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Croker Carbonate Slabs cSAC/SCI Initial Monitoring Report

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Summary

This monitoring report explores and describes the attributes of the Annex I habitat feature 'Submarine structures made by leaking gases' within the Croker Carbonate Slabs cSAC/SCI (and areas of the feature adjacent to the site boundary) to enable future assessments of feature condition under the EC Habitats Directive, and to fulfil wider monitoring needs under the Marine Strategy Framework Directive (MSFD). In addition to providing an initial monitoring dataset with which future monitoring data can be compared, the report will inform the development of an effective site and feature-specific monitoring approach for the Croker Carbonate Slabs cSAC/SCI.

This report primarily uses data acquired from a 2015 multidisciplinary partnership survey conducted by the Joint Nature Conservation Committee (JNCC) and the Centre for Environment, Fisheries and Aquaculture Science (Cefas). The survey was specifically designed to provide the first data point in a monitoring time-series for the Annex I feature 'Submarine structures made by leaking gases', in the form of Methane-Derived Authigenic Carbonate (MDAC). The feature was investigated both within the site (where it had previously been confirmed) and at an adjacent area of predicted 'hard substrate' beyond the site boundary, which was identified from acoustic data acquired in 2012 and 2013. This 2012/13 acoustic hard substrate model is compared qualitatively with the 2015 monitoring data, in addition to areas of MDAC delineated from acoustic and groundtruthing data acquired in 2008.

The 2015 acoustic and groundtruthing data provide evidence that the spatial extent of the MDAC both within and adjacent to the site boundary is greater than previously predicted (based on the 2008 data), and extends almost 1.4 nautical miles beyond the north east of the site boundary, culminating in a distinctive cliff feature alongside a channel. The new analytically confirmed areas of MDAC, and the new modelled extent of MDAC at or just below the seabed, correspond partially to the modelled extent of the predicted 'hard substrate' interpreted from the 2012/13 acoustic data. The 2015 and 2012/13 acoustic models did not correspond in the south east of the survey area. If MDAC is present in this area, it is thought likely to be buried under a thick veneer of sandwaves and/or clay.

Low lying 'pavement' forms of the MDAC feature were frequently observed from imagery within and adjacent to the site, with the exception of the south eastern area. More elevated, 'outcropping' forms of MDAC were less common and were generally recorded in the central areas of the site and beyond the site boundary, to the north east and south west. The presence of heterogeneous substrata and bedforms across the site confirms that the site lies within an area of high natural variability, characterised by a moderate to high energy hydrographic regime.

The 2015 multidisciplinary data provide strong evidence that active seepage of methane is ongoing within and adjacent to the site, and is likely to have occurred since the Last Glacial Maximum. It is thought likely that MDAC formation has continued to the present day, and that the regeneration of the habitat may counteract the natural erosion of the feature on a geological timescale.

Seabed imagery acquired in 2015 indicated the presence of seven broad habitat classes within the site, including examples of both pavement and outcropping forms of the MDAC feature. Multivariate analysis indicated that the assemblages associated with both forms of MDAC were markedly different to those observed in association with the other (largely sedimentary) habitat types present (with the exception of 'Subtidal coarse sediment'). Five epifaunal taxa (the soft coral *Alcyonium digitatum*, the hydroids *Nemertesia* and *Tubularia*,

the bryozoan *Cellaria* and the polychaete family Sabellidae) were typically associated with the MDAC feature and occurred more frequently in areas of the outcropping form.

The proposed management measures for the site involve the exclusion of all mobile demersal fishing gears. These measures are proposed to maintain the feature in or restore it to favourable condition, in line with the site conservation objective. In order to assess and monitor the efficacy of the management measures proposed at this site, and evaluate feature condition subject to natural change, a robust monitoring design (using a combination of both pressure-based monitoring and direct observation) is required. Recommendations for future monitoring activities are presented based on the findings of the report.

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1 Introduction

The Croker Carbonate Slabs candidate Special Area of Conservation / Site of Community Importance (cSAC/SCI) is part of a network of Natura 2000 sites designed to meet conservation objectives under the EC Habitats Directive. These sites will also contribute to an ecologically coherent network of MPAs across the North-east Atlantic agreed under the Oslo Paris (OSPAR) Convention, and other international commitments to which the UK is signatory. This particular site is designated as an example of the Annex I habitat 'Submarine structures made by leaking gases'.

Under the Article 17 of the Habitats Directive, Defra is required to present a report to Parliament every six years that includes an assessment of the degree to which conservation objectives set for Annex I habitat features are being achieved. In order to fulfil this obligation, Defra has directed the Statutory Nature Conservation Bodies (SNCBs) to carry out a programme of MPA monitoring. As the SNCB responsible for nature conservation offshore (between 12nm and 200nm from the coast), JNCC is conducting a programme of MPA monitoring within these areas. Where possible this monitoring will also inform assessment of the status of the wider UK marine environment; for example, assessment of whether Good Environmental Status (GES) has been achieved, as required under Article 11 of the Marine Strategy Framework Directive (MSFD).

This initial monitoring report primarily explores data acquired from the first dedicated monitoring survey of the Croker Carbonate Slabs cSAC/SCI, which form the first point in a monitoring time series against which feature condition can be assessed in the future. The specific aims of the report are discussed in detail in Section 2.

1.1 Feature description

As defined by the European Commission (2013) the Annex I habitat 'Submarine structures made by leaking gases' consists of slabs, pavements, and pillars up to 4m high. These structures are formed by the aggregation of carbonate cement, resulting from microbial oxidation of gas emissions (mainly methane). Two sub-types of this feature have been identified; 'bubbling reefs' consisting of extensive formations where gases have leaked from the seabed, and 'submarine structures associated with pockmarks', which are associated with depressions in soft sediment seabed areas, up to 45m deep and a few hundred meters wide. Benthic communities consist of invertebrate specialists of hard marine substrata and are different from the surrounding habitats. 'Submarine structures made by leaking gases' are typically referred to as Methane-Derived Authigenic Carbonates (MDAC). Further information on the formation of MDAC is provided in Annex 1.

Previous characterisation surveys have shown that the seabed within the Croker Carbonate Slabs cSAC/SCI is composed of extensive areas of the 'bubbling reef' form of MDAC, which are likely to extend outside of the site boundary[2] (Judd 2005, Whomersley *et al* 2010, Callaway *et al* 2015). These MDAC structures are typically observed in two topographical forms within the site; 'pavement' (low-lying, often continuous carbonate slabs) and 'outcropping' (structures which are distinctly elevated above the surrounding sediment, often by up to 2m). A cliff feature up to 8m in elevation and 500m long has also been recorded

[2] At the time of publication a proposed boundary amendment for the site was out for public consultation, which would extend the boundary to encompass this additional area.

slightly south of the site centre (Judd 2005, Whomersley *et al* 2010), with another occurring to the south east of the site (Judd 2005).

1.2 Site overview

A summary overview of the Croker Carbonate Slabs cSAC/SCI is presented in Table 1, including the site conservation objective and feature attributes, information on the structural and ecological characteristics of the feature, its distribution in the context of the UK MPA network, and the measures proposed to manage these activities.

Table 1. Croker Carbonate Slabs cSAC/SCI site overview.

Site name	Croker Carbonate Slabs
Designation	Candidate Special Area of Conservation (cSAC) / Site of Community Importance (SCI)
Location	Irish Sea
Depth	65 – 110m
Area	66km^2
Designated feature	Submarine structures made by leaking gases (Annex I habitat)
Monitoring status	Initial monitoring survey: Oct–Nov 2015 (Wood *et al* 2016)
Previous data acquisition	A preliminary survey of the region that would later become the Croker Carbonate Slabs cSAC/SCI was undertaken in 2004, as part of the Department of Trade and Industry (DTI) SEA6 survey. In this survey, sub-bottom profiler, side-scan sonar, multibeam echosounder (MBES) and video data were collected from sites referred to as Texel 10 and Texel 11, and the presence of methane-derived authigenic carbonate (MDAC) was confirmed by carbon isotope analysis of grab samples (Judd 2005). In 2008, JNCC undertook additional survey work to characterise the features and established the presence of MDAC over a wider area (Whomersley *et al* 2008). The feature was mapped using MBES and side scan sonar data, and the MDAC was validated using seabed imagery, grab samples and carbon isotope analysis. Further sampling took place within and adjacent to the site as part of the MB0120 Marine Conservation Zone (MCZ) site verification surveys of the North St George's Channel; a recommended Marine Conservation Zone (rMCZ), which overlapped the entire Croker Carbonate Slabs cSAC/SCI (Callaway *et al* 2015). A full coverage MBES survey was carried out by Osiris Projects in 2012, with groundtruthing and side scan sonar data being collected by Cefas in 2012 and 2013 (CEND03/12 & CEND05/13).
Feature network context	The Croker Carbonate Slabs cSAC/SCI is the only MPA in the UK network designated for the feature sub-type 'bubbling reefs' (although it

	is present in the Pen Llyn a'r Sarnau SAC in Welsh territorial waters, designated as Annex I Reef). Two sites in the central North Sea, Braemar Pockmarks SAC and Scanner Pockmark SAC, have been designated for the sub-type 'Submarine structures associated with pockmarks'. The carbonates at these sites are limited in extent, and comprise a much smaller proportion of the designated Annex I feature extent within UK waters. The UK MPAs designated for Annex I 'Submarine structures made by leaking gases' are displayed in Figure 1.
Conservation objective	The conservation objective for the site is: Subject to natural change, **maintain/restore** the submarine structures made by leaking gases in favourable condition, such that: – the natural environmental quality is maintained; – the natural environmental processes are maintained; – the **extent**, **physical structure, diversity, community structure and typical species** representative of submarine structures made by leaking gases in the Irish Sea are maintained (JNCC 2015).
Human activities and pressures	Low levels of demersal towed fishing are known to occur within the site. Vessel Monitoring System (VMS) data (processed using the method described in Church *et al* 2016), have historically shown negligible activity within the site boundary, although an increase has been noted in the north eastern area of the site since 2012. It has also increased in an area of the substrate thought likely to be MDAC outside the north eastern boundary. Demersal static fishing (e.g. potting) is also known to occur within the site, although the intensity is unknown, as sufficiently resolute positional information is not available for this activity. Fishing activities within the cSAC/SCI are associated with the following pressure (JNCC 2015): • Physical damage through physical disturbance and abrasion The feature is also exposed to low levels of obstruction by an inactive submarine cable which runs across the site (approximately east to west), three wrecks, and static fishing gear (JNCC 2015). These anthropogenic modifications to the seabed are associated with the following pressure: • Physical loss due to obstruction
Current and proposed management measures	At the time of reporting, no management measures for human activities are implemented within the site (beyond those which would apply to licensed development activities as part of the consent process). However, proposals for the management of demersal fisheries within the site have been shared with relevant EU Member States prior to development of joint management recommendations. The proposed management measures include the exclusion of demersal trawls, dredges and seine net fishing within the management boundary.

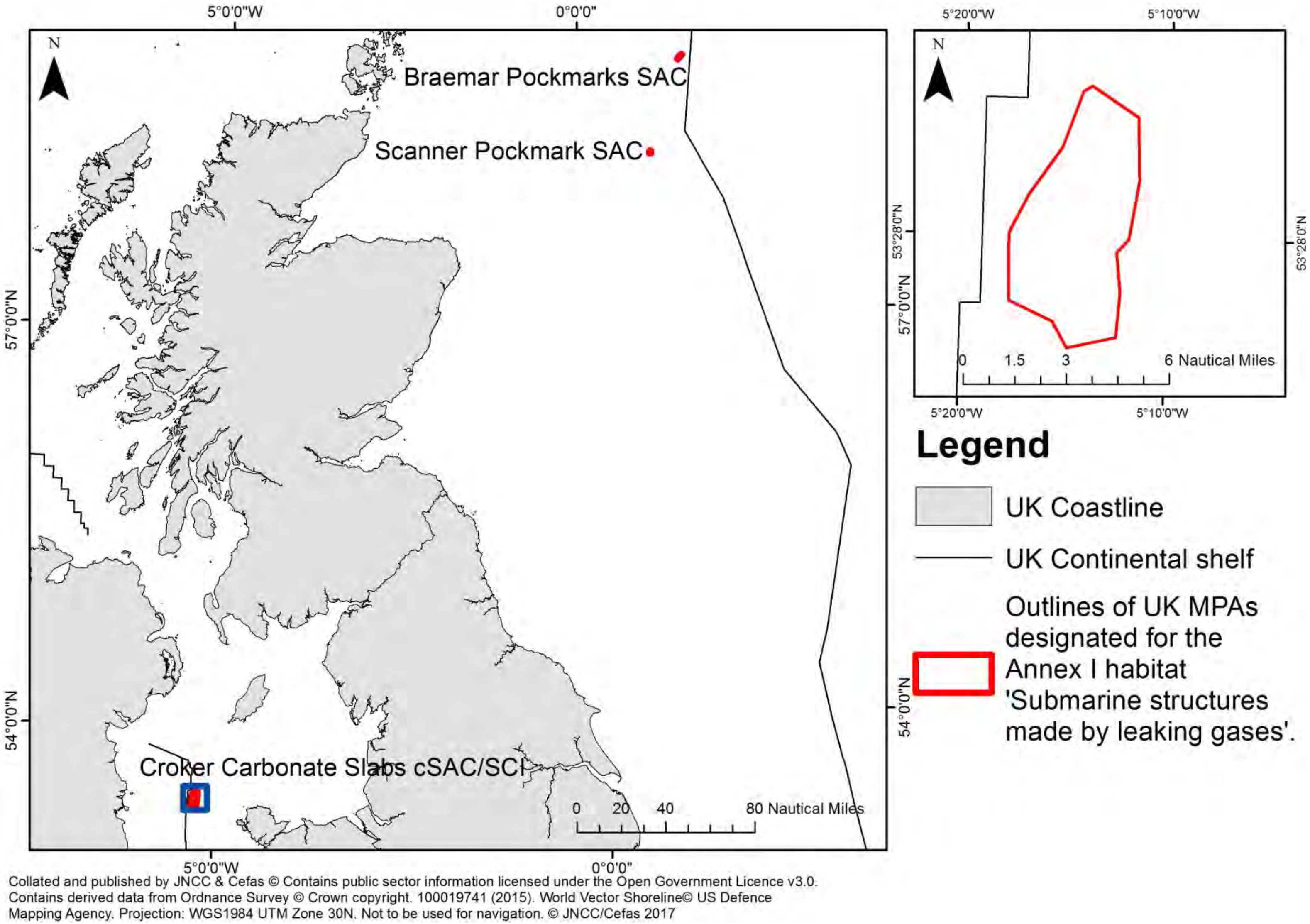

Figure 1. The UK network of Natura 2000 sites designated for the Annex I habitat feature ‘Submarine structures made by leaking gases’, with the Croker Carbonate Slabs cSAC/SCI boundary inset.

2 Monitoring aim and objectives

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

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

i) its natural range and the area it covers within that range are stable or increasing;

ii) the specific structure and functions, which are necessary for its long-term maintenance, exist and are likely to continue to exist for the foreseeable future, and;

iii) the conservation status of its typical species is favourable.

As stated in Table 1, the high-level conservation objective for Croker Carbonate Slabs cSAC/SCI is to **maintain/restore** the Annex I 'Submarine structures made by leaking gases' in/to favourable condition. The **maintain** aspect of the conservation objective implies that, based on our current understanding, the feature is generally considered to be in favourable condition across the site, subject to natural change. The **restore** wording has been added to the maintain objective to reflect the possibility that increased fishing activity in the north-east of the site in recent years may have impacted the structure and function of feature, and the associated biological communities in that area.

The primary aim of this monitoring report is to explore and describe the attributes of the feature within the Croker Carbonate Slabs cSAC/SCI (and likely areas of the feature adjacent to the site boundary) to enable future assessments of feature condition. The results presented will be used to develop recommendations for future monitoring, including the discussion of specific metrics which may indicate whether the condition of the feature has been maintained, improved or declined. The secondary aim of the report is to present evidence relating to MSFD Descriptors of Good Environmental Status (GES).

This report will primarily utilise data acquired from a partnership survey conducted in 2015 by JNCC and Cefas (CEND 23/15) which was designed to deliver the first benthic habitats monitoring dataset for the site. These data will be used as the initial dataset in a monitoring time-series from which the rate and direction of change can be inferred in the long-term, and will also inform the development of an effective site and feature-specific monitoring approach for the site. The data will be compared qualitatively with existing data acquired in 2004 (Judd 2005), 2008 (Whomersley *et al* 2010) and 2012/13 (Callaway *et al* 2015).

The specific objectives of this monitoring report are as follows (feature attributes defined in the site conservation objective (see Table 1) are in bold):

1) Describe the **extent** and **physical structure** of the Annex I feature 'Submarine structures made by leaking gases' within the Croker Carbonate Slabs cSAC/SCI, and in an adjacent area of hard substrate likely to constitute Annex I feature.
2) Describe evidence of ongoing methane seepage (indicating the potential for ongoing formation of the feature) within and adjacent to the Croker Carbonate Slabs cSAC/SCI.
3) Describe the **diversity, structure and typical species of the biological communities** associated with the Annex I feature within the Croker Carbonate Slabs

cSAC/SCI, and in an adjacent area of hard substrate likely to constitute Annex I feature.

4) Present any evidence of non-indigenous species (MSFD Descriptor 2) and marine litter (MSFD Descriptor 10) within and adjacent to the Croker Carbonate Slabs cSAC/SCI.

5) Recommend future monitoring approaches for the Croker Carbonate Slabs cSAC/SCI, and other sites containing comparable Annex I features.

3 Methods

3.1 2015 survey design (CEND 23/15)

The 2015 survey required a multidisciplinary approach to achieve the diverse monitoring objectives described in Section 2, and employed a number of gear types and data acquisition techniques. These included; acoustic data acquisition (MBES and single-beam bathymetry, side scan sonar, and sub-bottom profiling), grab sampling of seabed sediments, video and still imagery acquisition, MDAC sampling for verification and dating, and methane detection at the seabed and in the water column (using a combination of water sampling and a methane sensor deployed on the underwater imagery system).

The survey was designed with reference to the existing acoustic and groundtruthing datasets acquired in 2004 (Judd 2005), 2008 (Whomersley *et al* 2008; CEND 11/08), and 2012/13 (Callaway *et al* 2015; CEND 03/12 & CEND 05/13). A limited amount of acoustic data were acquired within the site in 2004 and 2008, with groundtruthing data allowing delineation of MDAC areas. Full MBES coverage of the site and surrounding areas was achieved in 2012/13 during a survey of the North St. George's Channel rMCZ, but the groundtruthing conducted was limited. This 2012/13 MBES data indicated (but could not confirm) the presence of substantial additional areas of hard substrate thought likely to be MDAC, both within and adjacent to the Croker Carbonate Slabs cSAC/SCI (Callaway *et al* 2015; see Figure 2). The 2015 survey was designed to allow acoustic investigation and groundtruthing of these areas of predicted hard substrate, in addition to revisiting areas of previously groundtruthed MDAC identified in 2004 and 2008. A number of stations were also planned to verify substrate composition in areas where MDAC was not predicted to occur. Where groundtruthing operations were not focused on areas of previously observed MDAC, sampling locations were systematically stratified between the hard substrate and sedimentary habitats modelled from 2012/13 acoustic data.

A basic overview of the distribution and extent of 2015 survey data is presented in Section 3.2, with more detailed information on survey design and data processing available in the cruise report (Wood *et al* 2016).

3.2 Data acquisition and processing

3.2.1 Acoustic data

Sub-bottom profiler data were acquired using a Geoforce deep-towed boomer, operating alternately at low (135J) and high (240J) power. Georeferencing, processing and interpretation of the data took place onboard during the survey to inform the prioritisation of groundtruthing activities. The boomer lines were positioned to cover known and potential MDAC features on the seabed, to improve understanding of the sub-seabed geology and to examine potential fluid transport pathways.

Singlebeam scientific echosounder (Simrad EK60) and multibeam echosounder (MBES; Simrad EM2040) were deployed continuously and simultaneously throughout acoustic survey operations. The singlebeam echosounder was used to identify water column targets thought to be attributable to gas seepage plumes, whilst the MBES provided information on seabed depth, seabed type and backscatter character, and the distribution of hard substrates.

Dual frequency side scan sonar data were acquired using an Edgetech 4800MP at 300/600kHz,to assess the texture and dimensions of hard substrata in potential areas of MDAC identified from the MBES backscatter data. The side scan sonar system was also used to investigate potential areas of gas seepage, with possible gas bubbles being visible in the water column data.

Acoustic data acquired from the 2015 survey were processed and quality assured by Cefas, with the exception of the sub-bottom profiler data, which were processed by Exploration Electronics Ltd. Further details on acoustic methods and data processing are provided in the cruise report (Wood *et al* 2016).

3.2.2 Seabed imagery

A total of 128 drop frame camera transects were conducted, each acquiring video and still imagery data. The imagery data were collected in accordance with MESH guidelines (Coggan *et al* 2007) using an STR SeaSpyder “Telemetry” drop camera system. They were processed by Seastar Survey Ltd., and underwent quality assurance by Cefas. Epifaunal taxa were enumerated, with abundance recorded for solitary forms and percentage cover recorded for colonial taxa. The entire dataset was also recorded according to the SACFOR abundance scale (see Turner *et al* 2016). The processing protocol is provided in Annex 2.

3.2.3 Grab sampling

Sediment samples for particle size analysis (PSA) and macrofaunal community characterisation were collected at 56 locations across the survey area using a 0.1m^2 mini Hamon grab. These Hamon grab samples were sub-sampled for sediment and sieved over a 1mm mesh to retain the macrofauna. In addition, grab samples were collected at three stations using a 0.1m^2 Day grab, which provided an undisturbed sample to allow meiofaunal sub-sampling. The Day grab samples were sub-sampled for PSA (using a full depth core) and meiofauna, to investigate potential symbiotic associations between nematodes and chemo-autotrophic microorganisms which could indicate ongoing release of methane. Suspected MDAC concretions present in the grab samples were retained for analysis following onboard carbonate testing using 10% hydrochloric acid. Sediment samples for PSA, macrofaunal community analysis, meiofaunal community analysis and verification of potential MDAC were processed onboard the vessel according to the methods described in the cruise report (Wood *et al* 2016).

PSA was conducted for a total of 53 sediment samples by Cefas, in accordance with NMBAQC standards (Mason 2011). Forty-six Hamon grab samples were processed for macrofauna by the Institute of Estuarine & Coastal Studies (IECS) according to NMBAQC standards (Worsfold & Hill 2010), with external quality assurance provided by APEM Ltd. Meiofaunal processing of the seven sub-samples acquired from the Day grabs was undertaken by Physalia Ltd according to the industry standard protocol detailed in Physalia 2016 (published alongside this report).

3.2.4 Mineralogical and petrographical characterisation, isotope analysis and dating

Samples of potential MDAC were retained from 30 locations within and adjacent to the Croker Carbonate Slabs cSAC/SCI boundary, and were submitted to the British Geological Survey (BGS) for petrologic, mineralogic and isotope ($\delta^{13}C$ and $\delta^{18}O$) analysis. Eight samples were selected for $^{87}Sr/^{86}Sr$ ratio analysis and nine for U/Th age dating.

X-ray diffraction (XRD) optical microscopy and backscatter scanning electron microscopy (BSEM - including energy-dispersive X-ray microanalysis (EDXA)) were undertaken primarily to ascertain the mineralogical composition of carbonate cements. Carbon ($\delta^{13}C$) and oxygen ($\delta^{18}O$) stable isotope analyses provided indications of the origin of the carbon in the cements, and the temperature of cement formation, respectively.

Strontium isotope ($^{87}Sr/^{86}Sr$) analysis of aragonite was conducted for five of the MDAC samples to establish the source of pore water fluids, and on three samples of late calcareous encrusting biota (e.g. serpulid and bryozoan encrustations) to provide a reference value for the $^{87}Sr/^{86}Sr$ of the marine Irish Sea seawater signature, against which the $^{87}Sr/^{86}Sr$ of the aragonite cements could be compared. Uranium-thorium age dating analysis was carried out on a subset of nine samples, previously analysed by petrographic and stable isotope methods, to provide a broad indication of the age of the deposit.

The procedures used in these analyses are detailed in the BGS reports (Field *et al* 2016a, 2016b & 2017), which are published alongside this report.

3.2.5 Methane concentration in seawater

A pump-driven molecular electronic transducer (METS) methane detector system was attached to the camera frame, and operated continuously to measure variations in methane concentration in the water column on 125 of 128 deployments. The sensor remained immersed in seawater to stabilise between deployments. Methane concentration data were collected by the METS sensor as voltages, which were then used to calculate methane concentration in nmol/L. Data were recorded at 5 second intervals for the duration of each camera transect, and average methane concentration per minute was calculated.

Additional water sampling was conducted at six stations, using a 10L Niskin bottle, to provide validation of the METS sensor readings. Three samples were collected within the Croker Carbonate Slabs cSAC/SCI at locations where METS sensor, singlebeam echosounder, MBES or visual data suggested possible gas seepage. In addition, three control locations were sampled; one adjacent to the site, and two outside the survey area. At each sampling location, three water samples were acquired; one just above the seabed, one in mid-water and one just below the sea surface. The samples were processed by Newcastle University using a modified version of the method detailed in Upstill-Goddard *et al* (1996). Further information on methane sampling procedures is available in the cruise report (Wood *et al* 2016).

3.3 Mapping the extent of MDAC

3.3.1 Current mapping

Object-based image analysis (OBIA) was conducted to produce an interpretation of the extent of MDAC, using data from the 2015 survey. MBES bathymetry and backscatter data (3m resolution) and derivatives of bathymetry including slope, orientation, rugosity and relative position were used as predictor variables. Seabed still imagery, classed as MDAC or sediment, were used to train the MDAC extent model.

Initial data exploration revealed little discriminatory power across all examined predictor variables, due to a large overlap in acoustic signatures between the MDAC and the surrounding sediment classes. The most likely explanation for this is a combination of small-scale spatial heterogeneity across the features, along with an inability to reliably delineate areas of MDAC covered by a thin sediment veneer from adjacent sediments using the acoustic data. Additionally, the MBES 'ping' may be able to penetrate thin sediment veneers, resulting in a mixed backscatter response with contributions from both the sediment veneer and the underlying MDAC. Therefore, a presence-only approach was selected for mapping, with only observations of MDAC from the seabed imagery data illustrated in the resultant feature map. Due to this, the final mapped product shows the interpreted extent of MDAC at *or just below* the seabed, alongside an assessment of confidence in the interpretation. A detailed account of the method is provided in Annex 4.

3.3.2 Previous mapping

As stated in Section 3.1, a previous delineation of MDAC had been made using the acoustic and groundtruthing data from CEND 11/08 (see Figure 2). This delineation was created using expert judgement to interpret side scan sonar data and MBES data processed at a resolution of 20m. The acoustic data showed a distinctive signature which corresponded to areas of MDAC observed from camera transects, and was manually delineated across the extent of the acoustic data (see Whomersley *et al* 2010).

In addition, as part of a study into the wider broad-scale habitats of the North St. George's Channel rMCZ, Callaway *et al* (2015) used full-coverage MBES from CEND 03/12, processed at a resolution of 5m, and imagery from CEND 05/13 (2012 & 2013 data) to produce an OBIA interpretation of hard substrate at the surface (Figure 2). It was thought likely that this substrate would also constitute MDAC, as observed in 2008.

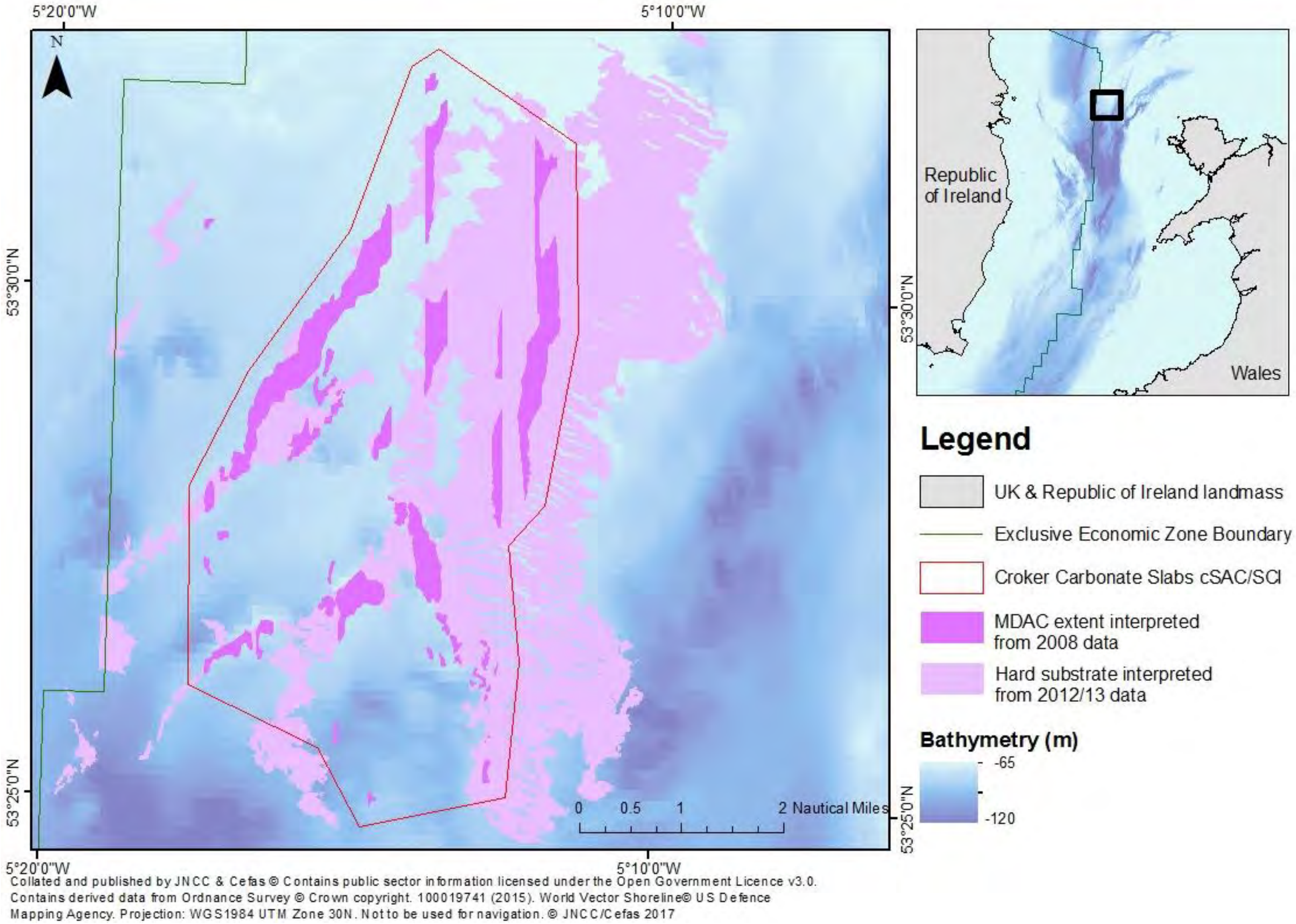

Figure 2. MDAC delineated from 2008 data (CEND 11/08) overlying area of hard substrate interpreted from 2012/13 data (CEND 03/12 & CEND 05/13; Callaway *et al* 2015)[3].

[3] All site plots in this report are overlain on Astrium bathymetry relative to Chart Datum based on 6 arcsec Defra Digital Elevation Map.

3.4 Biological data preparation, rationalisation and analysis

The procedures used to prepare, rationalise and analyse infaunal and epifaunal data are described in the following sections.

Prior to these processes macrofaunal and epifaunal taxon lists, from grab sample and still imagery data respectively, were cross-referenced with the World Register of Marine Species (WoRMS Editorial Board 2017) to ensure consistent nomenclature, and amended accordingly. The amended taxa were then cross-referenced against the MSFD Descriptor 2 list of non-indigenous species (Stebbing *et al* 2014, see Annex 6).

The number of stations at which meiofaunal samples were acquired was insufficient for statistical analysis, therefore no preparation or rationalisation of meiofaunal data was required.

3.4.1 Epifaunal data

Still seabed images were processed to allow semi-quantitative comparison of epifaunal communities between areas of MDAC and the surrounding sediments, and identification of taxa which could be considered as representative or typical of the MDAC feature. The visual quality of still images was extremely variable, with high levels of suspended sediment in the water column rendering a number of the images of insufficient quality for analysis, particularly when tidal currents were flowing. To ensure that the images were used appropriately in statistical analyses (e.g. only images of sufficient quality to identify epifauna were included), each image was assigned to one of three quality categories (Table 2).

Table 2. Quality categories and criteria assigned to still seabed images.

Category	Quality	Use	Assumptions	No. stills
Zero	Seabed is not visible across 0-25% of the image.	Not to be used for any purpose	n/a	798
Substrate ID	Seabed is fully or partially visible (across at least 25% of the image), and the substrate composition of the seabed can be reliably determined. The image quality is not sufficient for taxonomic analysis.	To determine whether MDAC is present or absent.	Assume that MDAC is not present in the areas of the image which are not visible.	973
Taxon ID	Seabed is fully visible to partially visible (across at least 50% of the image), and image quality is sufficient to identify and quantify epifauna according to the SACFOR scale (e.g. Excellent or Good quality according to NMBAQC guidelines; Turner *et al* 2016).	To determine whether MDAC is present or absent, and to be used for semi-quantitative analyses of biological communities.	Assume that the areas of the image which are not visible display a similar composition to those which are visible. If visibility is obscured across >50% of the image it will be assigned to the Substrate ID category.	2457

Conclusive identification of MDAC requires carbon isotope ($\delta^{13}C$) analysis of physical samples, however it was not feasible to collect and samples from all imagery locations. Where physical samples could not be acquired, areas of potential MDAC were identified with reasonable confidence from seabed imagery. The feature was recorded as present where the characteristic physical appearance of MDAC was noted from imagery, and appeared similar where MDAC could be reliably classified from samples (see Table 3 and Table 4), and where MDAC was indicated from acoustic data.

The topographical characteristics of MDAC varied along a continuum, with observations assigned to one of two classes; ‘outcropping’ or ‘pavement’ (see Table 3). These classes were analogous to the ‘high relief’ and ‘low relief’ MDAC categories described by Whomersley *et al* (2010).

Table 3. Description and example images of outcropping and pavement MDAC.

MDAC Type	Description	Example image
Outcropping	MDAC that outcropped from the surrounding sediment and showed distinct elevation.	
Pavement	MDAC that formed low-lying ‘pavement’ slabs and isolated chunks, which did not stand significantly above the surrounding seabed.	

It should be noted that MDAC may underlie surficial sediments in a large number of images where fauna appear to be attached to a hard substrate beneath a thin sediment veneer, or where large amounts of *Sabellaria spinulosa* obscured the seabed. To avoid over-estimating the extent of the MDAC, a conservative approach was taken and the substrates in these images have not been classified as MDAC.

Due to, 1) the highly mixed and dynamic nature of the sediments, 2) the patchiness of some areas of hard substrata, and 3) the variable quality of the seabed images, still images could not always be classified as MDAC with high confidence. Each image where MDAC was recorded was assigned to one of three confidence categories, as described and illustrated in Table 4.

Table 4. Description and example images of high, moderate and low confidence MDAC.

Confidence	Description	Example image
High (outcropping)	Hard substrate with the appearance of MDAC is directly visible, and/or relief from the seabed is substantial.	
Moderate (pavement)	Continuous hard substrate is present with noticeable relief from the seabed, but the substrate is obscured by epifaunal growth, or image quality is not sufficient to allow high confidence in feature classification.	
Low (pavement)	Hard substrate is present which may constitute MDAC, but the substrate is obscured by epifaunal growth or sediment. This category generally was applied when low-lying, isolated chunks of hard substrata were present, or small areas of exposed hard substrata were visible in areas of mobile sand.	

It was determined that the use of a data-driven approach to identify statistically significant multivariate groupings (e.g. using a cluster analysis) was not feasible, due to the large size of the epifaunal dataset (2457 still images of sufficient quality) and the highly variable nature of the sediments. This type of analysis is likely to have resolved an extremely large number of statistically significant groups, which would have been difficult to interpret. Each still image was therefore assigned 'a priori' to one of seven broad habitat categories, and multivariate analyses used to explore differences and similarities between the MDAC feature and other substrata. The majority of sediments observed in the seabed imagery were assigned to physical habitat categories at level 3 of the EUNIS classification (Davies *et al* 2004); however, this resolution was not sufficient to adequately characterise the full range of hard substrata within the survey area.

MDAC is not currently listed within level 3 of the EUNIS classification as a separate habitat. MDAC is represented in the EUNIS classification as A4.73 'Vents and seeps in circalittoral rock' under the level 3 category A4.7 'Features of circalittoral rock', however, it may also be considered to fall within the 'A4.2 Moderate energy circalittoral rock' category. An additional continuous hard substrate, blue-green clay, was observed within the survey area, and is also classified under A4.2 in the EUNIS hierarchy (as ‘A4.23 Communities on soft circalittoral rock’). As such, for the purpose of this report, occurrences of MDAC have been assigned to one of two separate groups, ‘outcropping MDAC’ and ‘pavement MDAC’ for analysis; both of which would be classified as A4.2 ‘Moderate energy circalittoral rock’ under the EUNIS hierarchy. Where MDAC was observed and recorded, this classification overruled other sediment types observed in the still image. The habitat groupings used for epifaunal analysis are provided in Table 5.

Table 5. Broad habitat groups used in statistical analysis of epifaunal data, including number of ‘Taxon ID’ quality still images available for analysis (including EUNIS classifications, Davies *et al* 2004).

A4.2 Moderate energy circalittoral rock			A5.1 ‘Sublittoral coarse sediment’	A5.2 ‘Sublittoral sand’	A5.4 ‘Sublittoral mixed sediment’	A5.6 ‘Sublittoral biogenic reefs’
Outcropping MDAC	Pavement MDAC	A4.23 ‘Communities on soft circalittoral rock’ (clay)				
67*	207*	48	1663	154	244	74

* Only includes images assigned as high and moderate confidence in MDAC classification.

Epifauna were recorded from still images using percentage cover for colonial taxa, or abundance values for solitary taxa. In order to retain information for the entire community (comprising both solitary and colonial taxa), abundance according to the SACFOR scale was selected as the appropriate metric for analysis (see Turner *et al* 2016). Use of this semi-quantitative score allowed all taxa to be grouped in a single analysis, and retained information on relative abundance, which would have been lost if a binary presence/absence transformation had been applied.

The percentage cover and abundance data were converted from SACFOR to a numerical score of 1 (rare) to 6 (super-abundant) for truncation purposes. In the process of merging taxa, only the maximum numerical score was retained, resulting in a potential underestimation of abundance. Therefore a precautionary approach was applied to merging of epifaunal taxa. Where different growth-forms of the same taxon were recorded, these were kept as separate taxa to reflect morphological diversity and avoid underestimation of abundance (e.g. arborescent, encrusting, papillate, and repent forms of Porifera). Where it was thought unlikely that a taxon could be consistently or reliably identified, even from ‘high quality’ images, it was merged to a lower taxonomic resolution (e.g. *Sabella pavonina*, *Myxicola* and *Bispira volutacornis* were merged to Sabellidae). Fish and cephalopods were removed from the dataset.

3.4.2 Macrofaunal data

Infaunal taxa which were likely to have been incompletely sampled as part of the >1.0mm fraction, or were problematic to identify (e.g. due to fragmentation), were removed from the dataset (e.g. Nematoda, Copepoda, Nemertea), in addition to eggs and algae. Fish and highly mobile fauna were also removed from the dataset, (with the exception of *Ammodytes marinus* due to their burrowing habit).

Expert judgement was used to rationalise the remaining taxa and reduce incidences of ‘double counting’ of given taxa in the dataset. Rationalisation of the macrofaunal data was conducted according to the protocol described by Downie *et al* 2016 (and summarised in

Annex 3). In addition to this protocol, juveniles were removed from the dataset when they were extremely numerous in comparison to adult specimens of the same species or genus, and were thought to represent an ephemeral component of the community (e.g. bivalve spat). In some instances, named species were recorded alongside records of members of the same genus, where the latter was not identified to species level. In such cases, the abundance of the taxa was examined in respect to the relative impact removal or merging would have on the outputs of analyses. Where merging to genus level would result in substantial loss of information at the species level, the genus level taxon was removed (e.g. where the genus level taxon occurred only a few times, in comparison to multiple occurrences of multiple species level taxa of the same genus). Where removing the genus level taxon would result in substantial loss of occurrences, the species-level taxa were merged to genus level.

3.4.3 Statistical analysis

Macrofaunal data

The truncated macrofaunal abundance data (including only solitary fauna) were imported into PRIMER v6 (Clarke & Gorley 2006) for multivariate analyses, and associated environmental and factorial data were assigned. The number of taxa (S) and total abundance of individuals (N) were calculated using the DIVERSE function. A fourth-root transformation was applied to the dataset to reduce the influence of numerically dominant taxa, and a resemblance matrix was generated using the Bray-Curtis similarity measure. A hierarchical cluster analysis was conducted on the transformed dataset, using group average linkage, and a SIMPROF test was applied with a significance threshold of 5%. A non-metric multidimensional scaling ordination (MDS) was generated for the dataset, which was overlain with the cluster groupings generated by the SIMPROF test, and additional environmental parameters to aid interpretation. Bubble plots were created to visualise the influence of continuous and ordinal environmental data on multivariate spatial patterns in community composition.

Epifauna

Statistical analyses of epifaunal data were undertaken on still images assigned to the highest image quality category (as described in Table 4). 'Low confidence' MDAC images were also removed to reduce uncertainty in the dataset.

Numerical SACFOR abundance data for each still image were imported into PRIMER v6 (Clarke & Gorley 2006), and analytical factors were assigned, including habitat groups. Analysis of similarity (ANOSIM) was conducted to test for statistically significant differences ($p \leq 0.05$) in overall epifaunal assemblage structure between the habitat groups presented in Table 5, and similarity percentage (SIMPER) measures were generated for each group to explore the contribution of different taxa to similarity within groups and dissimilarity between groups.

Mean SACFOR abundances of the taxa contributing the most to similarity within the outcropping MDAC category were compared to those generated for the other habitat groups. Five taxa were found to be substantially higher in association with the MDAC features, in comparison to the other habitat groups where MDAC was absent. Three of these taxa were judged to occur at sufficient frequency (were present in >20% of images within each group) to allow statistical comparison of their abundance in association with outcropping versus pavement forms of MDAC. Pavement forms were observed to be patchily distributed at a small spatial scale, and were more subject to inundation by sediment than outcropping forms of MDAC, due to their low-lying nature. It was postulated that the differences in abundance of MDAC-associated taxa observed between the two forms may be due to the more fragmented character of the pavement form, and therefore the lower overall extent of substrate available for faunal settlement. Further analyses were conducted to compare the abundances of the three taxa between outcropping and pavement forms of MDAC, using the permute.groups test (R emon package; Barry *et al* 2017) to investigate whether observed differences could be attributed to the increased patchiness of low-lying pavement MDAC (quantified as % cover from still images), as opposed to a genuine difference in colonisation of the two forms. This analysis was repeated three times, for; a) all images in which MDAC was observed to be present, b) images with ≥30% MDAC coverage, and c) images with ≥70% MDAC coverage.

3.4.4 Trait-based inferences

Biological traits information was compiled for the five epifaunal taxa observed in association with MDAC, to inspect the occurrence of characteristics that explain and thus further establish the association. This, in turn, will aid determination of whether each of these taxa are consistently representative of the MDAC feature (thus allowing subsequent assessment and monitoring of whether typical species of this feature have been adequately maintained). To this end, focus was placed on traits that relate to a taxon's substrate preference; mobility, living habitat, morphology and feeding type. This is not an exhaustive list of relevant traits, but rather a subset that was selected based on the availability of information. Information on the modalities taxa exhibit for each biological trait was obtained from a review of published literature and online sources, including the Marine Life Information Network (MarLIN 20016a) and the Biological Traits Information Catalogue (BIOTIC; MarLIN 2016b), the Genus Trait Handbook (Marine Ecological Surveys Limited 2007), and Marine Species Identification Portal (ETI Bioinformatics 2017).

In addition to studying the traits of epifauna observed to be associated with MDAC, the full list of epifauna was explored to identify any taxa that are particularly sensitive to smothering and/or physical disturbance. Sensitivity to smothering was considered as a single trait, for which information was sought directly. However, the occurrence of sessile taxa was also noted due to their inability to avoid, and therefore sensitivity to, high levels of sedimentation. Sensitivity to disturbance was inferred from the modalities that taxa exhibit across a suite of traits; high fragility, low flexibility, low mobility, large body size, and long lifespan. The abundance and spatial distribution of faunal assemblages (and their associated biological trait groupings) were then explored to identify any potential effects of the pressures of interest (namely sedimentation and/or physical disturbance arising as a result of mobile demersal fishing activities) on the biological assemblages present (and thus the conservation status of the Annex I feature).

4 Results

4.1 Wider environmental context

The Croker Carbonate Slabs cSAC/SCI is located in an area of high natural turbulence, where tidal currents are strong and sediments are subject to frequent suspension and redistribution (Whomersley *et al* 2010). This influence is particularly apparent to the east of the site, where a 3.5km wide and 24km long deep-water channel has formed, possibly as a result of the strong north-south prevailing current, resulting in the formation of large symmetrical sandwaves in the deeper parts of the channel (Figure 4). Sandwaves are also visible outside of the channel, to the east and south-east of the site, and in hollows within the site. In addition to mobilising sediments, the hydrodynamic regime has previously been noted to cause erosion of MDAC within the site. Whomersley *et al* (2010) observed that MDAC appeared to be eroded by currents and biological activity, and speculated that MDAC cliffs were likely to recede rapidly as the carbonate was undercut by water and sediment scour. The MDAC samples collected on the 2008 survey suggested an evolution from intact sheets of carbonate to sand and gravel sized fragments, through hydrodynamic and/or biological weathering.

The 2015 grab sample and seabed imagery data revealed a highly heterogeneous seabed within and adjacent to the Croker Carbonate Slabs cSAC/SCI, as would be expected given the hydrodynamic conditions. Results of the sediment PSA indicated that the majority of coarse sediment was located in the north of the site, with A5.2 ‘Sublittoral sand’ and A5.1 ‘Sublittoral coarse sediment’ being the predominant EUNIS level 3 habitats present. As illustrated in Figure 4, the sediment samples from the northern part of the site contained higher percentages of gravel and sand fractions, with some mud present in the A5.4 ‘Sublittoral mixed sediment’. The south of the site was dominated by A5.4 ‘Sublittoral mixed sediment’ and also contained the only stations classified as A5.3 ‘Sublittoral mud’ within the entire survey area. As the pie charts show in Figure 4, the percentage of mud was higher in the majority of southern stations compared to northern stations. This could be due to the variation in bathymetry, with the southern end of the site being deeper than the northern end, or a reflection of spatial heterogeneity. Well sorted sediments occurred predominantly in the north, and extremely poorly sorted sediments in the south. Well sorted sediments were exclusively classified as A5.2 ‘Sublittoral sand’, whilst A5.4 ‘Sublittoral mixed sediment’ was very poorly sorted or extremely poorly sorted. The physical and biological character of these sediments is discussed further in Section 4.4.1.

Seabed imagery revealed the widespread occurrence of a hard substrate throughout the survey area, both within and outside the cSAC/SCI, in addition to the sediments described above. This substrate was interpreted to comprise MDAC from still images, and thus assumed to constitute the Annex I ‘Submarine structures made by leaking gases’ feature for which the site is designated[4]. Evidence regarding the physical, biogeochemical and biological attributes of these features is presented in Sections 4.2, 4.3 and 4.4, and is discussed Section 5. In addition to the MDAC feature, blue-green clay was observed to outcrop from the seabed in a number of locations throughout the site (discussed further in Section 4.4.3), and lithified rocks of non-local origin (considered to be glacially-derived) were present in areas where the seabed was not characterised by mobile surficial sediments.

[4] As mentioned in Section 3.4.1, definitive identification of MDAC requires mineralogical and petrographical verification, which was not achievable for all areas of MDAC.

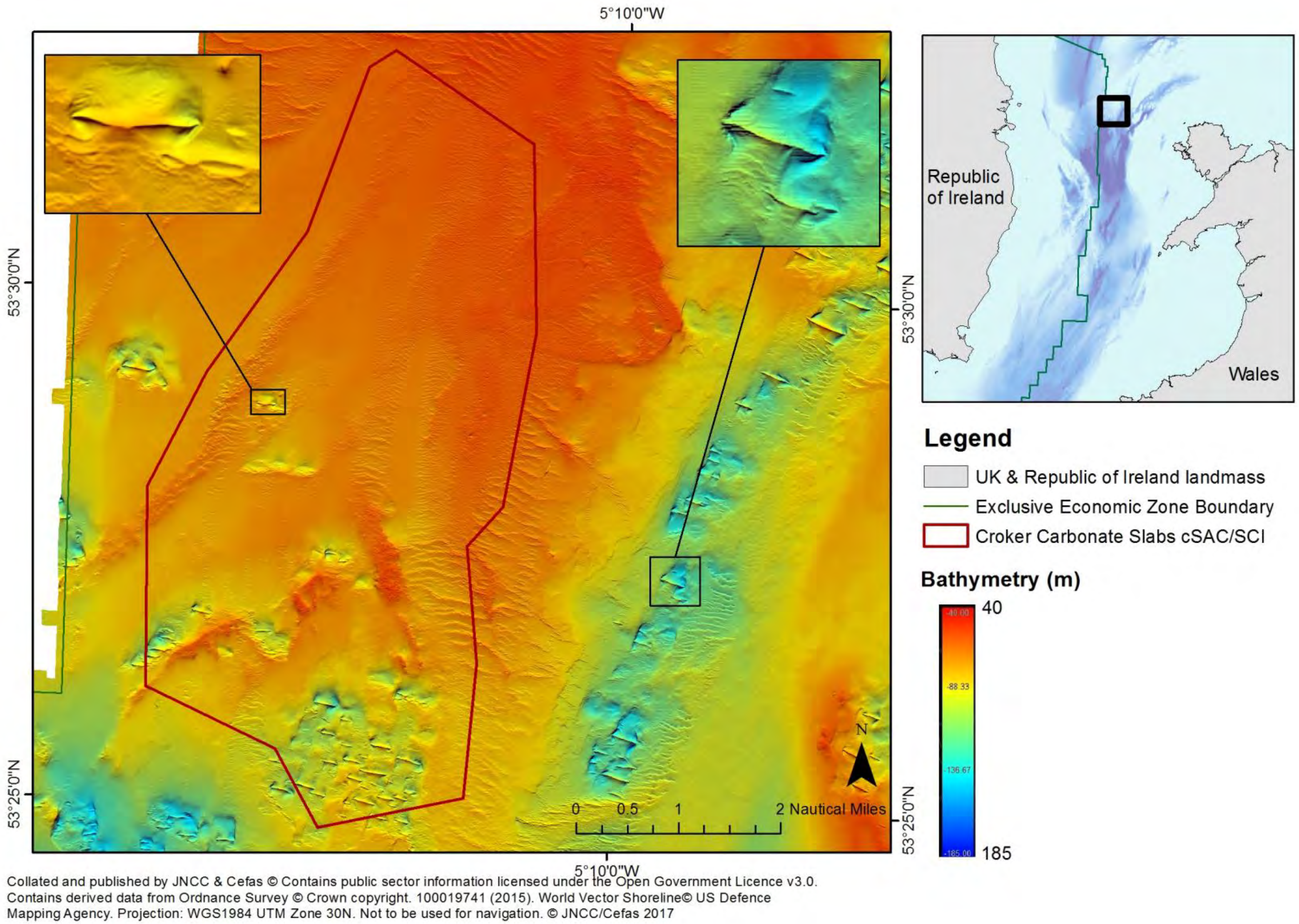

Figure 3. MBES data acquired for the 2012/13 North St George's Channel rMCZ, illustrating seabed topography. Inset images show sandwave features in hollows within the Croker Carbonate Slabs cSAC/SCI and in a channel to the east of site.

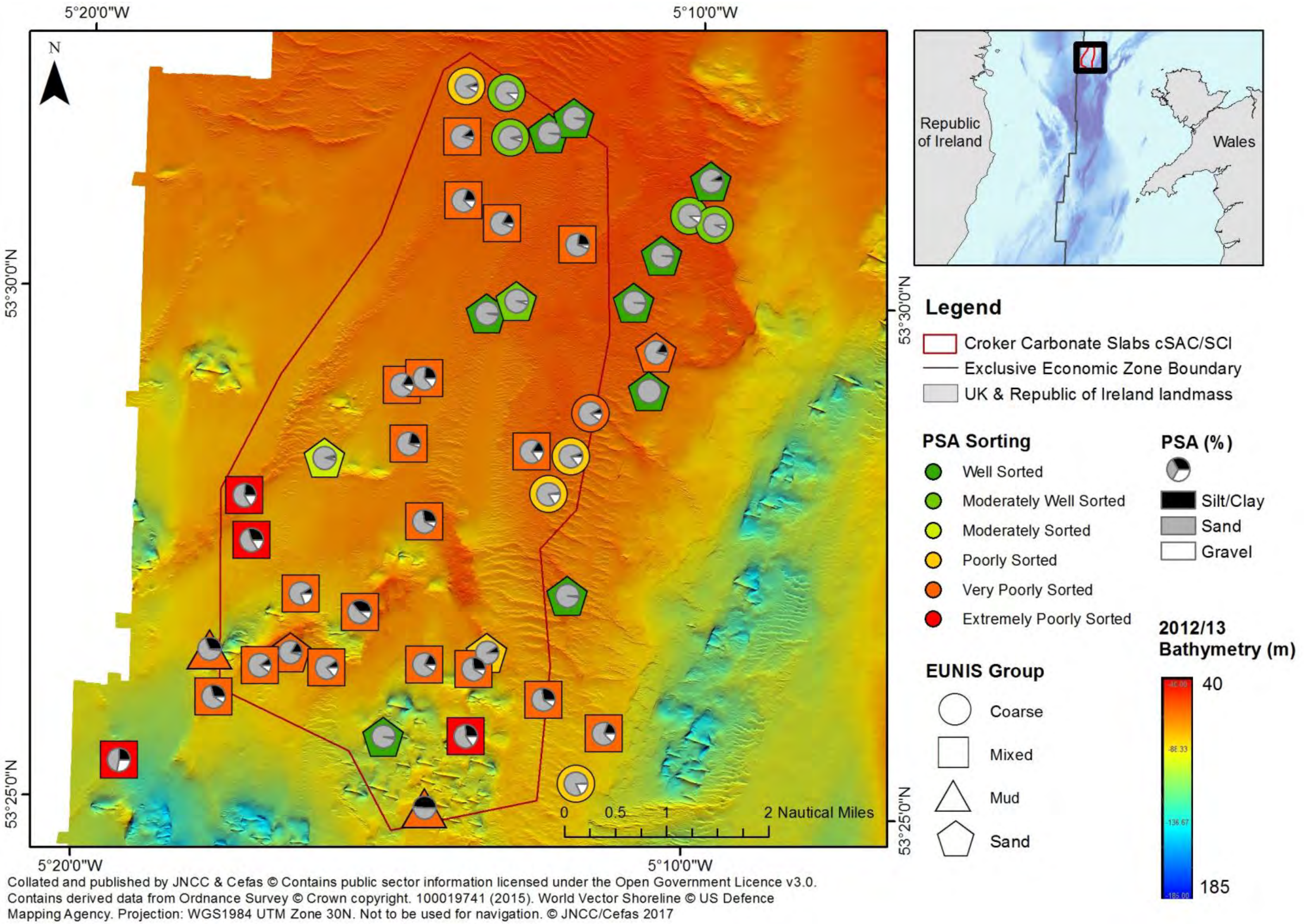

Figure 4. Grab stations with pie charts showing the results of particle size analysis, sediment sorting classifications and EUNIS level 3 habitat classifications, overlain onto 2012/13 MBES data.

4.2 Physical structure and spatial extent of MDAC

4.2.1 Mineralogical and petrographical verification, isotope analysis and dating

Of the 30 possible MDAC samples, 29 were determined to be carbonate-cemented and contain high-magnesium calcite, aragonite, or a combination of both cements. The remaining sample appeared to be a collection of random rock clasts, including igneous and other rock fragments. All of the aragonite and high-magnesium calcite cements were highly depleted in ^{13}C. $\delta^{13}C$ values were generally between -34 to -54‰ when compared with the Pee Dee Belemnite, a global standard, which is consistent with an authigenic origin in which carbonate precipitation is a consequence of anaerobic methane oxidation. Such $\delta^{13}C$ values are characteristic of MDAC described previously from this site (Judd 2005, Milodowski *et al* 2009) and elsewhere (Judd & Hovland 2007), including the UK sector of the North Sea (Milodowski *et al* 2013) and the Kattegat, where ‘Submarine structures made by leaking gases’ were first described (Jensen *et al* 1992). Therefore, this provides conclusive evidence that the samples recovered from both inside and adjacent to the Croker Carbonate Slabs cSAC/SCI are MDAC, and comprise the Annex I feature ‘Submarine structures made by leaking gases’.

Strontium isotope ($^{87}Sr/^{86}Sr$) analysis indicates the aragonite and calcareous encrusting biota were precipitated in seawater comparable to present day seawater (Elderfield 1986). U-Th dating was not possible for all samples, however aragonite extracted from two samples indicated that MDAC formation occurred during the period from approximately 17,000 (+/- 5,500 years) to 4,000 (+/- 200 years) years before present (Field *et al* 2017).

Figure 5 shows locations of the confirmed and refuted MDAC samples. Full details of the analysis and results are provided in the BGS reports published alongside this report (Field *et al* 2016a, 2016 & 2017).

4.2.2 Range and distribution of the MDAC feature

The still images acquired from 128 transects across the site were analysed to identify MDAC from its characteristic appearance. As described in Section 3.4.1, each observation of MDAC was categorised as ‘outcropping’ or ‘pavement’ forms of the feature, or where the topography of the MDAC could not be ascertained, as ‘unknown’. Each MDAC record was assigned a ‘high’, ‘moderate’ or ‘low’ confidence rating (see Section 3.4.1 for criteria). The pie charts in Figure 5 show the proportion of high and moderate confidence MDAC in the outcropping, pavement and unknown categories along each transect, whilst the low confidence MDAC records are presented in a separate class. The observations of MDAC assigned to the low confidence category indicate the presence of areas of hard substrate colonised by sessile epifauna, apparently attached to an unseen underlying hard substrate. Although this may consist of pavement MDAC, the visual evidence is insufficient to reliably determine whether or not it is. The veneer of sediment may periodically become mobilised to expose the hard substrate, in which case future surveys may be able to confirm (or refute) the presence of MDAC at these low confidence locations. It seems likely that MDAC occurs across much wider areas of the seabed than indicated by the moderate and high confidence images. It is evident from Figure 5 that MDAC, as identified from still images, is present across much of the Croker Carbonate Slabs cSAC/SCI, and extends beyond the site boundary, particularly in the north-east, but also the south-west of the survey area. The spatial distribution of MDAC appears to be more concentrated in the north-east and central areas of the cSAC/SCI, while the north-west and far south-east showed fewer recorded instances. The pavement form of the MDAC feature was observed consistently across the site, whilst outcropping MDAC was more prevalent in the southern-central area of the site.

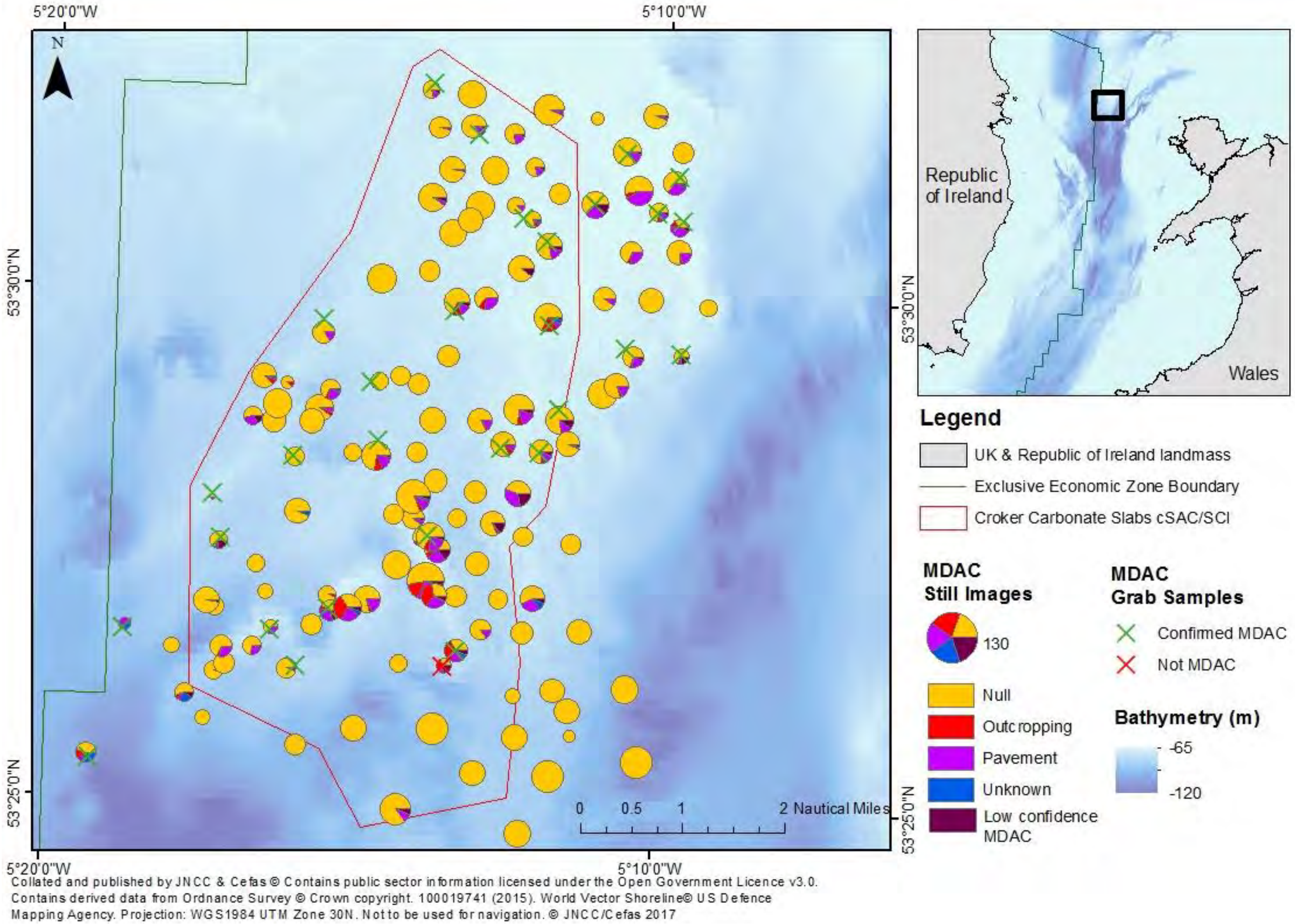

Figure 5. Still image analysis for presence of MDAC, and MDAC sample verification*. Each pie represents a transect and pie size increases with the number of still images per transect), and MDAC sample verification. *(Null = MDAC not observed; Outcropping = outcropping MDAC observed (high or moderate confidence); Pavement = pavement MDAC observed (high or moderate confidence); Unknown = MDAC topography could not be determined; Low confidence MDAC = unidentified hard substrate).

4.2.3 Interpretation of MDAC feature extent from 2015 data

The new spatial extent of MDAC occurring at or just below the seabed interpreted from the 2015 data (using OBIA) is presented in Figure 6, with a map of associated confidence supplied in Figure 7.

The still images and grab samples provided evidence that surficial sediment is present in the areas acoustically resolved as MDAC. However, it can be assumed that the sediment thickness is low and the thin veneer is discontinuous. Areas with thicker surficial sediment deposits, particularly sandwaves, have been excluded from the new interpretation of MDAC extent (e.g. in the far south-east of the site), as there is a lower likelihood of exposure at the seabed. As this is a dynamic sedimentary environment, it is possible that MDAC underlies these sediments, and may be revealed or covered over time as sandwaves move across the site.

The majority of the new interpretation scored three out of four according to the Lillis (2016) three-step confidence assessment framework (see Figure 7), whilst confidence scores of two were attained in peripheral areas where no samples were collected (see Annex 4 for criteria). It should be noted that a score of one for the sampling criterion was given when a polygon coincided with at least one MDAC sample, irrespective of the number of sediment samples. This means that MDAC is more likely to be found in areas assigned a total confidence of three and four.

4.2.4 Comparison of MDAC and hard substrate interpretations

The new mapped spatial extent of the MDAC occurring at or just below the seabed is presented in Figure 8, overlaying the area of predicted hard substrate identified from the 2012/13 MBES data, and the previous MDAC extent delineated from the 2008 survey data (Whomersley *et al* 2010) for comparison.

It is important to acknowledge the limitations associated with the combined use of these three interpretations. The interpretations cannot be assumed to provide a definitive present-day extent, as some areas delineated as MDAC in 2008 may have eroded, and/or been covered (or revealed) by a mobile veneer of surficial sediment. Also, the extent of the hard substrate delineated from the 2012/13 MBES data may include other hard substrata in addition to MDAC. The different data types, data resolutions, and methods used in the current and previous studies are also likely to influence the results (see Section 3.3, also Callaway *et al* 2015; Whomersley *et al* 2010). Reviewing the three interpretations in combination does, however, provide a more complete overview of where MDAC has been considered to be at or just below the seabed over time, and is therefore likely to persist in some form.

The results of the current mapping largely confirmed the spatial extent of the hard substrate identified by Callaway *et al* (2015), based on the 2012/13 survey data, in areas where the two studies overlapped. However, differences were observed in the south-east region of the site where Callaway *et al* (2015) indicated a more widespread occurrence of hard substrate. The presence of MDAC in this area is not confirmed by the 2015 sample data or the latest habitat mapping results, and it is possible that the underlying clay observed in this area may have contributed to the ‘hard substrate’ signature interpreted by Callaway *et al* (2015). To the east of the Croker Carbonate Slabs cSAC/SCI, the MDAC feature appears to be bounded by a discontinuous ‘cliff’[5] line of variable height adjacent to the channel. This ‘cliff’

[5] Judd (2005) used the term ‘cliff’ to describe a feature within the study area. Similar geomorphological features are found and mapped here. However, although the height of the ‘cliffs’ is substantial in places (up to 20m), the slope angle is typically <15˚. Therefore, the term ‘cliff’ might not be justified, but we retain it in line with Judd (2005).

line is relatively clearly expressed in the south-east, which might indicate the potential presence of MDAC, albeit covered by a more substantial sediment blanket, in the form of sandwaves. Comparison of the 2008 and 2015 interpreted MDAC feature maps and imagery confirms that the smaller 'cliff' feature to the south of the site centre, originally described by Judd (2005) and subsequently observed by Whomersley *et al* (2010), was still present in 2015. It was not possible to determine whether erosion of the cliff had occurred, due to differences in MBES resolution.

Where the 2008 and 2015 MDAC interpretations (as opposed to the 2012/13 'hard substrate') overlap, the degree of correspondence indicates that the previously mapped MDAC persists from 2008, although some areas may have been covered by mobile sediments. It should be noted that full acoustic coverage was not achieved for the entire cSAC/SCI during either the 2008 or 2015 surveys, and the spatial extent of MDAC is known to exceed that delineated from either of these datasets (e.g. MDAC has been observed from still imagery south-west of the site boundary). It is likely that many areas of MDAC which were not acoustically identified in 2008 or 2015 may align with the 2012/13 interpretation of hard substrate.

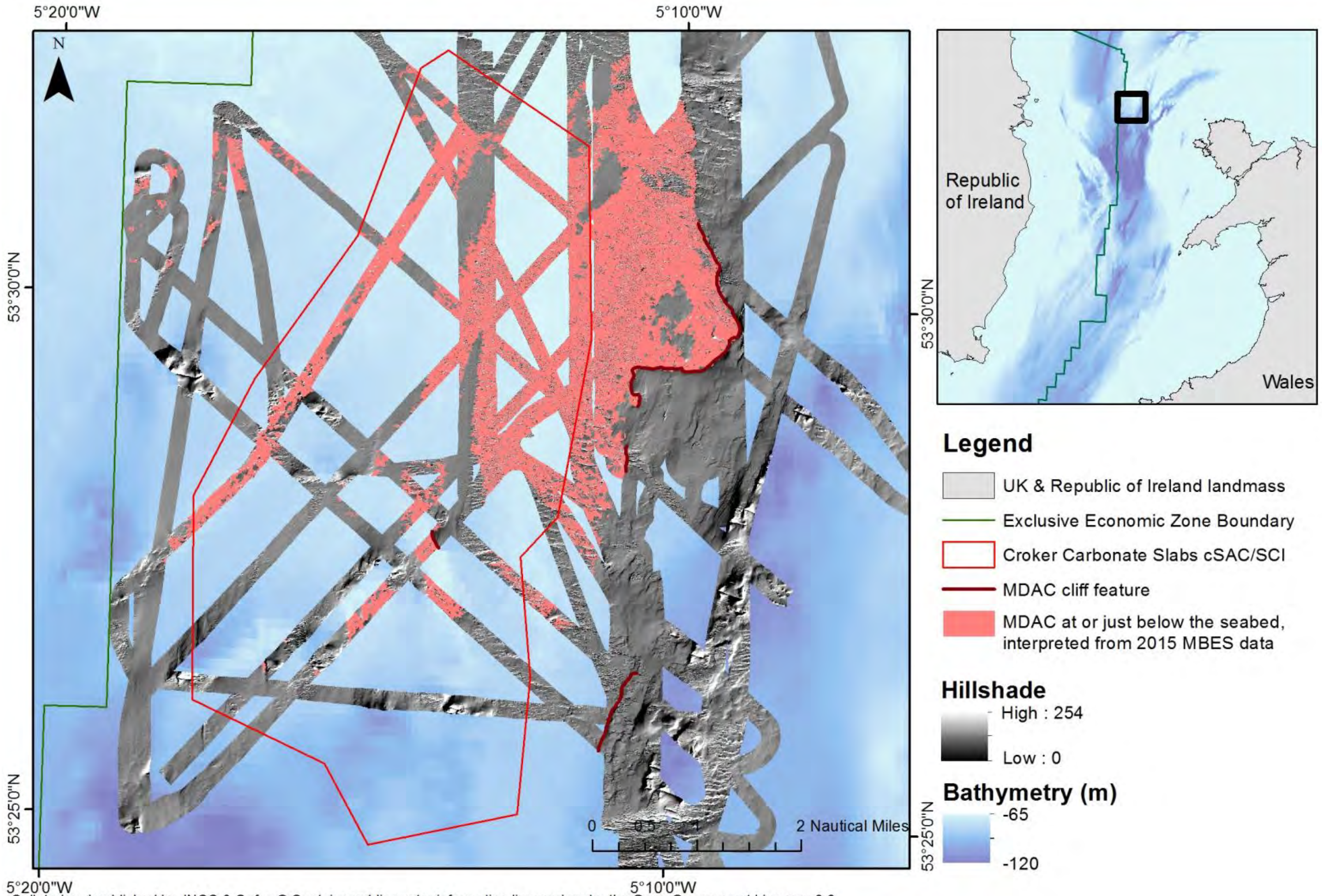

Figure 6. Mapped extent of MDAC at or just below the seabed, and MDAC cliff feature, as interpreted from the underlying 2015 MBES data.

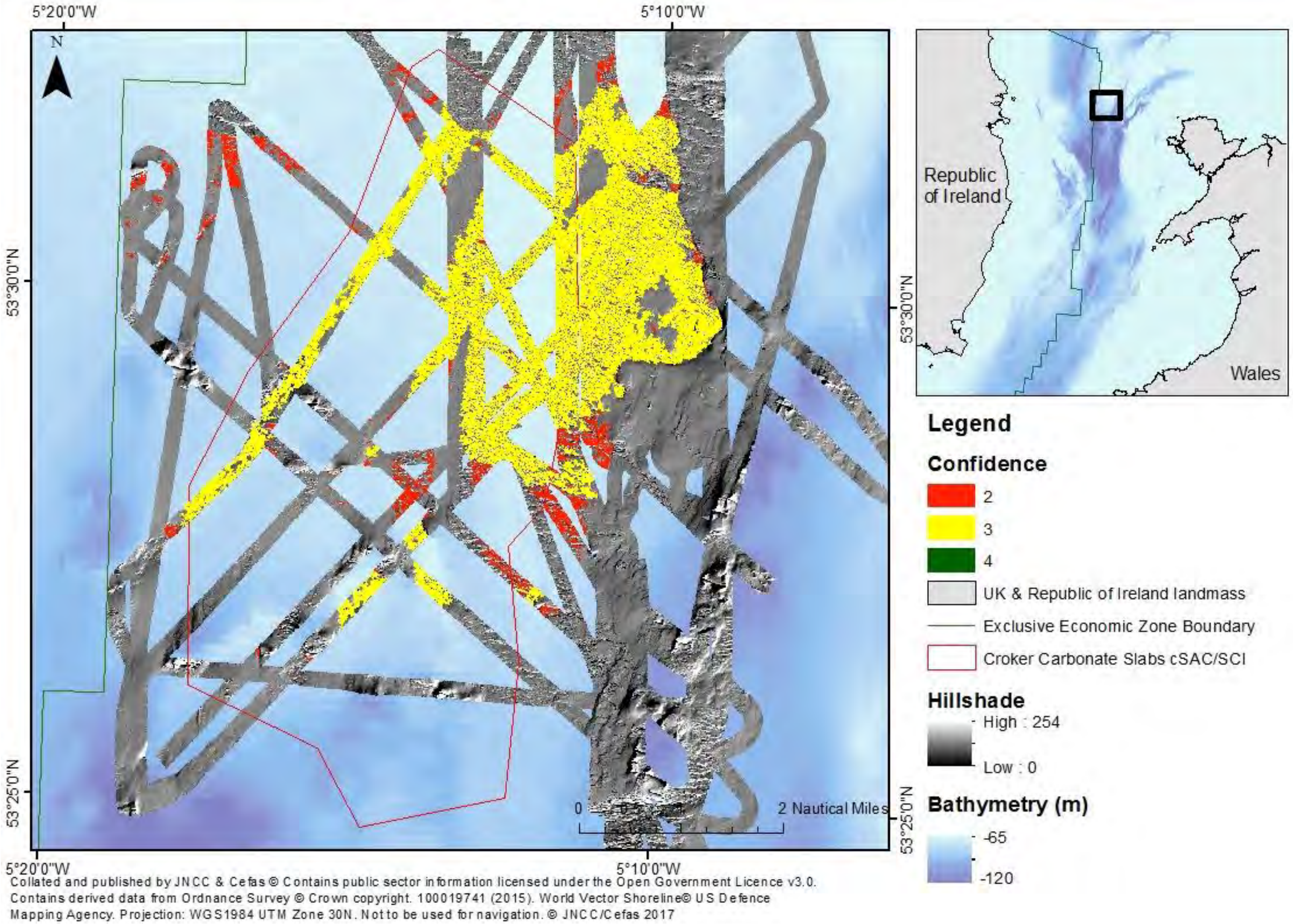

Figure 7. Confidence in predictions of MDAC at or just below the seabed from the underlying 2015 MBES data, based on a three-step confidence assessment (Lillis 2016). Possible scores range from 0 (no confidence) to 4 (high confidence).

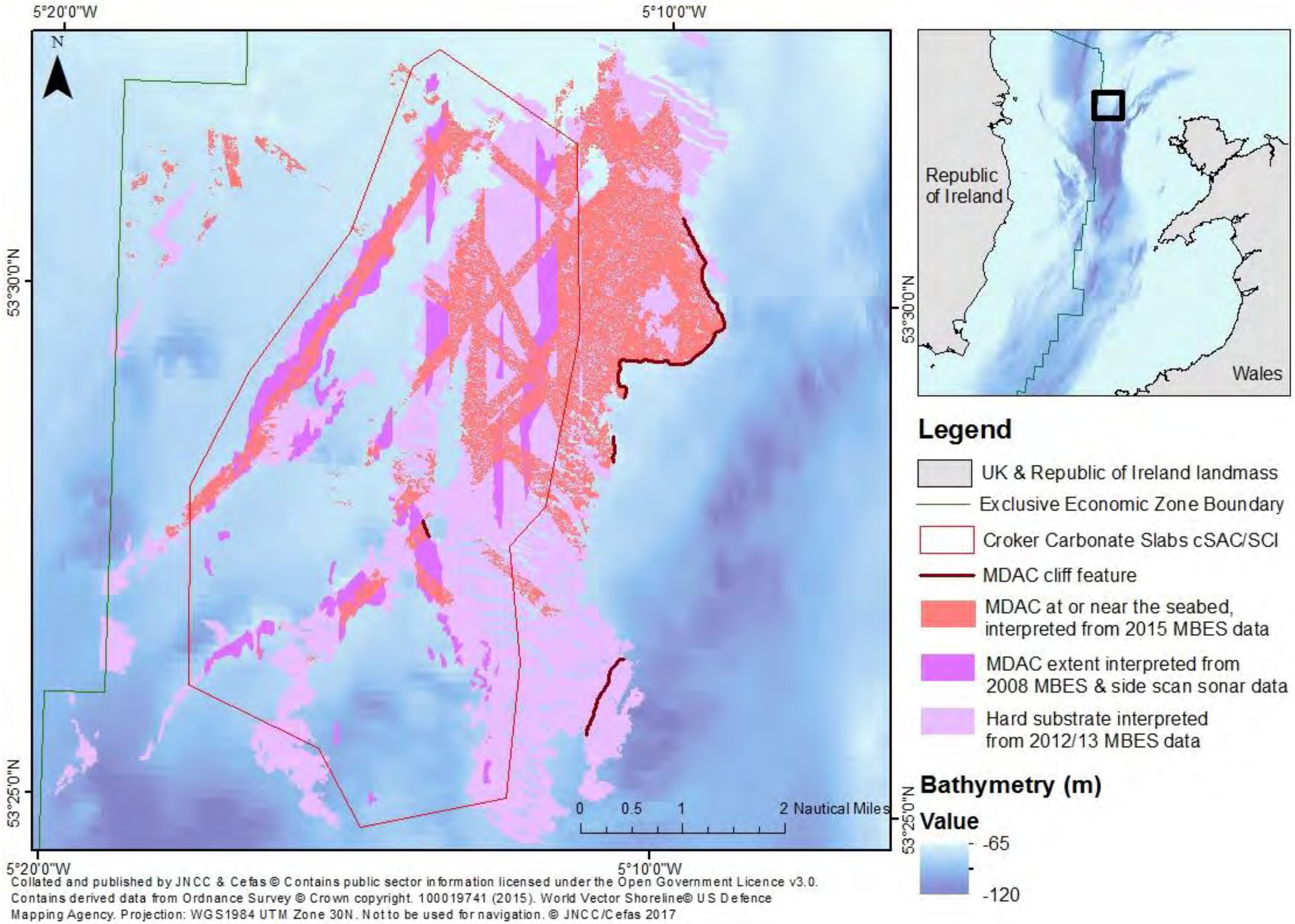

Figure 8. Comparison of modelled acoustic interpretations from 2008 MBES and side scan sonar data, 2012/13 and 2015 MBES data.

4.3 Evidence of ongoing methane seepage

The ongoing seepage of methane from the seabed, indicating the potential for further MDAC formation, was confirmed by various acoustic, visual, and physical data sources.

4.3.1 Seawater methane concentration

As described in Section 3.2.5, methane concentrations in the seawater were determined using a METS methane detector for the duration of each camera transect. Data were recorded at five second intervals, and averages of methane concentration per minute were then calculated (Figure 9). The concentrations observed within and adjacent to the site ranged between 1.23 and 26.48nmol/L, more than 98% of which were considered to be supersaturated (>3 nmol/L). The highest methane concentrations were recorded in association with delineated areas of MDAC, however they were not restricted to the interpreted spatial extents of MDAC (2008 & 2015 data) or hard substrate (2012/13 data), particularly in the south of the survey area. The calculated average methane concentration (nmol/L) from each 20-30 minute camera tow highlights the stations with higher overall methane in the bottom waters (Figure 10). The two stations where the highest average methane concentrations were recorded, also provided video evidence of methane seepage in the form of gas bubbles leaking from the seabed (see Figure 10 and Figure 15). Waters with methane concentrations >3nmol/L are considered supersaturated, whilst those measuring >4nmol/L are particularly noteworthy (van Landeghem *et al* 2015).

Anomalously high methane concentrations were also evident from the water sample data (Figure 11). Samples collected using 10L Niskin bottles from within the site boundary had considerably higher methane concentrations than at the control station five nautical miles south of the site, and validated the concentrations determined by the METS sensor. Bottom water concentrations did not differ greatly from surface values, indicating that the methane has dissipated throughout the water column; at five out of six sample locations, methane concentrations in surface waters are ‘particularly noteworthy’ according the van Landeghem *et al* (2015) criteria. Furthermore, the low near-seabed concentrations contrasted with high surface concentrations at the control site three nautical miles to the east, suggesting that methane from the site is being exported beyond the site by surficial water movement. It is probable that a proportion of this methane enters the atmosphere, as is the case at other sites of natural seabed methane seepage (Judd 2015).

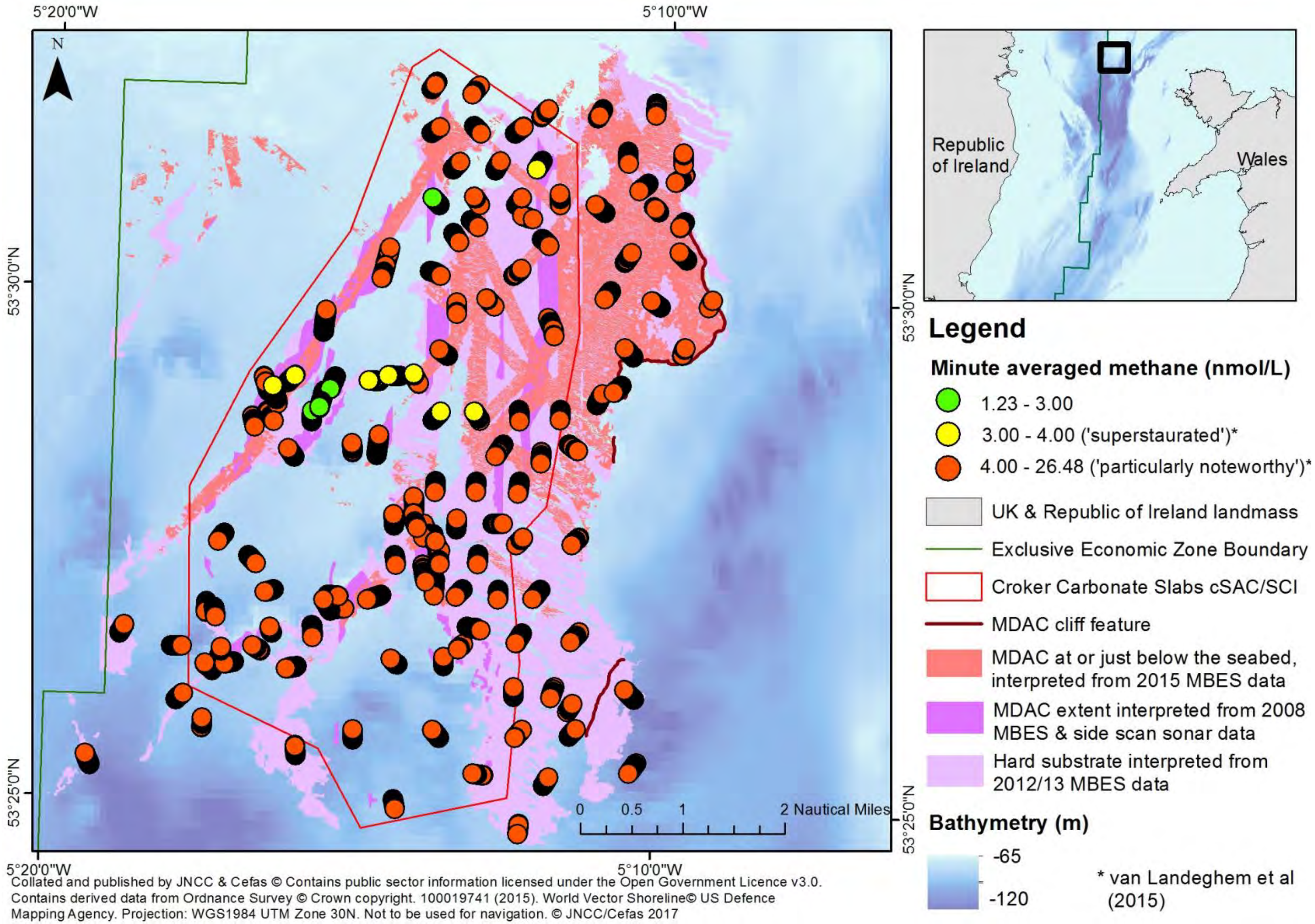

Figure 9. METS sensor average methane concentration per minute (nmol/L) from camera drop-frame deployments.

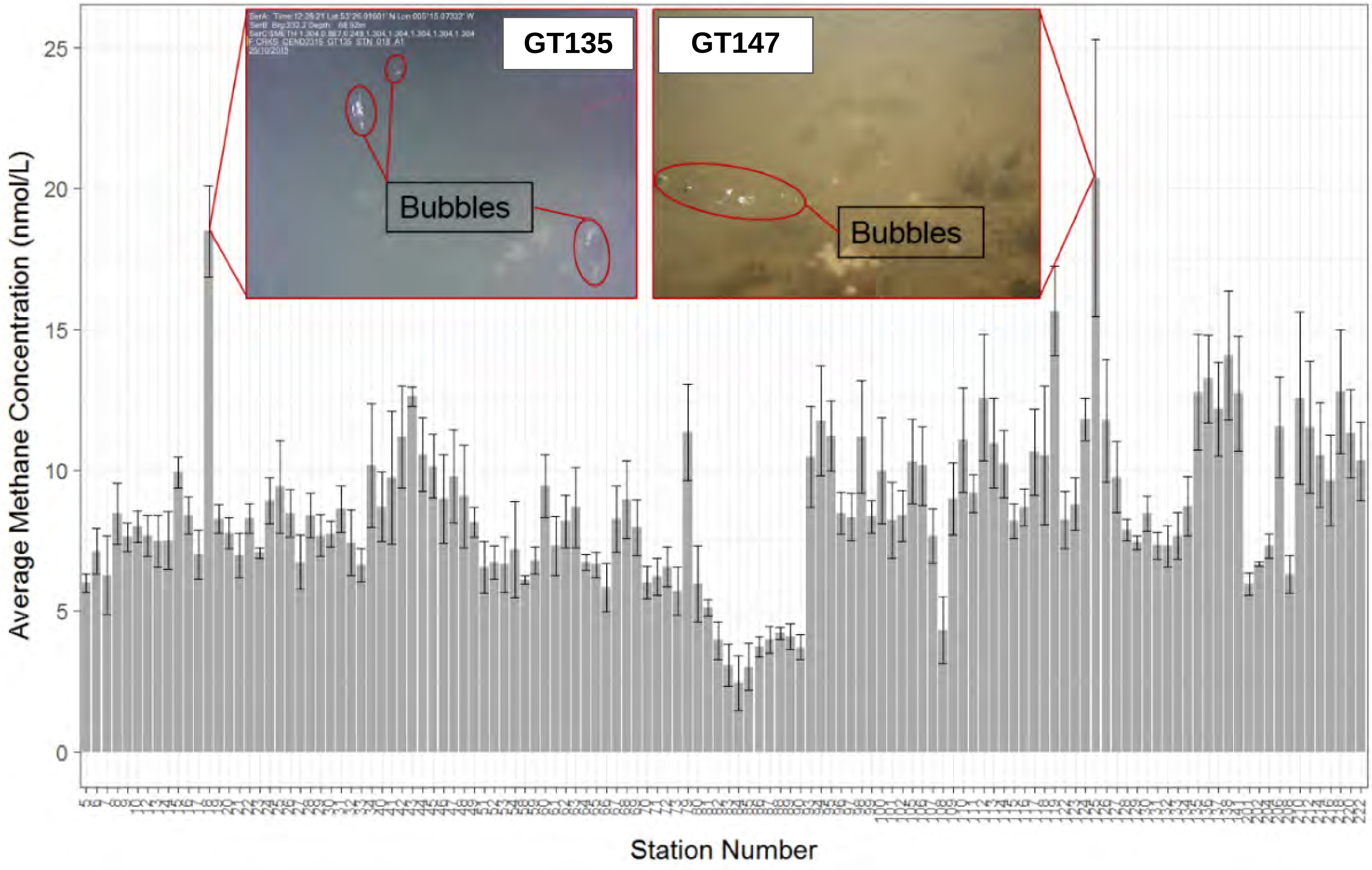

Figure 10. METS sensor average methane concentration (nmol/L), with standard deviation error bars. Images of bubble seepage were associated with the two highest averages.

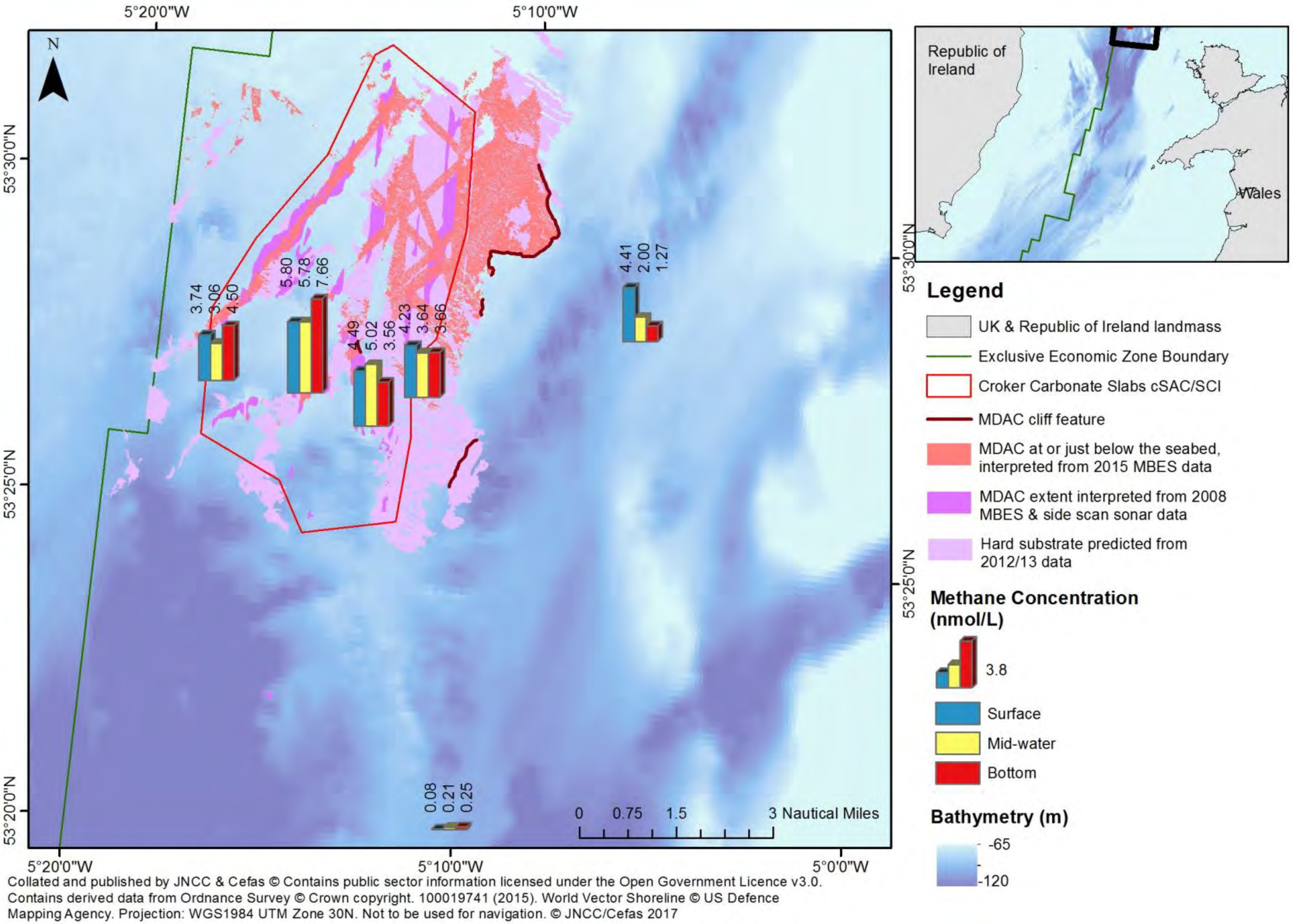

Figure 11. Methane concentration (nmol/L) from water samples acquired at surface, mid-water and bottom-water.

4.3.2 Seabed imagery

Still image and video data provided further evidence of ongoing methane release within the survey area, in the form of gas bubbles leaking from the seabed, black sulphidic patches close to MDAC substrates and white mats of possible thiotrophic bacteria (e.g. *Beggiatoa* spp.) at the edges of MDAC concretions (see Figure 12 for images and Figure 15 for locations).

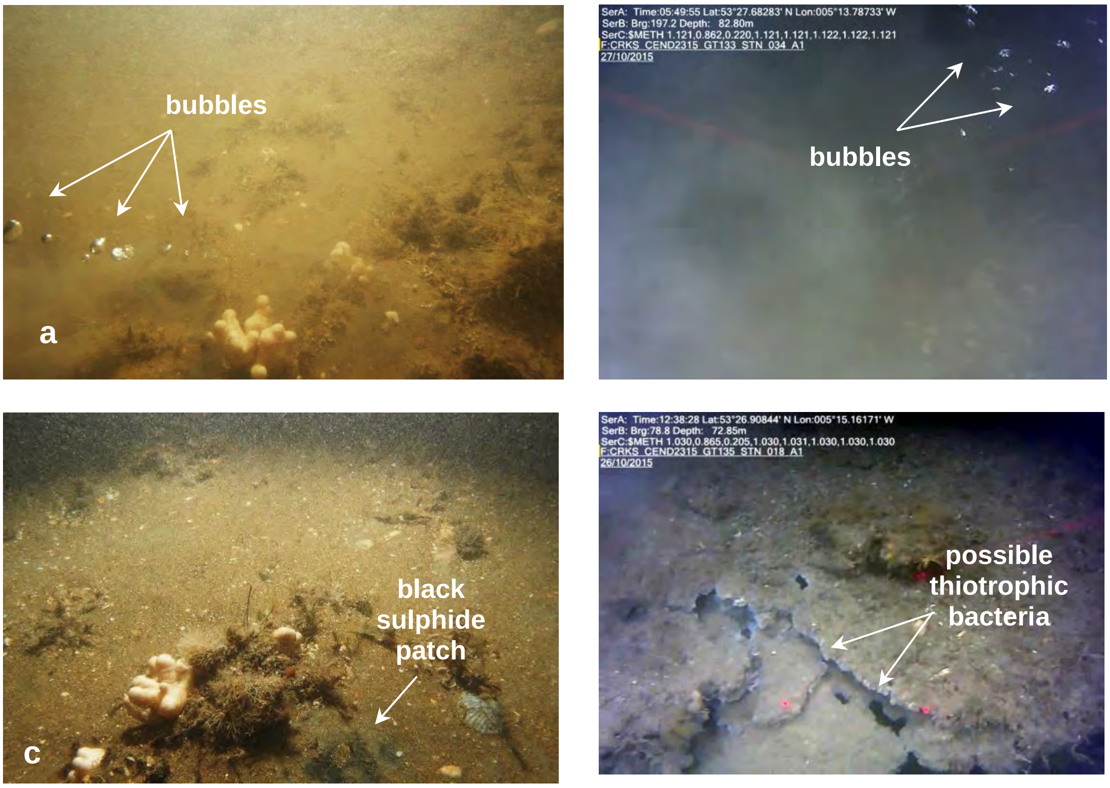

Figure 12. Example imagery evidence of active methane seepage. a) Still image of leaking gas bubbles (see also Figure 10) and MDAC; b) video screen grab of bubbles in water column; c) black sulphidic patch adjacent to MDAC; d) video screen grab of possible thiotrophic bacteria patches on MDAC.

4.3.3 Water column acoustic data

The singlebeam echosounder detected apparent streams of gas bubbles leaving the seabed (see example in Figure 13) at 21 different locations (Figure 15). The possibility that some of these water column targets reflect the presence of fish cannot be discounted, however their appearance as vertical or inclined, rather than horizontally extensive, is generally considered to indicate rising gas bubbles (Judd & Hovland 2007).

High reflectivity impedances in the water column were also interpreted from side scan sonar data (e.g. Figure 14) at five different locations within the survey area (see Figure 15).

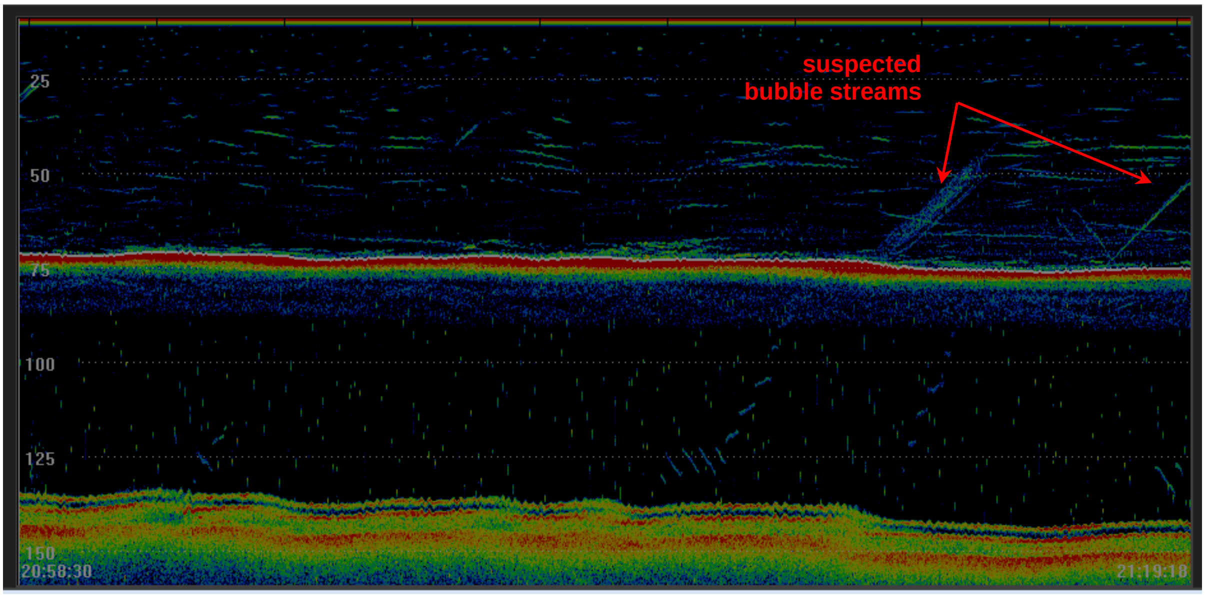

Figure 13. EK60 scientific singlebeam echosounder image of suspected bubble stream leaving seafloor.

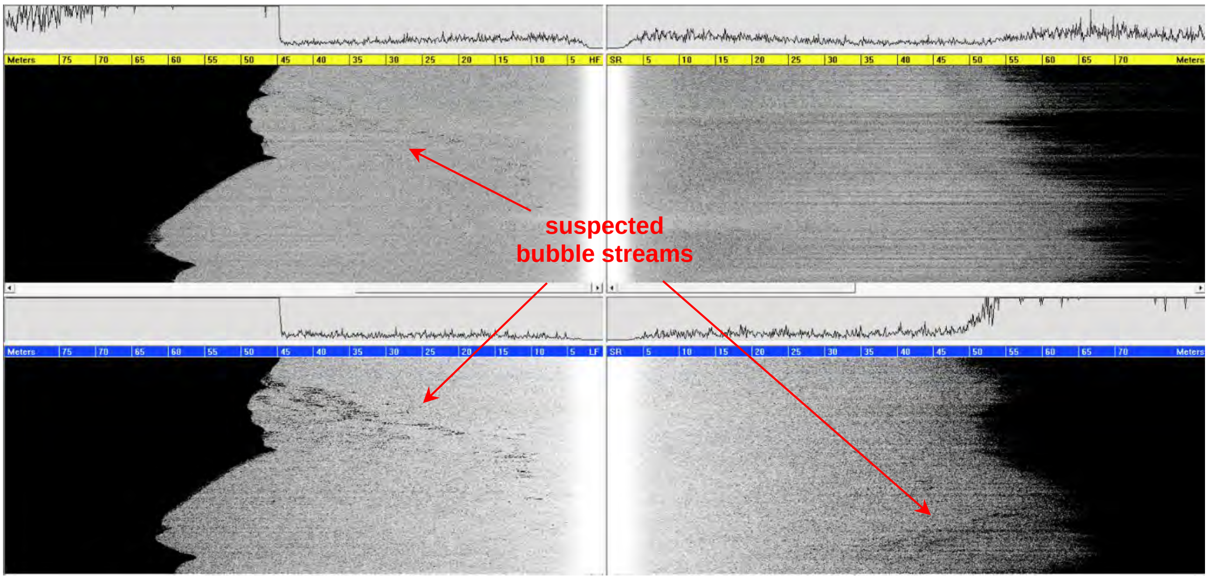

Figure 14. Example image of high reflectivity impedances (possible gas bubbles) observed on high and low frequency side scan sonar channels.

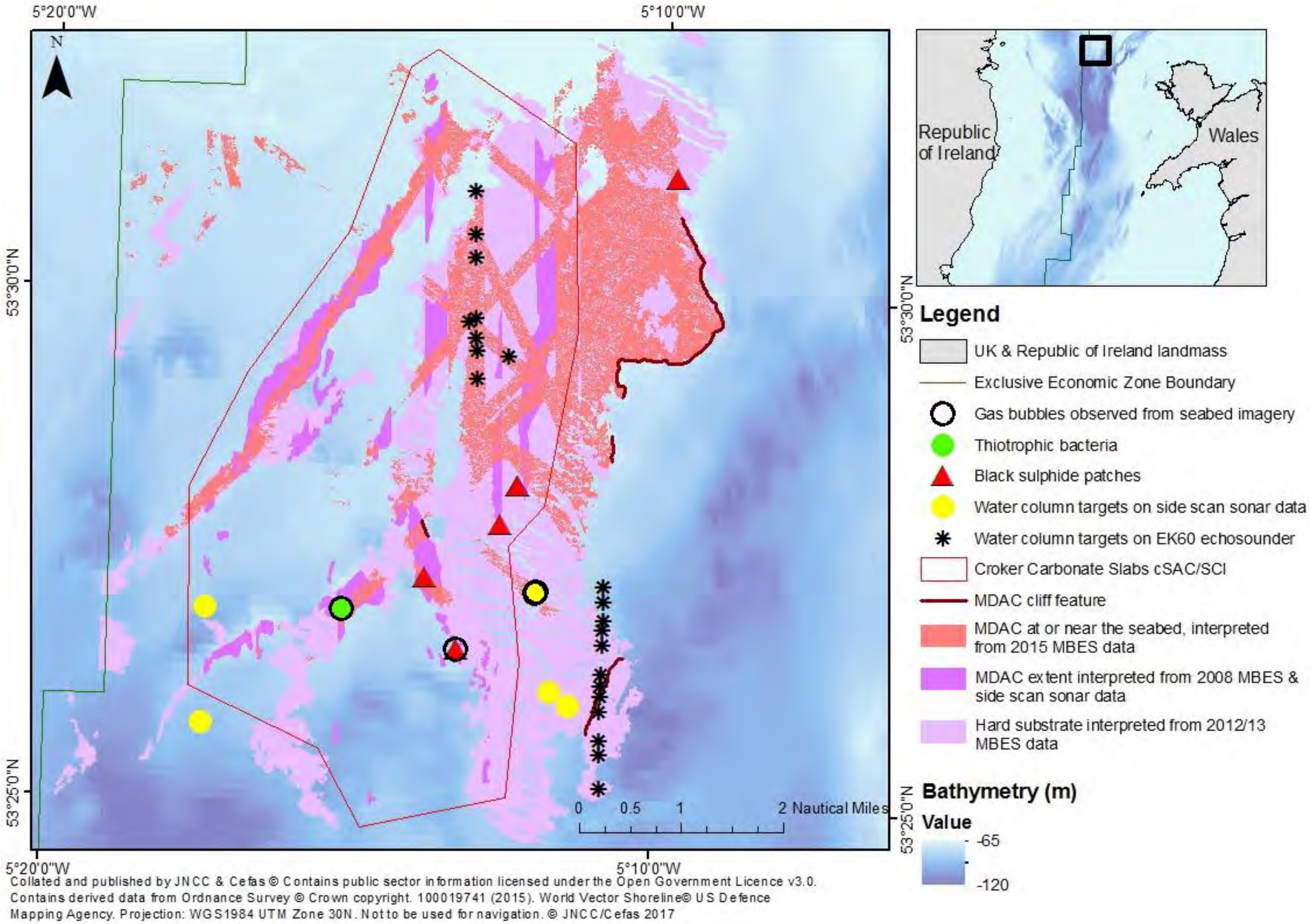

Figure 15. Supplementary water column and seabed evidence indicating gas seepage; water column targets evident from side scan sonar and EK60 data, still photographs and video evidence of gas seepage from the seabed, black sulphidic patches, and thiotrophic bacteria.

4.3.4 Sub-bottom profiles

The sub-bottom boomer profiles indicated the presence of two sediment packages (Units A & B) within the survey area, with evidence of a third unit (Unit C) beneath the deep-water channel to the east of the survey area (see Annex 5 for detailed seismic stratigraphy and sub-bottom profiles).

Unit B, the older (lower) package, present throughout the survey area, comprises multi-layered sediments, the layering being represented by numerous parallel internal reflections. These were thought to be caused by contrasts in the acoustic impendence (coarseness) of the individual layers. The internal reflections within Unit B are likely to be explained by the presence of inter-bedded relatively coarse (silty or sandy) and relatively fine (clayey) sediments. The clayey sediments provide impermeable barriers to vertical migration, forcing gas to migrate laterally and up dip.

The reflections evident on the profiles were obscured in places by acoustic turbidity (Figure **16**), which is commonly attributed to the presence of gas within sediments (Judd & Hovland 2007). The presence of gas is further indicated by the lateral variability in the amplitude of some individual reflections; this is referred to as gas brightening, and suggests an increased concentration of gas within the sediments. Together these features hinder the identification of individual reflections across the profiles. It is also noted that the signature of acoustic turbidity may be mistaken for signal starvation caused by the scattering of incident acoustic energy by sandwaves, and reductions in signal strength causing by marked acoustic impendence contrasts caused by a hard, shelly or pebbly seabed.

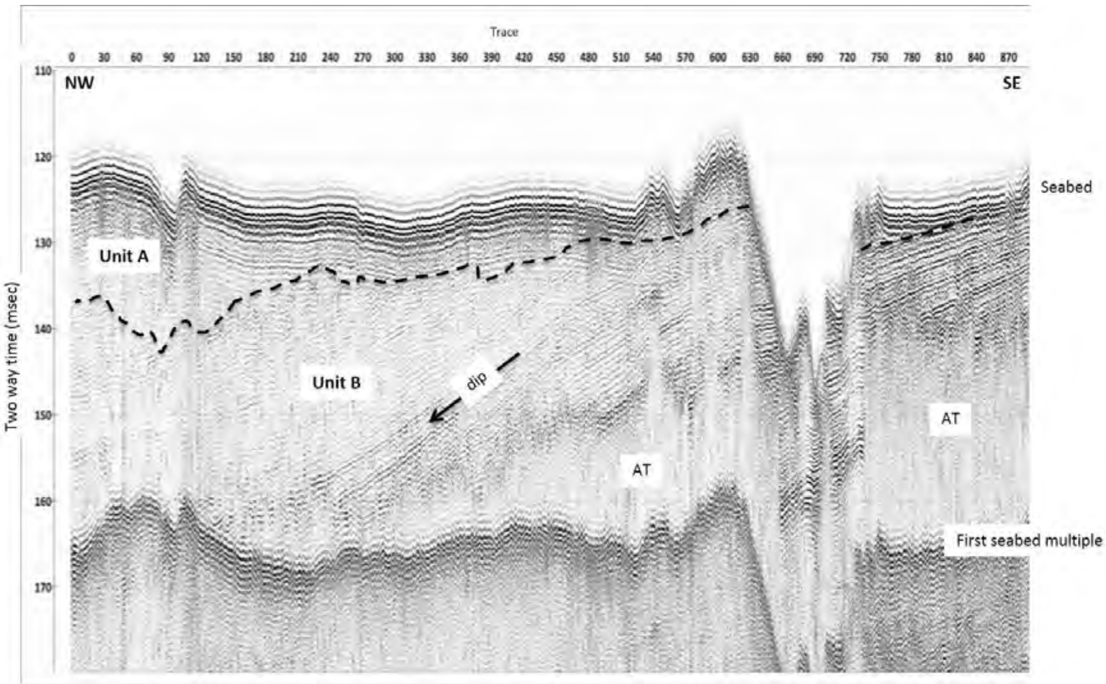

Figure 16. Boomer data example (SBP006): Unit A – Unit B unconformity (dashed line); acoustic turbidity (AT) interpreted as gas within sediments.

An indication of the widespread distribution of shallow gas in this area is provided in Figure 18. The boomer profiles provide evidence of gas migration, whereby gas from depth is channelled laterally and up dip through relatively permeable silty/sandy layers beneath relatively impermeable clayey layers. Where gas accumulates in the sediments, layering is obscured by acoustic turbidity. At certain locations, gas in these accumulations penetrates vertically towards the seabed, forming gas chimneys (vertical columns of acoustic turbidity).

Residual gas continues to migrate laterally and up dip, causing gas brightening. Some gas chimneys rise only part way to the seabed; others rise to, or close to it, apparently affecting the character of the seabed (Figure 17).

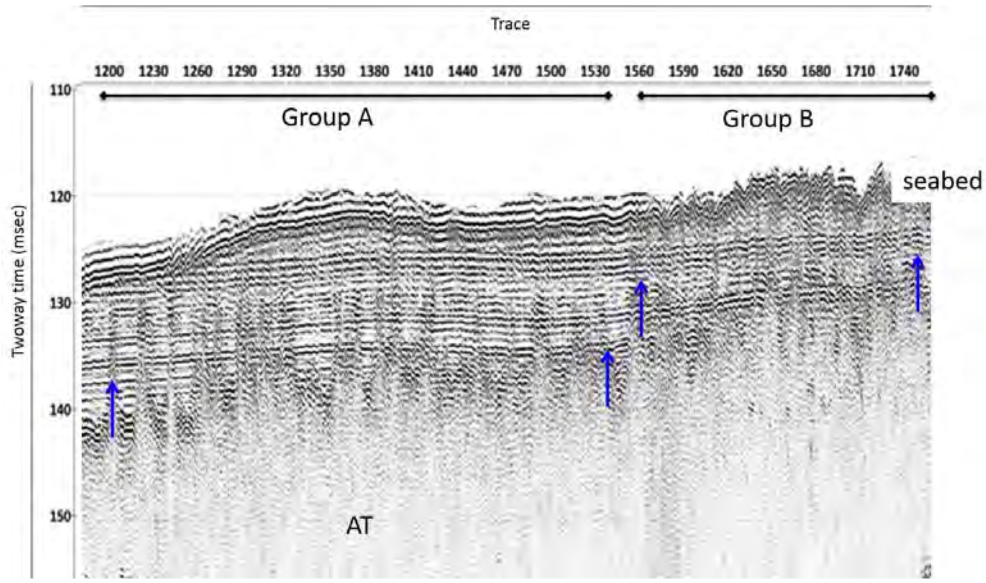

Figure 17. Boomer data example (SBP002): Acoustic turbidity (AT) and gas chimneys (some indicated by vertical arrows)*.

* Group A chimneys all terminate at a common horizon; Group B chimneys rise to, or close to the seabed. Note the effect on the seabed, which is smooth above Group A, but disturbed above the Group B chimneys.

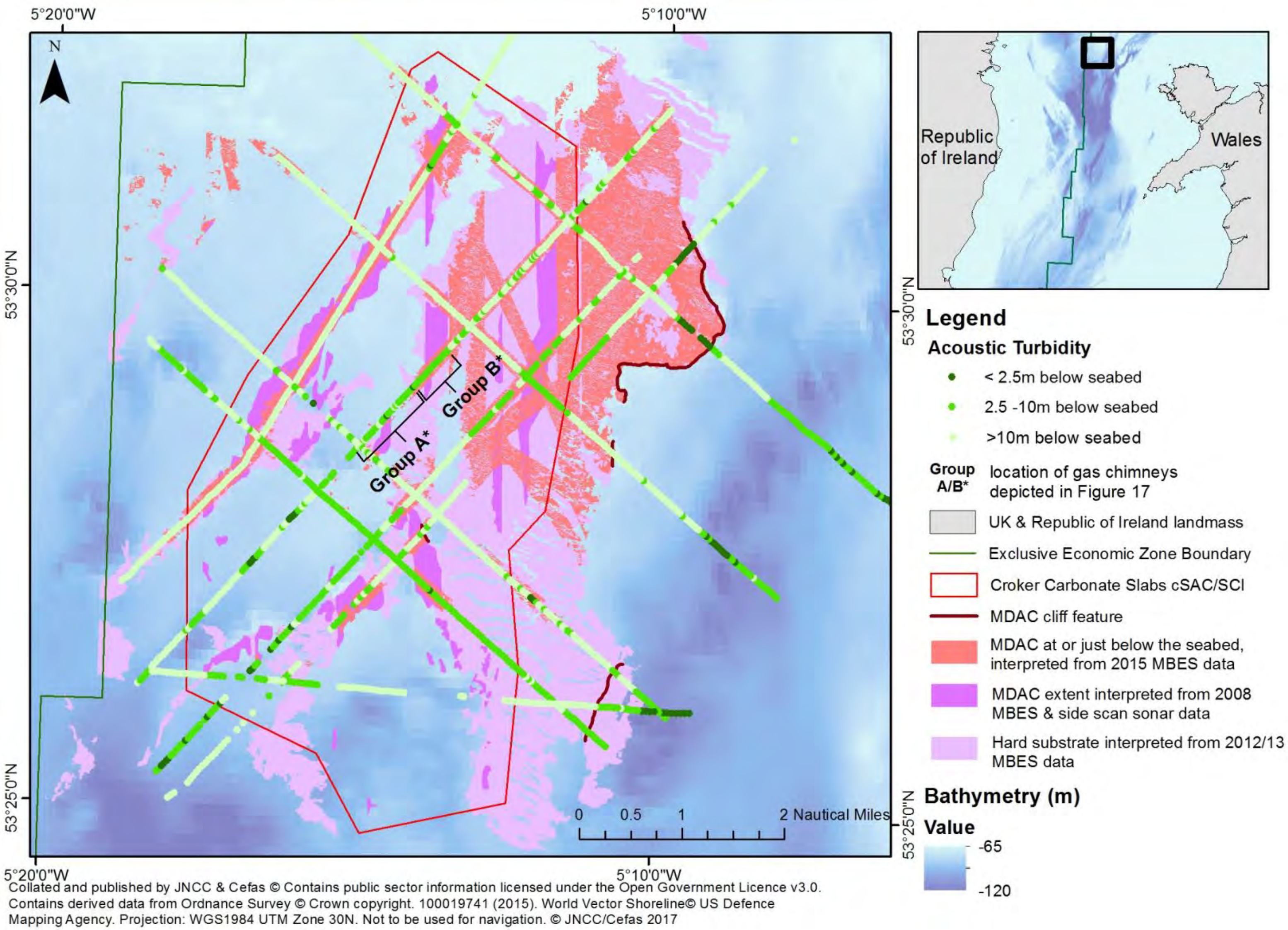

Figure 18. Boomer line plan showing acoustic turbidity below the seabed, and locations of gas chimneys.

4.3.5 Correlation of boomer data with other datasets

Selected sections of boomer profiles (see Annex 5) were investigated in order to consider whether the interpretation suggesting the presence of shallow gas was valid (see example in Figure 19), and justified the implication that gas is rising towards the seabed at other locations. These three examples demonstrate that the interpretation of sub-bottom acoustic turbidity as gas is reliably evidenced.

Example 1: Boomer profile SBP004SW is a strike section across an area with varied topography (see Figure 19). Most of the profile is underlain by acoustic turbidity, and there are several gas chimneys.

Four groundtruthing locations crossed the profile, and three more were close to it.

- Suspected MDAC was seen on all but one of these video transects (GT017), which crossed the base of a seabed depression underlain by a gas window (a gas-free zone).
- GT133 traversed the top of the Texel 11 'cliff', described from the SEA6 survey (Judd 2005) as a 6-8m high cliff with 'high-relief' MDAC (which equates to outcropping MDAC). Outcropping MDAC was also observed at GT135.
- Suspected MDAC was found in grab samples from GT005, GT014 and GT126; all were confirmed by carbon isotope analysis.
- Suspected bacterial mats, consistent in appearance with the seep-related thiotrophic bacteria, *Beggiatoa spp.*, were observed on video from GT135 (see Figure 12).
- Bubbles were observed in the water column at CT133.
- METS measurements of methane in the bottom waters were unusually high (maximum values >13 and >20nmol/L) at GT133 and GT135 respectively.
- The methane concentration in the bottom water sample at GT135 was 7.66nmol/L, the highest recorded in this study.

Example 2: Boomer profile SBP002 (Figure 17) crossed an area where gas was apparently rising towards the seabed and where several smaller areas of shallow gas (gas chimneys) were present. The section marked Group B coincides with the area indicated on Figure 18 as having MDAC at the seabed, whilst Group A does not. No evidence of MDAC (in either video or grab samples) was reported from GT142 (which crossed the boomer line), although possible MDAC was observed on the video transect at GT088 (directly adjacent to the boomer line). At GT148 possible pavement MDAC was observed on the boomer line from imagery, but the grab sample collected at this station did not contain any MDAC to enable validation of its presence. The methane concentration from a bottom water sample taken at this site was 4.5nmol/L

Example 3: 0.43km east of the site boundary, boomer profile SBP006 crossed station GT147. This station lies on a topographic high underlain by a gas chimney.

At GT147:

- Outcropping MDAC was observed on the video transect.
- The highest METS measurement (>27nmol/L) of methane concentration in bottom waters was recorded.
- Bottom water samples had a methane concentration of 3.66nmol/L.
- Gas bubbles were observed rising from the seabed.

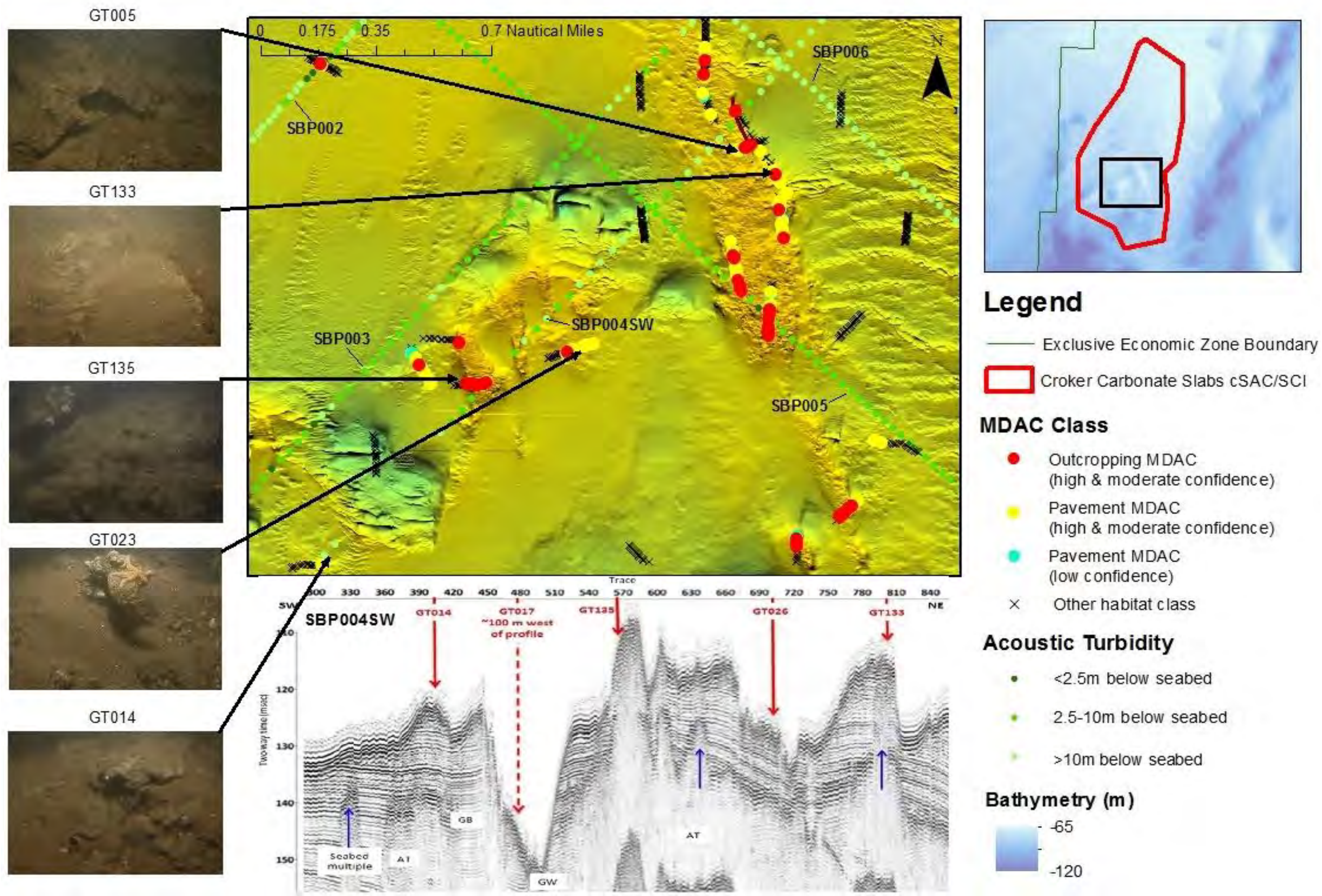

Figure 19. Boomer data (line SBP004SW) and multibeam data example with corresponding seabed imagery.

4.4 Diversity and structure of biological communities

The habitats and associated communities observed within and adjacent to the Croker Carbonate Slabs cSAC/SCI are described below, in relation to the broad habitat groups assigned for analysis in Section 3.4.1. Corresponding EUNIS codes are provided in the main text where they are available, whilst analogous Marine Classification for Britain & Ireland 04.05 codes (Connor *et al* 2004) are provided in brackets.

4.4.1 Sediment habitats

As described in Section 4.1, sediment habitats occurred across the survey area, being interspersed with areas of exposed MDAC. Although sediment still images have been grouped by EUNIS broadscale habitat for analysis purposes, it should be noted that these habitats are highly mixed, can show extreme within-group variability, and exist on a continuum as opposed to being clearly distinct habitats. It should also be noted that a relatively high amount of habitat class overlap may be present within individual images, limiting the ability to ascribe discrete epifaunal assemblages to each, and that low taxonomic resolution (due to image quality and inherent identification limitations) may obscure differences in community structure. For this reason, the mean number of epifaunal taxa have not been provided for each habitat class.

A5.1 'Sublittoral coarse sediment'

The broadscale habitat A5.1 'Sublittoral coarse sediment' (SS.SCS) was the most common substrate observed, occurring extensively across the full geographical range of the survey area (Figure 21) and along the majority of camera transects, with 62% of still images being classified as this habitat type.

This habitat was extremely variable in terms of lithic and sedimentary composition, with extremely large ranges recorded for the majority of different particle sizes (for instance, the estimated cover of pebbles from still images ranged from 1% to 82%, whilst empty shell cover ranged from 1% to 95%). Within this broadscale habitat a biotope complex, A5.14 'Circalittoral coarse sediment' (SS.SCS.CCS), and a biotope, A5.234 'Semi-permanent tube-building amphipods and polychaetes in sublittoral sand' (SS.SSa.IFiSa.TbAmPo), were interpreted from still images.

The epifaunal assemblage composition reflected the highly variable sedimentary mosaics within this habitat class; average similarity calculated for the group was just 15.2%. Polychaetes including Serpulidae contributed 63.3% of within-group similarity. In combination with hydroids (in clump/solitary and turf forms); these taxa accounted for almost 90% of within-group similarity. ANOSIM analysis showed a significant difference between the epifaunal assemblage structure of A5.1 'Sublittoral coarse sediment' and A5.2 'Sublittoral sand'. No significant differences existed between A5.1 'Sublittoral coarse sediment' and the remaining habitat classes (A5.4 'Sublittoral mixed sediment', A5.6 'Sublittoral biogenic reef', A4.23 'Communities on soft circalittoral rock', outcropping MDAC and pavement MDAC; see Table 6).

Of the 46 grab samples acquired for sediment PSA and macrofaunal analysis, nine (19.6%) were classified as A5.1 'Sublittoral coarse sediment'. The primary measures for the group reflected the high level of macrofaunal variability within grab samples, with the number of individuals ranging from 4 to 663, and the number of species ranging from 4 to 81. *Sabellaria spinulosa* comprised 34.2% of the fauna recovered from the sample containing 663 individuals. Within-group macrofaunal similarity was calculated at 25.5%, with the top contributing taxa consisting of terrebellid (*Lysilla nivea* and *Ampharete octocirrata*) and phyllodocid polychaetes (*Syllis* spp. and *Aglaophamus agilis*), peanut worms (*Nephasoma (Nephasoma) minutum*), pea urchins (*Echinocyamus pusillus*), the lesser sandeel (*Ammodytes marinus*), carditid and myid bivalves (*Goodallia triangularis* and *Sphenia binghami*).

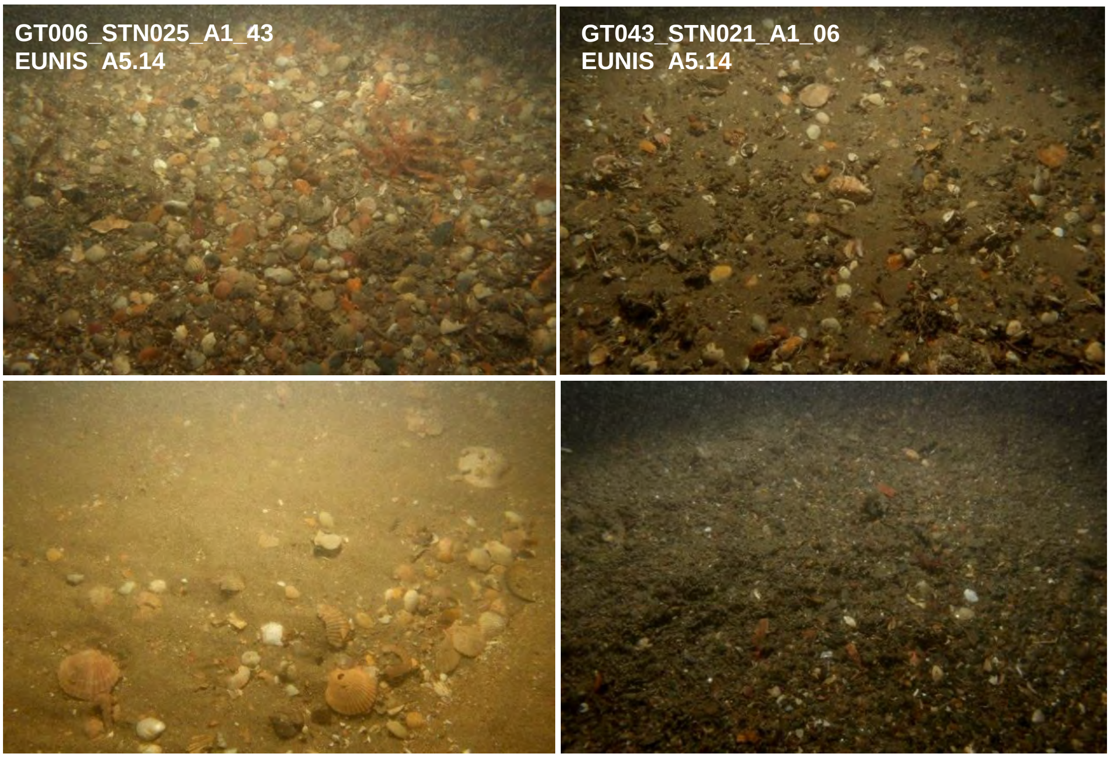

Figure 20. Example photographs of A5.1 'Sublittoral coarse sediment'.

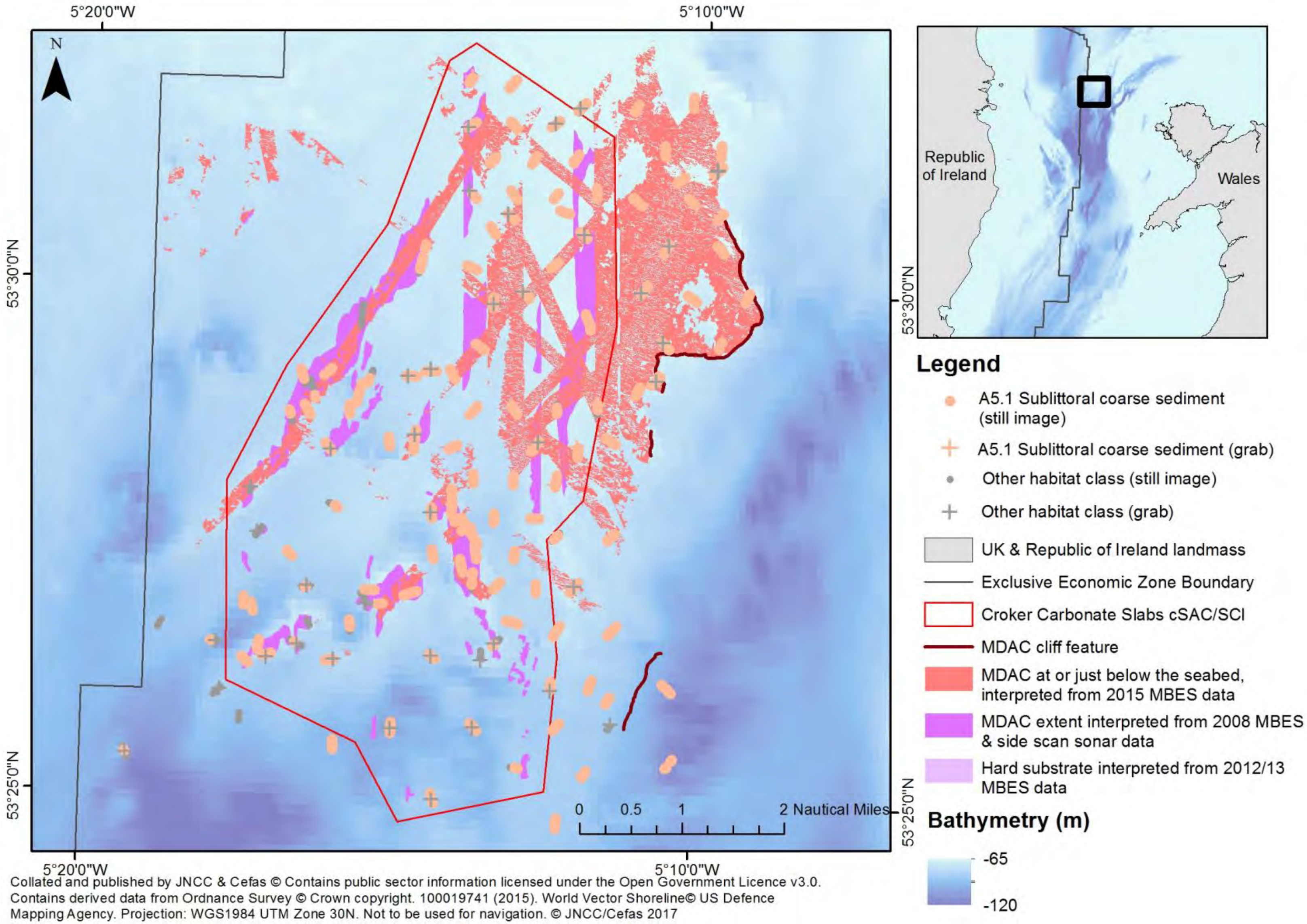

Figure 21. Distribution of A5.1 ‘Sublittoral coarse sediment’, as interpreted from still images and grab samples.

A5.2 'Sublittoral sand'

A5.2 'Sublittoral sand' (SS.SSa) was also widespread across the survey area, although this class accounted for just 5.1% of the still images (Figure 23). The observed extent of this habitat was generally limited to the east and south-east of the Croker Carbonate Slabs cSAC/SCI, and to the east outside of the site boundary. A5.2 'Sublittoral sand' comprised an absent to negligible coarse fraction, a small amount of shell and low mud content. The biotope A5.234 'Semi-permanent tube-building amphipods and polychaetes in sublittoral sand' (SS.SSa.IFiSa.TbAmPo), was also recorded in association with this broadscale habitat.

The A5.2 'Sublittoral sand' habitat was typically associated with a sparse epifauna, indicative of clean mobile sediments, which resulted in an extremely low within-group similarity of 4.4% (see Figure 26a). This majority of this similarity (>79%) was driven by the same top three taxa which contributed to similarity within the A5.1 'Sublittoral coarse sediment' class (Hydrozoa (clumps/solitary), Polychaeta and Serpulidae), whilst hermit crabs (Paguridae) contributed 6.1% similarity. Hydroids and serpulids require a hard substrate on which to attach, therefore it is expected that these taxa were associated with small amounts of coarse sediments on the seafloor, or that hydroids were attached to a hard substrate (possibly MDAC or clay) underlying the sand where the sediment veneer was sufficiently thin. ANOSIM analysis revealed significant differences between A5.2 'Sublittoral sand' and A5.1 'Sublittoral coarse sediment', A5.4 'Sublittoral mixed sediment', outcropping MDAC, and pavement MDAC (see Table 6). No significant difference existed between A5.2 'Sublittoral sand' and A4.23 'Communities on soft circalittoral rock'.

Of the 46 grab samples acquired for sediment PSA and macrofaunal analysis, 13 (28.3%) were classified as A5.2 'Sublittoral sand'. The macrofauna were typically sparse and impoverished, indicating an assemblage associated with high levels of natural disturbance, although there were exceptions within the group. The number of individuals ranged between 1 and 1053, whilst the number of species ranged from 1 to 96 per grab sample. The anomalously high count of 1053 invertebrates was primarily attributable to a small group of terrebellid polychaetes and bivalves (*Melinna elisabethae*, *Anobothrus gracilis*, *Ampharete octocirrata* and *Abra alba*), which were thought to be present due to the relatively high mud fraction within this particular sample (15.0%). A low level of within-group macrofaunal similarity was derived from the A5.2 'Sublittoral sand' samples (16.1%), with the top taxa including phyllodocid polychaetes (*Syllis* spp., *Glycera* spp. and *Pisione remota*), terrebellid polychaetes (*Polycirrus* and *Ampharete octocirrata*), the tubiculous polychaete *Sabellaria spinulosa*, pea urchins (*Echinocyamus pusillus*), the lesser sandeel (*Ammodytes marinus*), carditid and myid bivalves (*Goodallia triangularis* and *Sphenia binghami*).

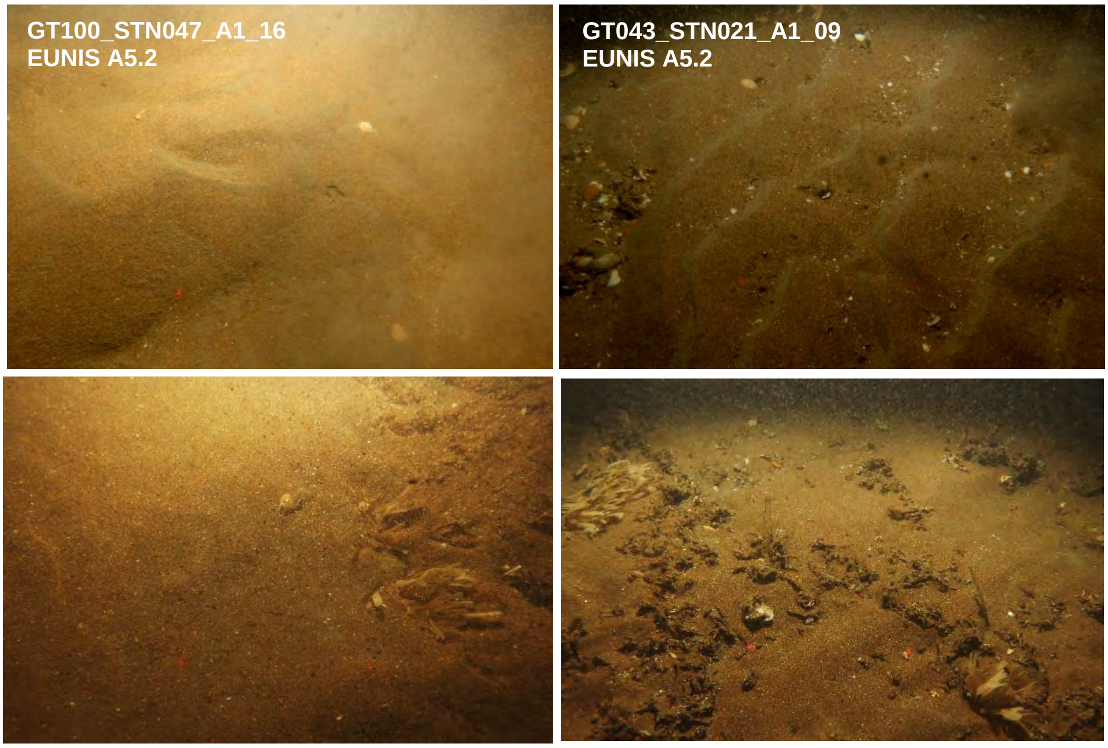

Figure 22. Example photographs of A5.2 ‘Sublittoral sand’.

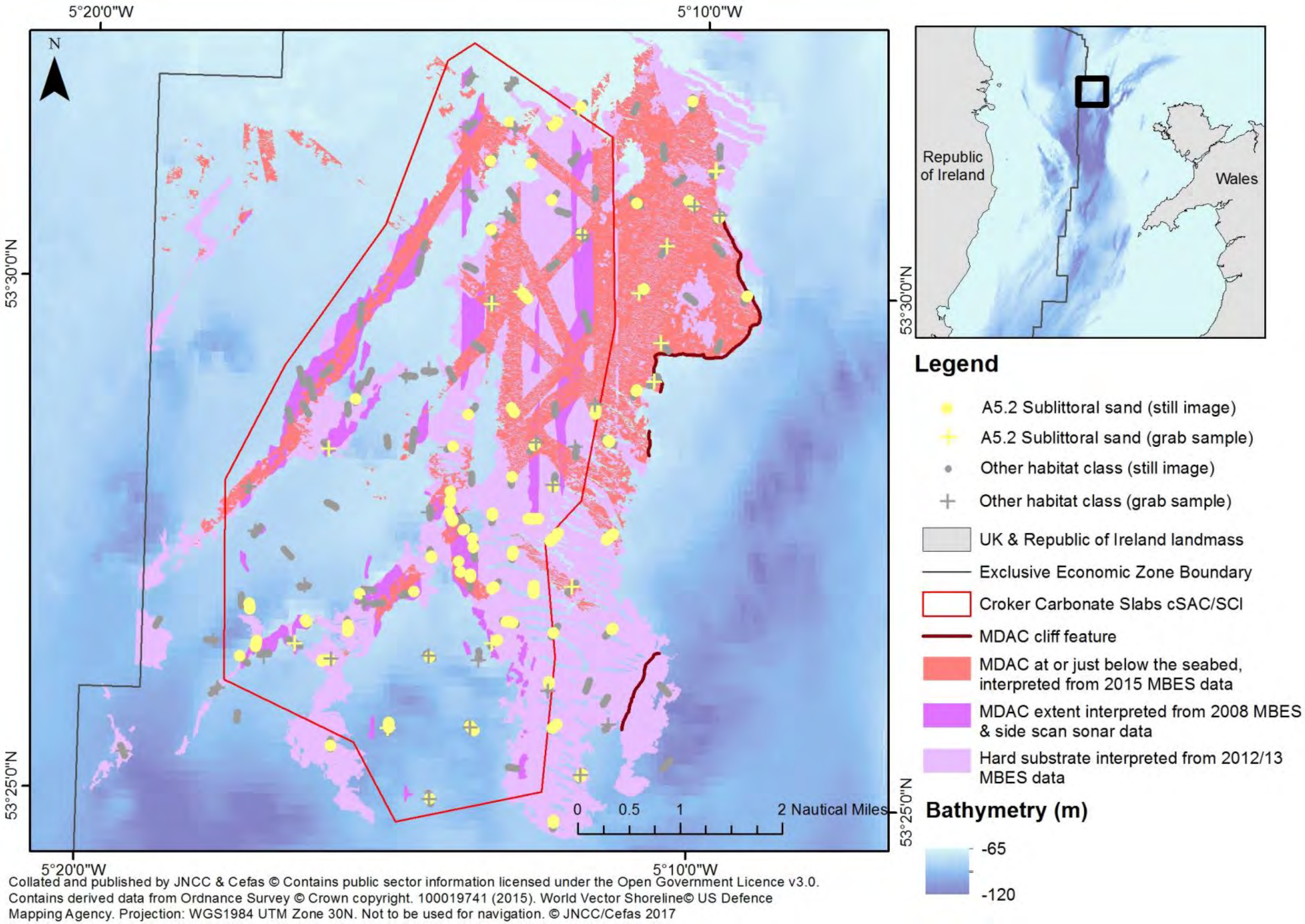

Figure 23. Distribution of A5.2 ‘Sublittoral sand’, as interpreted from still images and grab samples.

A5.4 'Sublittoral mixed sediment'

A5.4 'Sublittoral mixed sediment' (SS.SMx) was widely distributed across the survey area, although more frequently encountered in the west of the site, accounting for 11.9% of still images (Figure 25)

As observed for A5.1 'Sublittoral coarse sediment', the sediments within the group were extremely heterogeneous, with a variable lithic content and a typically higher mud fraction than the previous two sediment types (Figure 24). The biotope A5.234 'Semi-permanent tube-building amphipods and polychaetes in sublittoral sand' (SS.SSa.IFiSa.TbAmPo) was recorded. As observed for the similar habitat class A5.1 'Sublittoral coarse sediment', the epifaunal assemblage composition reflected the highly variable sediments, with average similarity calculated at 27.2%. The group shared the same top four taxa which contributed to similarity in the A5.1 'Sublittoral coarse sediment' class (Hydrozoa (clumps/solitary & turf), Polychaeta and Serpulidae), and which appeared to be ubiquitous across the survey area. These four taxa accounted for 77% of within-group similarity. ANOSIM analysis showed significant and highly significant differences between the epifaunal assemblage structure of A5.4 'Sublittoral mixed sediment' and A5.2 'Sublittoral sand', A4.23 'Communities on soft circalittoral rock', outcropping MDAC, and pavement MDAC (see Table 6). No significant difference was found between A5.4 'Sublittoral mixed sediment', A5.1 'Sublittoral coarse sediment' and A5.6 'Sublittoral biogenic reef'.

A5.4 'Sublittoral mixed sediment' was the most common EUNIS level 3 habitat identified from grab samples (47.8%), indicating that the mud fraction may be difficult to detect from imagery. Macrofaunal abundance and richness were both high and variable, indicating the heterogeneity and complexity of the sediments, with the number of individuals ranging from 126 to 1287, and the number of species from 47 to 127 per grab (see Figure 26b and c). The level of within-group macrofaunal similarity was high in comparison with the previously described sediment habitats (46.4%, see Figure 26a), with *Sabellaria spinulosa* dominating the similarity contribution ranking, and this gregarious taxon appearing to be spatially correlated with the increased fine sediment content of A5.4 'Sublittoral mixed sediment' (see Figure 26). *Sabellaria spinulosa*, in combination with terrebellid and spionid polychaetes (*Ampharete octocirrata* and *Laonice bahusiensis*), brittlestars (*Amphipolis squamata*), venerid and cardiid bivalves (*Timoclea ovata* and *Abra alba*), and gammarid amphipods (*Ampelisca diadema*), represented almost 25% of within-group similarity.

As detailed in Section 3.2.3, seven sub-samples from three grab stations were retained for meiofaunal analysis, specifically to investigate potential chemotrophic associations indicating active gas seepage (Physalia 2016). The majority of these samples were acquired from A5.4 'Sublittoral mixed sediments', and were composed of fine silts and carbonate debris, presumed to be fragmented MDAC debris.

The samples revealed assemblages of notable diversity, which were primarily composed of nematode species from a range of trophic groups (145 taxa recorded in total). This diversity was thought to be largely driven by the heterogeneity of microhabitats within the sampled sediments (Physalia 2016). The nematodes *Leptonemella* and *Catonema* were recorded, both of which browse on ectosymbiotic cyanobacteria which develop on their cuticles, which they in turn supply with reduced sulphur compounds and oxygen (Ott *et al* 1991). Whilst the presence of sulphidic materials associated with methane seeps may enhance conditions for *Leptonemella* and *Catonema*, they were not recorded in areas where evidence of gas seepage had been observed, therefore it was not possible to establish a direct link with active seepage. No examples of nematodes which had previously been noted in association with active methane seeps elsewhere in UK water were recorded (e.g. *Astromonema southwardarum*, which had been observed in high numbers at the Braemar Pockmarks SAC in 2013 (Physalia 2016), and also by Austen *et al* (1993) at the Scanner Pockmark SAC).

The entire macrofaunal taxon list (for the full set of 46 grab samples) was also reviewed by an experienced benthic taxonomist; however, no instances were recorded of taxa which were likely to be directly associated with MDAC or gas seepage.

Figure 24. Example photographs of A5.4 'Sublittoral mixed sediment'.

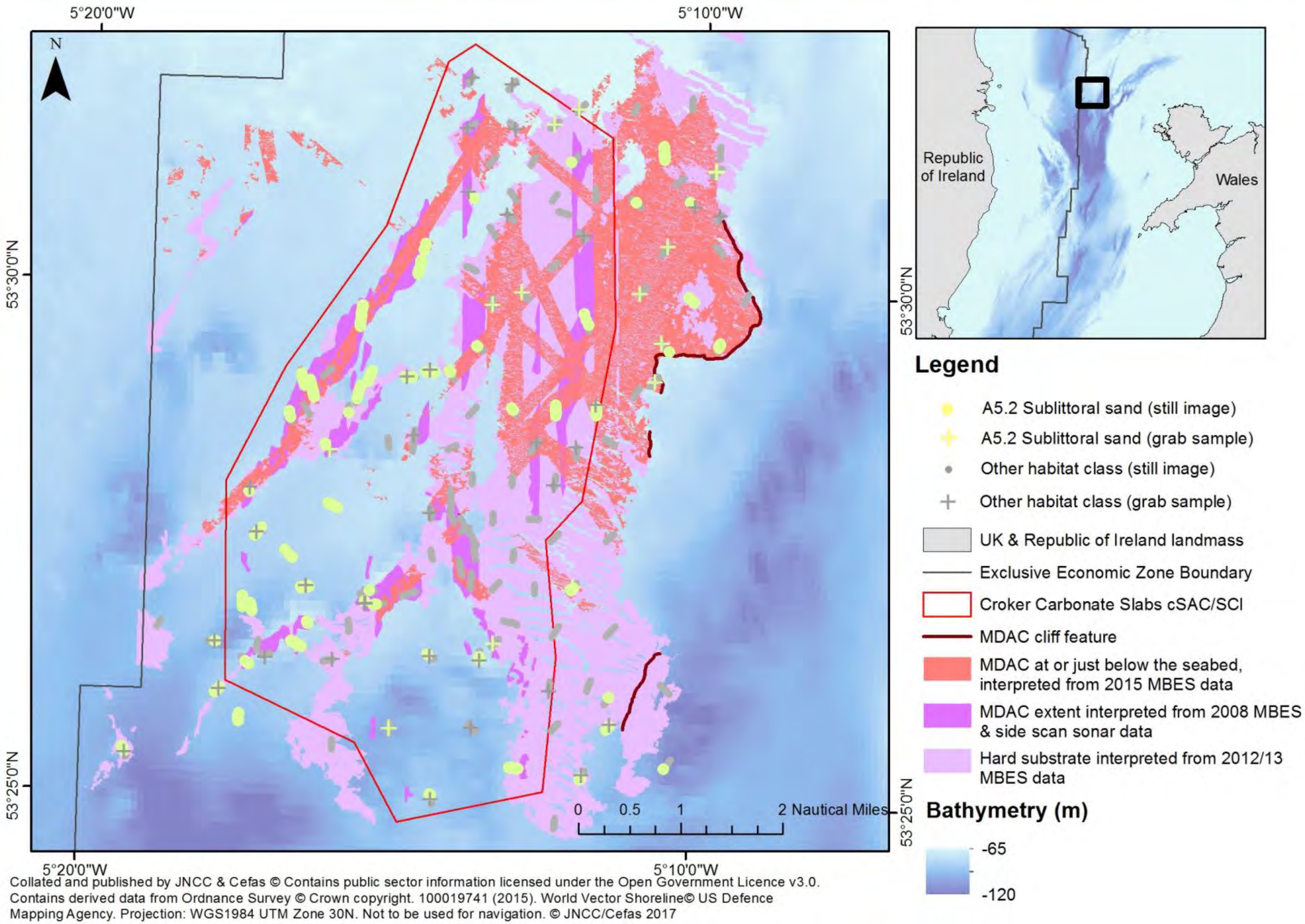

Figure 25. Distribution of A5.4 'Sublittoral mixed sediment', as interpreted from still images and grab samples.

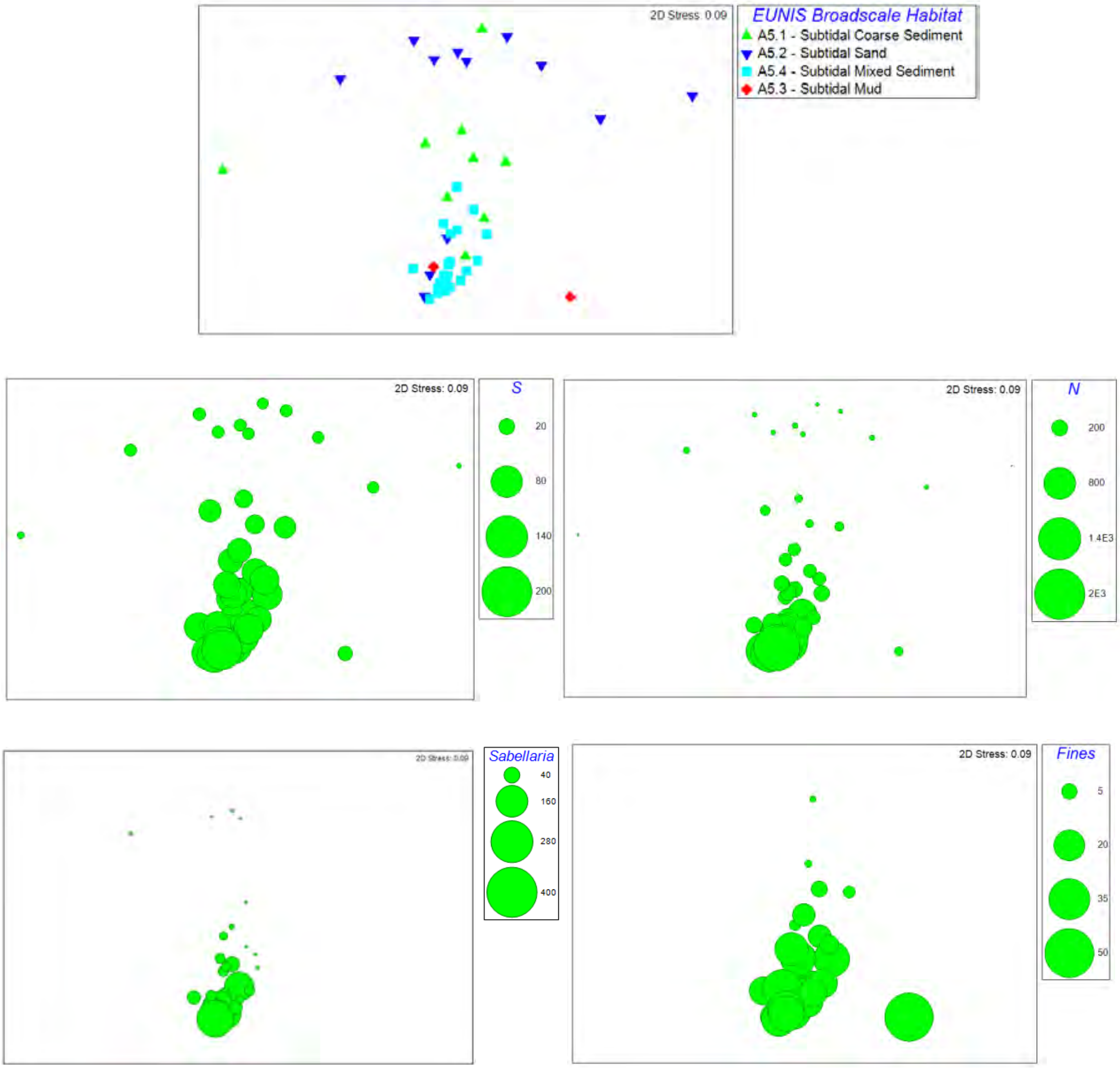

Figure 26. Multi-dimensional scaling ordinations (MDS) visualising multivariate similarity between macrofaunal samples, overlain with a) EUNIS broad-scale habitats, b) abundance of individual macrofauna, c) number of macrofaunal taxa, d) abundance of *Sabellaria spinulosa*, and d) percentage mud content.

4.4.2 A5.6 'Sublittoral biogenic reef'

A5.6 'Sublittoral biogenic reef' (SS.SBR), comprising aggregations of the tubiculous polychaete *Sabellaria spinulosa*, was recorded across the extent of the survey area. In general, this habitat was spatially correlated with areas of MDAC interpreted from the 2015 and 2008 data, indicating that *S. spinulosa* were likely to colonise areas of MDAC where the substrate was not directly visible (either due to the density of polychaete tubes, or burial by a surficial sediment veneer), or MDAC debris loose in the sediment (Figure 28). Still image records of A5.6 'Sublittoral biogenic reef' were correspondingly categorised as the biotopes A5.611 '*Sabellaria spinulosa* on stable circalittoral mixed sediment' (SS.SBR.PoR.SspiMx) and A4.221 '*Sabellaria spinulosa* on encrusted circalittoral rock' (CR.MCR.CSab.Sspi), where aggregations were directly observed colonising hard substrate. Where MDAC was clearly visible beneath the aggregations the MDAC classification took precedent over A5.6 Sublittoral biogenic reef for the purpose of analysis.

It should be noted that none of the areas of A5.6 ‘Sublittoral biogenic reef’ observed scored sufficiently against the *S. spinulosa* reefiness assessment criteria proposed by Gubbay (2007) to be considered Annex I Reef. The aggregations were typically present in the form of a patchy low-lying crust, and were not discernible from acoustic data. Average tube elevation was generally judged to be <2cm (‘not a reef’) or occasionally 2-5cm (‘low reefiness’), indicating that these aggregations do not constitute Annex I Reef features.

The epifaunal assemblages associated with A5.6 ‘Sublittoral biogenic’ reefs were similar to those observed for A5.1 ‘Sublittoral coarse sediment’ and A5.4 ‘Sublittoral mixed sediment’, as expected due to the necessity of coarse sediments for settlement by *S. spinulosa*. Unsurprisingly, group similarity was dominated by *S. spinulosa*, whilst Polychaeta, Serpulidae, Hydrozoa (clumps/solitary and turf), Ascidiacea, and the bryozoans *Alcyonidium diaphanum* and *Flustra foliacea* contributed almost 90% of the overall within-group similarity (43.5%). ANOSIM analysis showed no significant difference between the epifaunal assemblages of A5.6 ‘Sublittoral biogenic reef’, A5.1 ‘Sublittoral coarse sediment’ and A5.4 ‘Sublittoral mixed sediment’. Highly significant differences existed with A5.2 ‘Sublittoral sand’, A4.23 ‘Communities on soft circalittoral rock’, outcropping MDAC, and pavement MDAC (see Table 6).

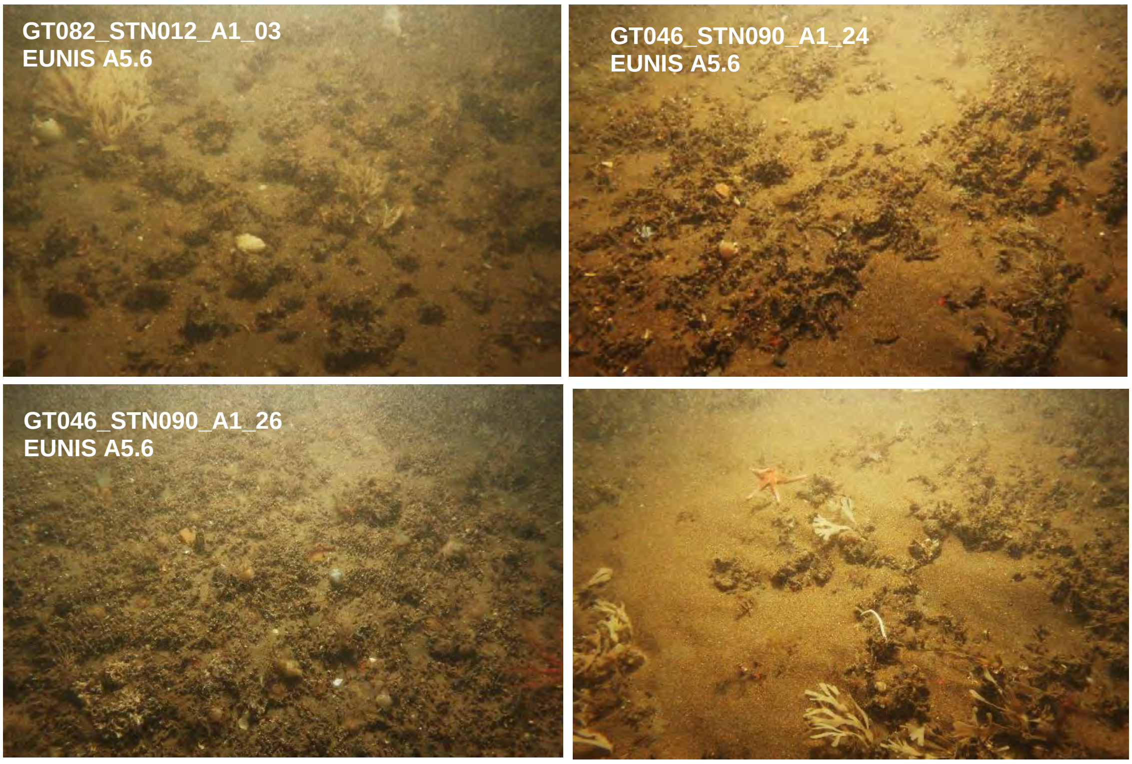

Figure 27. Example photographs of A5.6 ‘Sublittoral mixed sediment’.

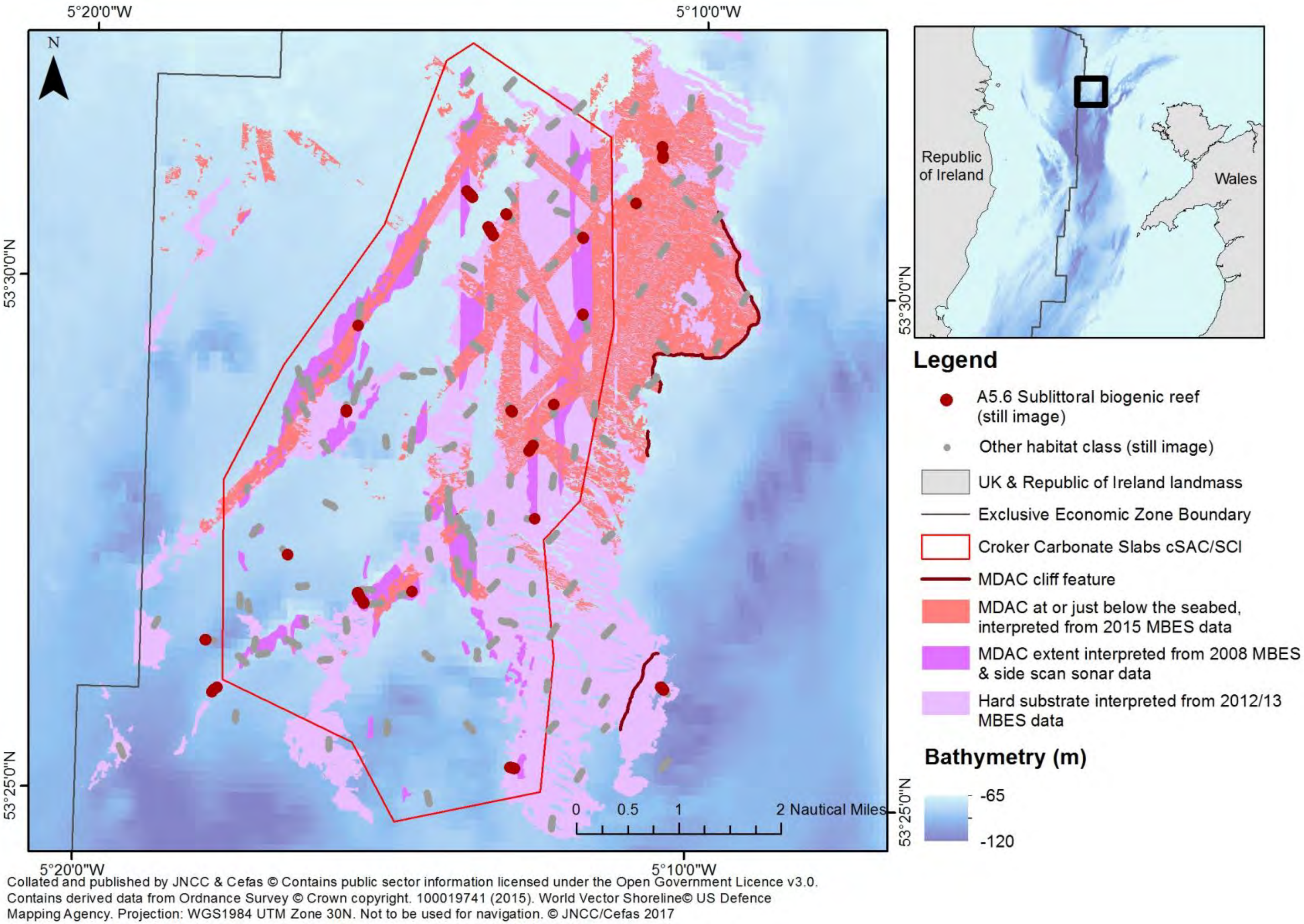

Figure 28. Distribution of A5.6 ‘Sublittoral biogenic reef’, as interpreted from still images.

4.4.3 A4.23 'Communities on soft circalittoral rock'

A4.23 'Communities on soft circalittoral rock', consisting of a blue-green clay, were observed across the latitudinal range of the survey area, being most commonly encountered in the south-east, and more sporadically in the north (Figure 30).

The clay was observed as raised continuous slabs and small exposures visible through a shallow veneer of mobile sands, particularly in the southern area interpreted to contain sandwaves (Figure 29). In places the clay was covered with a large number of small depressions; it is unclear whether they were attributable to bioturbation by boring organisms or weathering of the clay causing the release of lithic material from the fine matrix.

The clay itself was generally associated with a depauperate epifauna, but regularly interspersed with coarse sediments which are likely to have influenced the epifaunal assemblages recorded for images assigned to this habitat class. An average similarity of 35.1% was calculated for the A4.23 'Communities on soft circalittoral rock' class, with the most significant taxa contributing to similarity being similar to those recorded in the previously described habitats, and consisting of Polychaeta, Hydrozoa (turf) and Serpulidae, with the addition of tubiculous amphipods (possible *Ampelisca* sp.). ANOSIM showed significant and highly significant differences between A4.23 'Communities on soft circalittoral rock' and A5.4 'Sublittoral mixed sediment', A5.6 'Sublittoral biogenic reef', outcropping MDAC, and pavement MDAC (see Table 6). No significant differences existed with A5.1 'Sublittoral coarse sediment' and A5.2 'Sublittoral sand'.

Figure 29. Example photographs of A4.23 'Communities on soft circalittoral rock'.

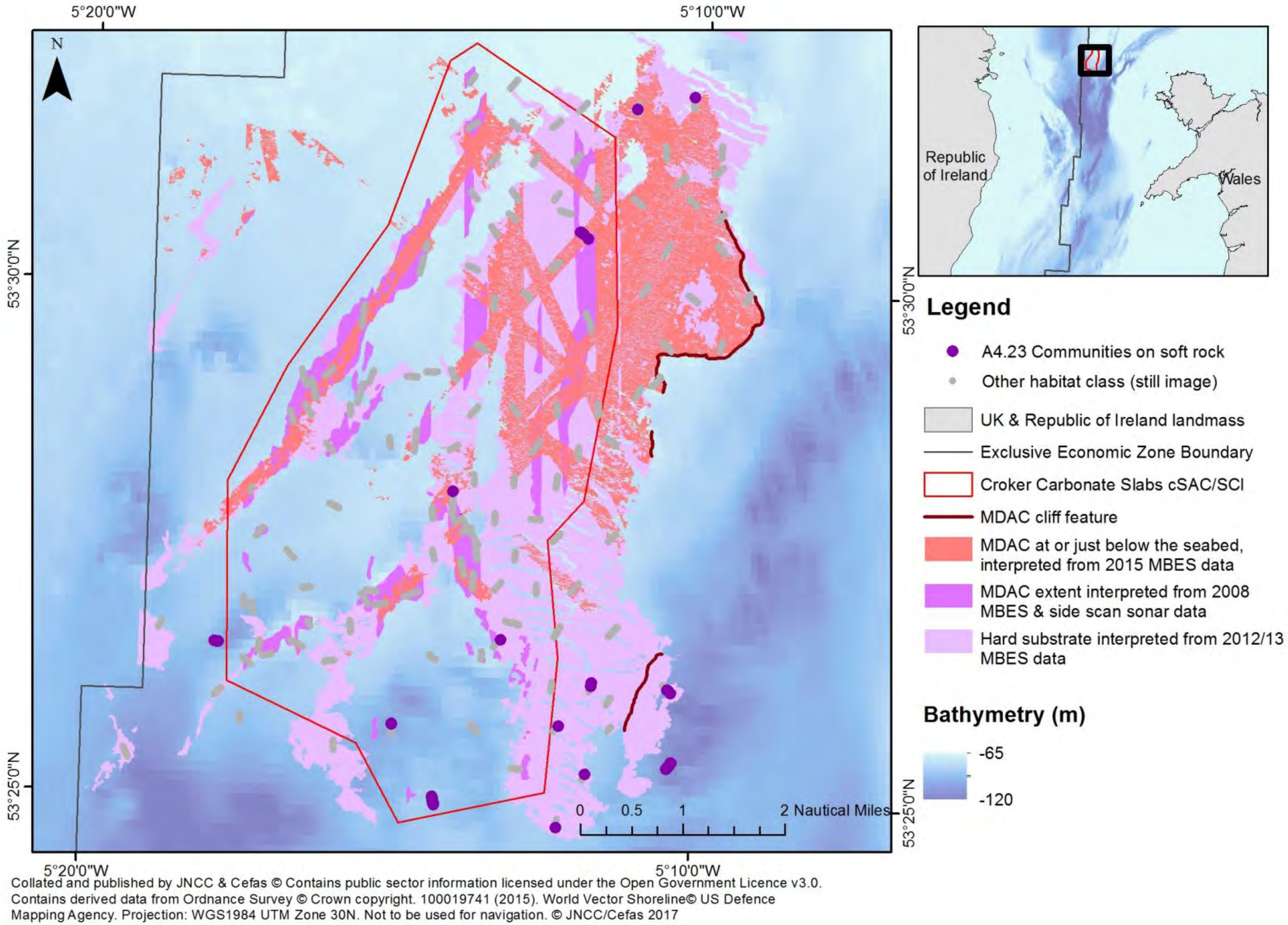

Figure 30. Distribution of A4.23 'Communities on soft circalittoral rock', as interpreted from still images.

4.4.4 Methane-Derived Authigenic Carbonates (MDAC)

As discussed in Section 3.4.1, epifaunal analyses were conducted using images of outcropping MDAC (68 stills) and pavement MDAC (207 stills) which were identified with high or moderate confidence. The distribution of these MDAC categories is displayed in Figure 32 and Figure 33.

Within the cSAC/SCI, both outcropping and pavement MDAC observations generally corresponded to the MDAC extent originally delineated and groundtruthed from 2008 data, whilst a number of new observations of both categories were made to the east, north east and south west of the site, on areas of hard substrate predicted by Callaway *et al* (2015).

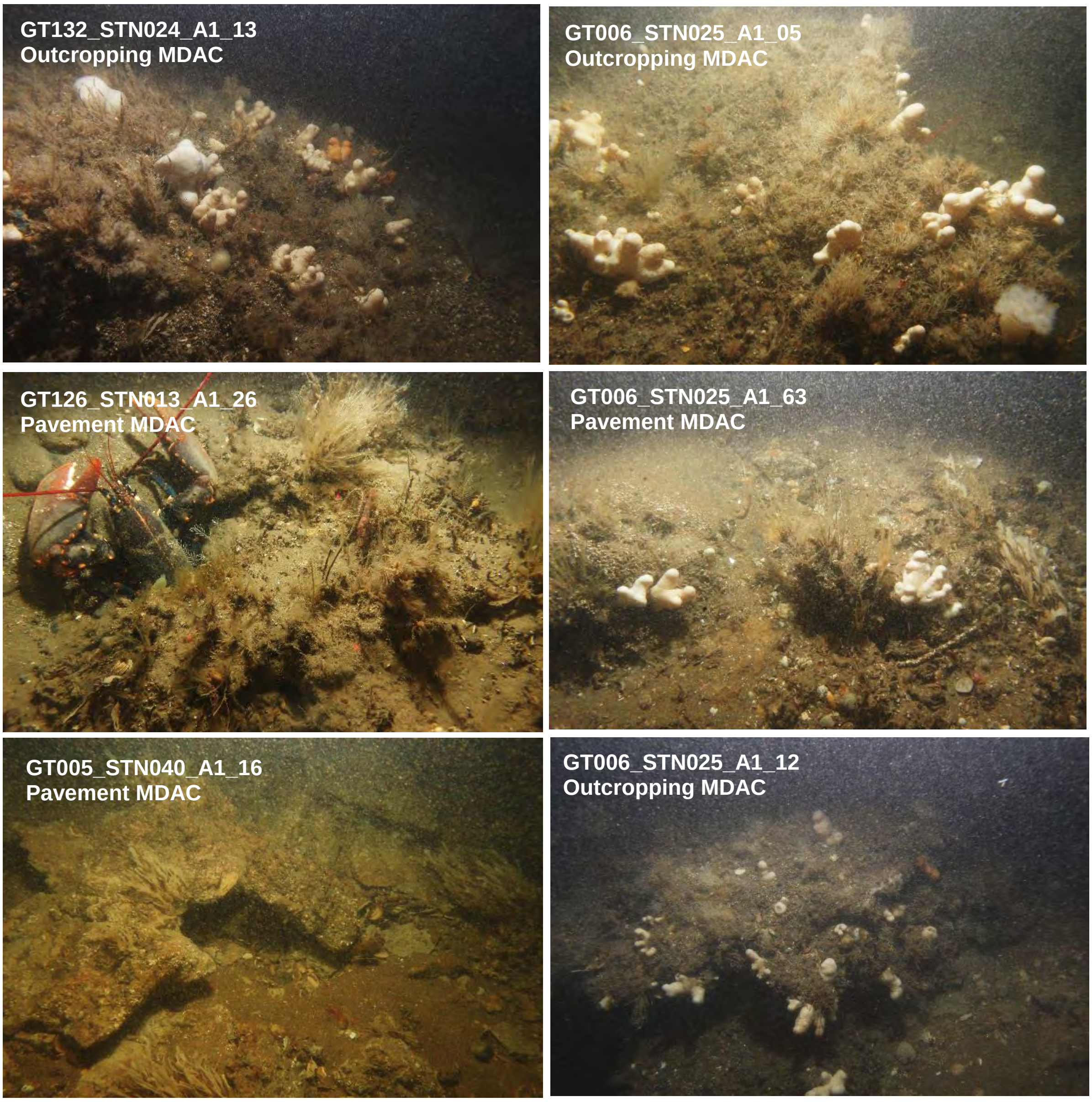

Figure 31. Example photographs of outcropping and pavement MDAC.

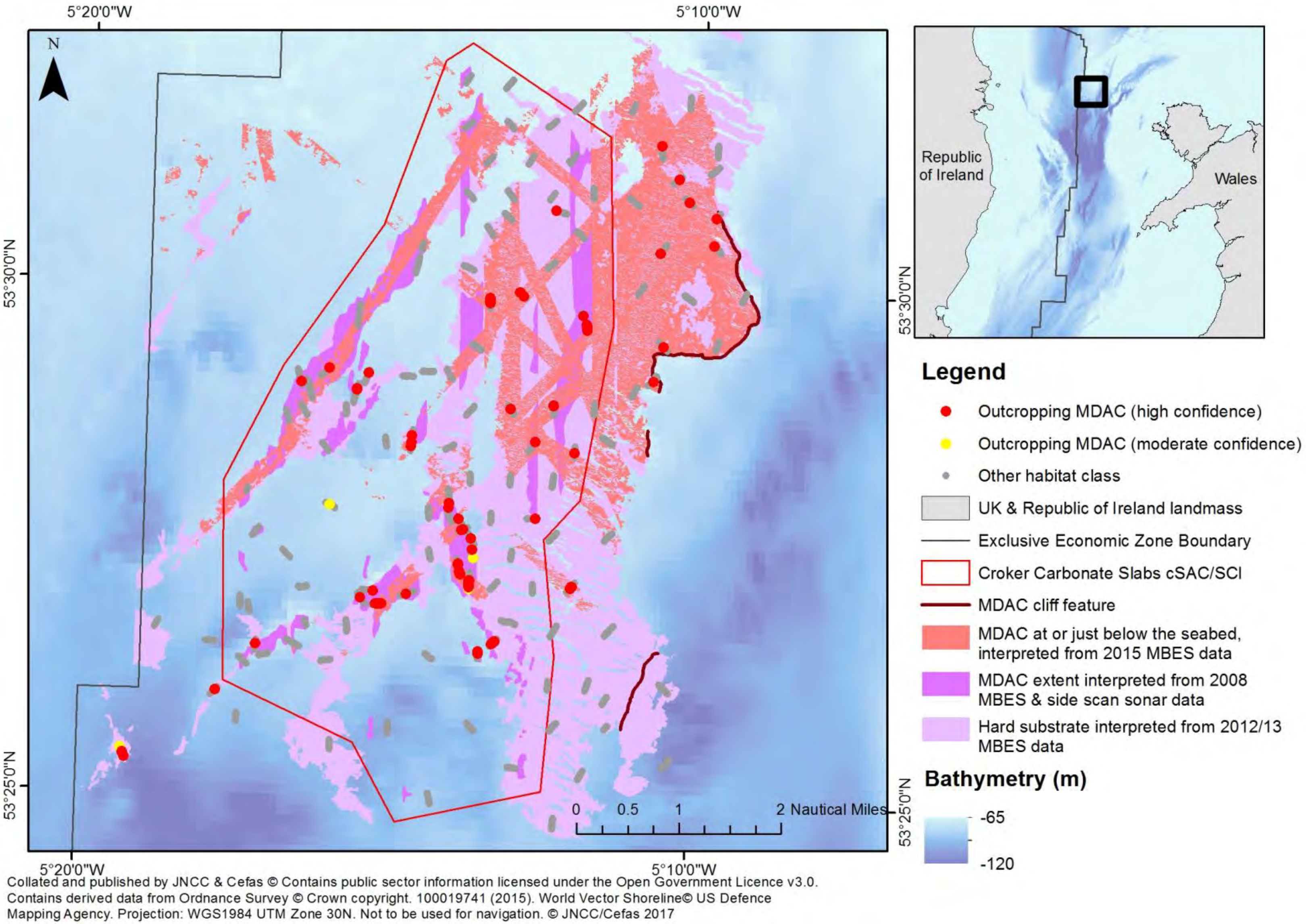

Figure 32. Distribution of outcropping MDAC (high and moderate confidence), as interpreted from still images.

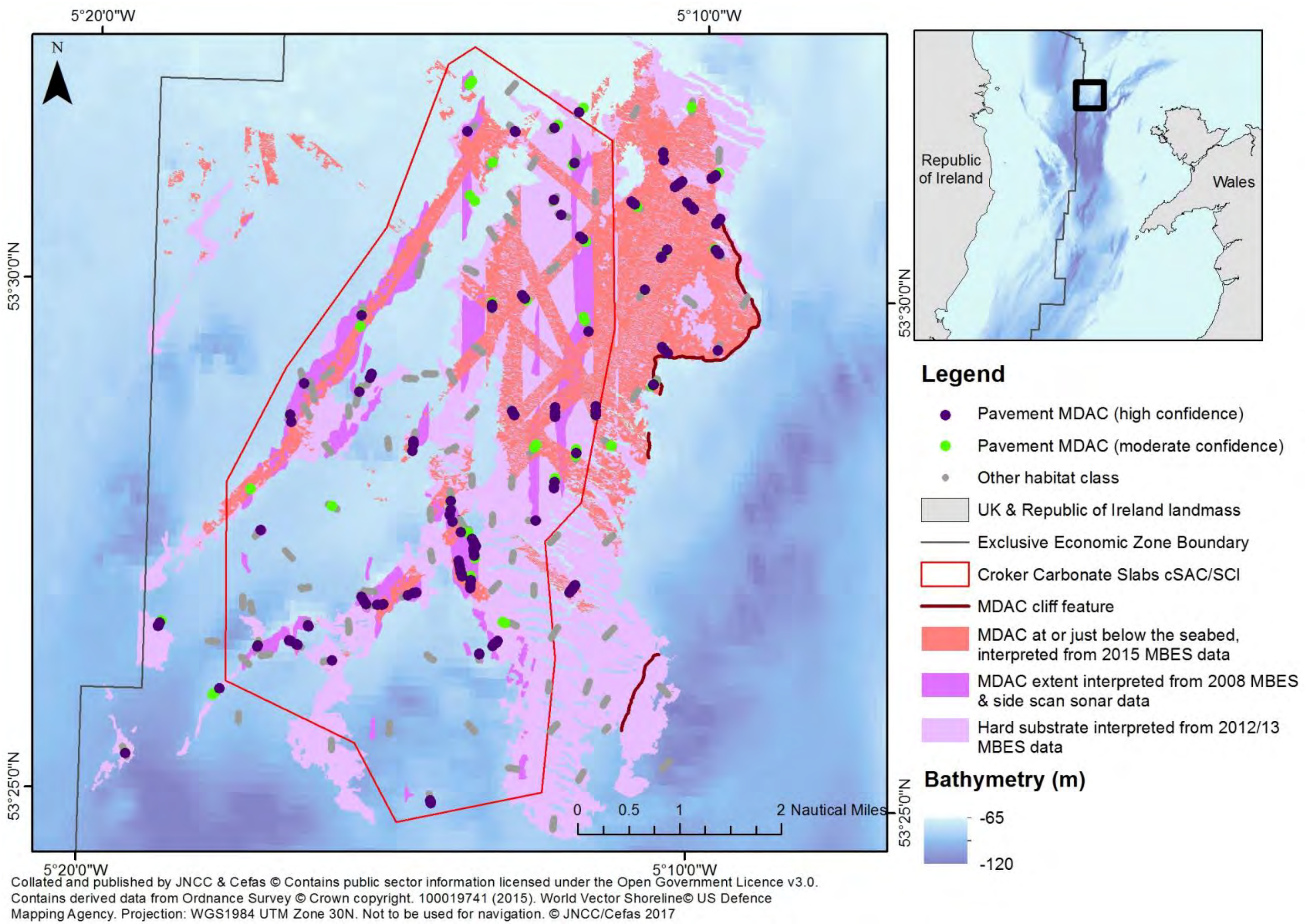

Figure 33. Distribution of pavement MDAC (high and moderate confidence), as interpreted from still images.

ANOSIM analysis revealed that the epifaunal assemblage composition of outcropping MDAC was not significantly different from that of pavement MDAC. Assemblages of both MDAC types were, however, highly significantly different to all other habitat classes, with the exception of A5.1 'Sublittoral coarse sediment' (see Table 6).

Table 6. Comparison of epifaunal assemblages between habitat classes (SACFOR abundance from still images) by ANOSIM analysis (R-values reported; * = significant difference, ** = highly significant difference).

	A5.1 'Sublittoral coarse sediment'	A5.2 'Sublittoral sand'	A5.4 'Sublittoral mixed sediment'	A5.6 'Sublittoral biogenic reef'	A4.23 'Communities on soft circalittoral rock'	Outcropping MDAC
A5.2 'Sublittoral sand'	0.226**					
A5.4 'Sublittoral mixed sediment'	-0.099	0.470**				
A5.6 'Sublittoral biogenic reef'	-0.152	0.140**	-0.067			
A4.23 'Communities on soft circalittoral rock'	-0.100	0.028	0.115*	0.369**		
Outcropping MDAC	-0.061	0.094**	0.142**	0.428**	0.473**	
Pavement MDAC	-0.096	0.464**	0.078**	0.120**	0.297**	0.019

The average similarity ranks for outcropping and pavement MDAC were both dominated by Hydrozoa (clumps/solitary), with other ubiquitous taxa (Polychaeta, Hydrozoa (turf) and Serpulidae) being amongst the most influential (see Table 7). Permutations of these four taxa had also dominated the similarity ranks for other habitat classes within the site.

Table 7. Average similarity ranks for epifaunal assemblages of outcropping and pavement MDAC classes, as derived from still image data by SIMPER analysis. Ave. abun. = average SACFOR abundance; Contrib% = percentage contribution to similarity; Cum% = cumulative contribution to similarity. Taxa in yellow occur ubiquitously within the top five dominating taxa of the majority of habitat classes.

Outcropping MDAC				**Pavement MDAC**			
Average similarity: 32.7%				**Average similarity: 29.7%**			
Taxon	**Ave. abun**	**Contrib. %**	**Cum. %**	**Taxon**	**Ave. abun.**	**Contrib. %**	**Cum. %**
Hydrozoa (clumps/solitary)	3.1	30.9	30.9	Hydrozoa (clumps/solitary)	3.2	42.9	42.9
Alcyonium digitatum	1.4	10.5	41.3	Serpulidae	1.5	13.5	56.4
Cellaria	1.4	10.3	51.7	Polychaeta	1.3	9.5	65.9
Polychaeta	1.2	6.9	58.6	*Cellaria*	1.0	6.6	72.5
Hydrozoa (turf)	1.4	6.4	65.0	Hydrozoa (turf)	1.2	6.3	78.8
Serpulidae	1.2	6.3	71.3	Sabellidae	1.1	3.4	82.2
Sabellidae	1.3	4.3	75.5	Sagartiidae	1.0	3.2	85.4

Outcropping MDAC				Pavement MDAC			
Average similarity: 32.7%				Average similarity: 29.7%			
Taxon	**Ave. abun**	**Contrib. %**	**Cum. %**	**Taxon**	**Ave. abun.**	**Contrib. %**	**Cum. %**
Bryozoa (turf)	0.7	3.2	78.7	Sertulariidae	0.8	2.2	87.6
Nemertesia	1.1	3.1	81.8	*Flustra foliacea*	0.6	1.9	89.4
Sagartiidae	1.0	2.8	84.6	*Alcyonium digitatum*	0.4	1.4	90.9
Sertulariidae	0.9	2.6	87.2	*Nemertesia*	0.7	1.4	92.3
Amphipoda tube	0.7	2.0	89.2	Bryozoa (turf)	0.4	1.4	93.7
Porifera (encrusting)	0.4	1.8	91.0	Amphipoda tube	0.4	0.9	94.6
Tubularia	0.6	1.5	92.5	Ascidiacea	0.4	0.6	95.2
Actiniaria	0.7	1.3	93.9	*Calliostoma zizyphinum*	0.3	0.6	95.8

Although many of the dominant taxa also dominated other habitat classes, the significant differences identified by ANOSIM analysis were evident in the ranks by the presence of a series of distinct taxa which did not feature in the dominant taxa of other habitats. The top 15 SIMPER similarity ranking taxa for each habitat were sorted according to the average SACFOR abundance of the top 15 taxa contributing to similarity within the outcropping MDAC class, to identify taxa that were exclusively or primarily associated with MDAC (see Table 8).

Table 8. Comparative average SACFOR abundance between habitat classes of the 15 taxa contributing most to similarity within the outcropping MDAC class, as derived from still image data. Colour-scaled from red (highest abundance) through to green (lowest abundance). Bold lettering indicates taxa which restricted to or notably more abundant on MDAC.

Taxa	Outcropping MDAC	Pavement MDAC	A5.1	A5.2	A5.4	A5.6	A4.23
Hydrozoa (clumps/solitary)	3.1	3.2	1.3	0.8	2.6	2.6	0.9
Alcyonium digitatum	1.4	0.4					
Cellaria	1.4	1.0		0.1		0.4	
Polychaeta	1.2	1.3	1.2	0.3	1.8	2.7	2.5
Hydrozoa (turf)	1.4	1.2	0.4		1.2	1.5	
Serpulidae	1.2	1.5	1.3	0.3	1.3	2.0	1.4
Sabellidae	1.3	1.1	0.2	0.1	0.4		
Bryozoa (turf)	0.7	0.4		0.1			
Nemertesia	1.1	0.7					
Sagartiidae	1.0	1.0	0.3	0.2	0.9	1.0	
Sertulariidae	0.9	0.8	0.2		0.6	0.9	0.5
Amphipoda tube	0.7	0.4					1.0

Taxa	Outcropping MDAC	Pavement MDAC	A5.1	A5.2	A5.4	A5.6	A4.23
Porifera (encrusting)	0.4						0.2
Tubularia	0.6						
Actiniaria	0.7				0.6	1.1	0.3

A5.1 = 'Sublittoral coarse sediment'; A5.2 = 'Sublittoral coarse sand'; A5.4 = 'Sublittoral mixed sediment'; A5.6 'Sublittoral biogenic reef'; A4.23 = 'Communities on soft circalittoral rock'.

Five taxa were identified from the SIMPER within-group similarity rankings which either only occurred in the top 15 characterising taxa of the outcropping and/or pavement MDAC classes, or have been recorded at notably higher abundances in association with MDAC as opposed to non-MDAC classes. Comparison of between-group dissimilarity ranks for MDAC classes and other habitats showed that taxa contributing the highest percentages were typically low resolution, and similar to those contributing to within-group similarity across the survey area (e.g. Hydrozoa, Polychaeta, Serpulidae), presumably due to differences in mean abundance between groups. The dissimilarity ranks also revealed that all five 'MDAC-associated' taxa identified from the similarity ranks were consistently listed amongst the top 15 taxa contributing to dissimilarity between MDAC classes and other habitats.

These five taxa comprised the soft coral species *Alcyonium digitatum*, branching bryozoans of the genus *Cellaria,* tubiculous polychaetes of the family Sabellidae (including suspected *Sabella pavonina*), and the hydroid genera *Nemertesia* (including *Nemertesia antennina* and *Nemertesia ramosa*) and *Tubularia* (including *Tubularia indivisa)*. All five taxa were widely distributed, with the exception of *Tubularia*, which was less commonly observed and appeared to be mainly limited to areas of outcropping MDAC (see Figure 37 to Figure 41). The graph presented in Figure 34 shows that each of the five taxa occurred in a substantially higher number of outcropping MDAC still images than in images of other non-MDAC habitat classes. The occurrence frequency of the taxa in the pavement MDAC class was also considerably higher than in non-MDAC habitat classes. All taxa were consistently recorded in a higher number of images for outcropping MDAC in comparison to pavement MDAC, although this difference was less pronounced for Sabellidae.

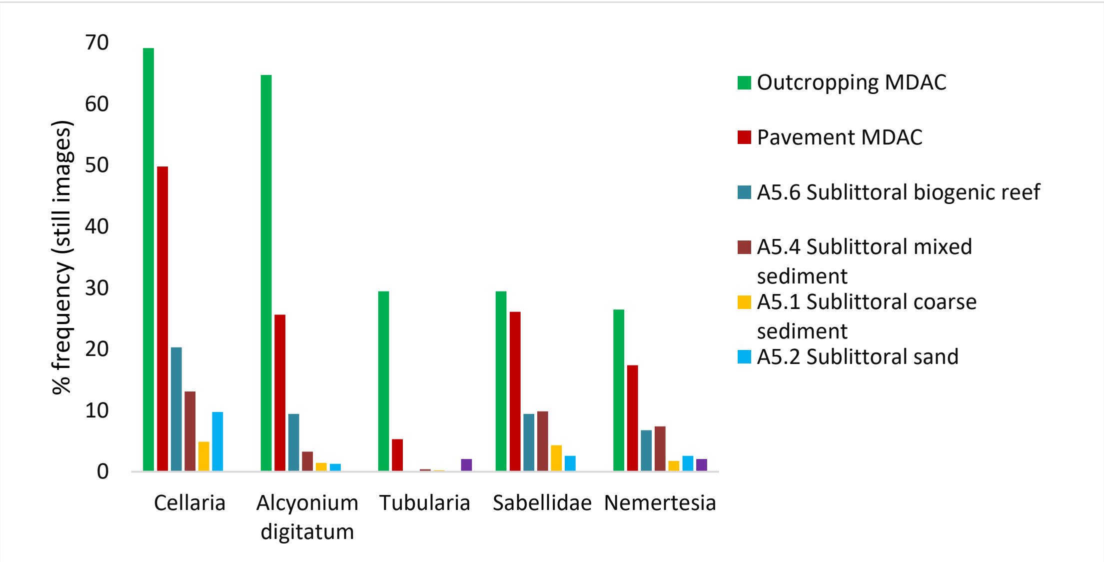

Figure 34. Percentage occurrence frequency of MDAC-associated taxa for different habitat classes, derived from still image data.

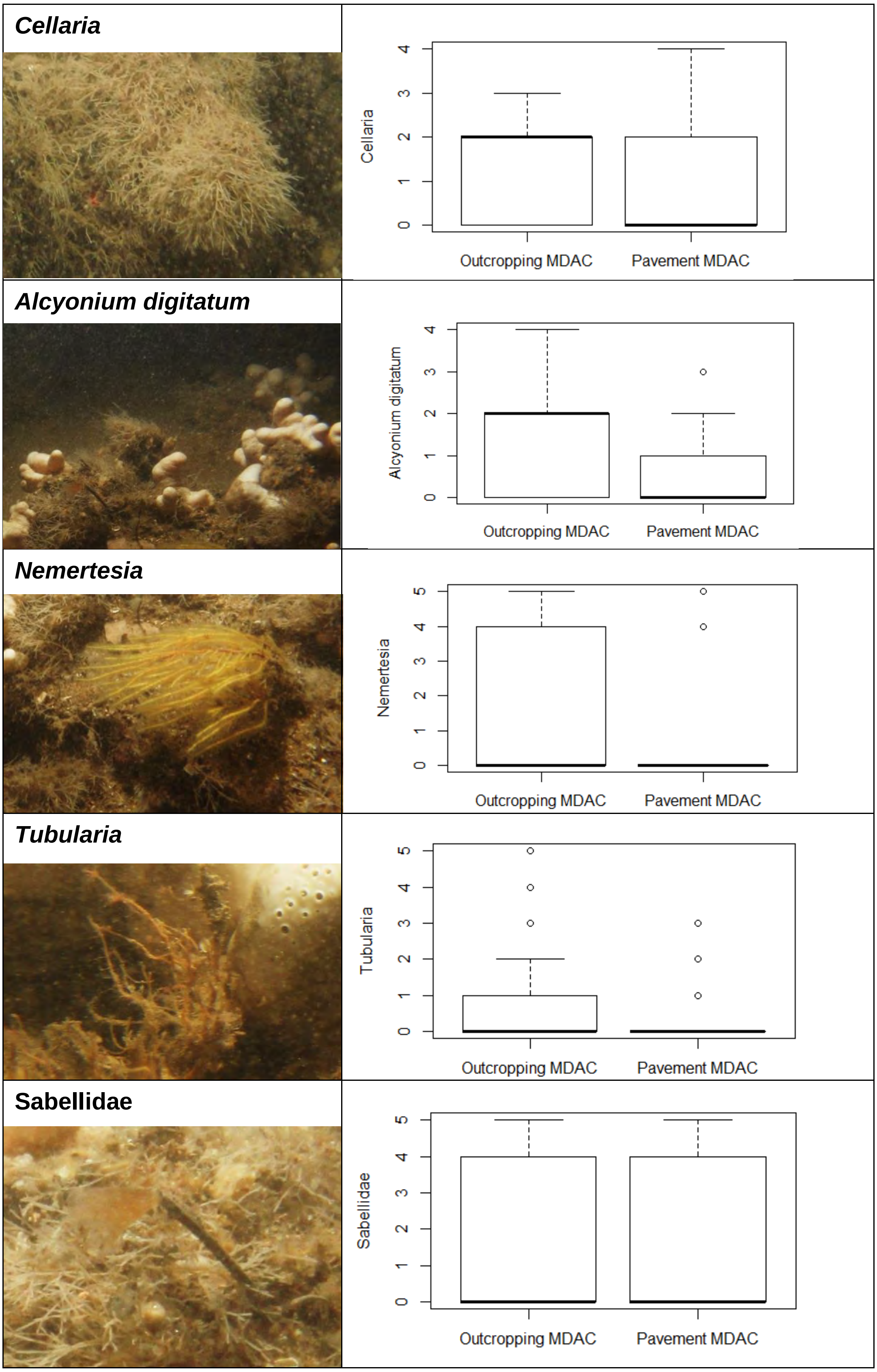

Figure 35. Box and whisker plot comparison of MDAC-associated taxon abundance (SACFOR) between outcropping and pavement MDAC. Bold lines = median, box limits = upper and lower quartiles (25th and 75th percentile), whisker = maximum value (excluding outliers lying >1.5x the length of the interquartile range).

The boxplots in Figure 35 compare median SACFOR abundance of each of the five MDAC-associated taxa between the two topographical classes of MDAC. The interquartile and upper ranges of *Alcyonium digitatum* abundance on outcropping MDAC are notably larger than those for pavement MDAC. With the exception of Sabellidae, all taxa were more abundant on outcropping MDAC than pavement MDAC, in particular *Alcyonium digitatum*.

Additional analyses were conducted to further investigate the differences observed in the abundance of three of the five epifaunal taxa (where the number of observations was sufficient) on outcropping and pavement MDAC. Additional analyses were performed to determine whether differences between the outcropping and pavement MDAC classes could be linked to the inherent disparities in MDAC % cover between the two forms, as opposed to the elevation and structure. As described in Section 3.4.3, the permute.groups test (Barry *et al* 2017) was conducted between the two MDAC groups for the taxa *Alcyonium digitatum, Cellaria* and Sabellidae, as the occurrence frequency of *Nemertesia* and *Tubularia* was too low. The test was performed with 10000 replications for; a) images with any coverage of MDAC, b) images with ≥30% MDAC, and c) images with ≥70% MDAC, testing the hypotheses presented below.

H_0 = There is no difference in taxon abundance between outcropping and pavement MDAC

H_1 = There is a difference in taxon abundance between outcropping and pavement MDAC

The results of the permute.groups analysis (displayed in Table 9) suggest that abundance of *Alcyonium digitatum* was significantly higher on outcropping MDAC than on pavement MDAC, regardless of the MDAC % cover (see Figure 36), whilst there was no significant difference in the abundance of Sabellidae between the two MDAC types in all categories of MDAC cover (see Figure 36). For *Cellaria*, a significant difference between outcropping and pavement forms was evident for any % cover of MDAC, but did not exist in the >30% and >70% categories. This suggests that *Cellaria* abundance may be more strongly related to the availability of hard substrate than to the elevation and structure of that substrate, and is similarly abundant on both types of MDAC (albeit with lower median values noted for pavement MDAC; see Figure 36).

Table 9. p-values for permute.groups tests (Barry *et al* 2017) conducted to detect differences between SACFOR abundance of three MDAC-associated taxa on outcropping MDAC and pavement MDAC, in three different categories of MDAC coverage, as derived from still image data.

Taxon	**Any MDAC cover**		**>30% MDAC cover**		**>70% MDAC cover**	
Sample size (n = no. of stills)	Outcroppping	Pavement	Outcroppping	Pavement	Outcroppping	Pavement
	67	207	58	97	37	58
Alcyonium digitatum	<0.001**		<0.001**		<0.001**	
Cellaria	0.014*		0.079		0.160	
Sabellidae	0.610		0.866		0.703	

* significant difference, ** highly significant difference

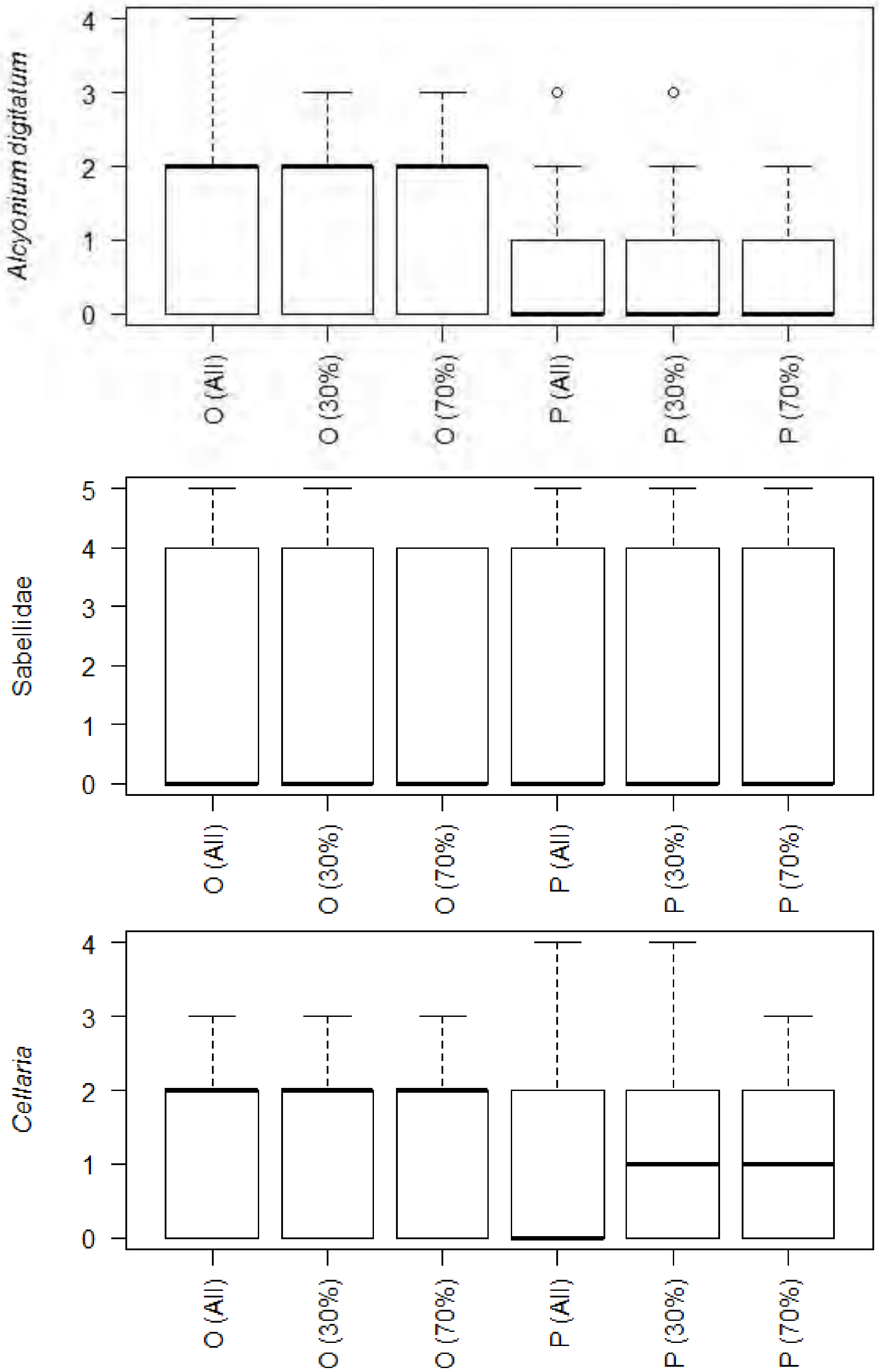

Figure 36. Boxplots of SACFOR abundance of *Alcyonium digitatum,* Sabellidae and *Cellaria* on outcropping MDAC (O) and pavement MDAC (P) with; any MDAC % cover (All), MDAC cover of ≥30% (30%), and MDAC cover of ≥70% (70%). Bold lines = median, box limits = upper and lower quartiles (25th and 75th percentile), whisker = maximum value (excluding outliers lying >1.5x the length of the interquartile range).

Biological traits analysis was undertaken for the five taxa to identify life history characteristics which may explain their association with the MDAC feature. The modalities exhibited by the taxa for biological traits relevant to substrate preference are presented in Table 10. All five taxa are sessile organisms, living either directly attached to the substratum or within a tube, and exhibiting pen-shaped or complex (bush/tree-like) morphologies. Each of these taxa obtains their nutrition through suspension-feeding. These characteristics are typical of fauna that inhabit hard substrates and reflect the constraints exerted on organisms by this habitat.

Table 10. The biological trait modalities of MDAC-associated taxa.

Taxon	Mobility	Living habit	Morphology	Feeding type
Cellaria	Sessile	Attached	Erect & complex	Suspension
Alcyonium digitatum	Sessile	Attached	Erect & complex	Suspension
Tubularia	Sessile	Attached	Erect & pen-shaped	Suspension
Sabellidae	Sessile	Tube-dwelling	Erect & pen-shaped	Suspension
Nemertesia	Sessile	Attached	Erect & complex	Suspension

None of the taxa recorded in the survey were found to be highly sensitive to smothering. However, 61 of the 91 recorded taxa (67%), including all those associated with MDAC (Table 10), were sessile organisms and are therefore vulnerable to severe sedimentation.

Alcyonium digitatum was identified as being highly sensitive to physical disturbance due to its high fragility, low flexibility, immobility, large body size (100-200mm) and long lifespan (>10 years). Various other taxa, including other MDAC-associated taxa, exhibited some trait modalities that cause sensitivity to disturbance.

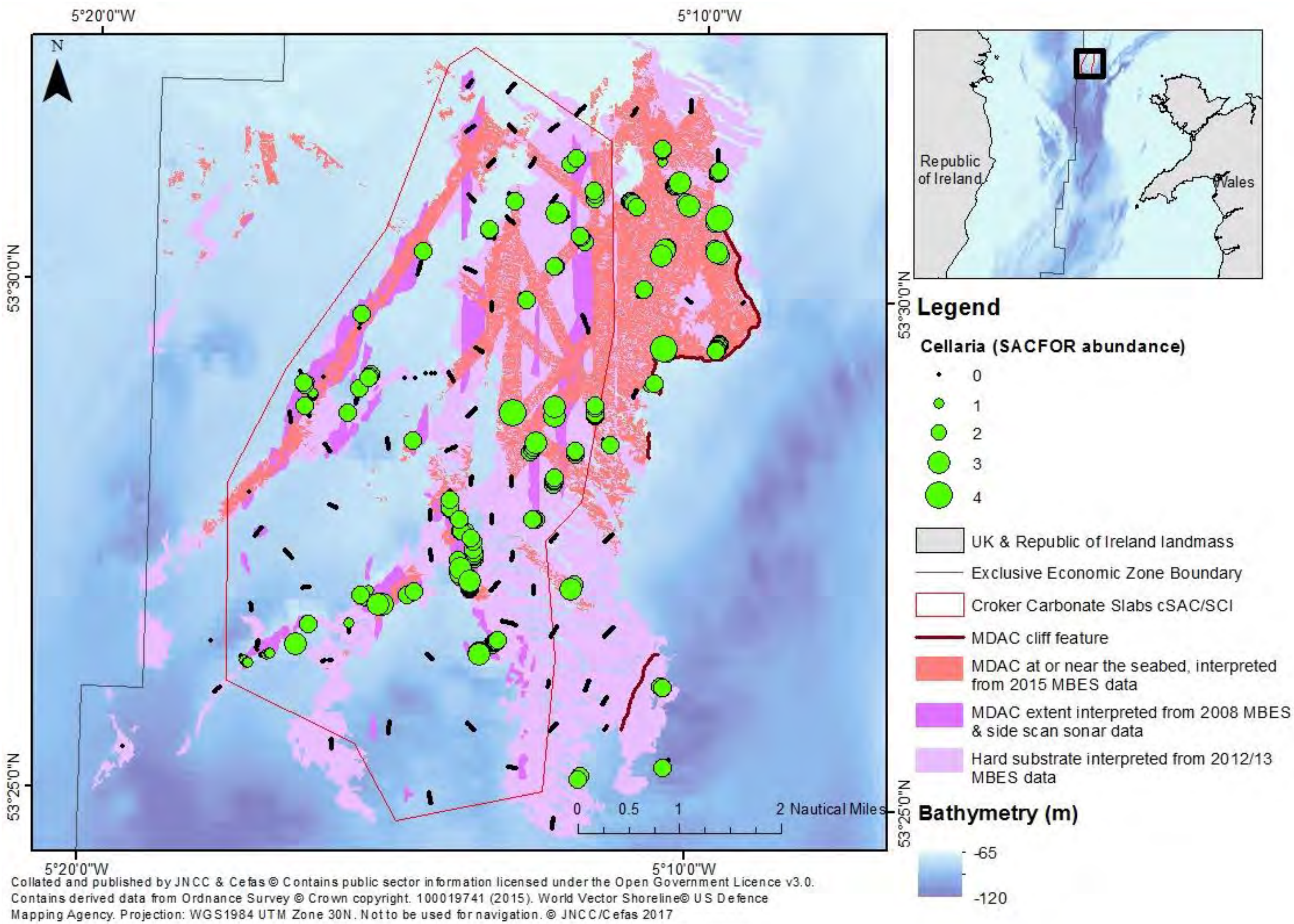

Figure 37. SACFOR abundance of the bryozoan genus *Cellaria*, as interpreted from still images. Bubble size increases with abundance.

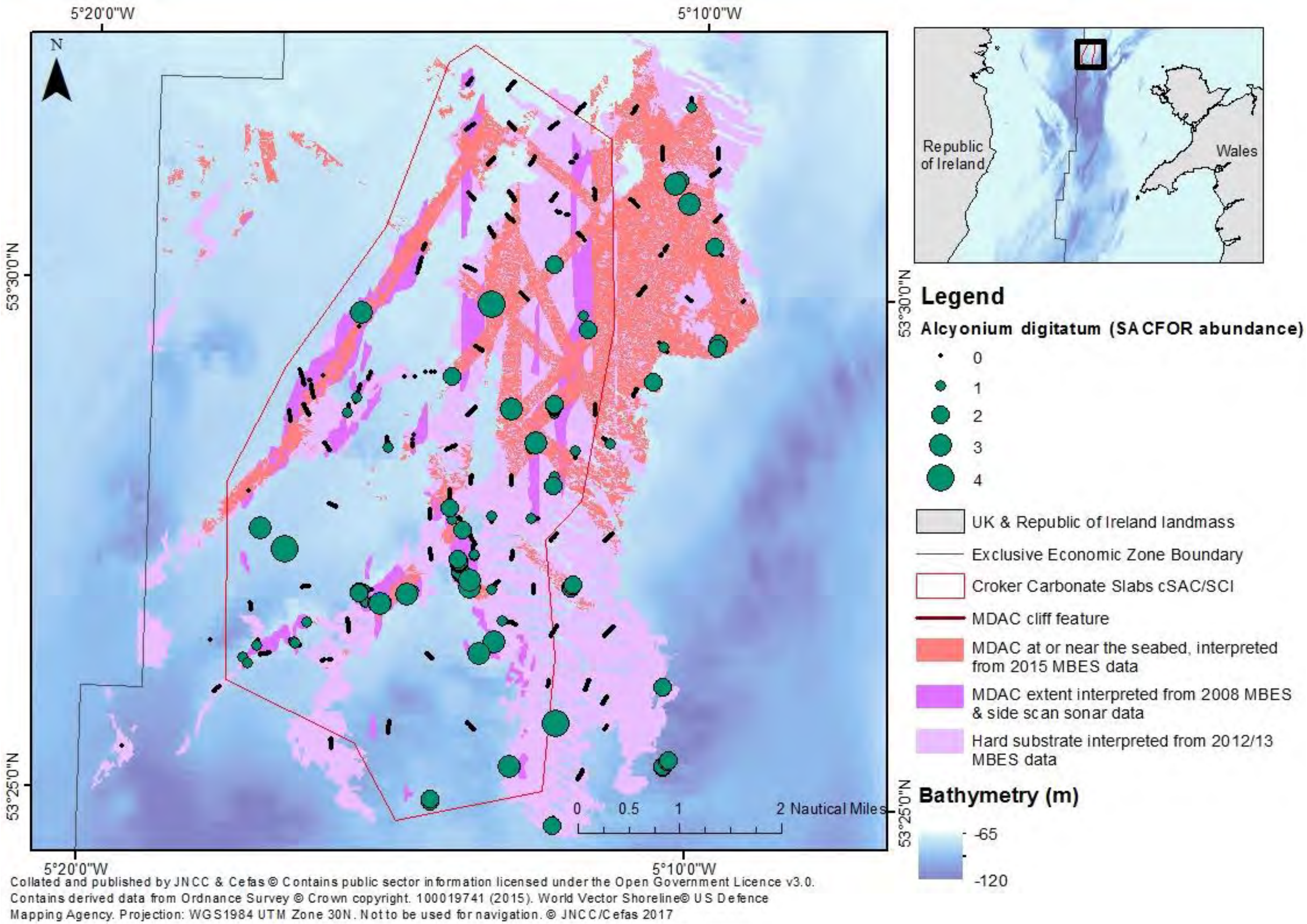

Figure 38. SACFOR abundance of the soft coral *Alcyonium digitatum*, as interpreted from still images. Bubble size increases with abundance.

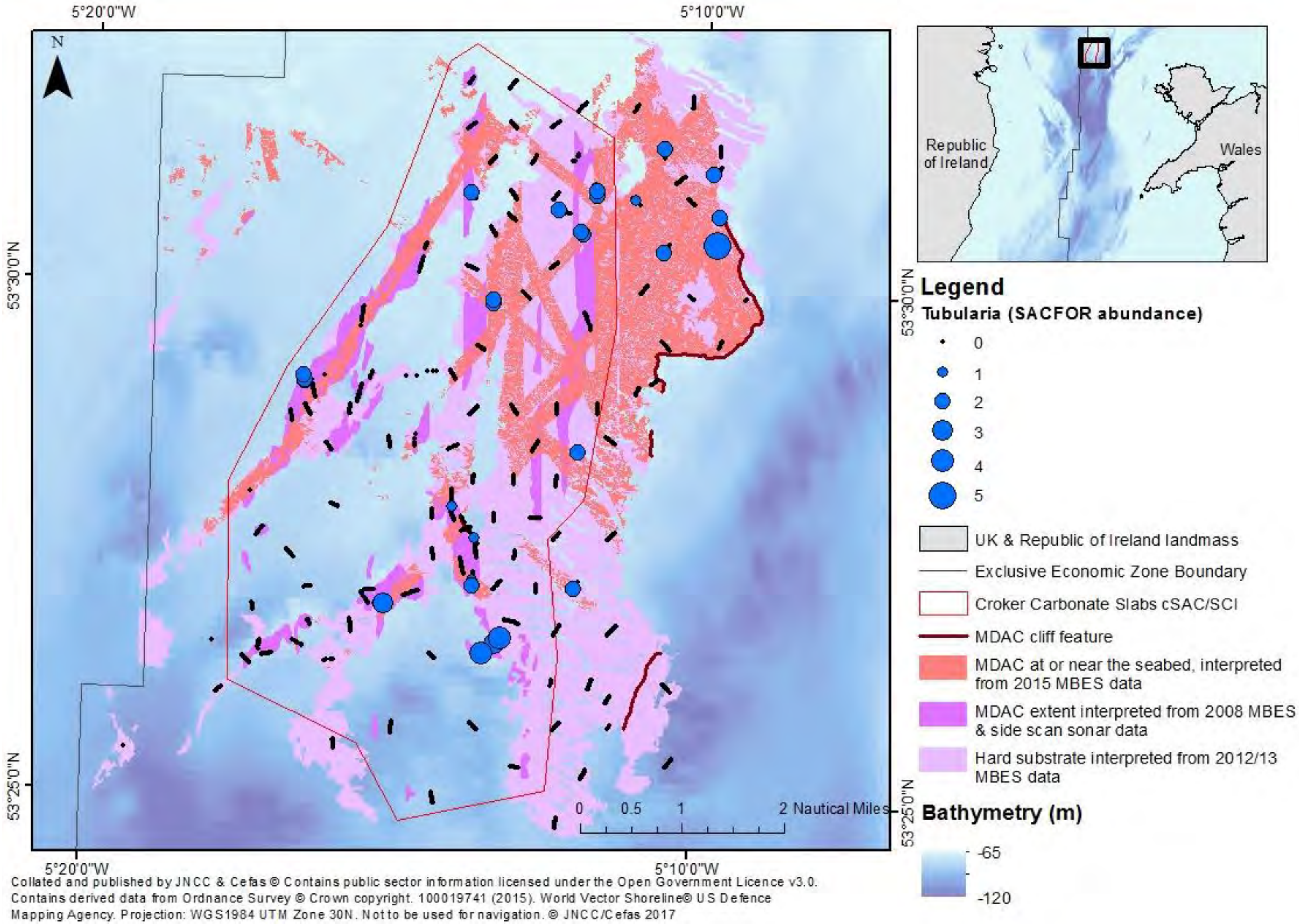

Figure 39. SACFOR abundance of the hydroid genus *Tubularia*, as interpreted from still images. Bubble size increases with abundance.

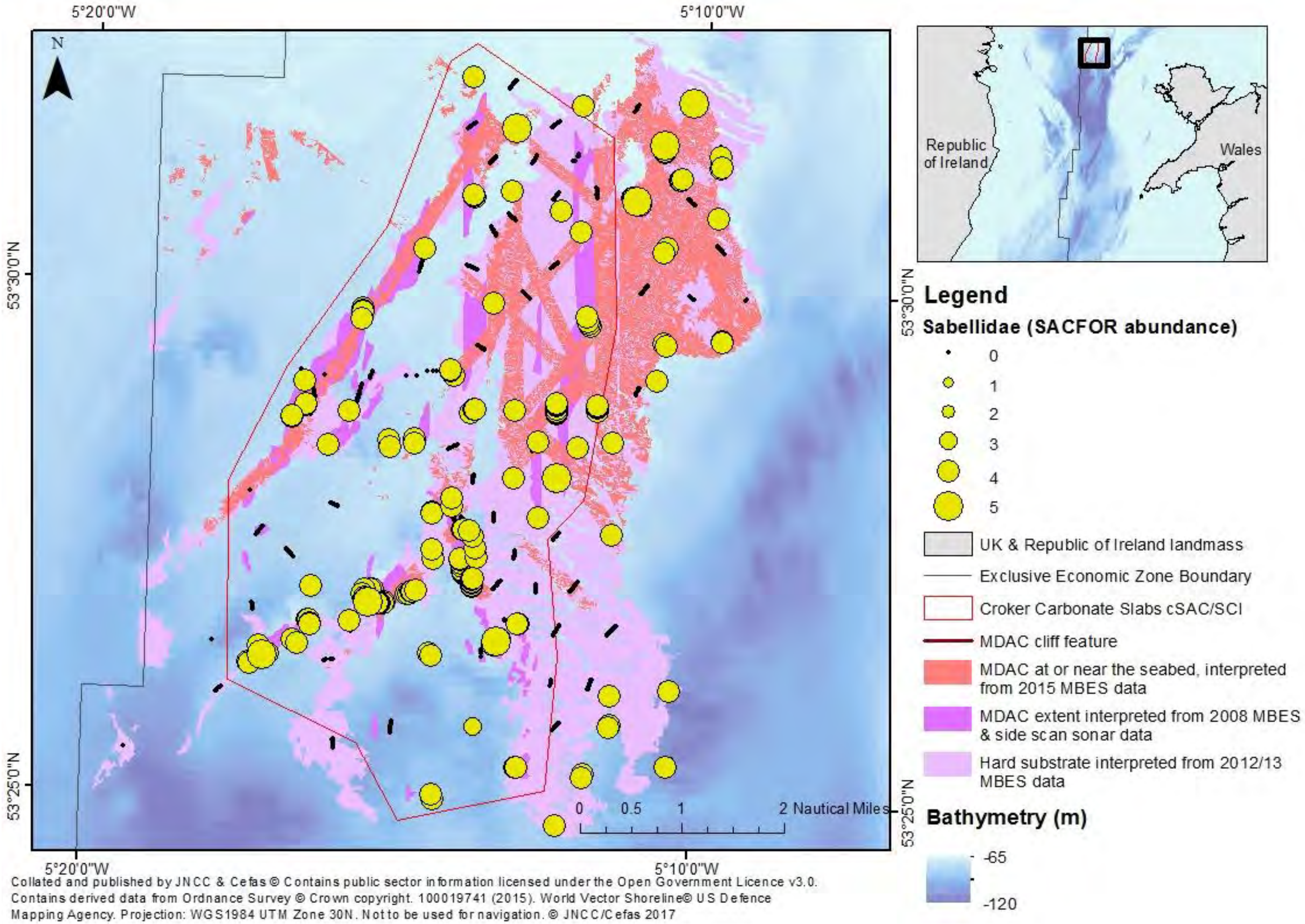

Figure 40. SACFOR abundance of the polychaete family Sabellidae, as interpreted from still images. Bubble size increases with abundance.

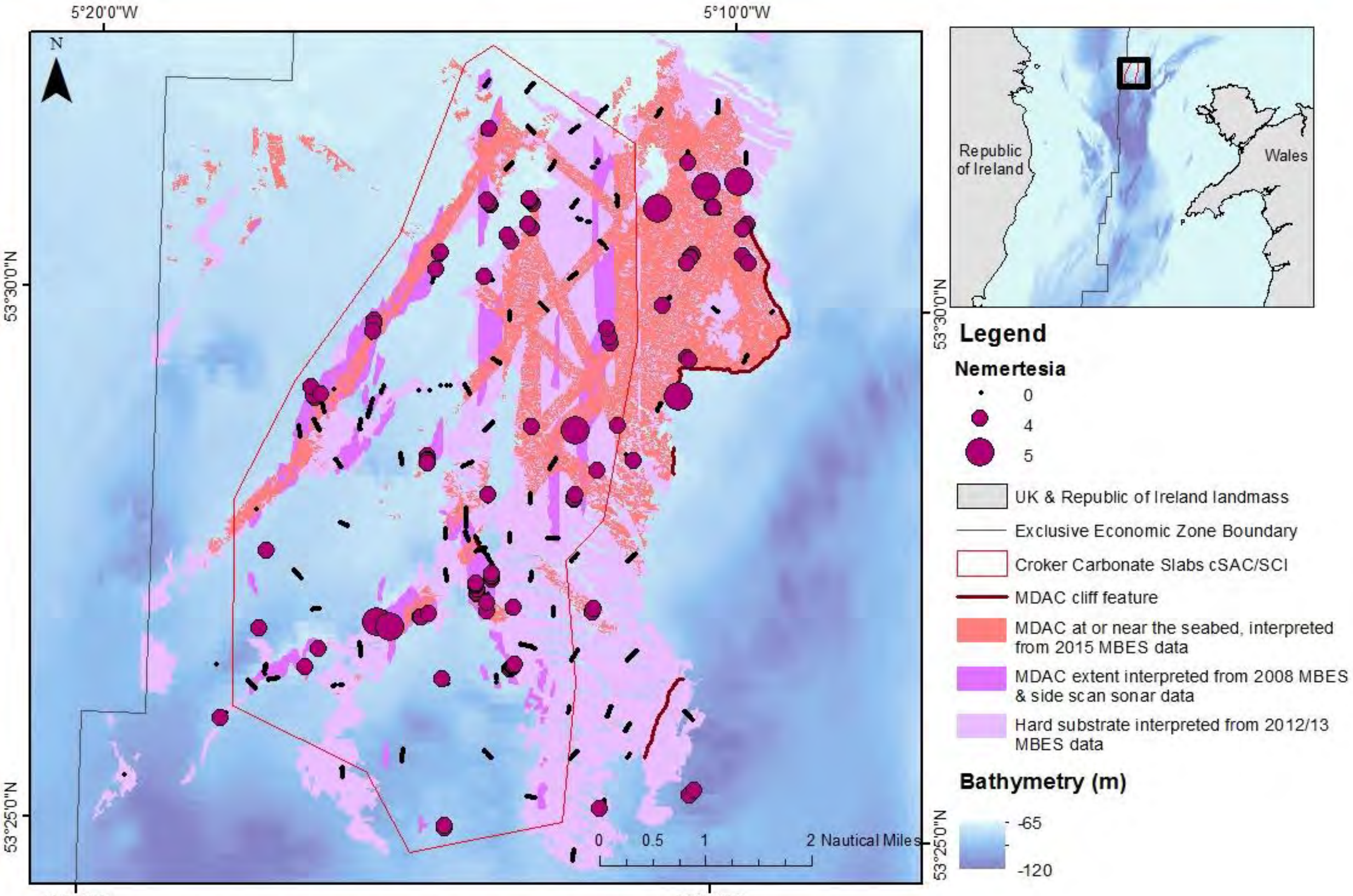

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Figure 41. SACFOR abundance of the hydroid genus *Nemertesia*, as interpreted from still images. Bubbles size increases with abundance.

4.5 Other monitoring requirements

There were no records of the non-indigenous species (NIS) listed under MSFD Descriptor 2 (Stebbing *et al* 2014) in the untruncated infaunal or epifaunal datasets.

Three items of litter listed under MSFD Descriptor 2 (see Annex 7) were observed on the seafloor; an adjustable spanner (B8 = Metals: other), and two ropes in separate locations. The material composition of the ropes is unclear; therefore, they could belong to either category A7 (Plastic: synthetic rope) or F3 (Miscellaneous: rope).

5 Discussion

5.1 Monitoring the physical structure and extent of MDAC

The Croker Carbonate Slabs cSAC/SCI represents an extremely valuable resource for both the EC Natura 2000 and UK MPA networks, providing an extensive area of the 'bubbling reefs' subtype of the Annex I 'Submarine structures made by leaking gases' feature. The Croker Carbonate Slabs are located in an area where continuous hard substrate does not otherwise occur, thus providing localised areas of relatively high biodiversity on the seabed (Jensen *et al* 1992). In the context of the UK Natura 2000 network, the Croker Carbonate Slabs are the sole example of the 'bubbling reefs' subtype of 'Submarine structures made by leaking gases' (see Figure 1). In addition, they are currently the only example of the Annex I feature in the UK MPA network generally considered to be in favourable condition. The data acquired for the initial monitoring event in 2015 (CEND 23/15 survey) have substantially improved on the prior understanding of the spatial extent and physical structure of the feature, both within and adjacent to the site, and form a robust first data point against which to assess whether favourable condition has been maintained in the future.

The predicted extent of MDAC exposed at or subcropping just below the seabed has increased significantly inside the cSAC/SCI and adjacent to it, following interpretation of new acoustic and groundtruthing data. Where the acoustic footprints overlapped, there was generally a high level of correspondence between the areas which had previously been delineated as MDAC from 2008 data, and those where MDAC had been modelled at or just below the seabed from 2015 data (Figure 8). For those areas where high-resolution data had not been interpreted for MDAC extent (e.g. within areas of 'hard substrate' delineated from 2012/13 data), it is now evident that the extent is far larger than had previously been estimated from the 2008 survey data. This is particularly apparent in the modelling and verification (through imagery and carbon isotope analysis) of a large area of MDAC to the north east of the site, which extends to almost 1.4 nautical miles beyond the site boundary, and culminates in a distinctive 'cliff' feature running alongside a channel. This verified area corresponds to the north east region of the 'hard substrate' delineated from 2012/13 data by Callaway *et al* (2015), which was predicted to comprise MDAC. It should, however, be noted that there are discrepancies between the 2015 model and the Callaway *et al* model. Following generation of the 2015 map, and examination of the seabed images, it is thought that MDAC may not occur at or just below the seabed in the south eastern area of 'hard substrate' delineated by Callaway *et al* (2015). Seabed imagery shows exposures of a blue-green clay inbetween mobile sandwaves in this south eastern area, and it is possible that the MBES data acquired in 2012/13 showed a similar signature for both clay and areas of MDAC. The continuation in the south east of the cliff feature identified from 2015 data does, however, indicate that MDAC may exist within this area, perhaps buried deeply under a more substantial veneer of mobile sediment and/or clay (Figure 8).

The outcropping and pavement MDAC sub-types occurred throughout the survey area, both within and outside of the cSAC/SCI. The physical characteristics of these two types of MDAC correspond to those described by Whomersley *et al* (2010) as 'high relief' and 'low relief' from the 2008 data. In both cases, the MDAC observations from seabed imagery almost consistently corresponded to areas where MDAC had been interpreted from 2008 and/or 2015 acoustic data. Low-lying pavement MDAC was frequently observed throughout the site, with the exception of the south eastern area, and was often interspersed and mosaiced with sediments. Outcropping MDAC features were less common, particularly in the north of the survey area, and were mainly confined to the central area of the site, and the north east and south west outside of the site boundary. Outcropping MDAC was recorded in several places at the edge of the newly interpreted north east 'cliff' feature (Figure 32) and also observed on the boomer profile SBP008 (see Annex 5). The delineation of this discontinuous 'cliff' substantially increases the known extent of this form of MDAC within the region, which was previously observed from features south of the site centre and south east of the site boundary (Judd 2005; Whomersley *et al* 2010).

The observed bedforms, features and substrates within the site indicated a seabed of considerable complexity, providing evidence of the moderate to high energy hydrodynamic regime. The water column was often extremely turbid with suspended sediment, mobile sandwaves and areas of sediment scour were observed from acoustic and groundtruthing data and low-lying pavement features were observed partially buried or inundated by veneers of surficial sediment. The mobility of surficial sediments within the area is likely to affect the MDAC feature in terms of; a) burial of hard surfaces by sediment, which may make it difficult to detect, and b) erosion of MDAC features into smaller fragments (as observed by Whomersley *et al* 2010). It is therefore expected that the exposed extent of MDAC is in a state of continual flux, with thin shifting surficial sediment veneers uncovering and burying MDAC across the cSAC/SCI at various temporal and spatial scales. It is thought likely that the hard MDAC substrate will persist beneath these veneers, unless erosion has been substantial, thus retaining the potential for exposure (and colonisation by fauna) at a future point. These findings strengthen the case for using the 2008 and 2015 extent maps in combination to investigate extent, with the 2012/13 interpreted 'hard substrate' to be used with awareness that the entirety of the extent delineated may not comprise MDAC at or just below the seabed. It is possible that deeply buried areas which are not currently delineated as MDAC at or just below the seabed (e.g. the possible MDAC cliff in the south east of the survey area) could become exposed if a significant hydrodynamic event or regime change occurs in the future.

Tidally-driven currents are thought to be the main causative factor in the erosion of MDAC, although it has also been postulated that methane gas escape may also have played a role in facilitating seabed erosion (Judd 2005; Judd *et al* 2007). The potential for erosion of MDAC features, and the rate at which this is likely to occur, are not well understood, and further research on sediment dynamics and MDAC erosion would be required to better understand this process. MDAC is a relatively hard substrate (in comparison to chalk, for instance) and it is thought likely that large outcropping MDAC features will erode gradually. This theory is supported by the persistence of the original 'cliff' feature observed in 2004 and 2008 (Judd 2005; Whomersley *et al* 2010), and outcropping and pavement forms in areas which were delineated as MDAC from the 2008 data. Given the natural processes occurring within the site, particularly with respect to sediment mobility, and the inherent difficulties in mapping the extent of MDAC acoustic signatures in the context of a highly mixed and complex seabed, the extent of MDAC should be used with caution as an indicator of feature condition. A reduction or apparent reduction in the extent of MDAC will not necessarily indicate a decline in condition, particularly in the case of pavement MDAC, which may have been covered by mobile sediments. A change from outcropping to pavement MDAC should also be interpreted with care; the two types are often interspersed and grade from one form to the other, and it can be difficult to return to exact locations with imaging equipment.

A wide range of multidisciplinary data sources (singlebeam echosounder, side scan sonar, sub-bottom boomer, METS sensor, water samples and seabed imagery) combine to provide a strong evidence base against which to conclude that seepage of methane is ongoing within and adjacent to the Croker Carbonate Slabs cSAC/SCI (Figure 14). Singlebeam echosounder and side scan sonar data revealed apparent streams of gas bubbles emanating from the seabed, which were also observed from imagery data, along with possible bacterial mats and black sulphidic patches. Interpretation of the boomer data indicated areas of sub-bottom acoustic turbidity and gas brightening which correlated with other evidence of gas seepage, and the vast majority of seawater methane concentrations were considered to be notably high (van Landeghem *et al* 2015). The evidence of present-day gas seepage suggests that MDAC formation continues today, and there is no reason to suppose that MDAC formation has not been continuous since the Last Glacial Maximum, given that the samples selected for strontium isotope analysis were determined to post-date this period. The rate at which MDAC is expected to form is unknown, although the U/Th dating analysis suggests that the formation of the Croker Carbonate Slabs has taken place over an extended period of time (between about 17,000 and 4,000 years before present). Strontium isotope ratios indicate that formation occurred in porewater sourced from seawater similar in composition to that of the present day.

As MDAC is likely to still be forming there may be potential for generation, regeneration and improvement of feature condition, which could counteract the natural erosion of the exposed MDAC. It is expected, however, that this regeneration would occur on a geological timescale, and would therefore be highly unlikely to mitigate any damage caused to the MDAC features by non-natural disturbances, such as abrasion by demersal trawling. The features are self-protecting to some extent, as demersal fishers are likely to avoid trawling over rocky substrate to avoid gear damage. The proposed management measures would also reduce levels of demersal abrasion by mobile fishing gears to the lowest possible level within the Croker Carbonate Slabs cSAC/SCI and the substantial newly verified areas of MDAC to the east and north east of the site (including the extensive ‘cliff’ features). Therefore, this pressure is thought unlikely be of concern following implementation of management.

5.2 Monitoring the typical species, diversity, structure of communities associated with MDAC

The seabed imagery and grab samples showed a highly complex seabed, with areas of outcropping and pavement MDAC interspersed with sedimentary, biogenic and soft rock habitats. The highly mixed and mosaiced nature of the seabed introduced a high degree of uncertainty into the habitat classification in terms of particle size composition, and whether sediment-covered hard substrates constituted MDAC. The substrates were, however, broadly classified as; EUNIS A5.1 ‘Sublittoral coarse sediments’, A5.2 ‘Sublittoral sand’, A5.4 ‘Sublittoral mixed sediment’, A5.6 ‘Sublittoral biogenic reef’, A4.23 ‘Communities on circalittoral soft rock’ (clay), outcropping MDAC and pavement MDAC.

Multivariate analysis indicated that the epifaunal communities associated with outcropping and pavement MDAC were significantly different from those of all other habitat classes, with the exception of A5.1 ‘Sublittoral coarse sediment’. Investigation of the top-ranking taxa contributing to similarity within habitat classes revealed that four taxa occurred within the top seven for each habitat (including outcropping and pavement MDAC), with the exception of A5.2 ‘Sublittoral sand’, which was dominated by three of these taxa. These dominant taxa could only be resolved to class (Hydrozoa clumps/solitary, Hydrozoa turf, Polychaeta) or family (Serpulidae), possibly reflecting the variable quality of the images (even within the highest image quality category), or the inherent challenges in identifying faunal turfs and tube worms reliably from imagery alone. We can only speculate whether the species present within these low-resolution taxa are consistent between habitat classes, but it appears that despite the significant differences between MDAC and the other habitat classes observed in analysis, the epifaunal assemblage composition of the MDAC classes show many similarities to other habitat classes within the survey area. This overlap of taxa is thought to be caused by a combination of classification artefacts, adaptability of the taxa and habitat heterogeneity within individual images. It is probable that some areas of MDAC buried by a thin surficial sediment veneer (and being colonised by typical sessile taxa) have been classified as a sediment habitat, and that many of the same taxa which colonise MDAC also attach to larger coarse fractions of the sediment, where hydrodynamic conditions allow. Either of these theories could explain the lack of significant difference in the epifaunal assemblage composition of MDAC classes and A5.1 ‘Sublittoral coarse sediment’.

Further investigation of the similarity ranks revealed five epifaunal taxa which were substantially more abundant on exposed MDAC and occurred in a far higher number of still images classified as MDAC, as opposed to other habitat classes where MDAC was absent. These were the soft coral *Alcyonium digitatum*, the hydroid genera *Nemertesia* and *Tubularia*, the bryozoan genus *Cellaria* and the polychaete family Sabellidae. These taxa also consistently made a substantial contribution to dissimilarity with other habitat classes, providing further evidence that these taxa are primarily associated with MDAC within and adjacent to the Croker Carbonate Slabs cSAC/SCI. The conservation objective for the site states that typical species representative of 'Submarine structures made by leaking gases' in the Irish Sea must be maintained/restored, in addition to their biodiversity and community structure (JNCC 2015). The five taxa were found to exhibit similar life history traits (Table 10), which are common among hard substrate-inhabiting invertebrates (Craig & Jones 1966, Sebens 1985), suggesting that they could possibly be considered typical of the MDAC within the survey area, although they are not necessarily restricted to MDAC and may be characteristic of other rocky substrates under similar environmental conditions. All five of the taxa were also noted in association with MDAC features from the 2008 survey data (Whomersley *et al* 2010) and *Tubularia, Nemertesia* and *Alcyonium digitatum* were also recorded at the nearby Codling Fault Zone SAC (NPWS 2015), in Republic of Ireland territorial waters. In the absence of perturbations to the ecosystem, future monitoring will establish whether this specific group of taxa do indeed constitute a representative MDAC epifaunal assemblage within the Croker Carbonate Slabs cSAC/SCI, or whether other taxa, possibly exhibiting similar traits to those highlighted here, are also associated with healthy MDAC reefs as opposed to surrounding coarse sediments. Such information will be important in determining whether the conservation objective of maintaining or restoring representative MDAC communities is consistently met in the future.

Multivariate analysis showed that there was no significant difference between the overall epifaunal assemblages of outcropping and pavement MDAC, and both showed associations with the five taxa discussed above. However, there were substantial disparities between the two topographical classes in terms of abundance and frequency of the taxa in still images. Outcropping MDAC showed consistently higher mean SACFOR abundances of all five taxa, with the difference being particularly pronounced for *Alcyonium digitatum*, and Sabellidae showing the lowest disparity in abundance. Each of the taxa occurred in a higher frequency of still images in the outcropping class, as opposed to pavement MDAC; *Alcyonium digitatum* and *Tubularia* occurrences were particularly disproportionate, whilst frequency of Sabellidae was comparable between outcropping and pavement forms of the feature. Univariate analysis was limited for some taxa due to the large number of zeros in the dataset, but it was possible to carry out comparisons of *Alcyonium digitatum, Cellaria* and Sabellidae SACFOR abundance between outcropping and pavement MDAC classes of varying percentage cover (per still image). The results of these analyses indicated that the disparity in *Alcyonium digitatum* abundance between outcropping and pavement forms remained constant regardless of MDAC percentage cover, whilst the difference in *Cellaria* abundance between the two MDAC forms appeared to be partially due to varying MDAC percentage cover, rather than linked to structural differences between outcropping and pavement MDAC. This implies that *Cellaria* can readily colonise pavement MDAC, but is less abundant where MDAC cover is <30%, possibly due to increased abrasion or smothering by mobile sediments, or competition for space.

Whilst *Alcyonium digitatum*, *Tubularia*, *Nemertesia*, *Cellaria* and Sabellidae were found to be associated with both outcropping and pavement MDAC, the colonisation rate and extent to which the two different forms sustain these taxa are clearly affected by environmental factors additional to the provision of hard substrate. The relatively high occurrence and abundance of these taxa on outcropping MDAC could be due to the greater elevation provided above the seabed, which could in turn protect these sessile animals from smothering or scouring by mobile sands close to the seabed. It is possible that currents are accelerated and strengthened when obstructed by outcropping MDAC. Subtle localised changes to the hydrodynamic regime may provide more food resources for these suspension-feeding taxa, and greater potential for colonisation by planktonic invertebrate stages (e.g. Eckman 1983), which could be more readily retained by the increased topographical complexity of the outcropping MDAC. This postulation would appear to be supported by the anecdotal observation of increased *Alcyonium digitatum* abundance at the edges of outcropping MDAC concretions. It appears that considering pavement and outcropping MDAC separately during monitoring will provide greater insights into the drivers of change within the cSAC/SCI; the former reflecting natural environmental fluctuations relating to sediment dynamics and the latter, being partially sheltered from such variability, possibly acting as a better indicator of other perturbations. That said, none of the taxa recorded in the survey were identified as being particularly sensitive to smothering, which implies that the fauna on the outcropping MDAC is likely to experience at least some sedimentation.

The maintain/restore conservation objective implies that the 'Submarine structures made by leaking gases' feature is generally considered to be in favourable condition within the Croker Carbonate Slabs cSAC/SCI (see Section 2); an assessment which reflects the relatively unimpacted state of the site due to the low levels of anthropogenic activity that have been observed from VMS data. In the absence of substantial human activity which causes a response in a single indicator metric, it is proposed that the entire epifaunal community should be quantified in future monitoring events, to verify the associations observed, with particular attention to the occurrence, abundance and distribution of the MDAC-associated species *Alcyonium digitatum*, *Nemertesia, Tubularia*, *Cellaria* and Sabellidae across the site. All these taxa appear to have different combinations of attributes which should be taken into account at the next monitoring event (see Table 11). In particular, *Alcyonium digitatum* and *Tubularia* can be used to assess whether typical occurrence rates and abundances have been maintained on outcropping MDAC compared to pavement MDAC; although *Tubularia* was observed to be almost exclusively associated with outcropping MDAC, and was therefore limited in its extent within the survey area. *Alcyonium digitatum* particularly shows potential for investigation due to its large body size, conspicuous shape and colour range. Given the turbid conditions typically encountered at the site this species could prove particularly valuable as a monitoring tool, as it can often be identified from sediment-obscured images and poor quality videos. This is particularly relevant for outcropping MDAC, as it is often necessary to employ avoidance tactics to prevent damage to the camera equipment.

Table 11. Characteristics of key taxa to consider for future monitoring of MDAC features, based on 2015 data.

Taxon	Visible in very poor quality imagery	More abundant and frequent on outcropping MDAC than pavement MDAC?*	Widely distributed across the site?
Alcyonium digitatum	✓	✓	✓
Nemertesia	×	?	✓
Tubularia	×	✓	×
Cellaria	×	×	✓
Sabellidae	×	×	✓

* based on statistical analysis and frequency graphs (Section 4.4.4).

Although little anthropogenic activity currently occurs within the Croker cSAC/SCI, future monitoring events could be required to detect the ecological effects of any future activities that may occur in association with the feature. *Alcyonium digitatum* was identified as being particularly sensitive to physical disturbance based on the suite of traits it exhibits. A decline in the abundance of this species could therefore be used as an early indicator of such perturbations to the system, should the onset of an activity that disturbs the seabed coincide with a reduction in its population density. The feasibility of using *Alcyonium digitatum* as an indicator species in this regard is verified by studies which have found reductions in this species to be one of the major differences that distinguish communities in bottom-fished areas of the seabed from unimpacted reference areas (e.g. Kaiser *et al* 2000; Cook *et al* 2013).

Despite an extensive infaunal grab survey, and the analysis of several meiofaunal samples, no chemotrophic organisms were found which were thought to be directly associated with ongoing methane seepage, and none were observed from seabed imagery with the exception of white patches thought to be the thiotrophic bacterium *Beggiatoa spp.*, observed on seabed images. Similarly, O'Reilly *et al* (2014) found no seep-specialist macrofauna at the Codling Fault Zone in the western Irish Sea. They noted that shallow water (0-200m) seep assemblages are less likely to contain seep-specialised taxa than deep water sites, and instead are expected to be out-competed by 'background' fauna typical to the region (Levin *et al* 2000; Rathburn *et al* 2000; Sahling *et al* 2003; Dando 2010), likely due to the dominant influence of photosynthetic carbon in shallow depths (Levin 2005). Dando *et al* (1994b) reported seep-specialist macrofauna (the pogonophore *Siboglinum poseidoni* and the bivalve *Thyasira sarsi*) near a seep in the Skagerrak, however, these were restricted to a very limited zone (measured in centimetres rather than metres) in the immediate vicinity of the seep. Dando *et al* (1994a) also found 'major differences' in nematode populations close to, and away from nearshore Kattegat seeps.

Given the lack of evidence for seep-specialist macrofauna or meiofauna in sediments which can be directly related to the presence of MDAC or gas seepage at this site, the value of grab sampling is limited and is unlikely to reflect the achievement of the site conservation objective or enable future assessments of MDAC condition. Targetted sampling of bacterial mats or seep-associated macrofauna or meiofauna could be conducted using a Remotely Operated Vehicle (ROV) or a camera-guided grab, to sample directly at a seep location. However, the benefits of acquiring such data must be weighed against the significant operational costs involved in the use of such equipment. Given the weight of evidence already available that methane seepage is ongoing within the site (and has continued since the Last Glacial Maximum), it is likely that the value of such data for assessment of the features against the site conservation objectives is minimal.

5.3 Other monitoring requirements

No non-indigenous species (NIS) were recorded in the macrofaunal and/or epifaunal datasets. Two occurrences of rope, and a spanner were observed, possibly relating to fishing or other shipping activities within the site.

6 Recommendations for future monitoring

The following recommendations are made for future condition monitoring of the Annex I habitat 'Submarine structures made by leaking gases' at the Croker Carbonate Slabs cSAC/SCI. It should be noted that these recommendations are site-specific and do not necessarily apply to similar features elsewhere.

6.1 Operational and survey design recommendations

- Anthropogenic pressures, with the potential to impact the condition of the MDAC features and associated biological communities (e.g. demersal trawling), should be monitored on a regular basis, in the form of analysing up-to-date pressures and activities maps. It is recommended that this is conducted at least once a year. This temporal scale is considered appropriate as the level of pressure within the site is historically low, due to avoidance of hard substrates by demersal fishers.

- The MDAC features should be directly monitored at an appropriate temporal scale, using a risk-based approach related to the monitoring of pressures that may affect the feature. The proposed management measures reduce the potential impact of demersal abrasion by towed fishing gear to the lowest practicable level. Frequent monitoring is therefore unlikely to be required, unless a new pressure to which the features are vulnerable is identified within the site. It is beyond the scope of this report to recommend an appropriate temporal interval for direct monitoring, as a risk-based approach will be used to determine a suitable frequency in the context of the wider UK MPA network (Kröger & Johnston 2016).

- Grab sampling for macrofauna and meiofauna will not be required for future monitoring of the MDAC feature at this site. Analysis of infaunal and PSA data allowed a more detailed characterisation of the wider sediments and assemblages within the site. However, no insights were gained from these data which would allow future assessment of the condition of the MDAC feature. To directly sample methane-associated meiofauna it would be necessary to take a sample directly at a seep location, an action which is impractical with a grab sampler. It may, however, be beneficial to acquire PSA grab samples at a series of fixed points to investigate changes in sediment composition or movement of veneers over time.

- Targeted sampling of bacterial mats or seep-associated meiofauna could be achieved using a Remotely Operated Vehicle (ROV) if such data are required for further investigation.

- It is recommended that the groundtruthing elements of future surveys of this site focus on acquisition of epifaunal data via video and still imagery, which will directly relate to the MDAC features themselves, as opposed to surrounding sediments (unless such data are required to achieve future monitoring objectives).

- Sampling effort directed at the acquisition of seabed imagery should be stratified between outcropping and pavement MDAC, to further investigate differences in epifaunal assemblage composition between the different forms of the feature. Sampling locations should be selected using interpreted video data from the 2008 and 2015 surveys, in the absence of a sufficiently resolute and accurate map of the outcropping and pavement forms of the MDAC feature.

- High definition (HD) camera technology should be utilised to increase opportunities for extraction of high resolution quantitative epifaunal data from video imagery. If HD imagery is used for future surveys, the improvement in image quality must be considered when comparing epifaunal assemblages to the current dataset.

- Autonomous underwater vehicles (AUV) provide an opportunity for simultaneous acquisition of imagery and bathymetry data, allowing photomosaics to be produced and linked to seabed features and topography. This technology could offer an efficient and accurate means of monitoring change in specific targeted areas of MDAC over time, if future budgets allow.

- Sub-bottom profilers such as chirp systems have been used effectively by JNCC and Cefas in the western Irish Sea, to investigate the depth of sediment veneers overlying rocky reef within the Pisces Reef Complex cSAC/SCI (Jenkins & Nelson 2017). Future monitoring events may benefit from the acquisition of chirp data to investigate sediment dynamics and MDAC burial and exposure over time. This technique could also provide clarity on the distribution of MDAC in the south east of the survey area.

- Repeated MBES data acquisition at fixed locations (in combination with groundtruthing) would improve understanding of sediment dynamics, and the erosion, exposure and burial of MDAC over time. Areas of particular interest would include; areas consistently identified as MDAC over time, the sandwave area to the south east, and the newly delineated ‘cliff’ feature to the north west to investigate the rate of erosion by the strong channel currents. MBES data acquired should be of sufficient resolution to monitor the migration of sandwaves, erosion of the MDAC cliff face, and modification of other bed forms.

- Future monitoring events should consider *in-situ* environmental monitoring, including tidal current meters and sediment traps, to improve understanding of hydrodynamics, erosion, sediment loads and deposition rates, and the associated impact on biological communities This information would allow a greater understanding of the amount of change which can be attributed to natural variability.

6.2 Analysis and interpretation recommendations

- Analysis of still and video imagery from future monitoring surveys should assign MDAC observations according to the topographical categories (outcropping and pavement) and confidence categories (high, moderate and low) detailed in this report, to enable accurate comparison of MDAC distribution and biological communities.

- Extent should be monitored, but it should be used with caution as an indicator of condition. An apparent reduction in MDAC extent does not necessarily indicate a decline in feature condition, as previously observed areas of sediment could have been covered by mobile sediment. An apparent change from outcropping to pavement MDAC should also be treated with caution, as it is difficult to revisit exact locations.

- The five MDAC-associated taxa are also known to associate with hard stable substrata such as rocky reefs, as opposed to being particularly associated with MDAC per se, and no seep-specialist biota were observed. Therefore, it is not recommended that a new biotope or habitat classification be created for MDAC features. MDAC within this site should be assigned to the A4.2 ‘Moderate energy circalittoral rock’ class.

- The entire epifaunal community should be identified, recorded and analysed, with particular attention to the occurrence, abundance and distribution of *Alcyonium digitatum*, *Nemertesia, Tubularia, Cellaria* and Sabellidae. Future monitoring data will verify whether these taxa may be considered as 'typical species' of MDAC, and be monitored in that context against the site conservation objective.

- *Alcyonium digitatum* is a conspicuous species and can be identified with more certainty in sub-optimal visual conditions than the other four MDAC-associated taxa, meaning it can be enumerated from lower quality video data, and when turbidity is high. It is recommended that future image quality categories take the five MDAC-associated epifauna into account. Future categories could consist of those supplied in Table 12 or similar.

Table 12. Suggested image quality categories for future monitoring.

Category	Quality	Use
Zero	Seabed is not visible across 0-25% of the image.	Not to be used for any purpose
MDAC	Seabed is fully or partially visible (across at least 25% of the image), and the composition of the seabed can be reliably determined.	To determine whether MDAC is present or absent, and whether it constitutes pavement or outcropping MDAC.
Alcyonium	Seabed is fully or partially visible (across at least 50% of the image), the composition of the seabed can be reliably determined, and the quality is sufficient for identification of *Alcyonium digitatum*.	To determine whether MDAC is present or absent, whether it constitutes pavement or outcropping MDAC, and to determine SACFOR abundance and % cover of *Alcyonium digitatum.*
All taxa	Seabed is fully visible to partially visible (across at least 50% of the image), and image quality is sufficient to identify and quantify epifauna according to the SACFOR scale (e.g. Excellent or Good quality according to NMBAQC guidelines (Turner *et al* 2016).	To determine whether MDAC is present or absent, whether it constitutes pavement or outcropping MDAC, and to be used for multivariate statistical analysis of the entire epifaunal community.

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Annex 1: Supplementary feature information

Methane-derived authigenic carbonate (MDAC) comprises normal seabed sediments, of whatever type, that are bound together to form a rock-like material by a carbonate cement. The cement forms as a result of the anaerobic oxidation of methane (AOM) close beneath the seabed. The essential ingredient, methane, rises from underlying sediments or more deeply-buried rocks.

In normal seabed sediments the sulphate concentration progressively decreases with depth as a result of utilisation by sulphate-reducing bacteria (SRBs). As sulphate concentrations decline so methane concentrations increase. The interface between sulphate-rich and methane-rich sediments is referred to as the sulphate-methane transition zone (SMTZ). The oxidation of methane rising into this zone is mediated by consortia of bacteria (SRBs) and archaea (Anaerobic Methanotrophs; ANME); together these constitute the AOMs (Boetius *et al* 2000).

The anaerobic oxidation of methane is summarised by the following generalised equation:

$$CH_4 + SO_4 \rightarrow CO + HS + H_2O$$

The CO combines with Ca to precipitate as $CaCO_3$, normally in the form of the minerals aragonite, high-Mg Calcite and/or dolomite, within the pore spaces between the mineral grains of the existing sediment of whatever type (including fine and coarse grained sediments) binding it together to form MDAC. The HS is represented in the sediments by H_2S which, as a result are typically blackened and smell of rotten eggs. The H_2S may be utilised by the thiotrophic bacteria such as *Beggiatoa sp.*, which may be present on the seabed as bacterial mats (typically filamentous and white in colour).

Any methane which is not utilised by the AOMs escapes from the seabed into the overlying water where it may be seen as bubbles (for example on video or photographs), or as water column targets on acoustic systems such as echosounders and side scan sonars. Any methane not removed in the water column by microbially-mediated oxidation, eventually escapes to the atmosphere.

Although MDAC has a characteristic appearance and suite of minerals, it is only the ratio of the carbon isotopes ^{12}C and ^{13}C that definitively demonstrates that the carbon in the carbonate is derived from methane. Consequently, carbon isotope analysis is an essential tool for MDAC investigations.

MDAC has been widely reported from methane seep areas in many parts of the world in water depths ranging from inter-tidal to the carbonate compensation depth (Judd & Hovland 2007). It takes the form of chimneys, blocks and slabs. which may be exposed at the seabed when the surface sediment is removed. MDAC occurs wherever methane rises to the seabed, consequently its distribution is a function of the distribution of methane sources beneath the seabed, and the availability of migration pathways that enable it to migrate, under the influence of its buoyancy, to the seabed.

The 'Submarine structures made by leaking gases', referred to in the EC Habitats Directive, were originally described from the Danish Kattegat where tall MDAC chimneys protrude from the seabed. In UK waters MDAC is present in diverse environments including the foreshore on either side of the Firth of Forth (Andrews 1998; Judd *et al* 2002), on sandy seabed around the Machar oil field in the central North Sea (Salisbury 1990), and in several North Sea pockmarks, including the Braemar and Scanner pockmarks.

Other MDAC occurrences in the Irish Sea include: carbonate mounds on the Codling Fault, and the Kish Bank Seep Mounds in the Irish sector (O'Reilly *et al* 2014; van Landeghem *et al* 2015), and Holden's Reefs in Cardigan Bay (Judd 2005, 2007; Judd *et al* 2007). Elsewhere, MDAC has been reported from many seabed methane seepage locations world-wide, including mud volcanoes.

'Submarine structures made by leaking gases' is thus a habitat that exists due to the juxtaposition of a geological source of methane, gas migration pathways, the presence of an SMTZ in which AOMs can operate, and the availability of benthic fauna requiring a hard substrate. Such locations are also likely to contain sulphidic sediments and thiotrophic bacterial mats, however these will be strictly confined to the immediate vicinity of methane migration pathways.

Annex 2: Data processing and laboratory analysis

A2.1 Seabed imagery

A total of 133 videos and 4238 still images from 128 transects were analysed by Seastar Survey Ltd., in accordance with the methodology below.

A2.1.1 Video analysis

The video analysis of each deployment started with an initial assessment to gain a broad understanding of the substratum, flora and fauna present, as well as the identification of any different habitats / biotopes on the seabed. The analysis was carried out ‘blind’ without any prior knowledge of the site, using a personal computer and software that allowed slow-motion, freeze frame and standard play analysis. During the initial assessment video footage was viewed at 2x - 4x normal speed in order to divide the footage into segments representing different substrata. The start and end time and position of each segment were recorded. Positional data and information regarding distance for each segment were calculated using the time codes on the video overlay and related back to the navigation data using spreadsheets provided to Seastar by Cefas. Brief changes in substratum type (considered to be less than 5m distance) were treated as incidental patches and were not recorded as separate segments. More detailed analysis of the video footage was then undertaken.

Detailed video analysis consisted of a description of the seabed and the identification of flora and fauna to the lowest practical taxonomic level. The abundance data were recorded using the SACFOR scale, with counts or percentage cover of taxa also recorded wherever possible, though the low quality of some of the videos (due to e.g. poor underwater visibility, irregular camera speed, camera being too far from seabed *etc*.) meant that estimates of numbers were likely under-representative. Sediment categories were assigned based on the Folk Trigon and Wentworth scale (see Leeder 1982), with boulders and cobbles being described within ‘gravel’, and ‘rock’ referring to bedrock. Observed sediment fractions were recorded as percentages and a broadscale habitat (BSH) type was subsequently assigned to each video segment. If applicable a Habitat Features of Conservation Importance (FOCI) category was also assigned. The presence of any Annex I habitats and associated sub-features were recorded. Any other features of interest, such as trawl marks or litter, were also noted.

Particular attention was paid to any potential methane-derived authigenic carbonate (MDAC) features, with details regarding their topography (outcropping or pavement) and coverage (isolated or continuous) recorded. Due to the nature of the habitats observed (see below) all rock and boulders were recorded as potential MDAC; ‘MDAC’ should therefore be read as ‘appears similar to MDAC but has not been confirmed as MDAC.’ Additional indicators of active anaerobic oxidation of methane, including black sulphidic patches, white bacterial mats and active gas seabed seepage (indicated by bubbles rising from the seabed) were also recorded.

A list of the encountered fauna was produced for each site using species reference numbers as cited in the Marine Conservation Society Species Directory (Howson & Picton 1997) with additional reference to the World Register of Marine Species (WoRMS Editorial Board 2016) to avoid problems in species nomenclature. Video segments were designated a biotope, and, where appropriate, a secondary biotope, according to Connor *et al* (2004) and a corresponding European Nature Information System (EUNIS) habitat classification code(s). All results of the video analysis were entered into a ‘proforma’ spreadsheet provided by Cefas.

A2.1.2 Still image analysis

The still images were analysed to provide a more detailed analysis than could be extracted from the moving video image. The still photography analysis was carried out using a personal computer. All still photographs supplied were analysed. The methodology was similar to the video analysis methodology and included a general description of the habitat present. The abundance data were recorded using the SACFOR scale, though counts or percentage cover of taxa were also recorded wherever possible. The substrata were described according to the Folk Trigon and Wentworth scale (see Leeder 1982), with boulders and cobbles being described within ‘gravel’, and ‘rock’ referring to bedrock. Observed sediment fractions were recorded as percentages and a broadscale habitat (BSH) type was subsequently assigned to each video segment. If applicable a Habitat Features of Conservation Importance (FOCI) category was also assigned. The presence of any Annex I habitats was recorded. Any other features of interest, such as trawl marks or litter, were also noted. As with the video analysis, details of potential MDAC features (again, all hard substrate was classed as potential MDAC) and potential indicators of active anaerobic oxidation of methane (black sulphidic patches, white bacterial mats and active gas seabed seepage) were also recorded. A list of the encountered fauna was produced for each still image using species reference numbers as cited in the Marine Conservation Society Species Directory (Howson & Picton 1997) with additional reference to the World Register of Marine Species (WoRMS Editorial Board 2016) to avoid problems in species nomenclature. Still images were designated a biotope, and, where appropriate, a secondary biotope, according to Connor *et al* (2004) and corresponding European Nature Information System (EUNIS) habitat classification code(s). All results of the video analysis were entered into a ‘pro forma’ spreadsheet provided by Cefas.

A2.1.3 Quality control

The Quality Control (QC) process involved an ongoing element and a post-analysis element, with ongoing collaboration with other Seastar staff to check species identification, sediment classification and biotope classifications during the process of analysis. A senior member of staff also checked any uncertain identification to ensure the highest possible level of quality in the data. The post-analysis QC process involved a re-assessment of 10% of the data, checking the faunal identification, habitat / biotope classification and data entry. A portion of the QC was performed immediately following completion of 10% of the video and stills, in order to allow identification of any errors at an early stage and to inform subsequent analyses. Any discrepancies were discussed between analysts and agreed on prior to finalisation of the results.

Some discrepancies in identification of taxa were identified, particularly in those videos and stills reassessed in the ‘early’ QC, including missed taxa and miscounts resulting in changes in SACFOR abundance. These corrections were made immediately, with the analyst(s) in question given detailed feedback to ensure that further errors were not made. In addition, all data analysed to that point were reassessed in order to amend any further potential errors. The QC following completion of the work revealed far fewer discrepancies, though some inconsistencies in the way different analysts recorded certain taxa were highlighted (e.g. classifying a taxon as a different size class). These inconsistencies were discussed and recording techniques ‘normalised’ (which included some minor reassessment of some still images) prior to finalisation of the results.

In some cases, MNCR biotopes assigned varied between analysts. This was primarily due to the difficulty in identifying mud from fine sand using photographic techniques alone, resulting in some changes between coarse and mixed sediment biotopes. In addition, some broadscale habitat assignments were altered to better fit the observed sediment type. As a result, all biotope and broadscale habitat designations were reassessed by senior analysts and amended as necessary. All discrepancies were discussed between analysts and agreed on prior to finalisation of the results.

A2.2 Meiofauna

Meiofaunal processing and analysis was conducted by Physalia Ltd., according to the protocol described in the following sections. The full report (Physalia 2016) is published alongside this document.

A2.2.1 Meiofaunal sample separation

Standard laboratory protocols developed and refined by staff at Physalia over the past 30 years were used for the extraction of the meiofauna. After re-coding of the samples, the volume of sediment in each sample was measured. The samples were then homogenised gently in approximately 800ml water. Initial separation was carried out using a modified, multiple Boisseau apparatus to elutriate the microscopic organisms from the bulk of the inorganic matrix. The first ("light") and subsequent ("heavy") meiofaunal fractions were collected on 38µm mesh sieves immersed in flowing tap water (Flegg & Hooper 1970). Pooled meiofauna/silt fractions for each sample were further concentrated by a polymer density separation technique with centrifugation and the meiofauna re-collected onto 38µm mesh sieves. The density separation technique was repeated and the separation efficiencies were estimated.

A2.2.2 Nematoda sample preparation and taxonomy identification

Modified nematological techniques based on those of Bührer (1949), Baker (1953) and Cairns and Tarjan (1955) were used to process, handle and examine the remaining meiofauna (primarily Nematoda – free-living roundworms). Specimens were processed to glycerol using a modified Seinhorst method (Seinhorst 1959) in Syracuse watch glasses at 40˚C. Taxonomic identification of meiofaunal specimens was carried out on prepared microscope slides using Zeiss and Nikon Nomarski DIC (differential interference contrast) compound microscopes. For the nematodes, the first 100 specimens encountered were identified and counted. Remaining animals were then counted enabling total densities of each species in each sample to be calculated and then recorded. Throughout the taxonomic analyses, standard taxonomic texts, including Platt & Warwick (1983 & 1988), and Platt *et al* (1998) were consulted along with the in-house Physalia reference materials.

A2.3 Methane-Derived Authigenic Carbonate (MDAC) verification and dating

MDAC verification and dating analyses were conducted by the British Geological Survey (BGS). The full methodology and results are published alongside this report in Field *et al* (2016a, 2016b & 2017).

Annex 3: Macrofaunal data truncation protocol

Truncation of the macrofaunal dataset was conducted according to the protocol detailed in The Manacles Marine Conservation Zone (MCZ) Baseline Monitoring Report (Downie *et al* In Prep, Annex 1), an extract of which is presented below. Truncation steps used for this report, but additional to the protocol below are detailed in Section 3.4.

Raw taxon-by-sample matrices can often contain entries that include the same taxa recorded differently, erroneously or differentiated according to unorthodox, subjective criteria, for example:

Each row should represent a legitimate taxon to be used in analytical software packages as a unit for the calculation of diversity indices and of similarity amongst groups of samples. An artificially inflated taxon list (i.e., one that has not had spurious entries removed) risks distorting the interpretation of pattern contained within the sampled assemblage. The truncation exercise aims to identify and neutralise such entries to reduce the risk of them supporting an artificial pattern in the assemblage.

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

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

In this way, the entries identified only to genus are not assigned to a level that is unsupported by the evidence, and the resulting single entry is representative of both original entries, albeit with a slight loss of information, but a loss that will not affect the pattern in the assemblage as a whole.

Additionally, taxa are often assigned as 'juveniles' during the identification stage with little evidence for their actual reproductive natural history (with the exception of some well-studied molluscs and commercial species). Many truncation methods involve the removal of all 'juveniles'. However, a decision must be made on how to avoid the issues discussed above while retaining valuable information within the multivariate data set. The term 'juvenile' is often used to refer to individuals which do not exhibit the morphological features to resolve them to species level. In this case, these records were removed from the analysis rather than lowering the taxonomic resolution of other species level identifications. When a species level identification was labelled 'juvenile' the record was combined with the associated species level identification, when present or the 'juvenile' label removed.

Annex 4: Mapping MDAC from 2015 data

This information relates to the production of the 2015 interpretation of MDAC at or just below the seabed, displayed in Figure 6, with associated confidence assessment provided in Figure 7.

A4.1 Data and predictor variables

MBES bathymetry and backscatter data (3m resolution, projected to UTM 30N) were the primary predictors available for mapping. Higher resolution (1m) MBES data were trialled, but did not result in improved results as artefacts became more prominent and processing time was significantly increased. Secondary predictor variables were derived from MBES bathymetry (Table 13). These included the recommended measures of slope, orientation, rugosity and relative position (Lecours *et al* 2017). A de-speckled backscatter intensity layer was also created using a Lee filter with a 5 x 5 kernel.

Table 13. Description of primary and secondary predictor variables.

Predictor variable	Type	Kernel sizes	Explanation
Bathymetry	Primary	n.a.	
Backscatter	Primary	n.a.	
De-speckled backscatter	Secondary	5 x 5	High-frequency noise (speckle) has been removed using a Lee filter. Used for segmentation only.
Slope	Secondary – slope	3 x 3	Maximum slope gradient.
Roughness	Secondary – rugosity	3 x 3	Difference between minimum and maximum of cell and its 8 neighbours.
Standard deviation	Secondary – rugosity	3 x 3	Standard deviation of cell and its 8 neighbours.
Bathymetric Position Index (BPI)	Secondary – relative position	3 x 3 5 x 5 10 x 10 25 x 25	Vertical position of cell relative to neighbourhood (identifies topographic peaks and troughs).
Classified BPI	Secondary – relative position	3 x 3	BPI classified using Jenks natural breaks. Classes are ‘negative’, ‘near zero’ and ‘positive’.
Northness	Secondary – orientation	3 x 3	Direction of steepest slope, expressed as Northness (cosine of aspect).
Eastness	Secondary - orientation	3 x 3	Direction of steepest slope, expressed as Eastness (sine of aspect).

Observational data consisted of seabed still imagery and grabs, classified as MDAC or sediment. Sampling effort was focused on areas likely to contain MDAC based on the map provided by Callaway *et al* (2015) (Figure 2). Initial data exploration revealed little discriminatory power across all examined predictor variables. The most likely explanation for this observation is small-scale heterogeneity with exposed MDAC and MDAC covered by a thin sediment veneer juxtaposed at scales that are not resolvable with ship-based MBES systems. Additionally, the MBES signal might be able to penetrate thin sediment veneer, resulting in a mixed backscatter response with contributions from the sediment veneer and the underlying MDAC. This signal might be less clearly distinguishable from backscatter returned from exposed MDAC.

A standard approach utilising sediment and MDAC samples yielded unsatisfactory results due to the large overlap in acoustic signatures for the two classes. Therefore, a presence-only approach was chosen and only observations of MDAC were used in the subsequent mapping.

A4.2 Mapping method

Software eCognition v9.2.1 was used to carry out object-based image analysis (OBIA). OBIA is widely used in terrestrial remote sensing applications (Blaschke 2010; Blaschke *et al* 2014), but has also been successfully applied for mapping benthic habitats (Lucieer 2008; Lucieer & Lamarche 2011; Lucieer *et al* 2013; Diesing *et al* 2014; Hill *et al* 2014). It has several advantages over traditional pixel-based image analysis approaches, for instance: (i) partitioning an image into objects is akin to the way humans conceptually organise the landscape/seascape to comprehend it; (ii) using image objects instead of pixels as basic units is less computationally intensive; (iii) image objects exhibit useful features (e.g., shape, texture, contextual relationships with neighbouring objects) that pixels lack; (iv) image objects are easily integrated into vector GIS (Hay & Castilla 2006, 2008).

OBIA is a two-step approach consisting of segmentation and classification. The aim of the segmentation is to divide the image into meaningful objects of variable sizes, based on their spectral and spatial characteristics. The resulting objects can be characterised by various features such as layer values (mean, standard deviation, skewness *etc*.), geometry (extent, shape etc.), texture and many others. Classification is then based on combinations of these image object features.

Segmentation was carried out using the multi-resolution segmentation algorithm in eCognition. This algorithm is an optimisation procedure, which locally minimises the average heterogeneity of image objects for a given resolution of image objects. Starting from an individual pixel, it consecutively merges pixels until a certain threshold, defined by the scale parameter is reached. The scale parameter is an abstract term that determines the maximum allowable heterogeneity for the resulting image objects. The object heterogeneity, to which the scale parameter refers, is defined by the composition of the homogeneity criterion. This criterion defines the relative importance of colour (the main information from an image) versus shape of objects. If a high weighting is given to colour, the object boundaries will be determined predominantly by variations in colour of the image (e.g., backscatter strength). The shape criterion is influenced by values representing smoothness and compactness, both of which can be weighted. A high value for smoothness results in smoother boundaries of the objects, whereas a high value for compactness increases the overall compactness of image objects.

A multiresolution segmentation was carried out on de-speckled backscatter and the classified BPI3 using a scale parameter of 10, shape of 0.1 and compactness of 0.5. Subsequently, small objects of less than 5 pixels in area were merged with neighbouring objects. In this way, the number of objects was significantly reduced.

Classification in eCognition can be carried out in two different ways: In the rule-based approach, the analyst's understanding of the imagery is used to formulate systematic rules that are applied to the imagery during the classification process. In the sample-based approach, observations are used to build up class descriptions based on suitable features or fed into data-driven machine learning algorithms (such as classification trees). In this instance, a combination of rule-based and sample-based approaches was used.

Mapping of MDAC was constrained to an area north and west of the St George's Channel depression and south of a subaqueous dune field. MDAC was mapped by extracting class descriptions from MDAC observations (still images). Various features were trialled and the following uncorrelated features were used: area (size of image objects), mean bathymetric roughness, mean backscatter, mean bathymetry, standard deviation of backscatter and standard deviation of BPI3 (Figure 42). In the sample-based approach, by turning the MDAC observations and their associated feature values into membership functions it is possible to assign a membership value between 0 and 1 to each image object. These membership values indicate the likelihood of MDAC being associated with an image object, with high values indicating high likelihood and vice versa. The default minimum membership value of 0.1 was used as the threshold to assign the class 'MDAC' to an image object. There is a difference between membership function (based on a feature, e.g. mean backscatter) and membership value (the final value assigned to an object). Although the class description might consist of several membership functions, only one (combined) membership value is computed by the eCognition software. Different logical operators (AND, OR and NOT) may be used to combine membership functions when calculating the membership value. In this case, the AND operator was used giving the minimum membership of all membership functions. For more information see Benz *et al* (2004).

A rule-based approach was subsequently used to map subaqueous dunes ('sand waves') based on a visual inspection of the imagery followed by the development and testing of rules that allow separation of dunes from all other seabed categories. This was achieved in a two-step approach. Initially, core areas were defined by the classified BPI3 ('positive'), the main direction (75° - 160°) and asymmetry (>0.85) of image objects. The latter two were subsequently relaxed (main direction: 50° - 160° and asymmetry >0.5) if image objects were next to previously identified dunes objects and had a positive BPI3. In this way, it was possible to further constrain the occurrence of MDAC. All objects classified as dunes or left unclassified were then assigned the class sediment. Finally, the results were simplified by reclassifying sediment objects fully or largely surrounded by MDAC (relative border to MDAC > 0.6) below a size of approximately $100m^2$ (Area <12 pixels) as MDAC. Only image objects classified as MDAC were exported and their confidence assessed.

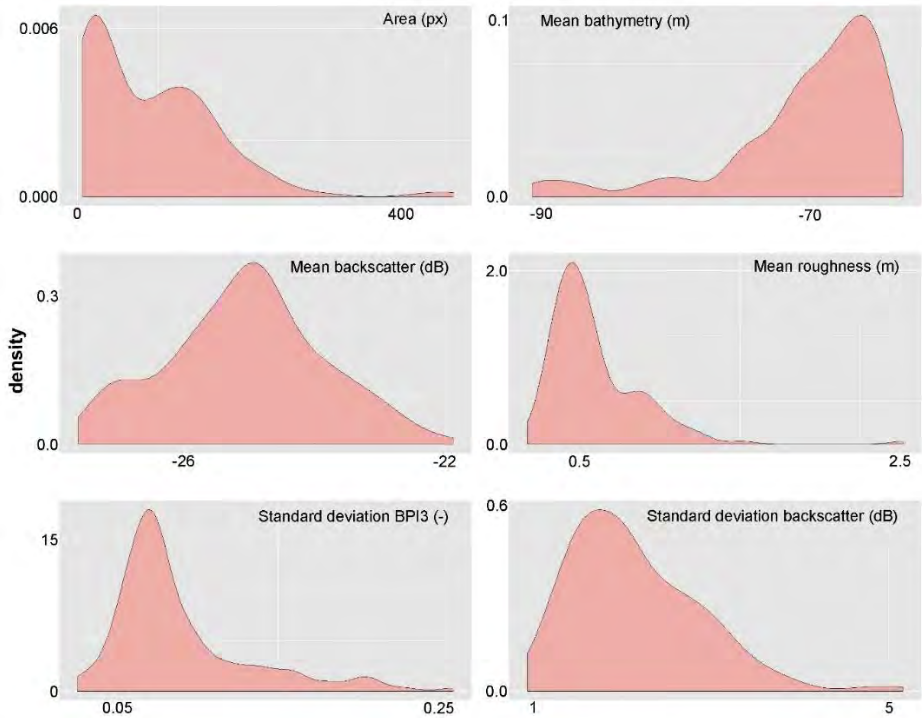


Figure 42. Density plots of the six features used for mapping MDAC. The resulting membership functions in eCognition have a similar, yet simplified form and y axes scaled between 0 and 1.

A4.3 Confidence assessment

The confidence assessment is based on a three-step confidence assessment framework produced by JNCC (Lillis 2016). The assessment was performed on a per-polygon basis due to the possible heterogeneity of inputs into the model across the output area. The method requires the assessor to follow the flow diagram shown in Figure 43 and score the polygon appropriately at each stage.

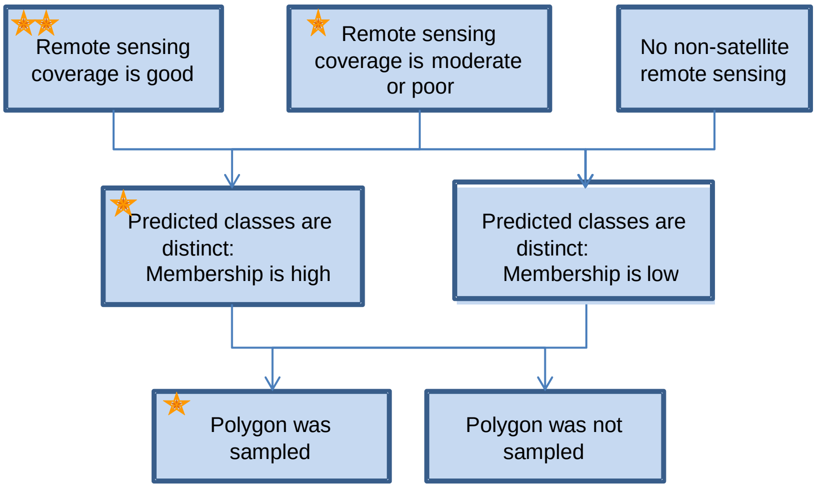


Figure 43. Three-step confidence decision tree; the assessor starts at the top and follows the arrows. Stars/points are awarded according to the answers given and the final score is the sum of the stars/points.

A maximum qualitative score of four can be achieved by a polygon. The final score should not be taken as a quantitative probability of the habitat's likelihood in extent or presence; the measurement is a qualitative score based on the data inputs, the membership values and the agreement between predictions and observations. The remote sensing coverage confidence was scored as two throughout, as MBES data were the input data. The distinctness of class boundaries criterion was scored in the following way: a score of one was attained where the membership value was larger than 0.5, and zero otherwise. In the case of the amount of sampling criterion, a score of one was given if a polygon coincided with at least one MDAC observation.

Annex 5: Sub-bottom profiling

A5.1 Boomer interpretation

On the nine boomer line profiles (Annex A5.3), the x-axis shows trace numbers (which can be cross-referenced to geographical locations along the profile) and the y-axis shows two-way travel time in milliseconds (msec). For ease of interpretation the profile images have been given a marked vertical exaggeration.

Profiles were interpreted assuming acoustic velocities of 1500m.sec^{-1} in seawater, and 1650m.sec^{-1} in near-seabed sediments. The low power (135 joules) profiles provided greater resolution than those recorded at higher power (240 joules; ~45cm compared to ~70cm); also, the higher power profiles produced more 'ringing' of the seabed reflection (i.e. more reflections close beneath the seabed were obscured). Sub-seabed penetration of up to 30m was achieved with low power, deeper penetration being prevented by the first seabed multiple. Theoretically the higher power profiles should achieve greater penetration, however, in practice this was not possible. Because of the range of water depths likely to be encountered along individual survey lines, it was decided to keep the towfish at a constant depth; this depth was not changed between low power and high power lines, so the first seabed multiple affected both sets of profiles at the same sub-seabed depth.

An interpreted section ("Section 1") through the Quaternary deposits of this study area was included on the BGS Quaternary Geology map (Wingfield *et al* 1990). Profile SBP009 was run specifically for comparison with this section.

A5.2 Seismic stratigraphy

The boomer profiles indicate the presence of two sediment packages within the survey area, divided by an unconformable, erosive boundary. These packages are referred to here as Units A and B. There is evidence of a third unit (Unit C) beneath the deep-water channel to the east of the survey area.

The youngest sediments, referred to herein as Unit A, lie unconformably on the underlying sediments of Unit B. Their thickness differs across the survey area, being generally thicker to the west of the site boundary. Over much of the area within the site boundary these sediments are either absent, or too thin to be identified on the boomer profiles. Unit A most likely represents the Wave and Bank Facies of the Surface Sands Formation (Holocene) indicated on Section 1 of Wingfield *et al* (1990), and comprises mobile sands, in places formed into sand waves and ribbons.

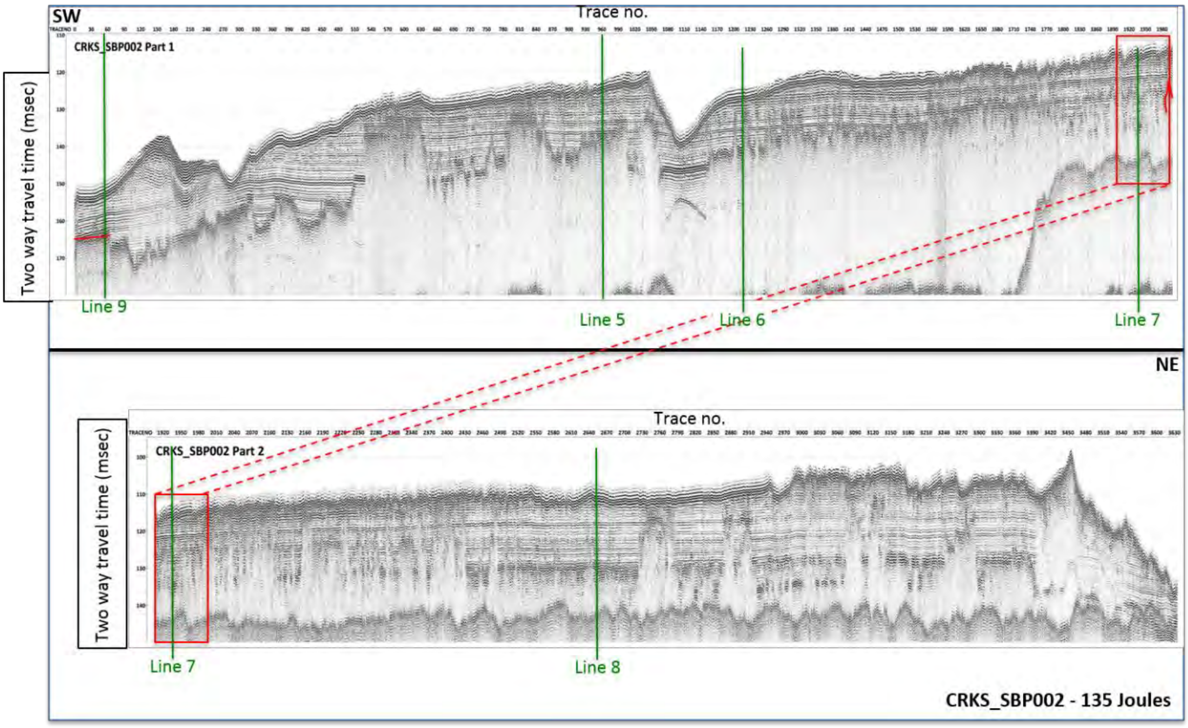
Unit B, the older (lower) package, is present throughout the survey area. It comprises multi-layered sediments, the layering being represented by numerous parallel internal reflections. These are probably caused by contrasts in the acoustic impendence (coarseness) of the individual layers. Lateral variations in the amplitude of individual reflections are likely caused by the presence/absence of gas within the sediment pore spaces (see below).

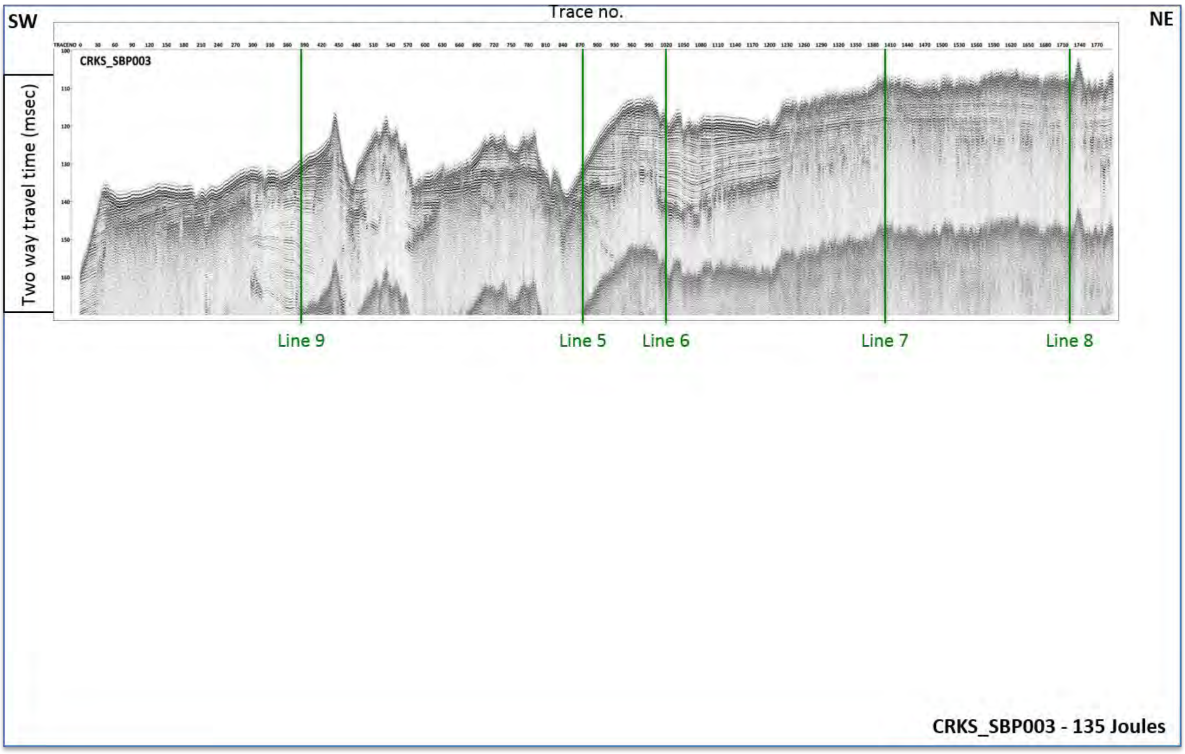
The Unit B reflections generally dip towards the NW, although the dip varies, being steeper on the western side of the area. The vertical thickness of Unit B visible on the profiles is at least 40m; however, the base of this sediment package is never visible. The top of this unit is truncated by the seabed, most clearly on the slopes of seabed depressions. Because of the NW dip, the youngest members of Unit B occur in the NW of the area; the oldest are exposed on the eastern side where the seabed slopes into a deep water channel.

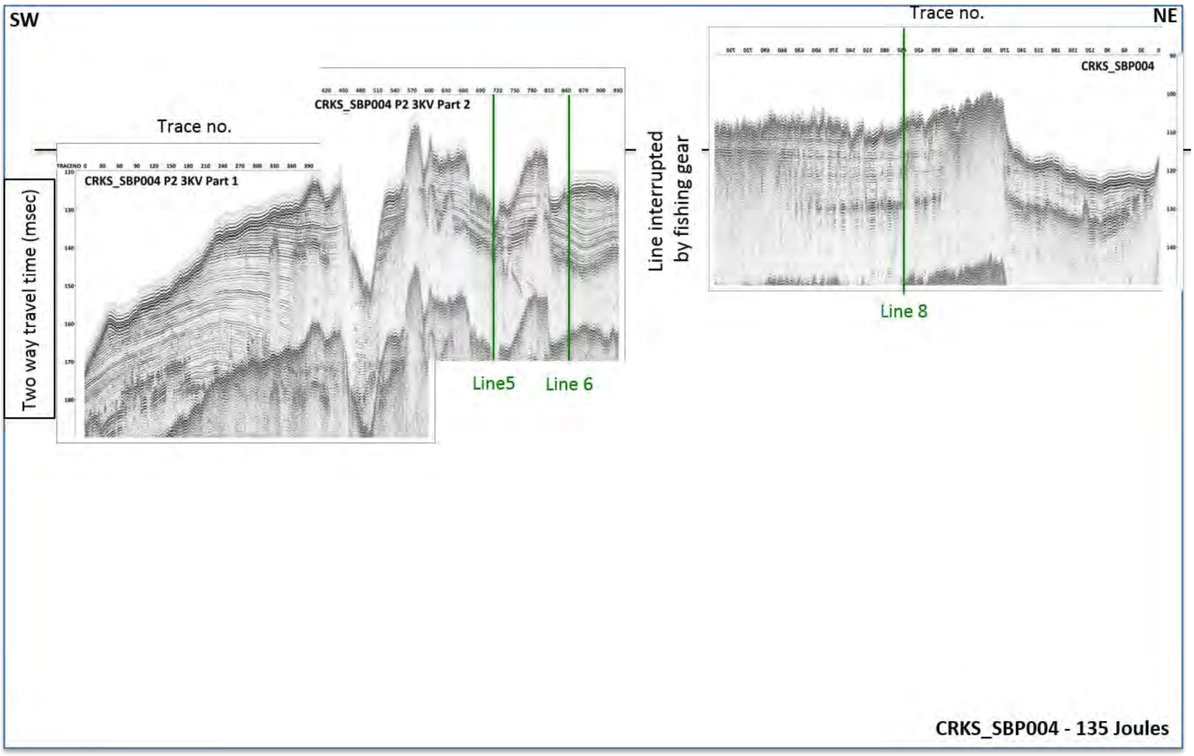
Because of the seismic character, thickness and geographical extent of Unit B, it is assumed that it represents the Prograded Facies of the Western Irish Sea Formation, as described by Wingfield *et al* (1990) and Jackson *et al* (1995).

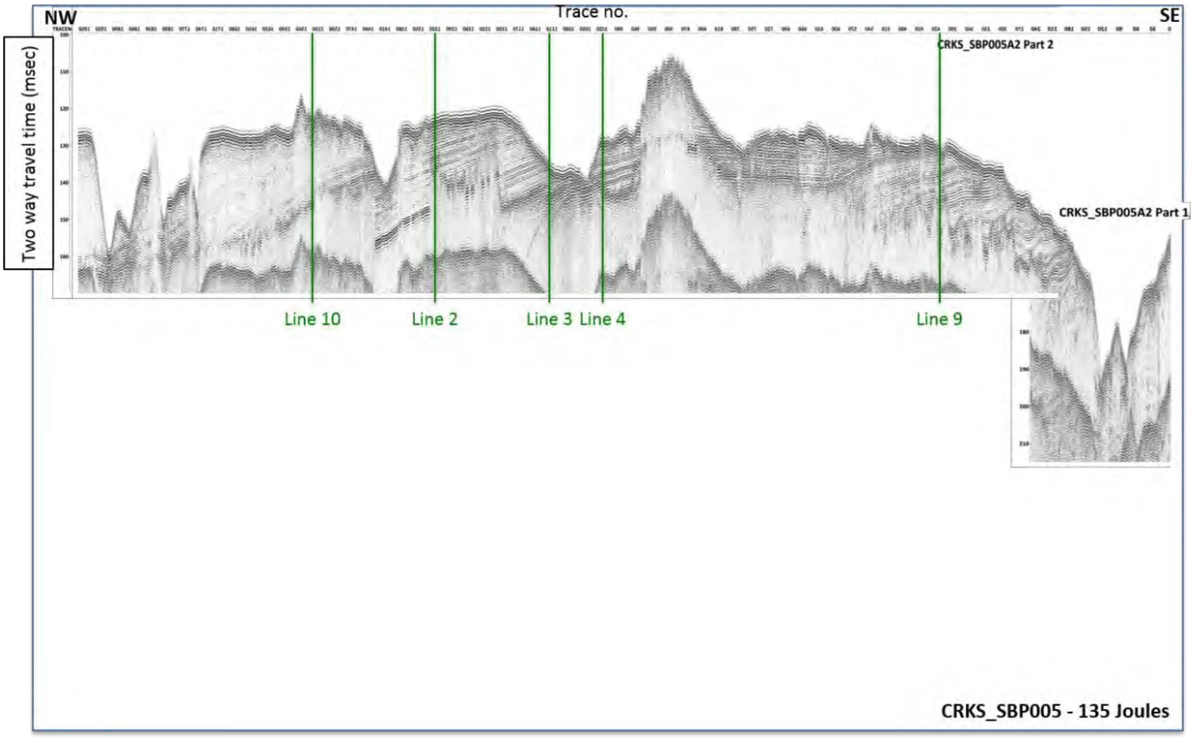
Beneath the deep-water channel, to the east of the area, a third sediment unit (Unit C) is present. Near the boundary between Units B and C there is a narrow area of multi-layered sediments. To the east of this Unit C is present immediately below the seabed, appearing on the profiles as a featureless unit with a strong (high amplitude) seabed reflection. This unit is probably equivalent to the Cardigan Bay Formation of Wingfield *et al* (1990) and Jackson *et al* (1995).

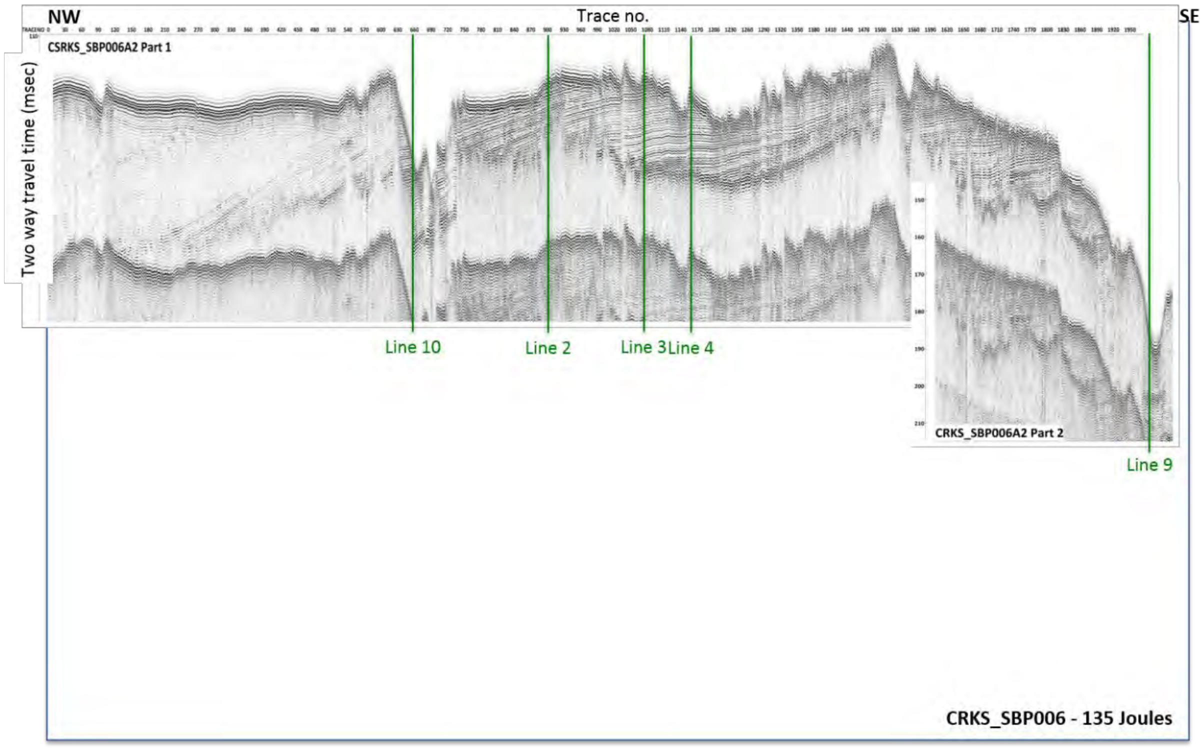
A5.3 Sub-bottom profiles

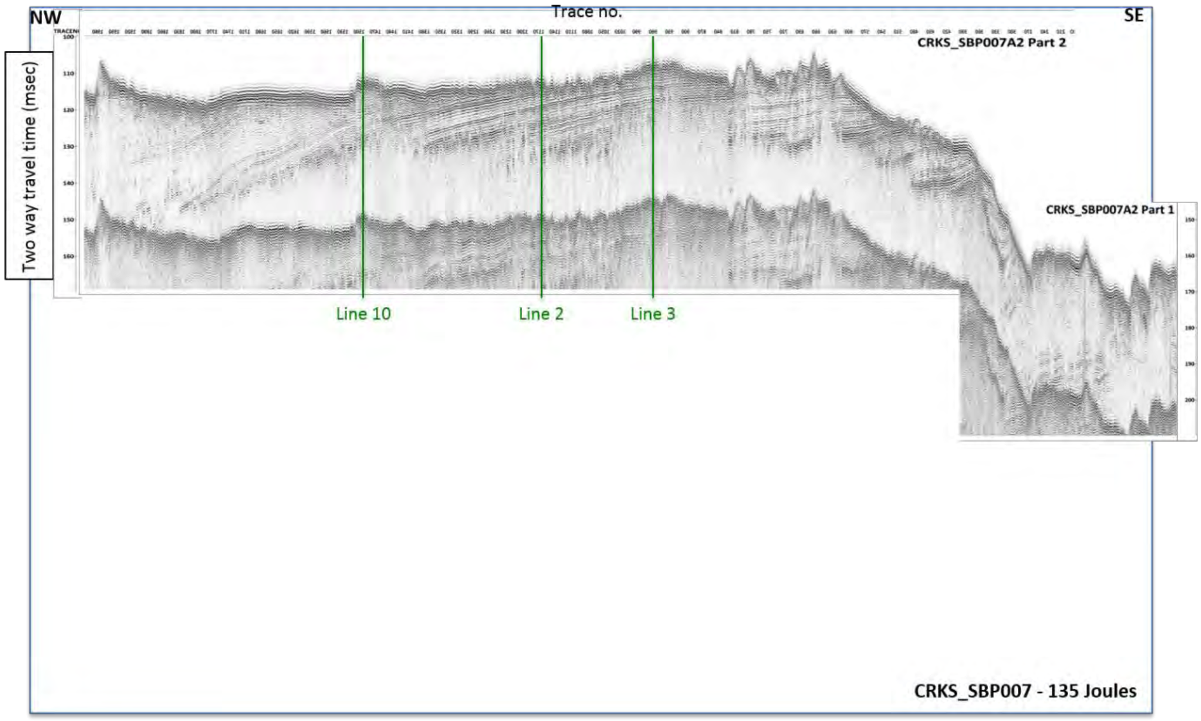


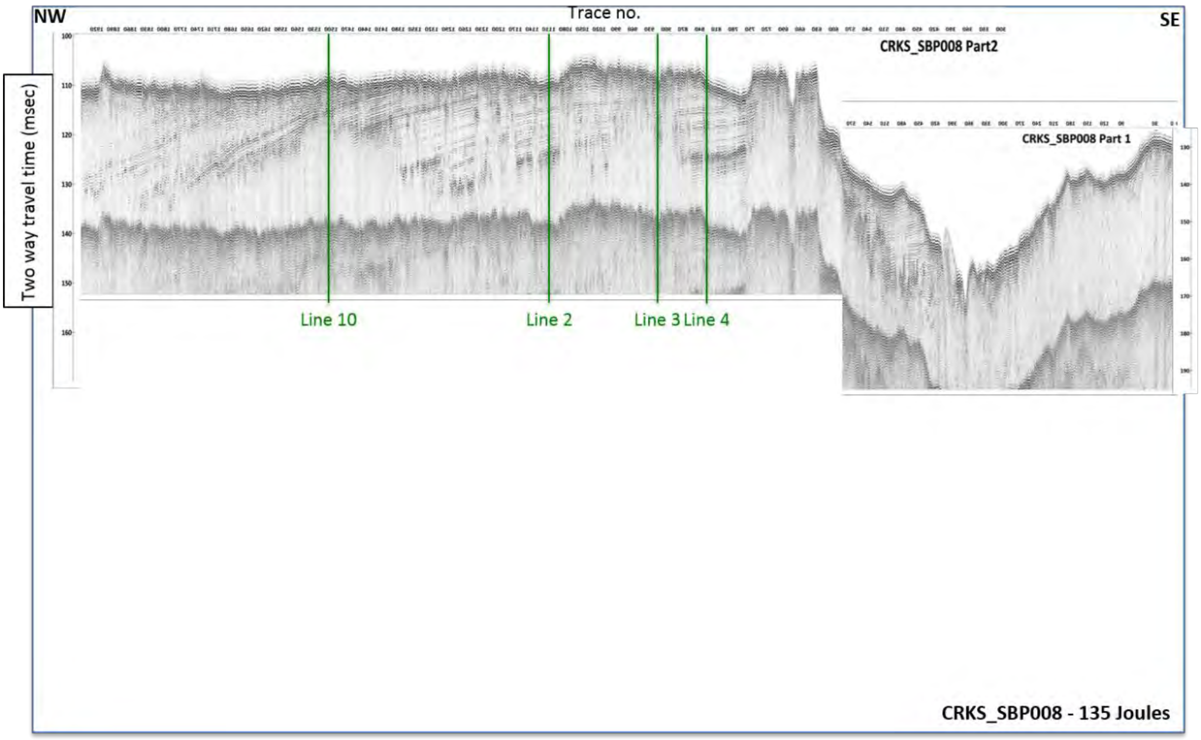

SW
Trace no.
NE
CRKS_SBP003
Two way travel time (msec)
Line 9
Line 5
Line 6
Line 7
Line 8
CRKS_SBP003 - 135 Joules

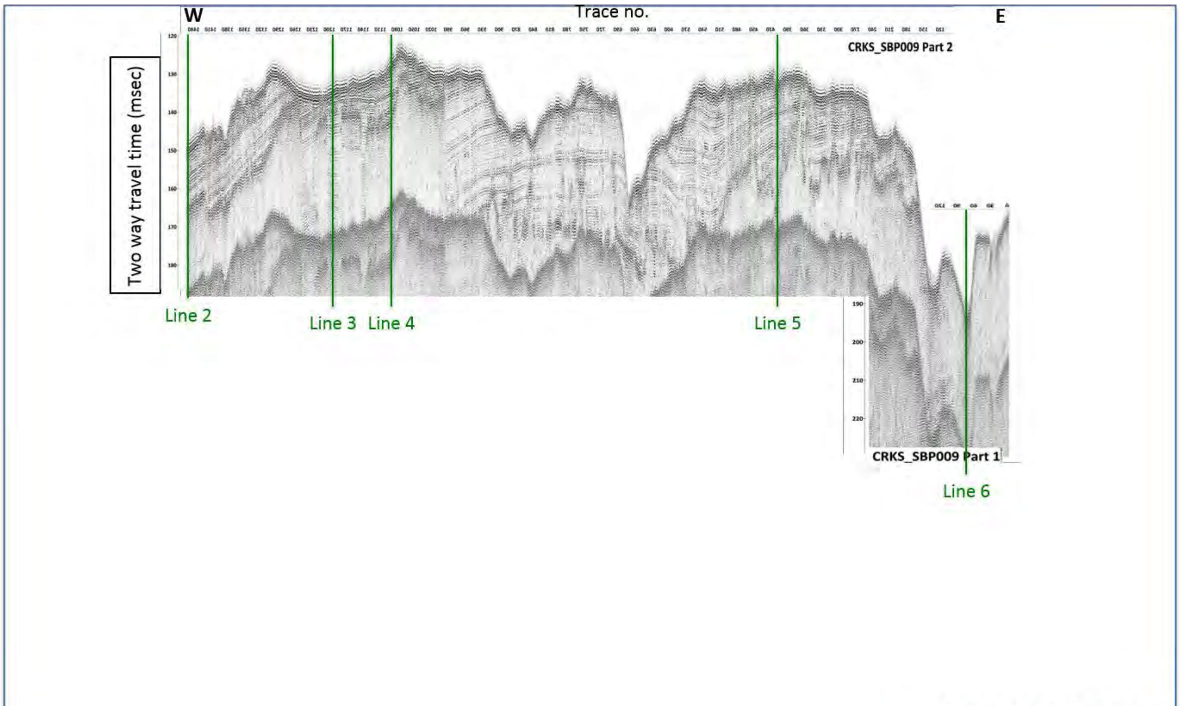

SW
Trace no.
NE
CRKS_SBP004 P2 3KV Part 2
Trace no.
CRKS_SBP004 P2 3KV Part 1
Two way travel time (msec)
Line5
Line 6
Line interrupted by fishing gear
CRKS_SBP004
Line 8
CRKS_SBP004 - 135 Joules


NW
Trace no.
SE
Two way travel time (msec)
CRKS_SBP005A2 Part 2
CRKS_SBP005A2 Part 1
Line 10
Line 2
Line 3
Line 4
Line 9
CRKS_SBP005 - 135 Joules

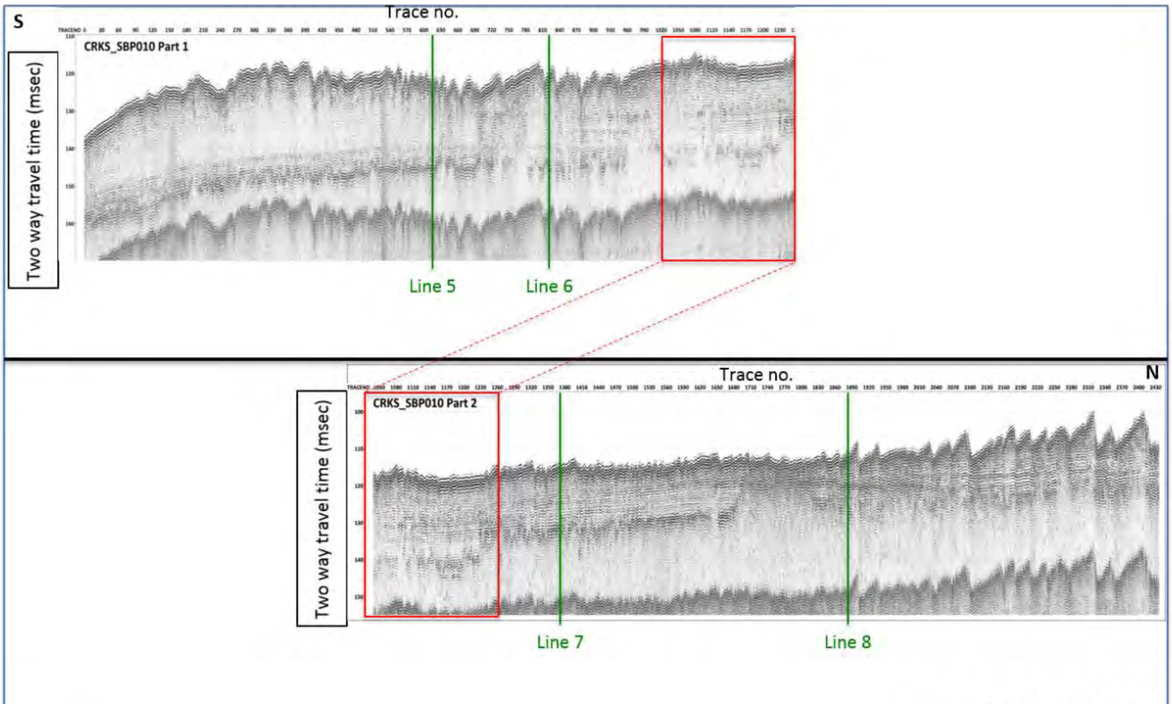

NW
Trace no.
SE
Two way travel time (msec)
CSRKS_SBP006A2 Part 1
CRKS_SBP006A2 Part 2
Line 10
Line 2
Line 3
Line 4
Line 9
CRKS_SBP006 - 135 Joules


NW
Trace no.
SE
CRKS_SBP007A2 Part 2
CRKS_SBP007A2 Part 1
Two way travel time (msec)
Line 10
Line 2
Line 3
CRKS_SBP007 - 135 Joules


NW
Trace no.
SE
CRKS_SBP008 Part2
CRKS_SBP008 Part 1
Two way travel time (msec)
Line 10
Line 2
Line 3
Line 4
CRKS_SBP008 - 135 Joules



CRKS_SBP009 - 135 Joules



CRKS_SBP010 - 135 Joules

Annex 6: Non-indigenous Species (NIS)

Table 14. Taxa listed as non-indigenous species which have been selected for assessment of Good Environmental Status in GB waters under MSFD Descriptor 2 (Stebbing *et al* 2014) (present = already present in UK waters, horizon = not currently present but of concern).

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

Annex 7: Marine litter

Table 15. Categories and sub-categories of litter items for Sea-Floor from the OSPAR/ICES/IBTS for North East Atlantic and Baltic. Guidance on Monitoring of Marine Litter in European Seas, a guidance document within the Common Implementation Strategy for the Marine Strategy Framework Directive, MSFD Technical Subgroup on Marine Litter, 2013.

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

Related size categories
A: ≤ 5*5cm = 25cm²
B: ≤ 10*10cm = 100cm²
C: ≤ 20*20cm = 400cm²
D: ≤ 50*50cm = 2500cm²
E: ≤ 100*100cm = 10000cm²
F: ≥ 100*100cm = 10000cm²

Annex 8: Version Control

Version	Date	Author	Reason/Comments
1.0	16/08/2017	TNJ	Finalised version for publication
1.1	01/02/2019	CMC	Corrections made: *Leptolaimella* updated to *Leptonemella*. Two instances total, p.45.

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