthic assemblages identified through multivariate analysis (Figure 7) and the composition of the sediments were investigated using routines in the PRIMER (v6) multivariate analysis programme. The standardised benthic abundance data were averaged across the assemblages and were compared with the raw PSD data which were also averaged across the faunal assemblages. Table 6 shows the result of a RELATE test that compares the resemblance between samples in terms of the fauna and the sediment. This confirms that there is a statistically significant relationship between the species present and the composition of the sediments at the 0.1% significance level.

Table 6. Summary of RELATE results carried out on benthic assemblage group averaged, standardised benthic abundance and raw PSD data.

RELATE Test Results	
Sample statistic (ρ)	0.619
Significance level of sample statistic	0.1 %
Number of permutations	999
Number of permuted statistics greater than or equal to ρ	0

The BIO-ENV routine was employed to investigate which components of the sediment, individually and in combination, correlate best with the patterns observed in the benthic assemblages (Table 7). The highest correlation ($\rho = 0.672$) was obtained from a five variable combination of; 16mm, 0.710mm, 0.355mm, 0.125mm, 0.063mm (gravel, coarse sand, fine sand and mud). The same particle sizes appeared throughout the BIO-ENV results indicating that a broad range of sediments are important in shaping the faunal assemblages in Scotland's seas.

Table 7. Summary of the BIO-ENV results carried out on benthic assemblage group averaged, standardised benthic abundance and raw PSD data.

No. of Variables	Correlation (ρ)	Variables
5	0.672	16 mm, 0.710 mm, 0.355 mm, 0.125 mm, 0.063 mm
	0.671	1 mm, 0.710 mm, 0.355 mm, 0.125 mm, 0.063 mm
	0.669	22.4 mm, 0.710 mm, 0.355 mm, 0.125 mm, 0.063 mm
3	0.603	0.355 mm, 0.125 mm, 0.063 mm
	0.596	0.5 mm, 0.125 mm, 0.063 mm
	0.595	0.355 mm, 0.125 mm, <0.063 mm
1	0.381	0.5 mm
	0.331	<0.063 mm
	0.330	0.125 mm

3.3.2 Environmental Parameters

Following the same steps described for the investigations into sediment composition, the influence of additional environmental parameters identified in Table 5, on the faunal assemblages were investigated. Percentage Gravel and Mean grain size were both found to be highly correlated with percentage sand ($\rho = -0.96$ and $\rho = 0.74$ respectively) as well as with each other ($\rho = 0.78$) and hence were removed from this analysis, with percentage sand representing all three parameters. Similarly, Latitude, Longitude and Area were found to be highly correlated with one another ($\rho > 0.8$) and Latitude and Longitude were excluded from the analysis leaving Area as the sole factor representing geographical location.

The standardised benthic abundance data were averaged across the assemblages and were compared with normalised environmental data which were also averaged across the faunal assemblages. Table 8 shows the result of a RELATE test that compares the resemblance between samples in terms of the fauna and the environmental data. This confirms that there is a statistically significant relationship between the species present and the environment in which they occur at the 0.2% significance level.

Table 8. Summary of RELATE results carried out on benthic assemblage group averaged, standardised benthic abundance and normalised environmental data (see Table 5).

RELATE Test Results	
Sample statistic (Rho)	0.527
Significance level of sample statistic	0.2 %
Number of permutations	999
Number of permuted statistics greater than or equal to Rho	0

The BIO-ENV routine was employed to investigate which environmental parameters, individually and in combination, correlate best with the patterns observed in the benthic assemblages (Table 9). The highest correlation ($p = 0.615$) was obtained from a five variable combination of; Area, Sorting, Kurtosis, Biological Zone, Energy. The same environmental parameters appeared throughout the BIO-ENV results indicating that these factors are all important in shaping the biological communities that occur across Scotland's seas.

The raw sediment granulometry (PSD) data correlated more strongly with the benthic assemblages than the environmental variables which is testament to the role that sediment composition plays in shaping benthic communities. Area (and by proxy latitude and longitude) featured as a significant correlate to the biological communities but it is worth noting that this is likely to be acting as a surrogate to other environmental parameters that were not included in this analysis such as sea temperature and oxygen departure zones.

Table 9. Summary of the BIO-ENV results carried out on benthic assemblage group averaged, standardised benthic abundance and normalised environmental data (see Table 5).

No. of Variables	Correlation (ρ)	Variables
5	0.615	Area, Sorting, Kurtosis, Biological Zone, Energy
	0.613	Area, Skewness, Kurtosis, Biological Zone, Energy
	0.605	Area, % Sand, Kurtosis, Biological Zone, Energy
3	0.614	% Mud, Kurtosis, Biological Zone
	0.599	Kurtosis, Biological Zone, Energy
	0.580	Kurtosis, Energy, Depth
1	0.490	Energy
	0.359	Biological Zone
	0.320	% Mud

To investigate how these environmental parameters relate to the faunal assemblages in more detail a LINKTREE was created (Figure 9). This routine provides quantitative information based on the environmental variables that best describe the difference between two groups. Using the LINKTREE we can start to understand the different niche requirements of the assemblages and how they relate to one another. For example split B in the LINKTREE is correlated strongly ($p = 0.52$) with Area reflecting the geographical patterns already observed in the data.

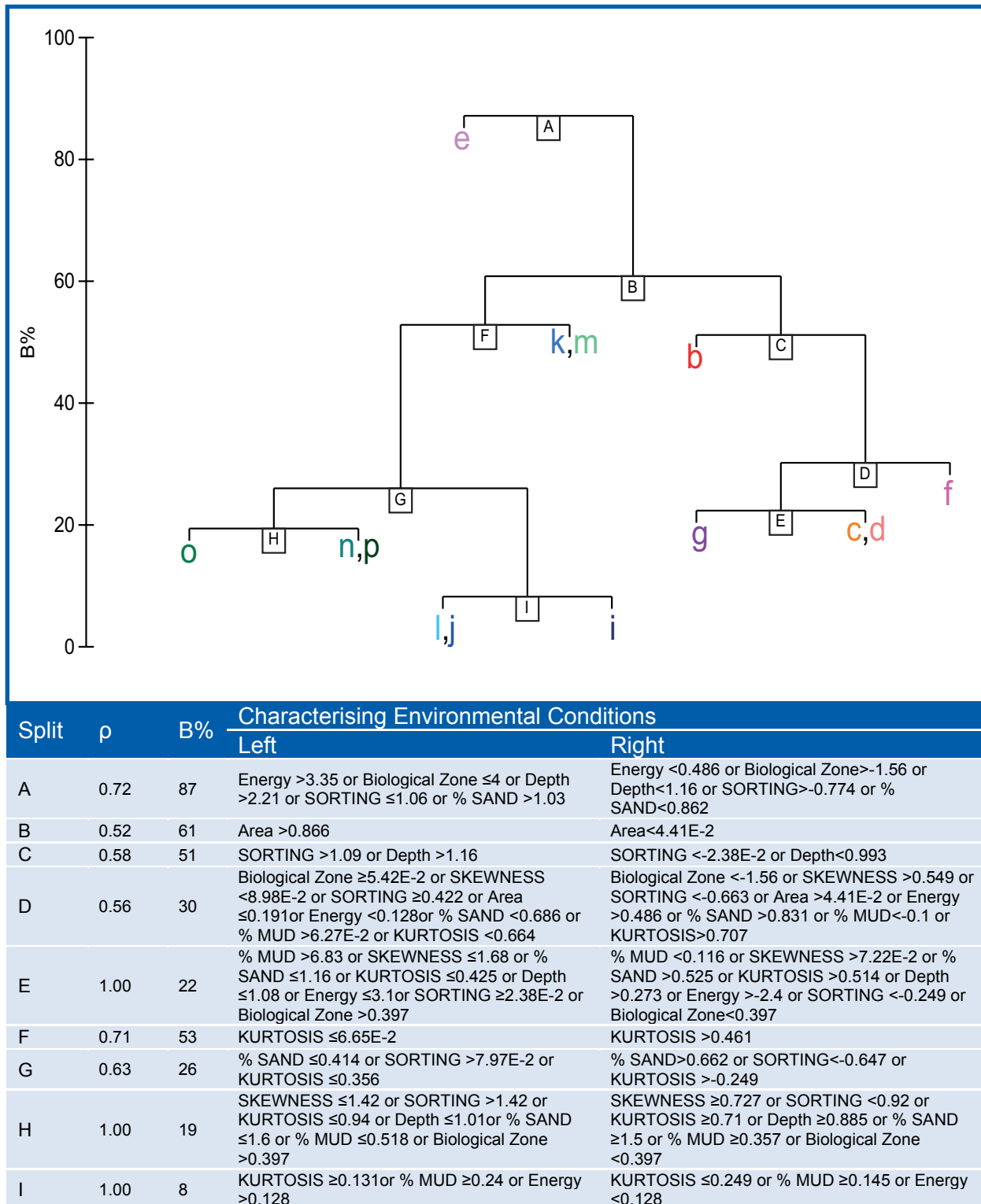


Figure 9. LINKTREE results carried out on benthic assemblage group averaged, standardised benthic abundance and normalised environmental data (see Table 5).

3.4 Biotope Assignment

The main aim of this study was to assign a biotope to each of the samples collected across Scotland's seas. Having investigated the faunal assemblages and their relationship with the physical environment the sample data were then compared with existing biotopes using multivariate statistical techniques and systematic manual checks.

3.4.1 Multivariate Biotope Matching

The benthic grab abundance data for each of the faunal assemblages identified through multivariate analysis (Figure 7) were translated to % occurrence data to enable comparisons with existing biotope data in multivariate space. The % occurrence data calculated for each of the faunal assemblages are provided in Electronic Appendix 5.3.9. The % occurrence data for each faunal assemblage were merged in PRIMER (v6) with the equivalent data for littoral and sublittoral biotopes and were subsequently plotted in an MDS ordination. A cluster was also created and a SIMPROF test was used to identify any faunal assemblages that were statistically inseparable from existing biotopes. The full results of this analysis are provided in Electronic Appendix 5.3.11 and are summarised below in Figures 10 and 11.

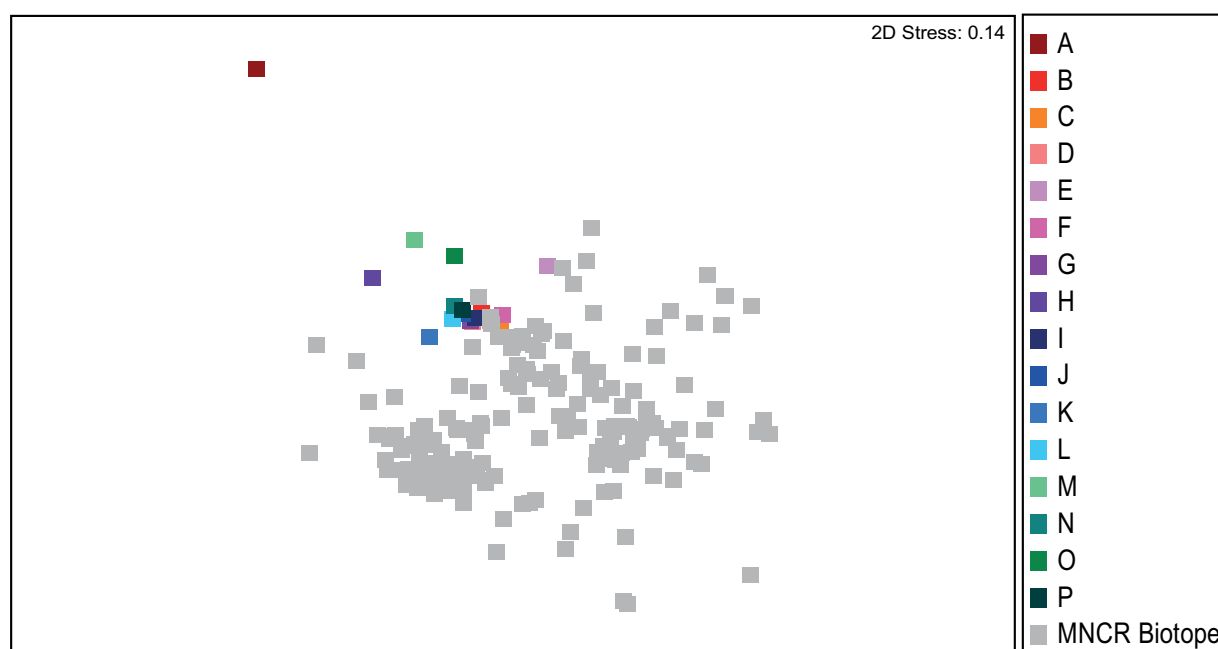


Figure 10. MDS plot showing the faunal assemblages derived from a 20% similarity slice through a cluster based on Bray-Curtis-similarity of standardised benthic abundance data collected from four surveys across Scotland's seas, and a sub-set of the existing MNCR Littoral and Sublittoral biotopes, based on % occurrence data.

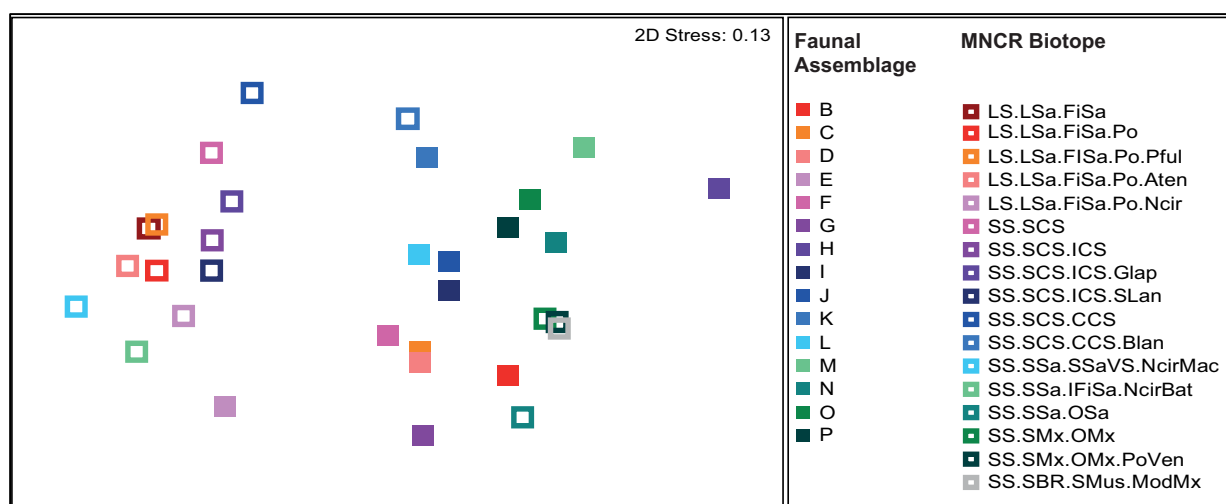


Figure 11. MDS plot showing a sub-set of the faunal assemblages derived from a 20% similarity slice through a cluster based on Bray-Curtis-similarity of standardised benthic abundance data collected from four surveys across Scotland's seas, and the existing MNCR Littoral and Sublittoral biotopes, based on % occurrence data.

Multivariate analysis revealed some overlap between the faunal assemblages identified in this project and existing MNCR biotopes although no assemblage was found to be statistically inseparable from an existing biotope using the SIMPROF test.

3.4.2 Manual Biotope Matching

The faunal assemblages were manually checked against existing MNCR biotopes using the JNCC website as well as the most up to date biotope correlation tables provided to the project team by the JNCC. The species contributing to 50% of the internal group similarity (limited to a maximum of five) were used to filter existing biotopes for possible matches. Those with the greatest number of matching taxa were examined more carefully by an experienced marine ecologist to determine whether or not there was good correspondence between the biotope described and the faunal group. Environmental data were also used to inform this process.

The full results of this exercise are provided in Electronic Appendix 5.4.1. The biotopes that were found to be the closest match to the faunal assemblages using the systematic manual matching protocol described in Section 2.2.2 are summarised below in Table 10.

Table 10. Summary of the faunal assemblages identified through multivariate analysis and the MNCR biotopes that were found to be the best match using the strategic manual checking protocol. Confident matches between faunal assemblages and biotopes are highlighted by a green box.

Faunal Assemblage	MNCR Biotope	Notes
A Sparse faunal assemblage including <i>Thelepus cincinnatus</i> and some hydroid species on deep circalittoral rock	CR.HCR.XFa.ByErSp.DysAct Mixed turf of bryozoans and erect sponges with <i>Dysidia fragilis</i> and <i>Actinothoe sphyrroseta</i> on tide-swept wave-exposed circalittoral rock	Some shared species but lacking the overall diversity of this biotope. This assemblage also occurs in a deeper, less exposed habitat.
b Mixed sands and gravels supporting a rich polychaete and epifaunal community including <i>Galathea intermedia</i>	SS.SMx.OMx Offshore circalittoral mixed sediment	Some species in common with this Level 3 biotope as well the Level 4 biotopes that sit within it, although none of these adequately describe the characterising fauna of this assemblage.
c <i>Spiophanes bombyx</i> aggregations in deep circalittoral sands	SS.SCS.CCS.MedLumVen <i>Mediomastus fragilis</i> , <i>Lumbrineris</i> spp. and venerid bivalves in circalittoral coarse sand or gravel	Some species in common with this biotope but the dense aggregations of <i>Spiophanes bombyx</i> are not adequately captured.
d <i>Paramphinome jeffreysii</i> , <i>Amphiura filiformis</i> and other echinoderms in offshore circalittoral sand	SS.SMx.CMx.MysThyMx <i>Mysella bidentata</i> and <i>Thyasira</i> spp. in circalittoral muddy mixed sediment	Some species in common but the main characterising species do not match. This assemblage also occurs in less muddy substrates.
e <i>Nephtys cirrosa</i> and <i>Bathyporeia</i> spp. in infralittoral sand	SS.SSa.IFiSa.NcirBat <i>Nephtys cirrosa</i> and <i>Bathyporeia</i> spp. in infralittoral sand	Very good match with characterising species and environmental conditions although there are some differences in the community as a whole, possibly indicating that this is a regional variant or that the existing biotope description needs to be expanded to capture the natural variation.
f Dense <i>Owenia fusiformis</i> aggregations in infralittoral coarse sediment and deep circalittoral sand.	SS.SCS.CCS.MedLumVen <i>Mediomastus fragilis</i> , <i>Lumbrineris</i> spp. and venerid bivalves in circalittoral coarse sand or gravel	Although the main characterising species of assemblage f (<i>Owenia fusiformis</i>) occurs in this biotope, it does not occur in the same high densities. There are a number of other shared species but overall this is not considered to be a good match.
g <i>Diplocirrus glaucus</i> , <i>Paramphinome jeffreysii</i> and <i>Amphiura chiajei</i> in offshore sandy mud	SS.SMu.OMu.MyrPo <i>Myrtea spinifera</i> and polychaetes in offshore circalittoral sandy mud	Some species in common although there are differences in the abundance of the characterising fauna. As this assemblage occurs in the deep circalittoral it could be a deeper water variant.
h <i>Ophiactis balli</i> on rocky substrate with encrusting bryozoans, saddle oysters and serpulids	CR.LCR.BrAs.NeoPro <i>Neocrania anomala</i> and <i>Prostanthera simplex</i> on sheltered circalittoral rock	Some species and numerous families in common but this biotope does not adequately describe the characterising fauna of this assemblage.
i <i>Owenia fusiformis</i> and <i>Galathea oculata</i> aggregations in offshore circalittoral sand	SS.SCS.CCS.MedLumVen <i>Mediomastus fragilis</i> , <i>Lumbrineris</i> spp. and venerid bivalves in circalittoral coarse sand or gravel	Some species in common with this biotope but the dense aggregations of <i>Owenia fusiformis</i> and <i>Galathea oculata</i> are not adequately captured.
j <i>Aponuphis bilineata</i> and <i>Echinocyamus</i> in polychaete-rich offshore sands	SS.SCS.CCS.MedLumVen <i>Mediomastus fragilis</i> , <i>Lumbrineris</i> spp. and venerid bivalves in circalittoral coarse sand or gravel	Some species in common but not all of the species that characterise this assemblage are represented in the biotope. This assemblage also occurs further offshore in the deep circalittoral zone.
k Sparse fauna in offshore circalittoral sands	SS.SCS.CCS.MedLumVen <i>Mediomastus fragilis</i> , <i>Lumbrineris</i> spp. and venerid bivalves in circalittoral coarse sand or gravel	Some species in common but this assemblage lacks the diversity of SS.SCS.CCS.MedLumVen and a number of the characterising species.
l <i>Angulus pygmaeus</i> (<i>Moerella pygmaea</i>) and sparse polychaetes in offshore gravelly sands	SS.SCS.CCS.MedLumVen <i>Mediomastus fragilis</i> , <i>Lumbrineris</i> spp. and venerid bivalves in circalittoral coarse sand or gravel	Numerous species in common but this assemblage lacks the diversity of polychaetes reported for SS.SCS.CCS.MedLumVen.
m Offshore circalittoral gravelly sand with sparse fauna including pagurids	SS.SBR.SMus.ModCvar <i>Modiolus modiolus</i> beds with <i>Chlamys varia</i> , sponges, hydroids and bryozoans on slightly tide-swept very sheltered circalittoral mixed substrata	Some species in common but assemblage m lacks the characteristic molluscs of this biotope (<i>Modiolus modiolus</i> and <i>Chlamys varia</i>).
n <i>Placostegus tridentatus</i> and <i>Galathea intermedia</i> on faunal encrusted gravelly sands and sandy gravels	SS.SCS.CCS.MedLumVen <i>Mediomastus fragilis</i> , <i>Lumbrineris</i> spp. and venerid bivalves in circalittoral coarse sand or gravel	Some species in common but assemblage n is lacking the venerid bivalves that are characteristic of this biotope
o Sipunculid community in deep circalittoral sandy gravel	SS.SCS.OCS.GlapThyAmy <i>Glycera lapidum</i> , <i>Thyasira</i> spp. and <i>Amythasides macroglossus</i> in offshore gravelly sand	Numerous species in common but this biotope lacks the sipunculid community that characterises this assemblage.
p <i>Aponuphis bilineata</i> and <i>Echinocyamus</i> in faunal encrusted polychaete-rich offshore gravelly sand and sandy gravel	SS.SCS.CCS.MedLumVen <i>Mediomastus fragilis</i> , <i>Lumbrineris</i> spp. and venerid bivalves in circalittoral coarse sand or gravel	Some species in common but lacking some of the species that characterise the assemblage.

3.4.3 Expert Moderation

Table 10 shows that the vast majority of faunal assemblages identified in this study did not correspond well to existing MNCR biotopes. This was in part, an artefact of the methods employed since matches were identified essentially on the basis of co-occurring species with little consideration of ecological functioning. This becomes particularly problematic where biotopes are only described qualitatively or do not yet encompass the full range of natural variability. Expert moderation was therefore applied to the process to identify biotopes that showed some correspondence to the faunal assemblages but were missed by the multivariate analysis and manual matching. The additional matching biotopes identified using this process are summarised below in Table 11.

Table 11. Summary of the faunal assemblages identified through multivariate analysis and the MNCR biotopes that were found to be the best match through expert moderation. Confident matches between faunal assemblages and biotopes are highlighted by a green box. Brackets around faunal assemblages indicate that this match applies to a part of that assemblage only.

Faunal Assemblage	MNCR Biotope	Notes
(b) (Mixed sands and gravels supporting a rich polychaete and epifaunal community including <i>Galathea intermedia</i>)	SS.SBR.PoR.SspiMx <i>Sabellaria spinulosa</i> on stable circalittoral mixed sediment	The fauna associated with the reef are quite different from those listed as being typical for this biotope. Most notably this reef supports high abundances of the ascidian sea squirt, <i>Asciidiella aspersa</i> and moderate abundances of <i>Galathea intermedia</i> . However, more samples would be required to determine whether or not this is a regional variant or a sub-biotope.
c <i>Spiophanes bombyx</i> aggregations in deep circalittoral sands	SS.SSa.IFiSa.TbAmPo Semi-permanent tube-dwelling amphipods and polychaetes in sublittoral sand	There is a partial match with this biotope although assemblage c lacks the characteristic tubicolous amphipods and occurs in deeper waters
d <i>Paramphinoe jeffreysii</i> , <i>Amphiura filiformis</i> and other echinoderms in offshore circalittoral sand	SS.SMu.OMu.PjefThyAfil <i>Paramphinoe jeffreysii</i> , <i>Thyasira</i> spp and <i>Amphiura filiformis</i> in offshore circalittoral sandy mud	This assemblage has similar fauna to SS.SMu.OMu.PjefThyAfil but occurs in coarser sediments and lacks the characteristic bivalves belonging to the genera <i>Thyasira</i> .
f Dense <i>Owenia fusiformis</i> aggregations in infralittoral coarse sediment and deep circalittoral sand.	SS.SSa.OSa.OfusAfil <i>Owenia fusiformis</i> and <i>Amphiura filiformis</i> in offshore circalittoral sand or muddy sand	Although the brittlestar <i>Amphiura filiformis</i> was not always recorded in this community, unidentified ophiuroids were present, so this was considered to be a good match.
h <i>Ophiactis balli</i> on rocky substrate with encrusting bryozoans, saddle oysters and serpulids	CR.MCR.EcCr.FaAlCr.Bri Brittlestar bed overlying coralline crusts, <i>Parasmittina trispinosa</i> and <i>Caryophyllia smithii</i> on wave exposed circalittoral rock	This assemblage is similar to CR.MCR.EcCr.FaAlCr.Bri but occurs in the deep circalittoral zone and therefore lacks the algal component. This biotope is also notably more diverse which may be a reflection of the increased stability of this habitat.
i <i>Owenia fusiformis</i> and <i>Galathowenia oculata</i> aggregations in offshore circalittoral sand	SS.SSa.OSa.OfusAfil <i>Owenia fusiformis</i> and <i>Amphiura filiformis</i> in offshore circalittoral sand or muddy sand	<i>Amphiura filiformis</i> is not present in high abundances in this community, but otherwise there is a very good match with SS.SSa.OSa.OfusAfil. The high abundance and regular occurrence of <i>Galathowenia oculata</i> could be cause to recommend this as a sub-biotope if more records of this assemblage were identified. However, as the biotope SS.SSa.OSa.OfusAfil is itself only qualitatively described there is not sufficient information available to determine whether or not the two should be separated.

A total of four matches were identified between the faunal assemblages identified in this study and existing MNCR biotopes. No matches were identified using multivariate statistics, one was identified through a systematic manual matching protocol and the remaining three through expert moderation.

3.5 New Biotopes Proposals

As illustrated by Tables 10 and Table 11 the majority of the faunal assemblages identified in this study were not found to correspond well with existing MNCR biotopes and it was therefore necessary to propose new biotopes. This is unsurprising as although the EUNIS scheme (which now encompasses the MNCR classification) is periodically updated to accommodate new biotopes, it is known to contain significant gaps. In recognition of this, recommendations for significant revisions were recently made as part of the outcome of a Mesh Atlantic workshop on the EUNIS scheme (Galparsoro *et al* In Press). One of the most significant gaps identified during the course of this workshop was in offshore habitats which are not well represented in the current EUNIS and MNCR schemes (Howell 2010). Most of the samples examined in this study would fall into this category and hence new biotopes have been proposed as a means of improving the biotope classification scheme in this area.

A number of the faunal assemblages were found to occur across a number of the habitat classes specified in the MNCR and EUNIS classification schemes and it was therefore necessary to split these in order to place them correctly within the biotope hierarchy. Where a split was necessary the differences in faunal composition between the different habitat types were investigated using the SIMPER routine. The results of this analysis are provided in full in Electronic Appendix 5.3.12 and are summarised below in Table 12.

Table 12. Summary of the splits made to align faunal assemblages identified in this study with the MNCR and EUNIS biotope hierarchy.

Faunal Assemblage	Habitat Split	Revised Assemblage
b	Mixed sands and gravels supporting a rich polychaete and epifaunal community including <i>Galathea intermedia</i>	Coarse
		Mixed* Mixed sediments supporting a rich polychaete and epifaunal community including <i>Galathea intermedia</i>
		<i>Sabellaria spinulosa</i> <i>Sabellaria spinulosa</i> reef with <i>Asciidiella scabra</i> and <i>Galathea intermedia</i> on gravelly sand
c	<i>Spiophanes bombyx</i> aggregations in deep circalittoral sands	Sand <i>Spiophanes bombyx</i> aggregations in deep circalittoral sands
		Coarse <i>Spiophanes bombyx</i> aggregations in deep circalittoral coarse sands
d	<i>Paramphinome jeffreysii</i> , <i>Amphiura filiformis</i> and other echinoderms in offshore circalittoral sand	Sand <i>Paramphinome jeffreysii</i> , <i>Amphiura filiformis</i> and other echinoderms in offshore circalittoral sand
		Coarse <i>Astrorhiza</i> and oweniid polychaetes in coarse offshore sands
f	Dense <i>Owenia fusiformis</i> aggregations in infralittoral coarse sediment and deep circalittoral sand.	Deep circalittoral sand <i>Owenia fusiformis</i> beds in deep circalittoral sand
		Infralittoral coarse sediments Dense <i>Owenia fusiformis</i> in infralittoral gravelly sand
j	<i>Aponuphis bilineata</i> and <i>Echinocyamus</i> in polychaete-rich offshore sands	Sand <i>Aponuphis bilineata</i> and <i>Echinocyamus</i> in polychaete-rich offshore sands
		Coarse <i>Aponuphis bilineata</i> and <i>Echinocyamus</i> in polychaete-rich offshore gravelly sand
l	<i>Angulus pygmaeus</i> (<i>Moerella pygmaea</i>) and sparse polychaetes in offshore gravelly sands	Sand <i>Angulus pygmaeus</i> (<i>Moerella pygmaea</i>) and sparse polychaetes in offshore circalittoral sands
		Coarse <i>Angulus pygmaeus</i> (<i>Moerella pygmaea</i>) and sparse polychaetes in offshore gravelly sands
n	<i>Placostegus tridentatus</i> and <i>Galathea intermedia</i> on faunal encrusted gravelly sands and sandy gravels	Rock <i>Placostegus tridentatus</i> on faunal encrusted rock
		Coarse <i>Placostegus tridentatus</i> and <i>Galathea intermedia</i> on faunal encrusted gravelly sands and sandy gravels
		Mixed <i>Placostegus tridentatus</i> and <i>Galathea intermedia</i> on faunal encrusted mixed sediments
p	<i>Aponuphis bilineata</i> and <i>Echinocyamus</i> in faunal encrusted polychaete-rich offshore gravelly sand and sandy gravel	Sand <i>Aponuphis bilineata</i> and <i>Echinocyamus</i> in faunal encrusted polychaete-rich offshore gravelly sand and sandy gravel
		Coarse <i>Echinocyamus pusillus</i> in polychaete-rich, deep circalittoral sands with some faunal encrusted gravel

In most cases splitting the faunal assemblages did not change the main characterising species, confirming the internal structure of the groups identified in this study. However, assemblage d was notable in its divisions as this revealed a very unusual faunal community associated with the coarser sediments and dominated by the large foraminifera *Astorhiza*. This community shows strong similarities with that reported by Buchanan and Hedley (1960) off the Northumberland coast and it is likely therefore to be a distinct biotope. The evidence from this study alone however is not sufficient to recommend this as a new biotope as this community was only observed at one station, albeit in three samples.

Once the faunal assemblages were aligned with the MNCR and EUNIS biotope hierarchies it was possible to identify those which did not correspond with any existing classifications and which presented sufficient evidence to form the basis of a new biotope proposal (>3 stations and >5 samples).

In total, ten new biotopes have been proposed based on the data analysed in this study and these are listed below in Table 13, full details including the physical environmental parameters under which they occur can be found in Electronic Appendix 5.5.1 and all of the data relating to each of the samples analysed in this study, including the biotopes that were ultimately assigned can be found in Electronic Appendix 5.5.2.

Table 13. Summary of the new biotopes proposed. Brackets are used to highlight temporary codes assigned to illustrate indicative placing of these new biotopes within the current MNCR and EUNIS biotope classification schemes.

EUNIS Code	MNCR Code	MNCR Description
A5.15[5]	SS.SCS.OCS.[PoGintBy]	Polychaete-rich <i>Galathea</i> community with encrusting bryozoans and other epifauna on offshore coarse sediment
A5.15[6]	SS.SCS.OCS.[Sbom]	<i>Spiophanes bombyx</i> aggregations in offshore coarse sands
A5.15[7]	SS.SCS.OCS.[PtriGintFaCr]	<i>Placostegus tridentatus</i> and <i>Galathea intermedia</i> on faunal encrusted gravelly sands and sandy gravels
A5.15[8]	SS.SCS.OCS.[AbilEpusFaCrPo]	<i>Aponuphis bilineata</i> and <i>Echinocyamus pusillus</i> in faunal encrusted polychaete-rich offshore circalittoral gravelly sands and sandy gravels
A5.27[4]	SS.SSa.OSa.[Sbom]	<i>Spiophanes bombyx</i> aggregations in offshore sands
A5.27[5]	SS.SSa.OSa.[AbilEpusPo]	<i>Aponuphis bilineata</i> and <i>Echinocyamus pusillus</i> in polychaete-rich offshore circalittoral sands
A5.27[6]	SS.SSa.OSa.[MoePo]	<i>Moerella pygmaea</i> and sparse polychaetes in offshore circalittoral sands
A5.27[7]	SS.SSa.OSa.[PjefAfilEc]	<i>Paramphipnomus jeffreysii</i> , <i>Amphiura filiformis</i> and other echinoderms in stable offshore circalittoral sand
A5.27[8]	SS.SSa.OSa.[EpusFaCrPo]	<i>Echinocyamus pusillus</i> in polychaete-rich, deep circalittoral sands with some faunal encrusted gravel
A5.45[2]	SS.SMx.OMx.[PoGintBy]	Polychaete-rich <i>Galathea</i> community with encrusting bryozoans and other epifauna on offshore circalittoral mixed sediment

The distribution of biotopes, existing and proposed, derived from benthic grab data analysed in this study are illustrated overleaf in Figure 12.

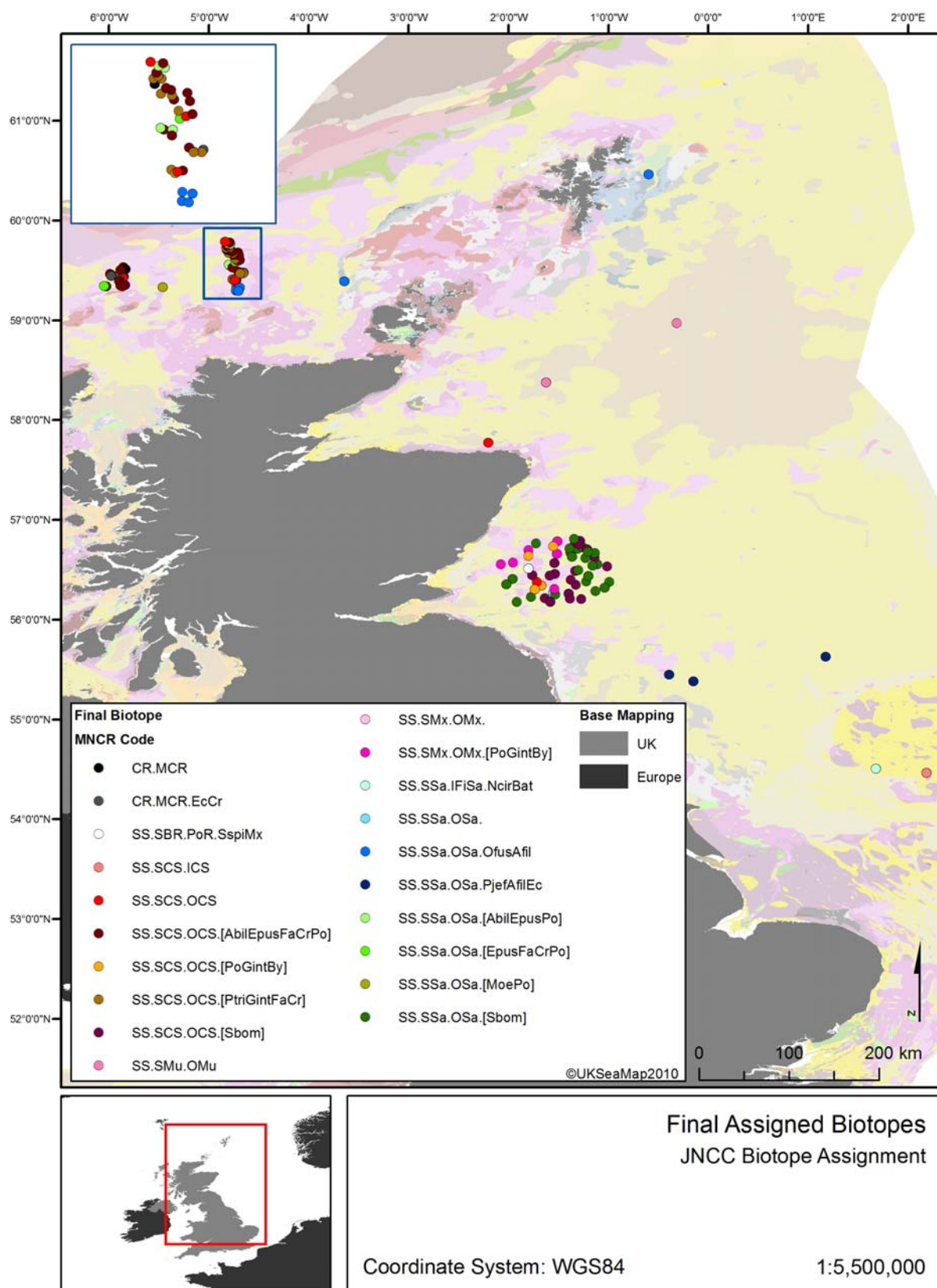


Figure 12. Chart showing the distribution of biotopes derived from benthic grab data collected from four sites across Scotland's seas.

3.5.1 Comparison between Biotopes Assigned to Grab and Video/Stills Data

Once biotopes had been assigned to each of the benthic station it was possible to compare these to biotopes assigned to collocated video and stills images. The closest video and still image was selected for each sample using a GIS spatial query. On a few occasions the closest video or still image to a sample, had not been assigned a biotope and in this instance the nearest video/still with a biotope assigned to it, was used for comparative purposes. In each instance the distance between grab sample and video/still was calculated. The full comparison is appended to this report in Electronic Appendix 5.4.2 and the results are also summarised below in Tables 14 and 15.

The grab to video/stills comparison was undertaken at both the physical habitat level (EUNIS Level 3) and at the final biotope level. Unsurprisingly, there was a better match between the physical biotopes assigned to grabs and video/stills images than to the biological ones, although the differences were still significant. Just under half of the habitat level biotopes matched the biotope (or a biotope within a matrix) assigned to the nearest collocated video and just over a third matched the biotope assigned to the nearest still image. The remainder were assigned to different sediment classes which is almost certainly an artefact of the different scales of sampling, and the level of small-scale habitat heterogeneity. The distance between the samples being compared was also fairly significant at times, ranging from a few metres to over a kilometre.

Table 14. Summary of the matches between habitat level (EUNIS L3) biotopes assigned to grab samples on the basis of physical parameters and the habitat level biotopes assigned to the closest video and still image.

Habitat Comparisons		
	Video	Stills
Match Habitat Biotope	85	112
Match Mosaic	52	1
Sediment recorded was different	154	178
Total	291	291

Around a third of the biotopes assigned to the grab samples matched those that had been assigned to the corresponding video footage or stills image. In the vast majority of cases the mismatch was because the sediment recorded was different, again highlighting the difference in the scale of observation and the level of local heterogeneity. The rest of the mismatches between the different sampling types were attributed to different biological zones being assigned. Biotope assignment of the video footage and still images appears to have been driven primarily by the visible fauna and in a number of instances a circalittoral biotope has been assigned to images taken in the deep circalittoral. There is every possibility that the same assemblage occurs across two biological zones but this certainly warrants further investigation as there may be enough evidence to propose a new biotope or an alteration to an existing one.

Table 15. Summary of the matches between the biotopes assigned to grab samples and the biotope assigned to the closest video and still image.

Biotope Comparisons		
	Video	Stills
Habitat Match	53	79
Mosaic Match	54	1
Sample assigned to a different biological zone	32	34
Sediment recorded was different	152	177
Total	291	291

3.6 Features of Conservation Interest

Another aim of this study was to identify features of conservation interest that occur within the four survey areas. This has been achieved by looking at evidence for Annex I Reef features, MPA search features and finally by screening the benthic grab data for the presence of rare and alien species.

3.6.1 Annex I Reef Features

The fauna recorded from each benthic grab sample was screened for significant abundances of biogenic reef forming species as well as those species that are indicative of stony or rocky reef e.g. sponges, bryozoans and hydroids (see Section 2.3.1 for full details of the reef assessment criteria).

High abundances (≥ 20 per grab sample) of the biogenic reef forming species, *Sabellaria spinulosa* were recorded in three samples collected from a single station in the Firth of Forth survey area (Station FF056). The abundance of *S. spinulosa* and the diversity of fauna present in these samples are indicative of *S. spinulosa* reef. However, information regarding the topographical height, the extent and ideally the longevity of the aggregation would be needed before it could be assessed against the EC Habitats Directive reef criteria (Hendrick and Foster-Smith 2006; Gubbay 2007). No other biogenic reef forming species were identified in the course of this study.

The cup coral *Caryophyllia (Caryophyllia) smithii* was identified in a single sample also collected from the Firth of Forth (FF005-B). It is highly unusual to sample a species like this using a grab as they tend to grow attached to hard surfaces including rock faces and artificial structures, though they are also reported to occur on shell fragments (Gregory, 2008). *Caryophyllia (Caryophyllia) smithii* is a solitary coral and as individuals may be quite widely spaced it is impossible to assess whether the occurrence of this species is indicative of a conservation feature. The grab sample in which *C. smithii* was identified was found to contain some fauna recorded in the biotopes CR.MCR.EcCr.CarSwi, CR.MCR.EcCr.CarSp and CR.MCR.EcCr.FaAlCr, although all of these biotopes are described on the basis of conspicuous epifauna present so it is difficult to determine the degree of overlap. Since all of these biotopes fall under the EC Habitats Directive definition of reef, examination of the video footage and still images collected at this site would be advisable.

One hundred samples were found to support fauna indicative of rocky or stony reef and a further 35 samples were found to support some fauna indicative of stony and rocky reefs. However, only two of these samples were taken in substrates indicative of stony and rocky reef ($> 10\%$ Cobbles ($> 45\text{mm}$)). However, it is not normal practice to retain cobbles greater than 45 mm for PSD analysis as a very large volume of sediment, far exceeding the volume of a grab sample, would be required to accurately analyse the granulometry (British Standards Institution, 1996; Passchier, S. 2007). As neither the field notes describing the sediments or the photographic records of the samples were available for the purposes of cross-referencing we have highlighted all samples that support faunal communities indicative of coarse sediments for the purposes of further investigation. The faunal composition of grab samples alone does not provide sufficient evidence of the presence or otherwise of a rocky or stony reef.

The distribution of samples containing evidence of Annex I reefs are shown in Figures 13-16. Significant numbers of samples collected from the Windsock survey area supported fauna indicative of rocky and stony reefs. This area is generally characterised by very stable coarse sediments that support a high diversity of infauna and epifauna.

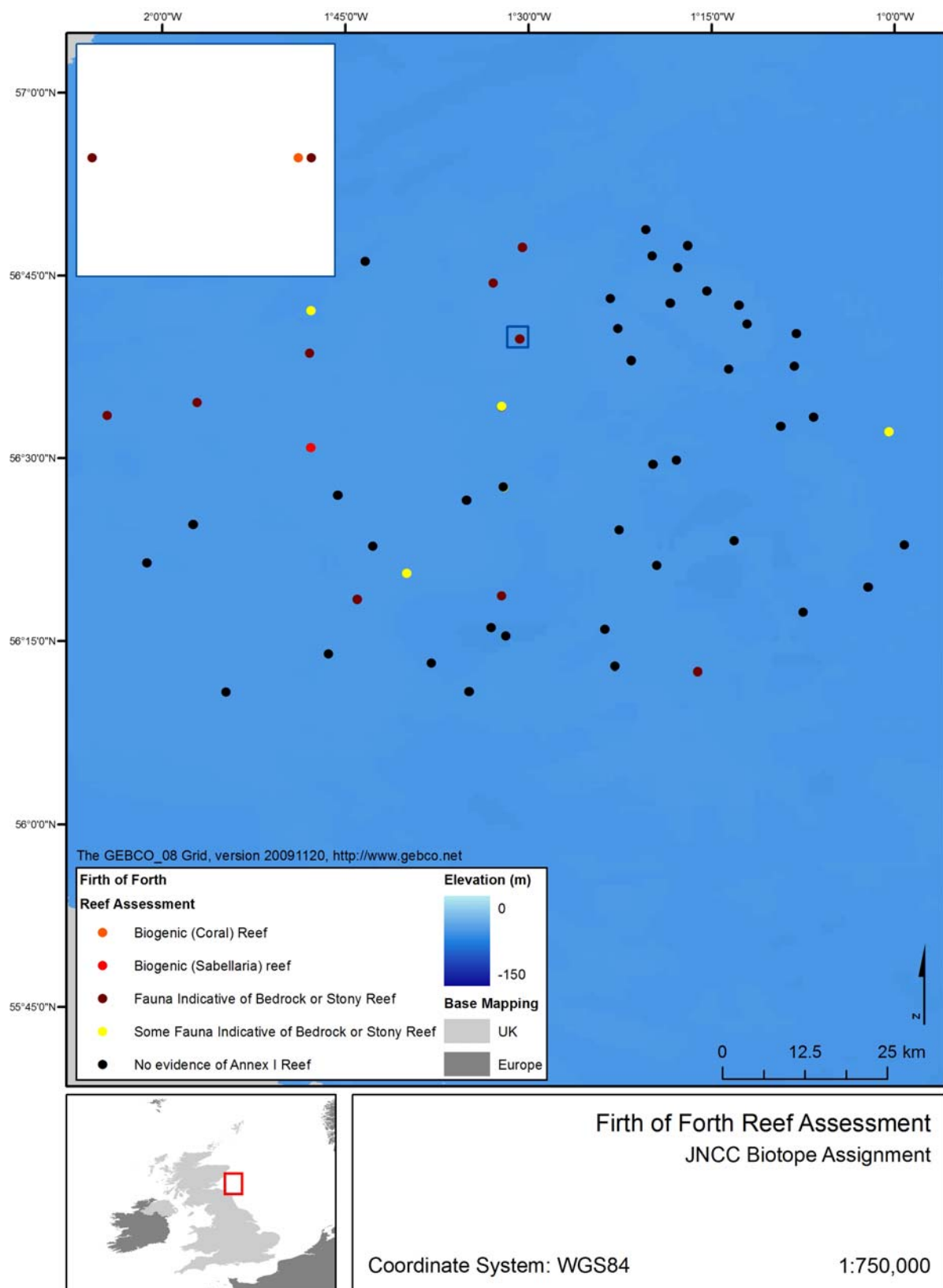


Figure 13. Chart showing the distribution of stations identified as supporting fauna typical of Annex I Reef Features within the Firth of Forth survey area.

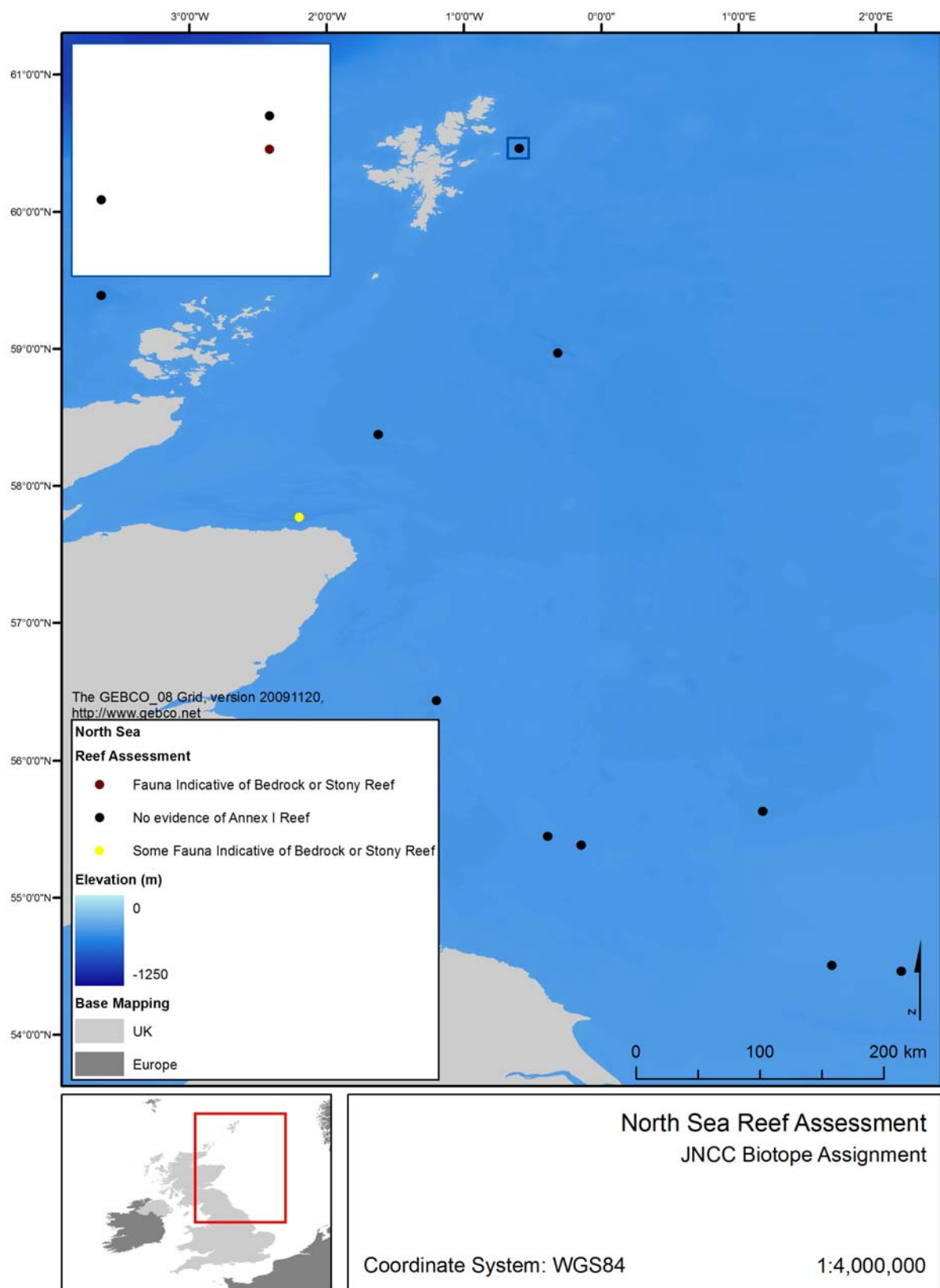


Figure 14. Chart showing the distribution of stations identified as supporting fauna typical of Annex I Reef Features within the North Sea survey area.

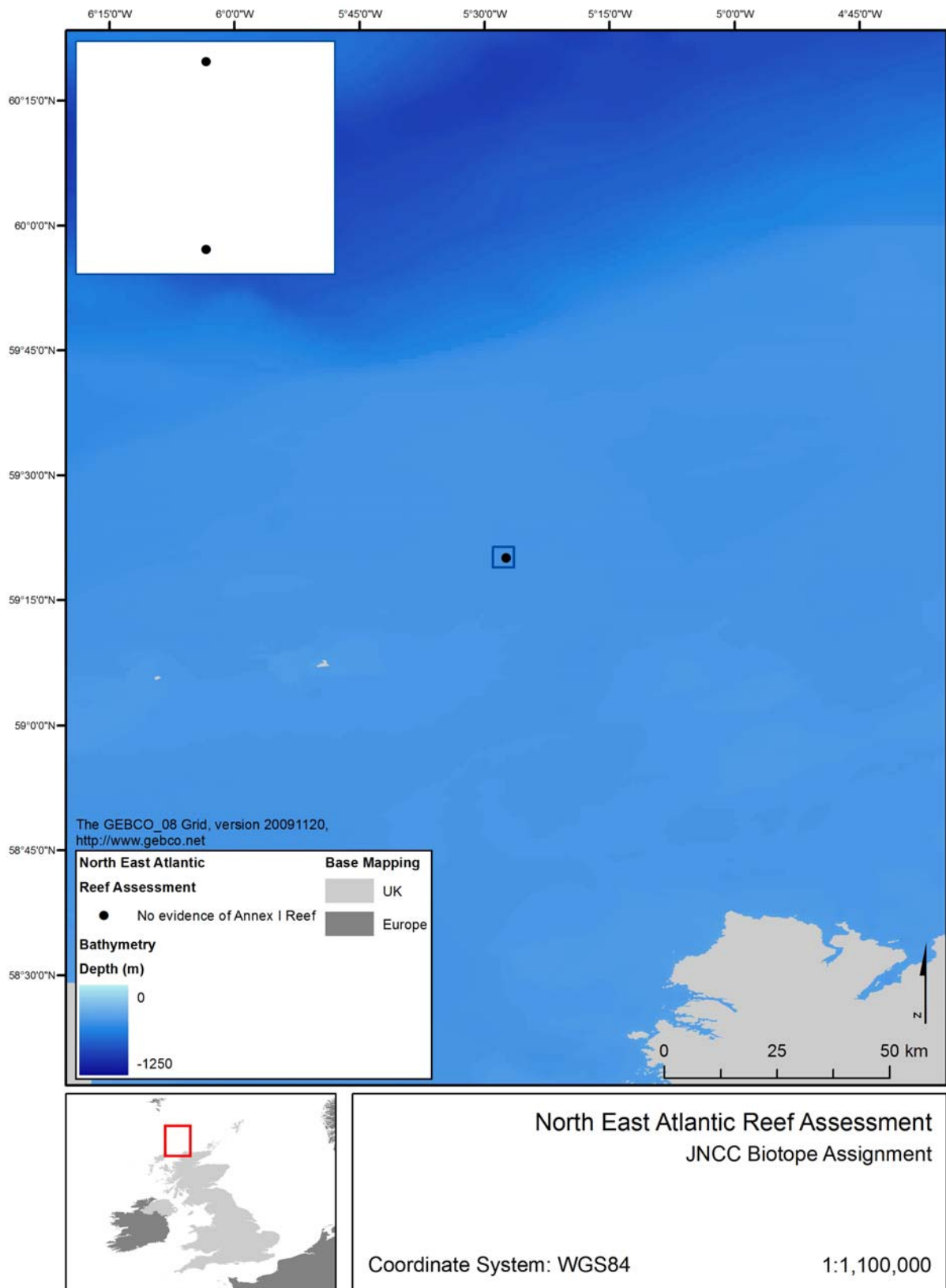


Figure 15. Chart showing the distribution of stations identified as supporting fauna typical of Annex I Reef Features within the North East Atlantic survey area.

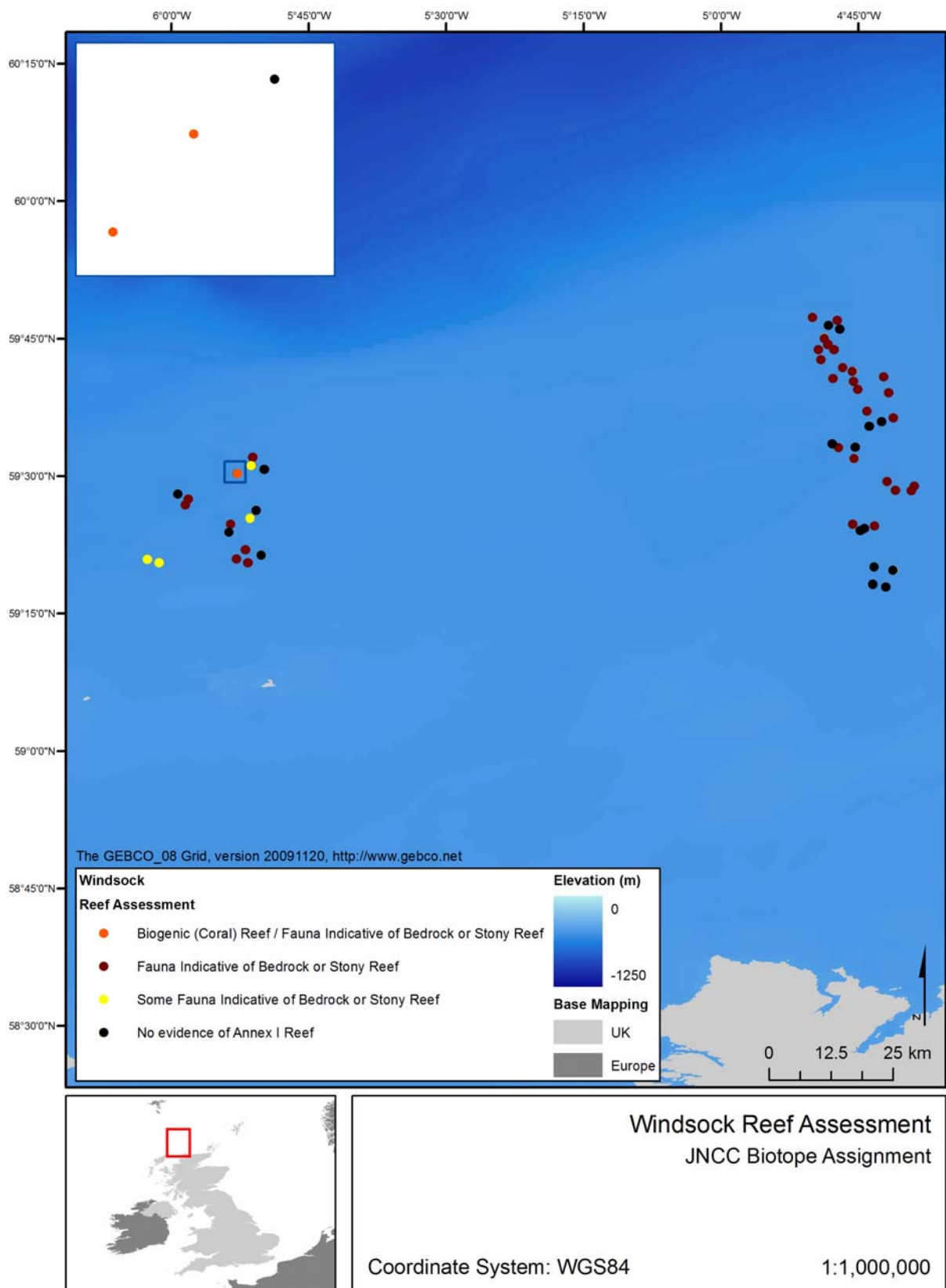


Figure 16. Chart showing the distribution of stations identified as supporting fauna typical of Annex I Reef Features within the Windsock survey area.

3.6.2 MPA Search Features

The data examined as part of this study were screened for the presence of MPA search features including “*seabed habitats and their component biotopes and species*” and “*limited mobility species*” which highlighted a number of features that should be considered during the development of the Scottish MPA network.

Seabed Habitats and their Component Biotopes and Species

Northern Sea Fan and Sponge Communities

Whilst no sea fans were recorded in the benthic grab samples used for this study, a number of sponge species were recorded and a single cup coral was also identified in sample FF005-B. These species are not likely to be sampled very well with a grab as they require a hard surface upon which to grow and occur mainly on bedrock. Whilst not absolute confirmation of the occurrence of these MPA search features, the presence of these species is unusual in grab samples and these stations may therefore warrant further investigation.

The presence of two component biotopes of the MPA search feature of Northern Sea Fan and Sponge Communities could warrant further consideration as follows;

- *Caryophyllia smithii* and *Swiftia pallida* on circalittoral rock (CR.MCR.EcCr.CarSwi)

Station FF005-B contains *Caryophyllia smithii*, and whilst only one specimen was recorded the environmental conditions and fauna present show some similarity to those described for this biotope. Examination of the collocated video footage and stills images would however be necessary to confirm the presence of this biotope as the biotope description is based almost entirely on the presence of conspicuous epifaunal which are not adequately samples using a benthic grab.

- Mixed turf hydroids and large ascidians with *Swiftia pallida* and *Caryophyllia smithii* on weakly tide-swept circalittoral rock

Stations WS009-D, WS027-B, WS038-C, WS042-D and WS045-C were identified as being circalittoral rock and all were encrusted with turf hydroids (e.g. *Lafoea dumosa*, *Abietinaria abietina* and *Campanularia hincksii*) and ascidians (e.g. *Cliona* and *Pyura tesselata*) and although no sea fans or cup coral were recorded in these samples there is some indication that this biotope may be present and close examination of the video footage collected at these sites would be recommended.

Offshore Deep Sea Muds

Within the samples examined as part of this project there were five (NS002-A and NS008-A-D) that were identified as being offshore muds (SS.SMu.OMu) which would fall under the MPA search feature of Offshore Deep Sea Muds. These samples were not assigned to a biotope although the nearest match was considered to be SS.SMu.OMu.PjefThyAfil which is a recognised component biotope of this MPA search feature.

Offshore Subtidal Sands and Gravels

The majority of the samples analysed as part of this study fall under the MPA search feature of Offshore Subtidal Sands and Gravel of which SS.SCS.OCS, SS.SSa.CFiSa and SS.SSa.OSa are all component biotopes. SS.SMx.OMx might also reasonably be considered under this search feature.

Low or Limited Mobility Species

Ocean Quahog Aggregations

The only low or limited mobility species that was identified during the present study was the Ocean Quahog, *Arctica islandica*. *A. islandica* is one of the longest living bivalves, reported to reach over 200 years in age (Ropes and Murawski 1983). They grow very slowly and sometimes not at all, therefore individuals of a similar size may vary greatly in age (Cargnelli *et al* 1999). *A. islandica* also matures slowly, with the average age at maturity reported to be anywhere between seven and 14 years (Rowell *et al* 1990; Thompson *et al* 1980). These traits make the ocean quahog particularly vulnerable to anthropogenic impacts and they are now seen as an indicator for environmental health (Rees and Dare 1993; Aquasense 2001).

The ocean quahog is not characteristic of any particular habitat and is known to occur in a range of sediments from coarse clean sand to muddy sand, and over a range of depths from 4m to 400m. Within Scottish territorial waters, *A. islandica* is found around all coasts but their offshore distribution is concentrated in the North Sea. Particularly high densities have been recorded from the North Sea Fladen Grounds (Whitbaard & Bergman 2003).



Ocean Quahog, *Arctica islandica* © seasurvey.co.uk

A total of 79 *A. islandica* were recorded in the grab samples analysed as part of this study, most of which were sampled from the Firth of Forth and the North Sea. *A. islandica* were present in the low abundances (1-3 individuals per grab sample) typical of this species (Cargnelli *et al* 1999; Witbaard & Bergen 2003). More than one individual per grab sample is thought to constitute the search feature “ocean quahog ‘aggregations’” (SNH 2012). A total of 22 sites fall into this classification as illustrated overleaf in Figure 17.

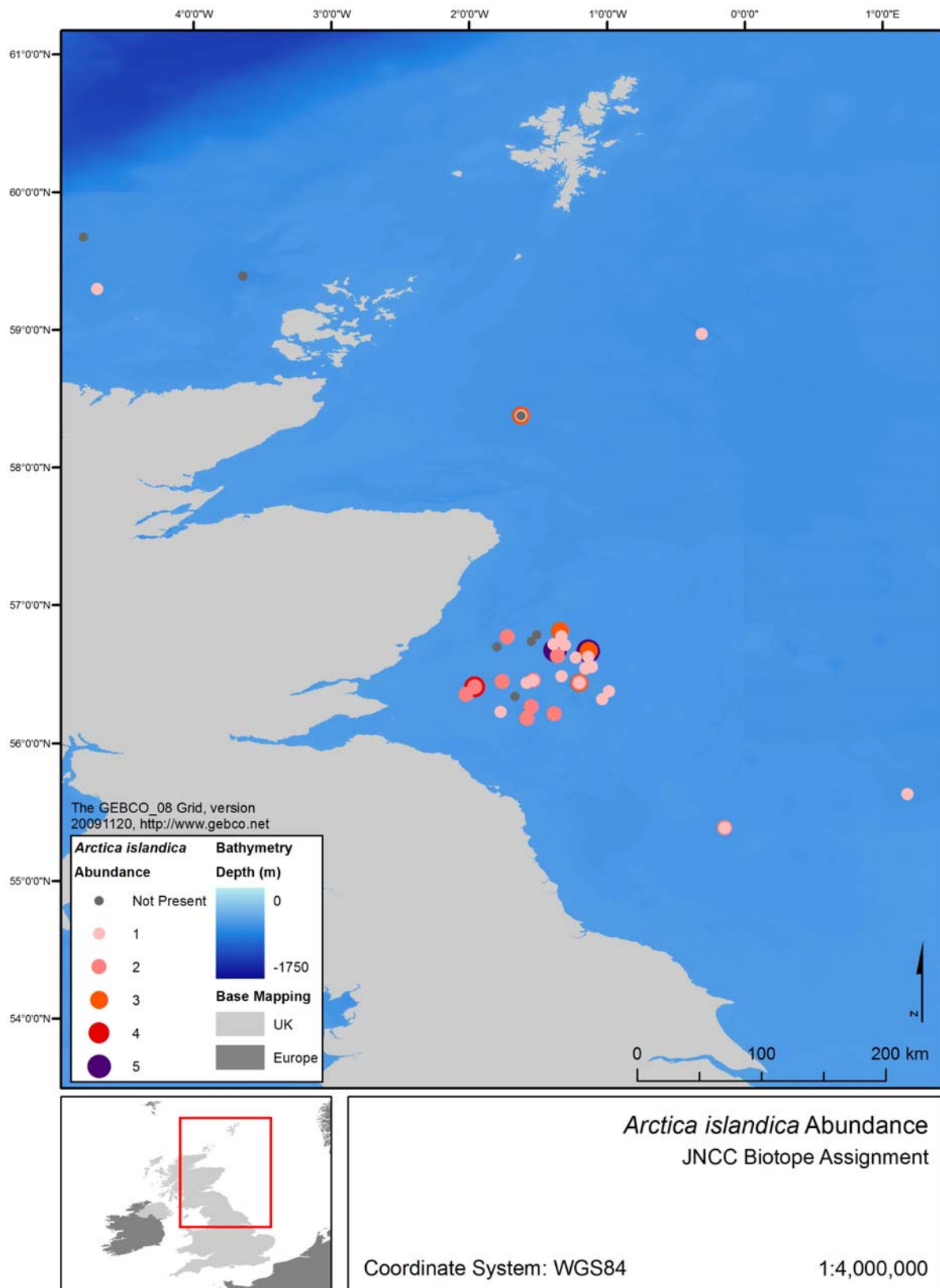


Figure 17. Chart illustrating the distribution of *Arctica islandica* records across the four study sites across Scotland's seas.

3.6.3 Rare and Alien Species

The rarity of species is a key factor determining the risk of extinction and since one of the main objectives in nature conservation is the avoidance of species extinctions, records demonstrating the presence and distribution of rare species are of key importance. The Joint Nature Conservation Committee (JNCC) developed criteria for the assessment of the rarity of marine benthic species in 1996 (Sanderson 1996). This identified “Nationally Rare Species” as; those species which have been recorded in eight or fewer of the 1,546 10km² squares of the Ordnance Survey national grid, and “Nationally Scarce Species” as those species occurring in nine to fifty-five squares. A number of marine species are also listed in the OSPAR List of Threatened and/or Declining Species and Habitats (OSPAR Commission 2008) as well as the International Union for the Conservation of Nature and Natural Resources (IUCN) Red List of Threatened species (IUCN 2012).

Another objective of nature conservation is to control the impacts of introduced species. Thousands of marine animals, plants and algae are transported from their natural range to ‘new’ areas through the transport and discharge of ballast water, as fouling organisms on ships hulls or through aquaculture. Environmental changes also give rise to new introductions, for example climate change is responsible for the extension of the natural range or distribution of numerous species. The term ‘alien’ is given to non-native species which have established self-maintaining populations in the UK. Where non-native species have not established self-maintaining populations or their origin is not clear they are classified as cryptogenic. This classification is particularly useful as it prevents introduced species being described as new or rare species. A number of rare and alien species were identified in grab sample taken across Scotland’s seas and these are summarised below in Table 16 and overleaf in Figure 18.

Table 16. Rare and alien species identified in grab samples collected across Scotland’s Seas.

Species	Common Name	Status	Abundance				Total
			Firth of Forth	North East Atlantic	North Sea	Windsor	
<i>Arctica islandica</i>	Ocean Quahog	OSPAR Listed	61	~	17	1	79
<i>Okenia leachii</i>	A sea slug	Nationally Rare	1	~	~	~	1
<i>Paradulichia typica</i>	Amphipod	Nationally Rare	~	~	1	~	1
<i>Escharoides mamillata</i>	Bryozoan	Nationally Rare	~	~	~	2	2
<i>Tamariscia tamariscia</i>	Sea Tamarisk	Nationally Scarce	2	~	~	~	2
<i>Harpinia laevis</i>	Amphipod	Nationally Scarce	1	~	~	~	1
<i>Paracentrotus lividus</i>	Purple Sea Urchin	Nationally Scarce	1	~	~	~	1
<i>Eriopisa elongata</i>	Amphipod	Nationally Scarce	~	~	3	~	3
<i>Mendicula pygmaea</i>	Venerid Bivalve	Not found in UK	~	~	14	~	14

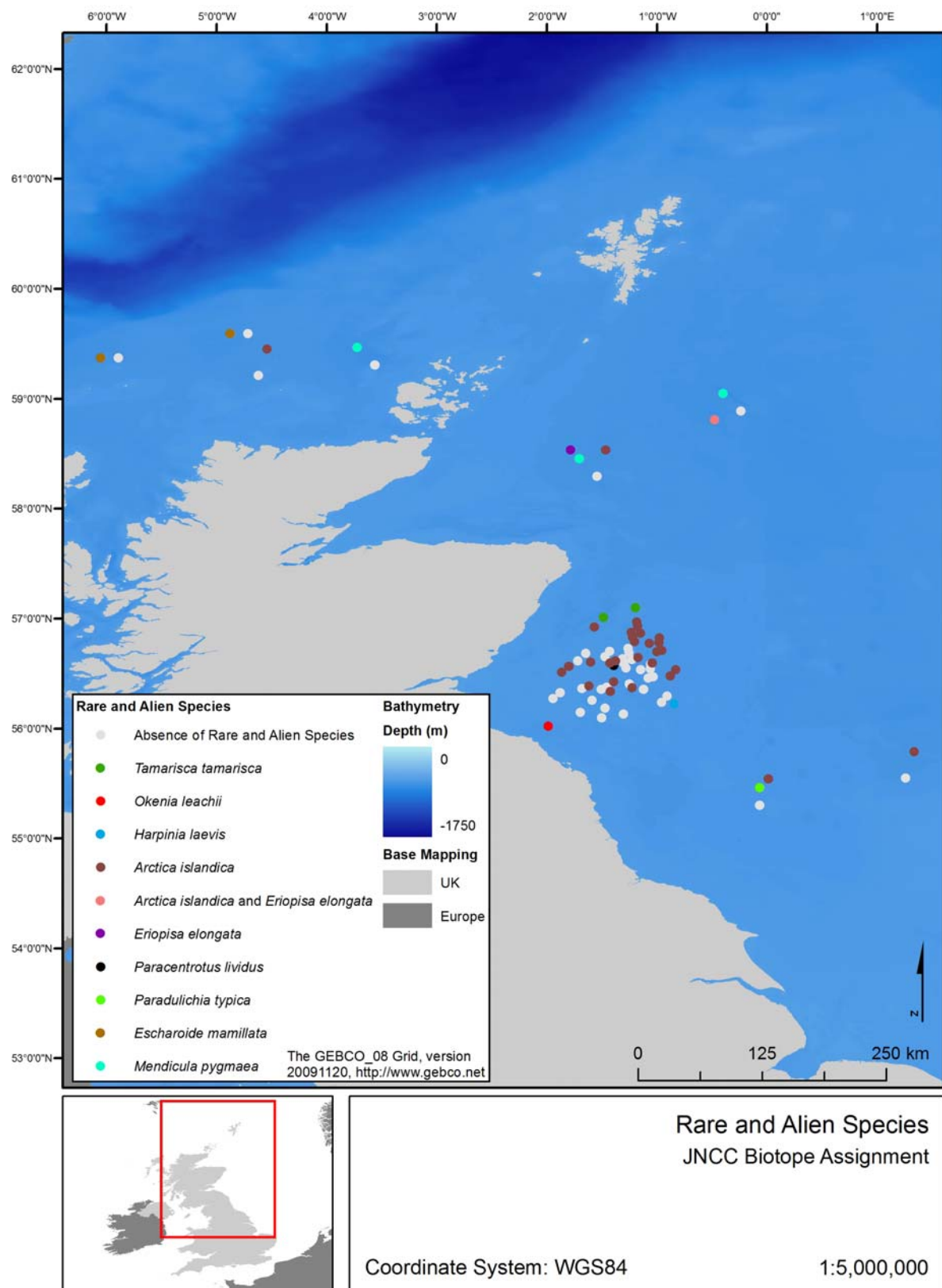


Figure 18. Chart illustrating the distribution of rare and alien species records across the four study sites across Scotland's seas.

OSPAR Listed Species

Only one species was identified in the benthic data collected across Scotland's seas, that appears on the OSPAR List of threatened and/or declining species and habitats (OSPAR Commission, 2008) and that is the Ocean Quahog, *Arctica islandica*. *A. islandica* was nominated for inclusion in the OSPAR list because significant declines were observed in the Greater North Sea (OSPAR Region II) populations between 1979 and 1986 and as a very long lived species it is vulnerable to direct and indirect anthropogenic impacts (Rachor, 2009). As this species is also an MPA Search feature its distribution and life-history are discussed in some detail in Section 3.6.2.

Nationally Rare Species

Three nationally rare species were identified across the study areas, although none of them were present in high abundances.

Okenia leachii

The sea slug *Okenia leachii*, was originally described from a single specimen from the Shetland Isles (Picton & Morrow 2010). There are more recent records from the Celtic sea, and from the Shiant Isles and Skye on the west coast of Scotland, however, the presence of this species in the Firth of Forth is to our knowledge the first record of this species on the east coast of Scotland. *O. leachii* has previously been recorded on muddy sand in deep waters (below 25m) (Picton & Morrow 2010; Place 2007) but was observed here on gravelly sand at a depth of 48m (Station FF001) indicating that the environmental niche requirements of this species may be broader than first thought.

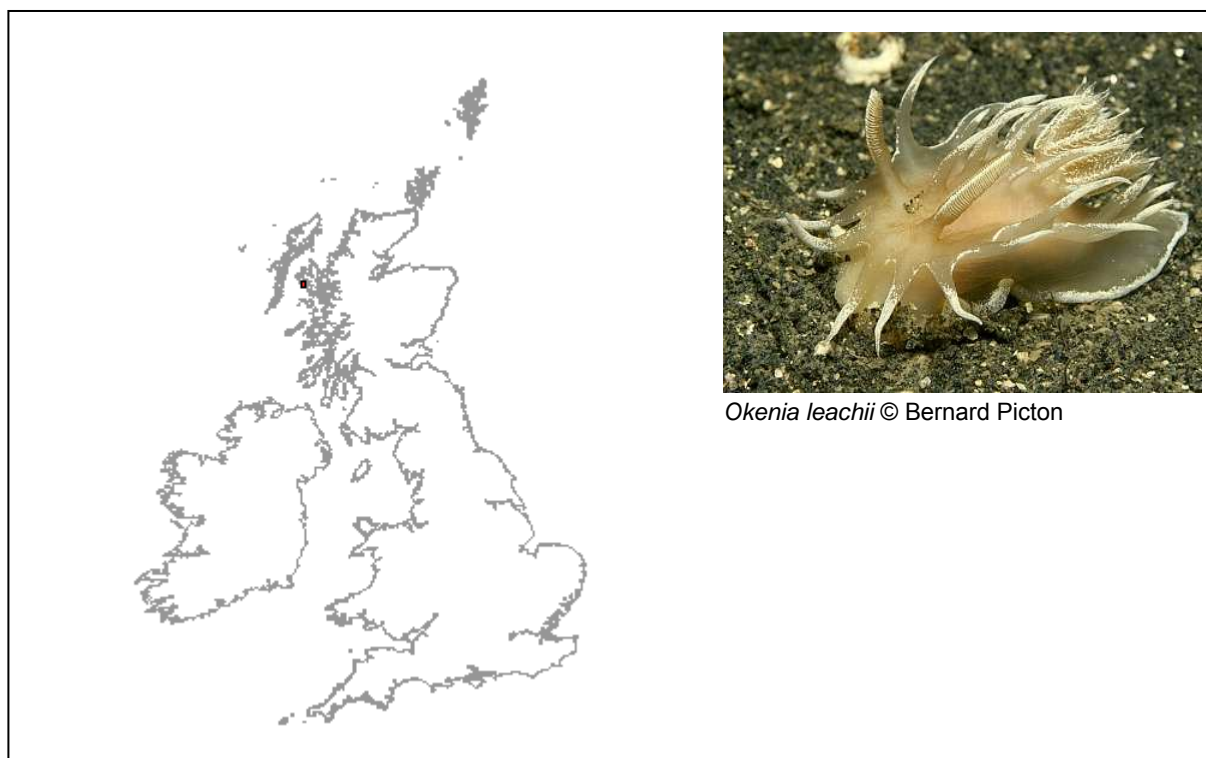


Figure 19. Chart showing the records of *Okenia leachii* held in the National Biodiversity Network (NBN) Database © Crown copyright and database rights 2011 Ordnance Survey [100017955].

Paradulichia typica

Very little is known about the caprellid amphipod *Paradulichia typica*, which is better known from studies in Greenland (Ortmann 1901; Stranksy and Brandt 2010) and Canada (Atkinson & Percy 1992). *P. typica* is known to form an important component of the diet of the Arctic alligatorfish *Ulcina olrikii* in Canada where this species is far more prevalent than in the UK (Atkinson & Percy 1992). It is unlikely that this species is an important component of the diet of fish in the UK given the low abundances in which it occurs. *Paradulichia typica* was recorded in just one sample in the North Sea survey (NS018-D) on sand at a depth of 67m.

Escharoides mamillata

Escharoides mamillata is an ancient bryozoan species that has been recorded from coralline crag deposits dating back to the Neogene (23 - 2.8 million years ago) (Bishop & Hayward 1989; Lagaaij 1852; Taylor 2012). Recent examples of this species have however, been recorded from the Orkneys in Scotland, Northern Ireland and the Gulf of Marseilles in the Mediterranean Sea (Hayward & Ryland 1999) as well as the Isle of Man (Figure 20).

Escharoides mamillata was recorded in two samples collected during the Windsock survey, WS024-A which was collected from an area of muddy sandy gravel at a depth of 117m and WS041-B which was collected from an area of sandy gravel at a depth of 132m.

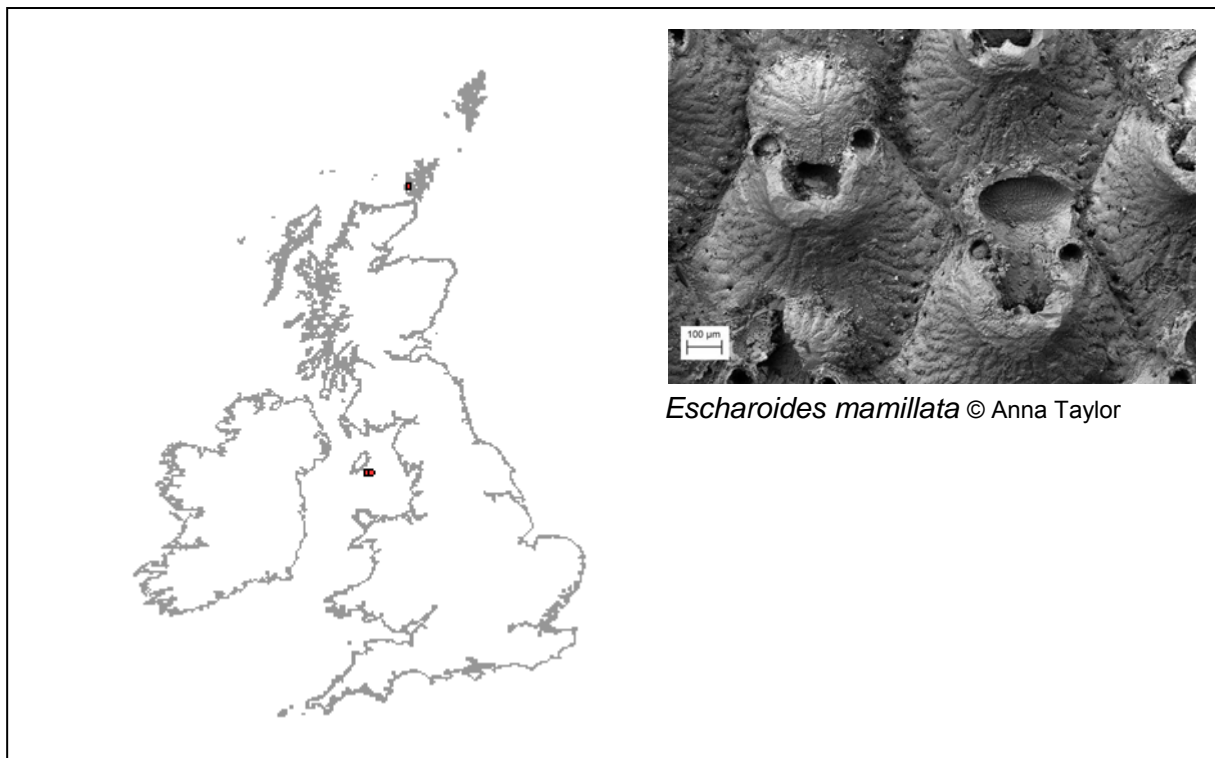


Figure 20. Chart showing the records of *Escharoides mamillata* held in the National Biodiversity Network (NBN) Database © Crown copyright and database rights 2011 Ordnance Survey [100017955].

Nationally Scarce Species

Four nationally scarce species were identified across the study areas, although none of them were present in high abundances.

Tamarisca tamarisca

Tamarisca tamarisca is a sertulariid hydroid which has previously been recorded in Iceland (Schuchert 2000), Bermuda (Calder 1998) as well in the North Sea, the Irish Sea and the west coast of Scotland (Figure 21 and Jennings *et al* 1999). Very little is known about the life-history of this species although it has been recorded at depths between 10 and 250m in UK (Hayward & Ryland 1998). *T. tamarisca* was recorded in two samples collected from the Firth of Forth, FF008-A which was collected from an area of slight gravelly sand at a depth of 62m and FF009-B which was collected from an area of sandy gravel at a depth of 56m. These are, to the best of our knowledge, the first records of this species in the Firth of Forth area.

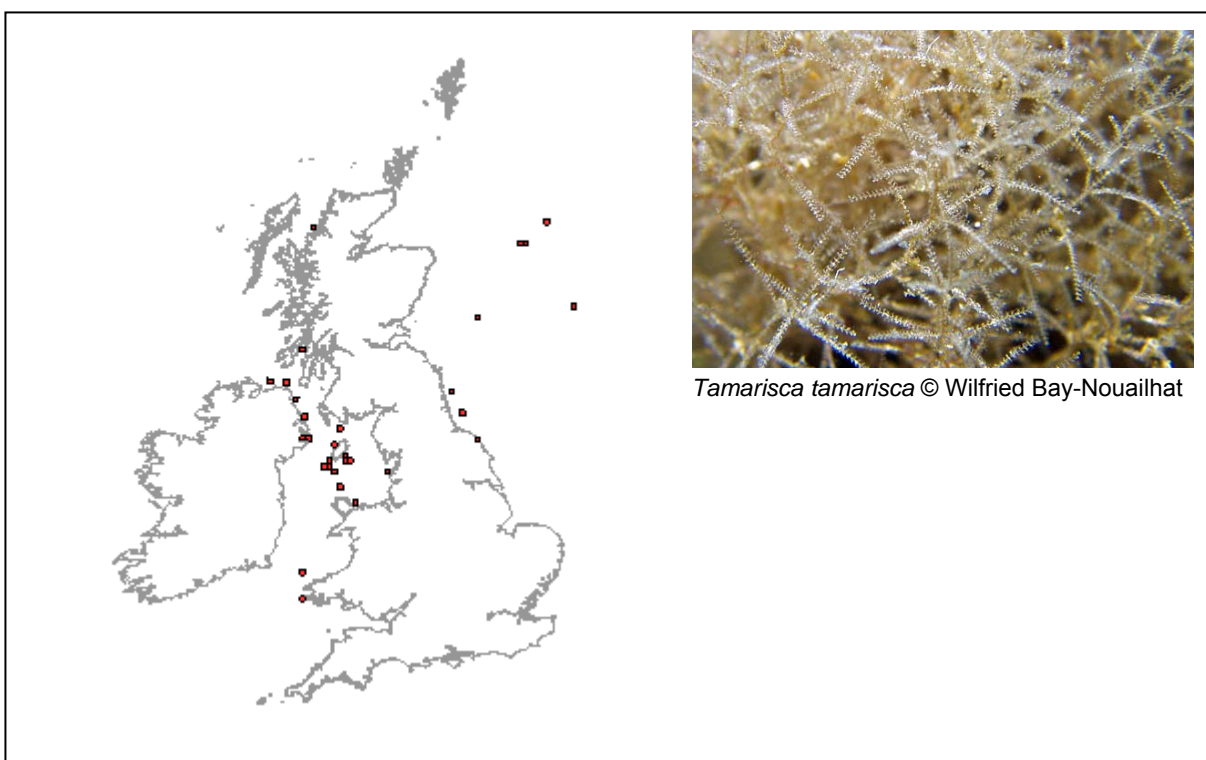


Figure 21. Chart showing the records of *Tamarisca tamarisca* held in the National Biodiversity Network (NBN) Database © Crown copyright and database rights 2011 Ordnance Survey [100017955].

Harpinia laevis

Harpinia laevis is a phoxocephalid amphipod which is known to occur in the North East Atlantic and North Sea between Norway and West Ireland (King *et al* 2004). There are numerous records of this species in Scottish waters in the NBN database (Figure 22), although these records are mostly offshore. *Harpinia laevis* was recorded from just one sample collected during the Firth of Forth survey (FF048-C) at a depth of 68m in an area of sand. Male specimens of *Harpinia* are less frequently recorded from benthic samples than their female counterparts (Karaman 1993) and it is thought that this may be because males live a more pelagic lifestyle. Males also become sexually mature, and hence morphologically distinct, at a relatively late stage meaning that they will naturally be less numerous than females and juveniles combined.

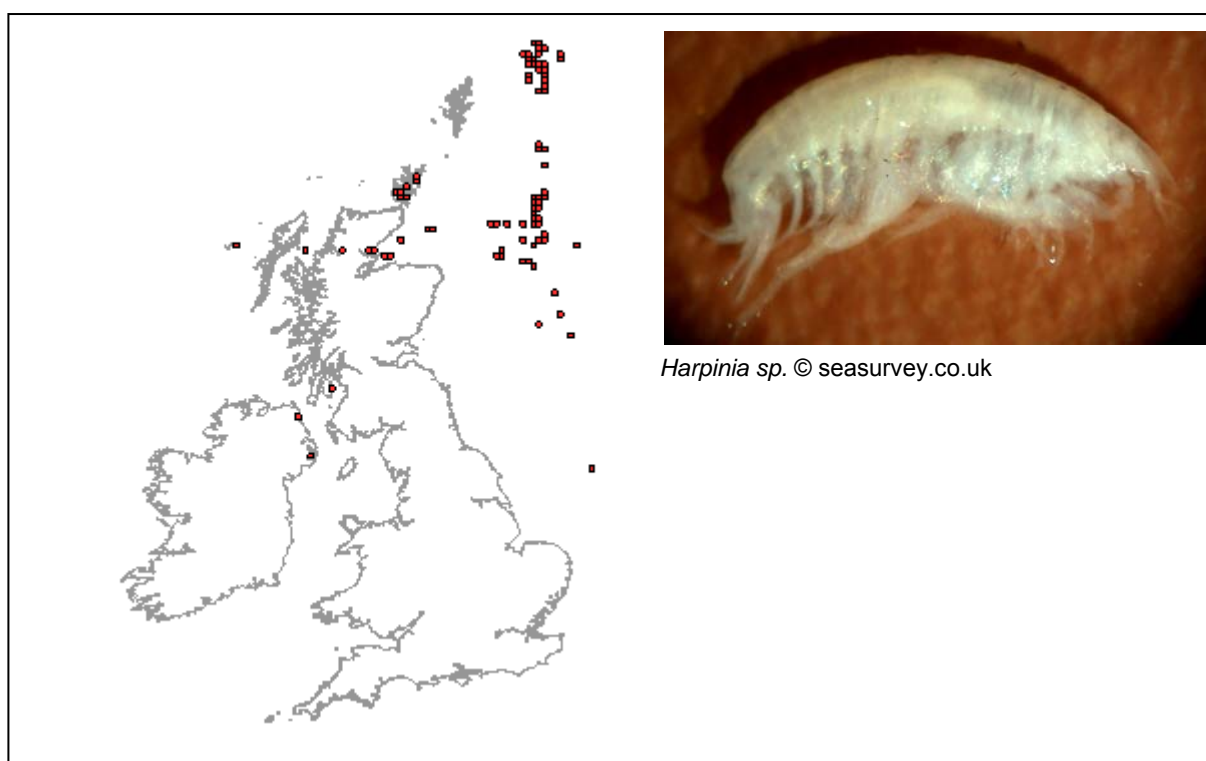


Figure 22. Chart showing the records of *Harpinia laevis* held in the National Biodiversity Network (NBN) Database © Crown copyright and database rights 2011 Ordnance Survey [100017955].

Paracentrotus lividus

The purple sea urchin, *Paracentrotus lividus*, is found in the Channel Islands, in a few locations in western Scotland and occasionally on the south west coasts of England. It is common on the west coast of Ireland and is abundant in areas such as County Clare (Pizzolla 2007). Despite this, there are only a small number of records for this species in the NBN database (Figure 23).

The purple sea urchin is typically found on the lower rocky shore in rock pools and in the shallow sublittoral down to depths of 3 m, but may be found deeper (Pizzolla 2007). It can also be found higher up on the middle/upper shore in rockpools, especially in Scotland. It uses its spines and teeth to bore into soft rocks, its burrow providing protection from both wave action and desiccation at low tide. The urchin increases the size of its burrow as it grows. They are also sometimes found in beds of the seagrass *Zostera* spp. In this study *P. lividus* was recorded in a single sample collected from an area of gravelly sand at a depth of 52m in the Firth of Forth (FF010-A).

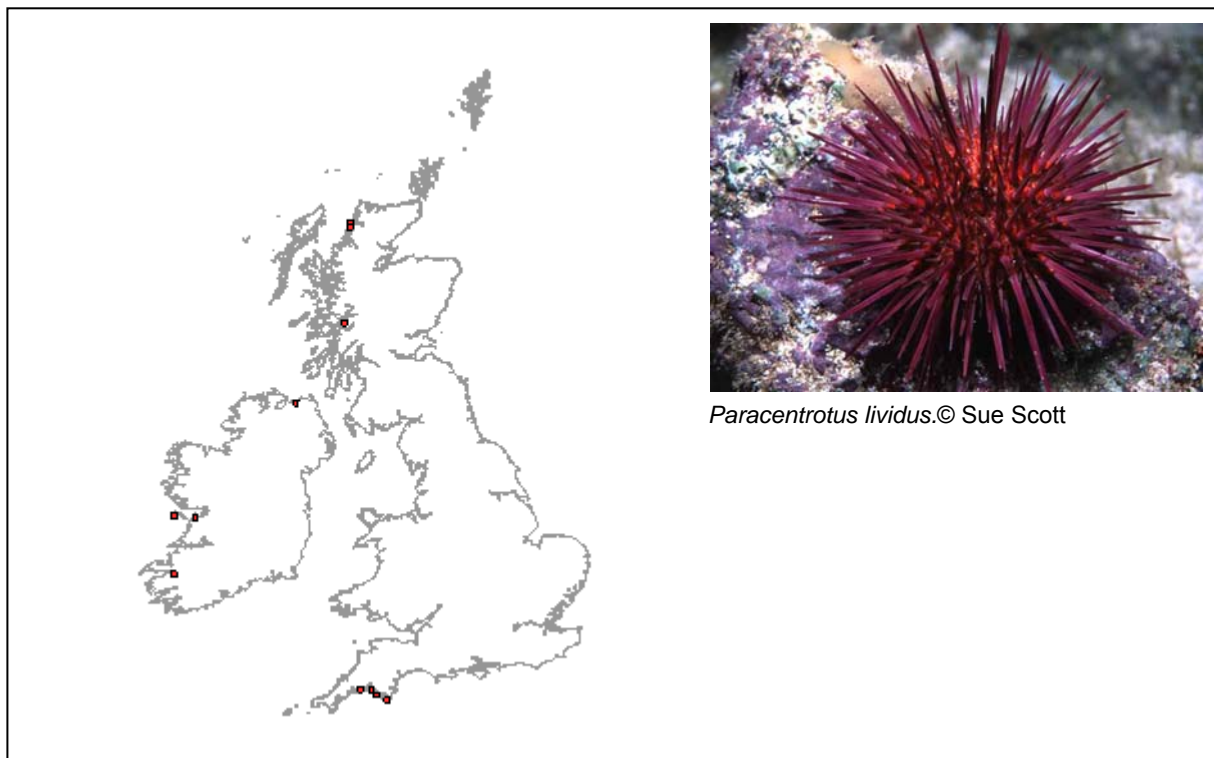


Figure 23. Chart showing the records of *Paracentrotus lividus* held in the National Biodiversity Network (NBN) Database © Crown copyright and database rights 2011 Ordnance Survey [100017955].

Eriopisa elongata

Eriopisa elongata is an amphipod belonging to the family Melitidae which has been recorded in high abundances in some locations in offshore areas of the North Sea including the Dogger Bank (Künitz 1990) and the northeast of Scotland (Figure 24). *Eriopisa elongata* was recorded in two samples collected during the North Sea survey, NS002-A which was collected from an area of sand at a depth of 125m and NS008-D which was collected from an area of sandy mud at a depth of 135m. Very little information is available regarding the life history and niche requirements of this species and so it is not known whether these conditions fall within the expected distribution for this species.

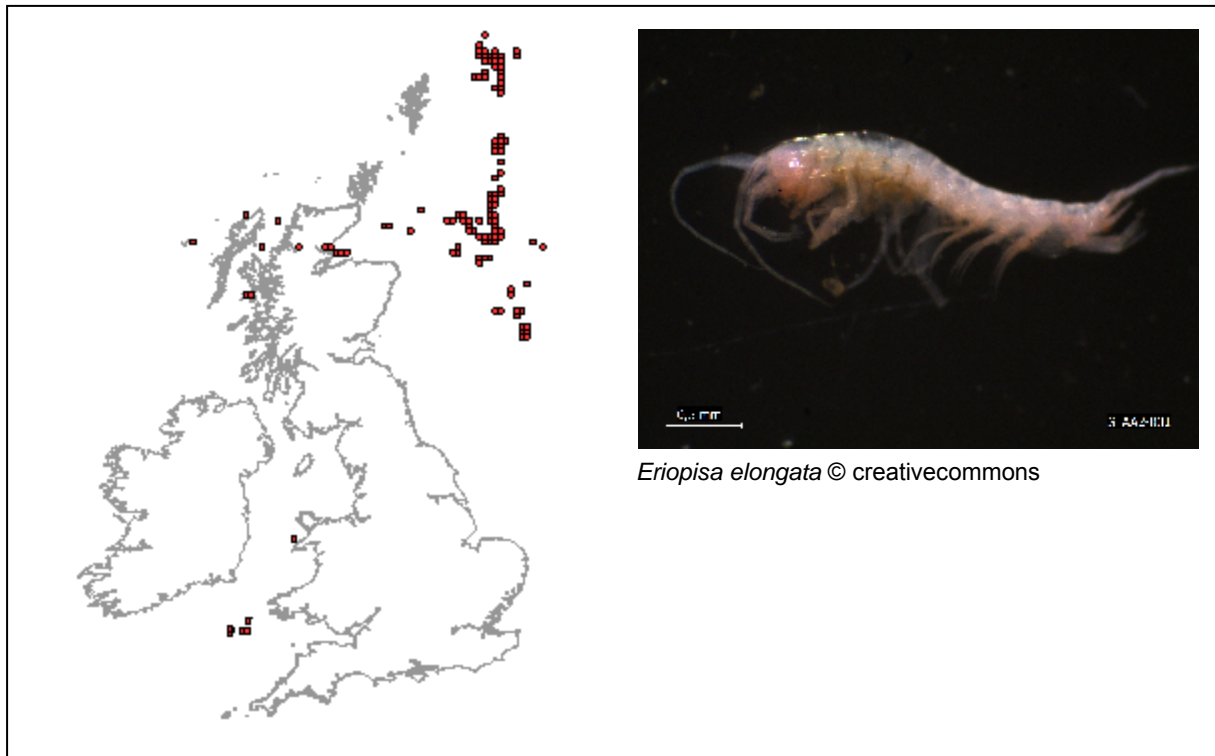


Figure 24. Chart showing the records of *Eriopisa elongata* held in the National Biodiversity Network (NBN) Database © Crown copyright and database rights 2011 Ordnance Survey [100017955].

Alien Species

No established alien species were identified in the data collected across the four sites in Scottish waters. However, the venerid bivalve, *Mendicula pygmaea*, which was listed in TREx as being “Not in UK Waters” was identified at seven sites within the North Sea survey area. Despite being listed as a potential non-native species, *M. pygmaea* has been observed, often abundantly, in samples from oil fields as far south as Fulmar in depths ranging from 85 to 161m (Oliver & Killeen 2002). This species has not been recorded from the oil fields in deeper water towards the shelf edge, but has been recorded on the west coast of Scotland, from Raasay Channel and in the Firth of Lorn where it is locally common in muddy gravel at depths of 20 to 100m (Oliver & Killeen 2002). *M. pygmaea* was recorded in seven samples collected during the North Sea survey from areas of sand and sandy mud in depths from 124m to 179m (NS002-A, NS008-B, NS008-D, NS005-A – NS005-D).



Mendicula pygmaea © National Museum of Wales

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5 Electronic Appendices

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