Environmental Summary

UK Seabed Resources
UK1 Contract Area
For the Calendar Year 2020

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Table of Figures and Tables:

Table of Tables

UKSR Environmental Survey Overview

UKSR's survey activities to date consist of three UK1-focused research expeditions to the Clarion Clipperton Zone: the first, UK1-AB01 taking place in 2013; and the second UK1-AB02 in collaboration with Ocean Minerals Singapore taking place in 2015. The most recent and third expedition was in two parts, RC01 Leg 1 (RC01-L1), February-March 2020 and the second part, RC01 Leg 2 in May 2020, Figure 1.

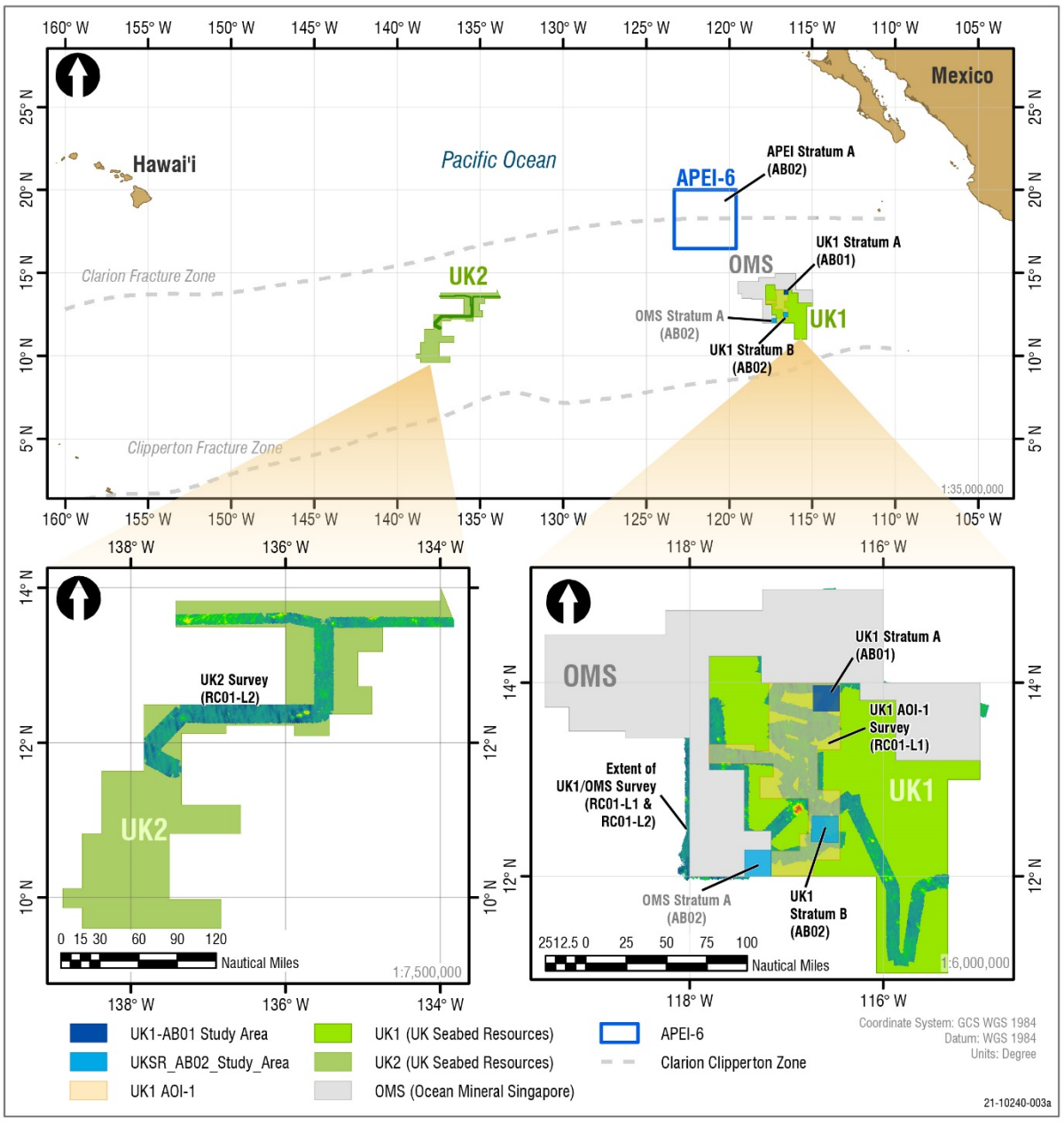

Figure 1. Reference maps of UKSR and OMS RC01 Expedition (2020) joint survey areas and relationship to 30 km x 30 km sampling strata from the AB01 (2013) cruises, AB02 (2015).

The primary objective for the initial two cruises was collection of data to develop an environmental baseline for UK1, although mineral resource samples were also collected on all cruises.

An overview of the sample and data collection achieved to date is presented below at Figure 2.

Figure 2. Overview of UKSR CCZ exploration activities.

RC01 was initiated in in February-March of 2020. This effort was primarily oriented toward mineral resource survey work, with additional biological analysis of box core samples and ROV footage, as well as deck observations. RC01 also incorporated important equipment upgrades, including novel survey technologies such as simultaneous multi-AUV deployment and HiSAS, neither of which had previously been applied to a nodule environment, as well as upgrading from 50 cm x 50 cm to 75 cm x 75 cm box cores. The high-quality capability provided by the RC01 operations vessel and staff allowed for the first known simultaneous deployment of an ROV to document a box core operation and sampling at >4100 m depth as shown in Figure 3.

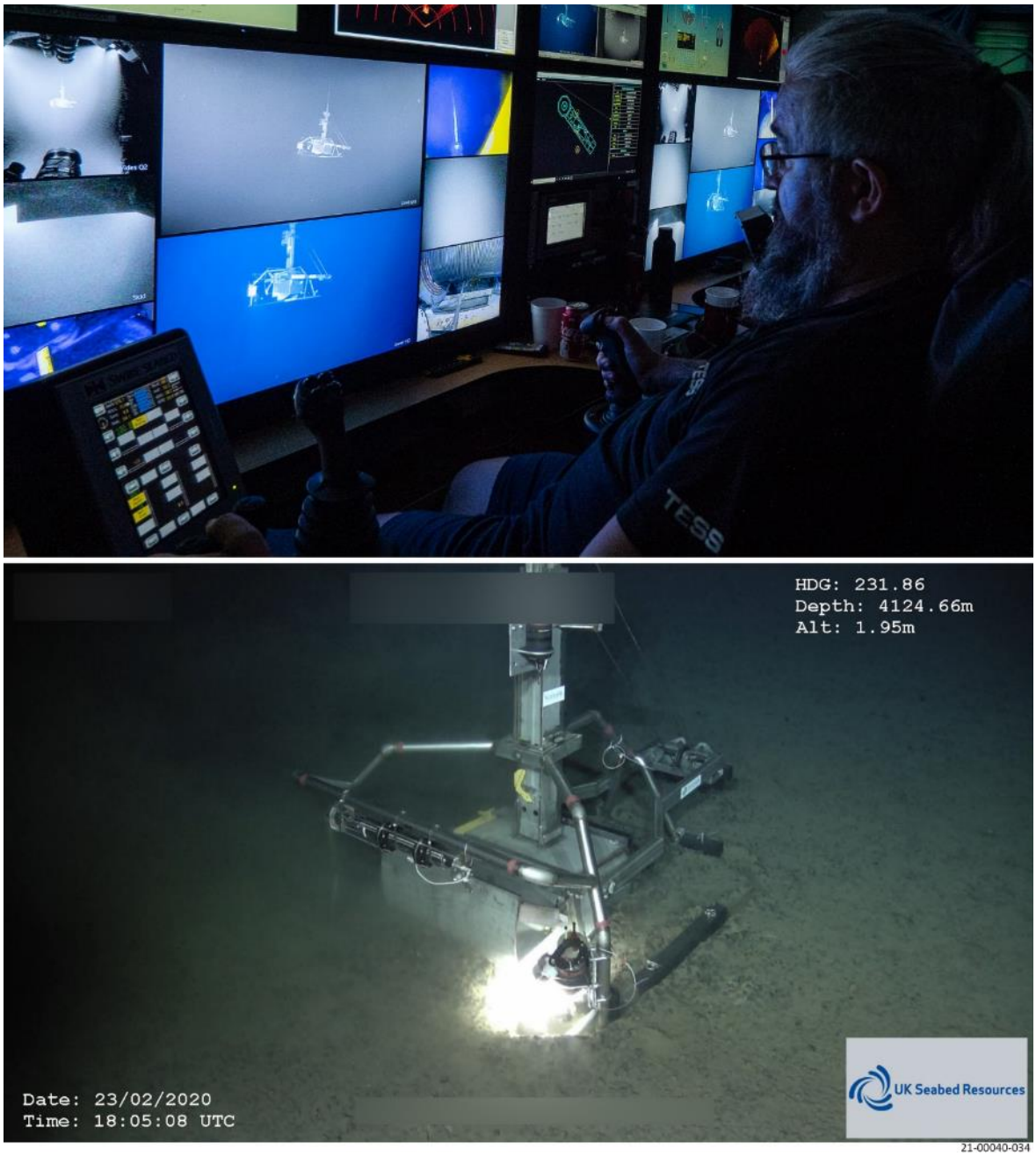

Figure 3. First known simultaneous deployments of an ROV to document a box core operation and sampling during RC01 survey work.

The analysis of the environmental samples collected from the AB01 (2013) and AB02 (2015) cruises in the UK1, OMS contract areas and APEI6 continued at several leading institutions around the world in 2020. The UKSR Environmental Baseline Scheme utilises a requirements/sampling matrix set out at Table 1 below. The current list of published research derived from analysis of UKSR samples is available from the UKSR website.

Table 1. UKSR Environmental Baseline Scheme.

Class	Benthic Megafauna	Benthic Macrofauna			Benthic Meiofauna (non foram)	Benthic Foraminifera[2]	Larvae of Benthos	Water Column and Benthic Microbes	Ecosystem Function		Demersal Ichthyofauna/ Scavangers
Subgroup		Polychaetes	Crustaceans	Total Macrofauna	Multi*				Sinking Particle Flux	Sed. Comm Respiration	
Abundance/Amount		N/A									
Diversity		Richness	Richness	Evenness & Richness					N/A	Macrofauna	
Species Composition								N/A	N/A	Macrofauna	
Community Structure							N/A	vs. Depth	N/A	Macrofauna	N/A
Population Connectivity[1]				N/A			N/A	N/A	N/A	N/A	
Relation to Nodule Abundance/Size					Sediment + Nodules	Sediment + Nodules	N/A	N/A	N/A	N/A	
Relative Abundance		N/A						N/A	N/A		
Species Range				N/A			N/A	N/A	N/A	N/A	
Taxonomy				N/A			N/A	N/A	N/A	N/A	
Sampling Tool	AUV/ROV/ Epibenthic Sled	Box Core/ Epibenthic Sled	Epibenthic Sled	Box Core	Multi-Core	Multi-Core/ ROV/ Box Core/ Epibenthic Sled	ROV Towed Plankton Net and Near-Bottom Sediment Trap	Multi-Core	Near-Bottom Sediment Trap	Sediment Respiro-meter	Baited Cameras and Traps

* copepods, isopods, cumaceans, tanaids
1: Studies of Population Connectivity and CCZ-Wide Gene Flow are the same thing.
2: Foraminiferan span a variety of size classes, but they are studied as a single faunal group because of the extraordinary specialized knowledge required to work with them

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Note. Dark green indicates the sampling apparatus will provide the definitive samples/information for a particular organism class and metric, light green indicates will provide supporting information.

2020 Environmental Baseline Study Objectives

The initial RC01 expedition environmental objectives for 2020, having been defined in 2019 planning workshops, were:

- To conduct biological analysis of mineral samples for environmental baseline.
- Additional detailed characterisation and sampling in the two Areas of Interest, AOI-2 & AOI-3 for potential pilot test site locations.
- Colonisation experiment deployment.

Throughout the expedition biological analysis was conducted on retrieved box-cores as opportunities presented themselves. The primary objectives were achieved in 2020 for sample collection, although at a smaller scale than anticipated due to AUV operational impacts on box core collections during RC01-L1. The colonisation experiments were placed in-situ and are expected to be retrieved in the future for analysis. More details are provided below.

In addition to the survey work the ongoing laboratory analysis at the Natural History Museum (NHM) – UK and the Norwegian Research Centre (NORCE) on the previously collected UKSR samples was ongoing as well. This activity was impacted by the pandemic to varying degrees throughout the year. Two new peer reviewed publications appeared in the 2020 reporting year, one additional manuscript is in review stage and a fourth is in advanced stages of preparation. An additional major publication on regional patterns in CCZ biodiversity and connectivity is in advanced stages and is expected to be published in due course. Some delays to publications have resulted owing to COVID-19 but in general work has progressed well with the RC01 cruise terminating just prior to international lockdowns in Late-March 2020. The majority of work since that time has been completed with staff at home, working on data already collected. The NHM/NORCE team was also able to present 8 talks at workshops and conferences in which they highlighted UKSR data from the CCZ.

In 2020 the NHM/NORCE work focused on:

- Continued detailed analysis and publication of environmental baseline data relating to the UK1 and OMS contracted areas in the CCZ and updating of data to be submitted to the contractors in the format requested by the International Seabed Authority,
- Preparation, mobilisation and biological sampling leadership on the UKSR RC01 Joint Expedition in Feb- April 2020
- Further project development and planning of the baseline work including long-term integration with NERC SMARTEX project. Deliverables for work periods in 2020 were met and those for the end of the 2020-2021 contract period (June 2021) are on target.

Methodologies and Equipment

RC01 Leg 1 Biological Sampling, Procedures and Techniques

The following describes the sampling protocol, samples and imagery collected, experiments placed and preliminary analysis (distribution of samples) from the RC01 expedition to the UK1 and OMS exploration areas for the Natural History Museum (NHM), Norwegian Research Centre (NORCE), and National Oceanography Centre (NOC) teams. (PIs: Dr Adrian Glover, Dr Thomas Dahlgren, Dr Daniel Jones).

Work was undertaken in accordance with the planned statements of work with each research institution. All deliverables were submitted, and additional tasks carried out including the emplacement of three abyssal nodule colonisation experiments for planned recovery in 2022 (NERC SMARTEX project), and examination of opportunistic ROV video and samples for megafauna. All box core samples recovered by the RC01 team (n=44) were examined by the authors and samples taken for future environmental baseline analyses.

Box core samples were studied in close cooperation with the RC01 Biology team from National University of Singapore (NUS) and complementary collections and analyses took place to maximise the potential environmental baseline data (see below for further details).

Boxcore Protocols and Approach

The Bio teams made use of the box core and ROV for sampling and survey operations. Additional data from the ship multibeam, AUV acoustic data (multibeam, sidescan and HISAS), AUV imagery data (if available) will be used in future biological analyses as data becomes available from SRDL.

Box core sampling. A 75 x 75 cm Ocean Instruments BX-750 (United States Naval Electronics Laboratory type) was used for all seabed sampling. Launch and recovery of the box core deployment and recovery was the responsibility of the vessel contractor. The onboard UK biology (UKBio) team followed established DNA-taxonomy protocols (Glover et al 2016) for the collection of samples and data at-sea from the box-core samples. Two types of box-core sample were taken:

1. Semi-Quantitative (SQ) samples (n=34) in which the core, after recovery was sampled quantitatively for megafauna, nodule fauna and macrofauna, but with macrofaunal animals limited to the nodule-wash (animals retained on muddy nodules) and sub-sections of the core taken in 15 x 15 cm squares (either 1 or 2 15 x 15 cm samples) with these sections sampled at 0-2 cm and 2-5 cm intervals. All samples in this type of core were subject to live-sorting, preliminary ID and imaging, with almost all specimens individually preserved in barcoded sample jars linked to a database.
2. Fully-Quantitative (FQ) samples (n=10) in which a 50 x 50 cm frame was lowered into the core immediately after recovery, with the entire section within the 50 x 50 cm frame treated as a quantitative sample and the outer section sampled as per SQ cores, an example is shown in Figure 4. Full details of the box core sampling protocols including methods for FQ and SQ preservation are detailed below in this report.

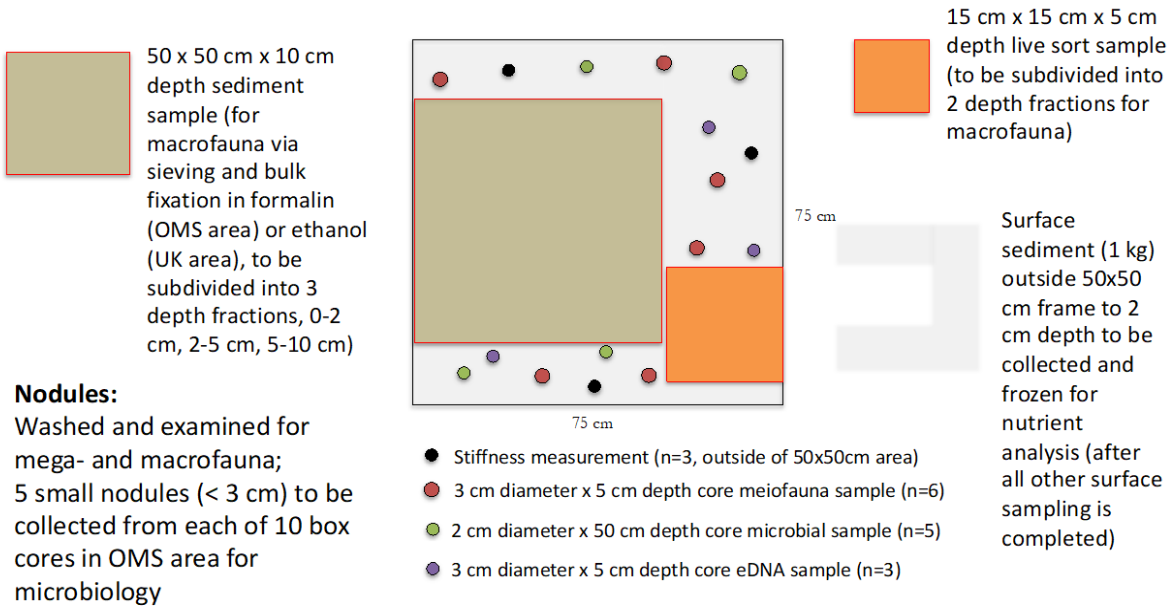

Figure 4: RC01 Expedition full quantitative (FQ) box core sampling plan utilised.

ROV sampling. Opportunistic ROV sampling for megafauna took place both with video observation and sampling using scoop net when possible (See section: ROV Imagery Results). In addition, the ROV was used to emplace colonisation experiments (n=3) (See section: Colonisation Experiment).

Opportunistic sampling. Some samples were also taken opportunistically for DNA analysis, e.g. cnidaria tentacles found on the box cores, flying fish found on the deck.

Sample preservation. All live-sorted specimens were preserved in 80% ethanol (96% non-denatured ethanol mixed with distilled water) and kept at -20C while on board (later at room temperature). All FQ bulk fixed samples were sieved and preserved in chilled 96% ethanol and kept at -20C on board, after 24 hrs the ethanol was replaced, and the jars agitated. Finally, these, bulk fixed samples were sieved again in pure 96% ethanol and preserved in smaller jars with minimal ethanol to enable air-freight shipping (special provision A180 ethanol packing). 3 samples of a Neanthes polychaete were preserved in RNAlater for transcriptomic studies of vision (to be passed to Aida Verdes, NHM). Some shark teeth were dried and preserved for transfer to the NHM palaeontology collection.

Complementarity of UKBio and NUS team sampling. A decision was taken during planning phases of the project to divide sampling efforts to maximise the complementarity of sampling and data analyses between the different institutions. This reflected taxonomic expertise in different institutions (which was highly complementary) as well separating samples for FQ analysis based on position either within the OMS area (samples sent to NUS) or UK1 area (samples sent to NHM London). In summary samples were divided as follows:

UKBio (NHM/NORCE/NOC) responsible for, and shipped to London (from all UK/OMS samples):

- Annelida (polychaete worms) (excl Glyceriformia -> NUS)
- Echinodermata (e.g. seastars, brittle stars, sea cucumbers)
- Cnidaria (e.g. nodule corals)
- Bryozoa (nodule animals)
- Brachiopoda (small nodule animals)
- Amphipoda
- Pycnogonida (sea spiders)

- Cirripedia (barnacles)
- Chordata (fish, shark, whale bone and teeth) from UK1 only
- Fully Quantitative (FQ) material from UK1 samples (ethanol fixed)

NUS responsible for, and shipped to Singapore (from all UK/OMS samples):

- Annelida (Glyceriformia only)
- Mollusca (clams, snails)
- Porifera (sponges)
- Copepoda
- Tanaidacea
- Isopoda
- Decapoda (e.g. penaeid shrimp)
- Foraminifera (incl xenophyophore)
- Fully Quantitative (FQ) material from OMS samples (formalin and ethanol-fixed)

RC01 Biology Protocols – Box core (BC) Sampling

Overview

There are 3 BC protocols for the BIO team (institutes: NHM, NUS, NOC, NORCE). These are:

Type FQ: Quantitative BCs that will be processed for quantitative BIO and GEO

Type SQ: Semi-quantitative BCs that will be processed for BIO and GEO

Type Geo: GEO only cores (sampled for BIO megafauna only)[1]

Type FQ: Quantitative Box Core Protocol for BIO

NOTE: The GEO team controlled the sample log sheets (latitude, longitude, depth, notes, sample distribution recording.)

- **Box core on deck.** Measure temperature of top water and record > data to sample log sheet.
- **Insert 50x50 cm frame, drain topwater.** Use hoses to drain top water from 50x50 cm into 300- and 250-micron sieves for quantitative sort. Simultaneously drain topwater from outside 50x50 cm onto 300 micron sieve for live sort. **Do not disturb sediment-water interface!** Wash topwater samples into 0-2 cm buckets (quantitative or live sort).
- **Box core moved to shade.**
- **Remaining top water.** Carefully removed with turkey basters into quantitative 0-2 cm (RED) or live sort 0-2 cm (BLUE) buckets.
- **Remove frame, Photography. Replace 50 x 50 cm frame.**
- **Microbiology.** Nodule, sediment taken from outside 50 x 50 cm area. **Care for contamination!**
- **Megafauna.** Carefully remove any megafauna e.g., large sponges, xenophyophores to BIO labs. From both outside (LIVE SORT) and inside (Quantitative) 50 x 50 cm (to be kept separate). Quantitative samples > NUS for OMS sites, >NHM for UK sites.
- **Shear strength.** Outside 50 x 50 cm.

[1] Note: Although initially planned, no 'Geo only' boxcores were collected on RC01-L1 Expedition. All box cores collected were FQ and SQ types.

- **eDNA sampling.** Small sediment samples taken for eDNA from outside 50 x 50 cm.
- **Forams/Xenophyophores.** Large xenophyophores removed from nodules. From both outside and inside 50 x 50 cm area. KEEP TRACK OF NODULES USING BLUE=LIVE SORT, RED=QUANT.
- **Meiofauna.** Push cores from outside 50 x 50 cm.
- **Nodules picking Quantitative.** Picked from 50 x 50 cm quantitative – washed into 0-2 cm RED bucket layer. Nodules with animals (NOT forams) on > BIO Quantitative nodule dishes (RED). Nodules without animals > GEO buckets (grey 1).
- **Nodules picking LIVE SORT.** Nodules picked from non-quantitative area – washed into 0-2 cm live sort (NHM and NUS) (BLUE) buckets sample. Nodules with animals (NOT forams) on > BIO LIVE SORT nodule (BLUE) dishes. Nodules without animals > GEO buckets (grey 2).
- **Mud Slicing Quantitative.** 50 x 50 cm area sliced into 0-2 cm, 2-5 cm, 5-10 cm quantitative RED buckets. **Care taken to not lose mud!** Sub-surface nodules with animals (NOT forams) on > BIO Quantitative nodule dishes. Nodules without animals > GEO buckets (grey), organised into 0-2 cm, 2-5 cm and 5-10 cm sections.
- **Mud Slicing LIVE SORT.** One 15 x 15 cm, area selected from outside 50 x 50 cm area and sliced into 0-2 cm and 2-5 cm and taken to 0-2 cm, 2-5 cm live sort buckets (small BLUE buckets). Any nodule found to GEO bucket (grey 2)
- **Sieving Quantitative.** Sieve Quantitative samples (0-2, 2-5, 5-10) on 250- and 300-micron sieves > OMS ones to NUS (formalin fix for AOI-6, ethanol fix for AOI-2), UK ones to NHM (ethanol fix in AOI-2/AOI-3).
- **Sieving LIVE SORT.** Sieve one 15 x 15 cm live sort sample from outside 50 x 50 cm box, into 0-2 cm and 2-5 cm – sieve and > Bio lab.
- **Laboratory Nodules Quantitative.** Image and sample animals on quantitative nodules from inside 50 x 50 cm area and return to GEO grey buckets.1
- **Laboratory Nodules LIVE SORT .** Image and sample animals on non-quantitative nodules from outside 50 x 50 cm area and return to GEO grey buckets 2.
- **Laboratory Mud Animals LIVE SORT.** Both labs to sort their mud samples to phylum and distribute samples in dishes with labels as agreed in taxonomic sorting).
- **Laboratory Mud Animals Imaging/ID.** Both BIO labs to ID, image and preserve animals picked from the live sort samples in chilled 96% ethanol.
- **Nutrients.** All remaining surface mud outside 50 x 50 cm area taken to depth of 2 cm for nutrients/Geochem

Type SQ: Semi-Quantitative BC Protocol for BIO

NOTE: The GEO team will control the sample log sheets (latitude, longitude, depth, notes, sample distribution record)

NOTE: All sieving done gently in COLD FILTERED SEAWATER with SIEVES SUBMERGED (where possible)

- **Box core on deck.** Measure temperature of top water and record > data to sample log sheet.
- **Drain topwater.** Drain topwater onto 300 micron sieve for live sort. **Do not disturb sediment-water interface!** Wash topwater samples into 0-2 cm live sort buckets.
- **Box moved to shade.**
- **Remaining top water.** Carefully removed with turkey basters into live sort 0-2 cm buckets.
- **Photography.**
- **Microbiology if flagged for collection.** Nodule, sediment taken. **Care for contamination!**

- **Megafauna.** Carefully remove any megafauna e.g. large sponges, xenophyophores to BIO labs.
- **Shear strength.**
- **eDNA sampling if flagged for collection.** Small sediment samples taken for eDNA.
- **Forams/Xenophyophores.** Large xenophyophores removed from nodules.
- **Meiofauna.** Push cores.
- **Geochemistry.** Push cores/syringes.
- **Nodules picking LIVE SORT.** Nodules picked and washed into 0-2 cm live sort (NHM and NUS) buckets sample. Nodules with animals (NOT forams) on > BIO LIVE SORT nodule dishes. Nodules without animals > GEO buckets (grey).
- **Mud Slicing LIVE SORT.** Two 15 x 15 cm areas selected and sliced into 0-2 cm and 2-5 cm and taken to NUS and NHM 0-2 cm, 2-5 cm live sort buckets (small BLUE buckets-).
- **Sieving LIVE SORT.** NHM to sieve one 15 x 15 cm live sort, into 0-2 cm and 2-5 cm – sieve and > NHM lab. NUS to sieve second 15 x 15 cm live sort sample, into 0-2 cm and 2-5 cm > NUS lab.
- **Laboratory Nodules LIVE SORT.** Image and sample animals on nodules and return to GEO grey buckets.
- **Laboratory Mud Animals LIVE SORT.** Both labs to sort their mud samples to phylum and distribute samples in dishes with labels as agreed in taxonomic sorting.
- **Laboratory Mud Animals Imaging/ID.** Both BIO labs to ID, image and preserve animals picked from the live sort samples in chilled 96% ethanol.

Type GEO: 'Geology only' Box Core Protocol for BIO

N.B. The GEO team will control the sample log sheets (latitude, longitude, depth, notes, sample distribution etc)

- **Box core on deck.** Measure temperature of top water and record > data to sample log sheet.
- **Drain topwater.** Drain topwater, discard. **Do not disturb sediment-water interface!**
- **Box core moved to shade.**
- **Remaining top water.** Carefully removed with turkey basters, discard.
- **Photography.**
- **Megafauna.** Carefully remove any megafauna e.g., large sponges, xenophyophores to BIO labs.
- **Shear strength.**
- **Laboratory.** Imaging and preservation of megafauna.

ROV Imagery Protocols and Approach

Opportunistic examination of megafauna through ROV imagery was carried out on the RC01-L1 Expedition. In addition, a total of 3 specimens were collected using a scooping net with the ROV manipulator arm during ROV operations on Dives 119 and 121. The main camera used to obtain video footage during ROV operations, with 1920 x 1080 px HD video resolution, was an *Imenco Spinner II Shark Wide Angle HD SDI zoom*. Camera specifications are provided in Table 2, and Figure 5 depicts the configuration of the HD camera within the ROV frame. Although most of the video collected with this camera was obtained from an oblique view to the seabed, the recording angle constantly changed within each Dive, as did the ROV altitude above the seabed (approx. range: 0.5 - 4.5 m), and the camera zoom (max: 30x).

Table 2. Imenco Spinner II Shark Wide Angle HD SDI zoom camera technical specifications mounted on ROV utilised for macrofauna imaging.

Parameters	Values
Video output	HD SDI
Resolution	1080p@60fps
Sensitivity (lux)	1.4
View angle in water diagonally (deg)	73
View angle in water horizontally (deg)	64
Optical zoom	30x
Depth Rating (msw)	4,000 / 6,000
Diameter (mm)	140 / 117
Length (mm)	227

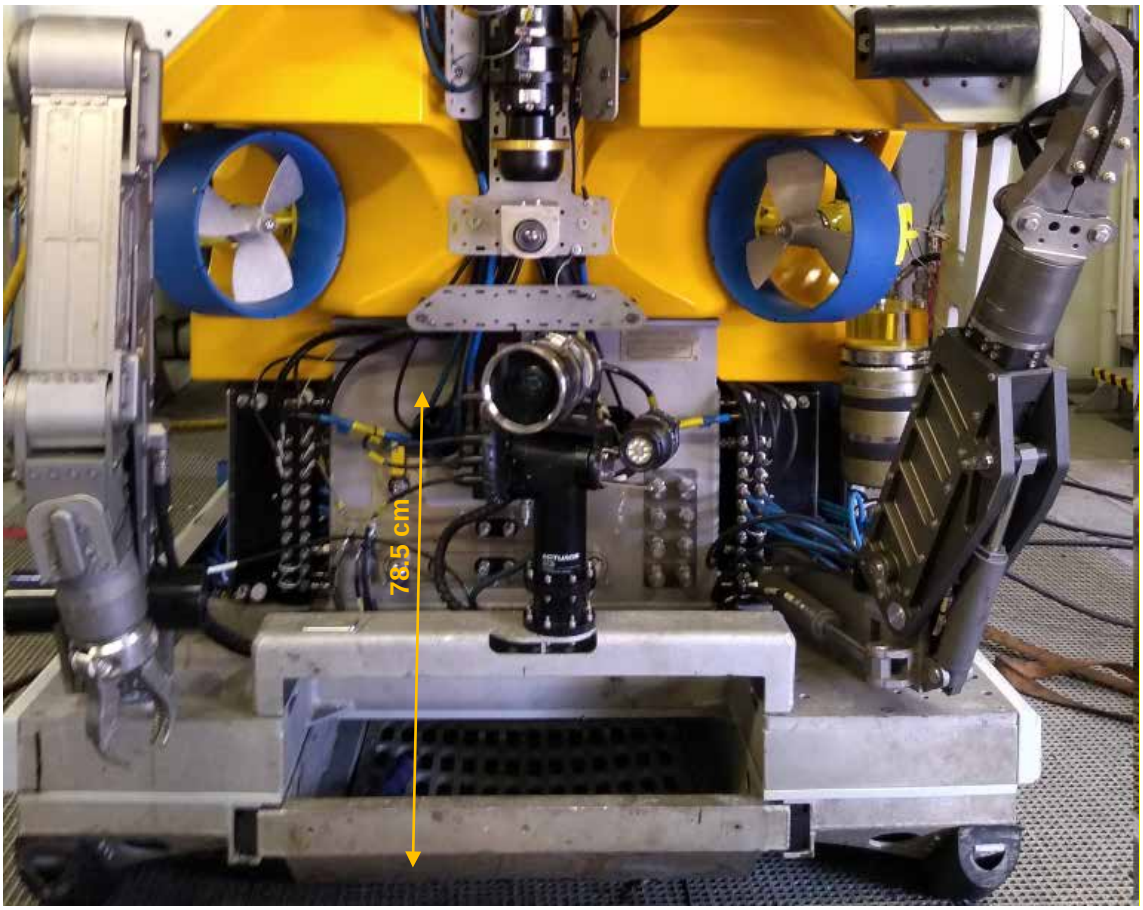

Figure 5. ROV frontal frame configuration and camera position.

Detail on the setup (e.g. height of the camera from ROV base) of the Imenco Spinner II Shark Wide Angle HD SDI zoom camera.

Colonisation Experiment

During RC01 project planning workshop in November 2019, a proposal for a simple colonisation experiment was developed. This was made possible, as an additional funded project is due to work in the UK1 area in 2022, (UK National Environmental Research Council (NERC) 'SMARTEX': principal investigators, Dr Daniel Jones, Dr Adrian Glover). A simple hypothesis was proposed to test if nodule-fauna are able to colonise similarly-sized basaltic blocks to nodules within a 2-3 year time frame. It is unusual to get an opportunity like this in deep-sea science with such a long period of time for the experiment to run.

Basalt blocks from the East Pacific Rise, collected by DSV Alvin (Woods Hole Oceanographic Institution) were provided to the UK biology team and used to construct an ROV-deployable experimental rig during project mobilisation in Panama. 50 cm x 50 cm frames were constructed from PVC plastic pipe each with 10 basalt blocks held on a grid by cable ties. Above the frames, floating polypro line lead to a piece of syntactic foam and bucket lids, equipped with ROV-light reflecting tape. Together, the bucket-lids and foam make a reasonable sonar-target on the seabed for subsequent experiment location, Figure 6.

The UK RC01 biology team constructed and planned deployment of these experiments in three localities during RC01, two sites in AOI-2 and one site in AOI-3 as detailed in Figure 7 below. The design was created to enable the basalt blocks to sit as near to the surface of the seafloor as possible, with as minimum amount of corrosive materials and to allow easy ROV deployment and recovery.

Figure 6. Colonisation Experiments on deck pre-deployment.

The colonisation experiments were deployed successfully with the vessel's KystDesign 6000 m rated ROV. The ROV pilots were able to place them carefully on the seafloor in areas of reasonable nodule abundance and image them subsequently. The specific sites shown in Figure 8, Figure 9 and Figure 10 below.

The colonisation experiments are planned to be recovered for analysis in 2022 on the NERC 'SMARTEX' project (Principal Investigators: Dr Daniel Jones, NOC, Dr Adrian Glover, NHM).

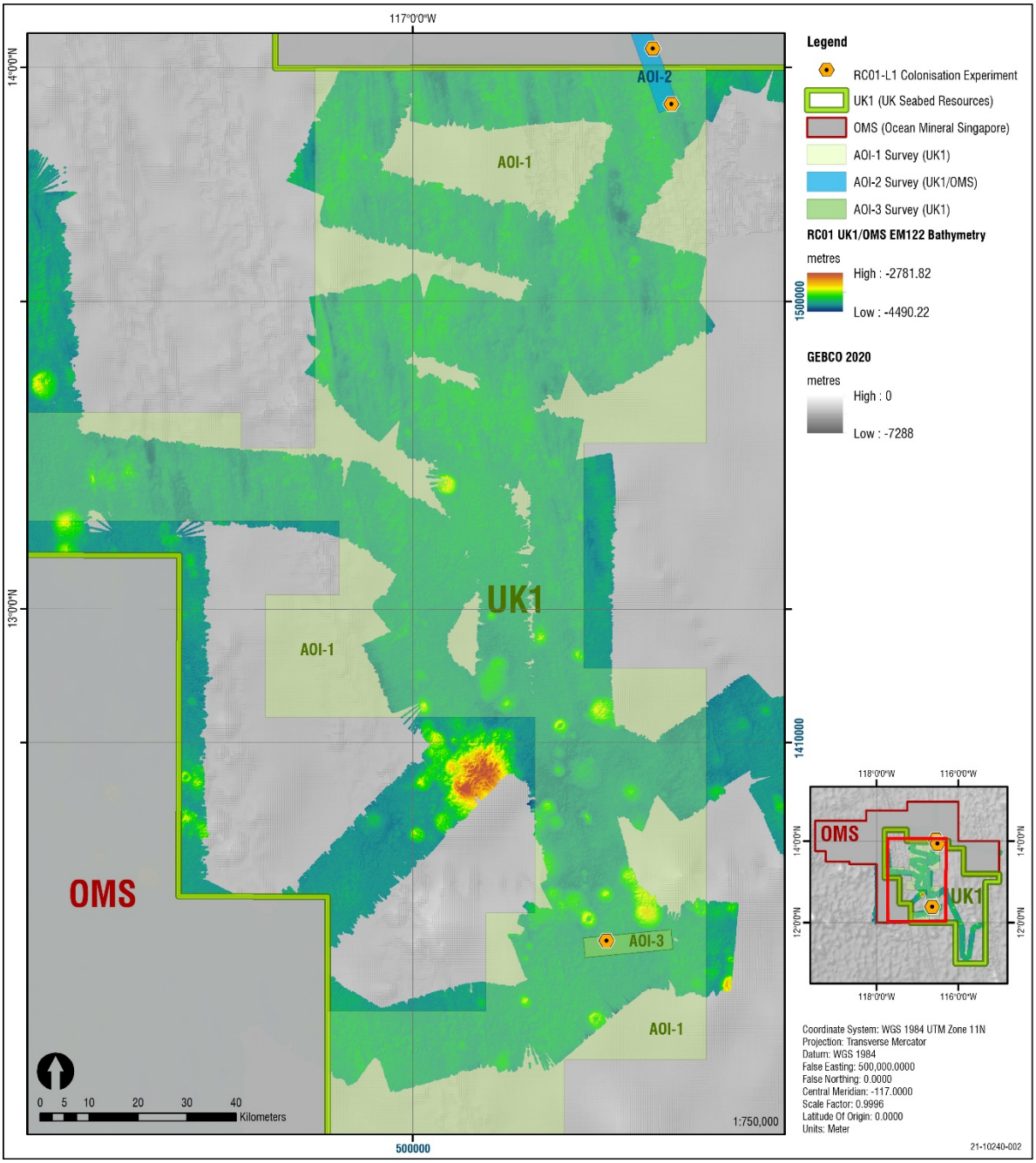

Figure 7. Deployment locations of RC01-wL1 colonisation experiments.

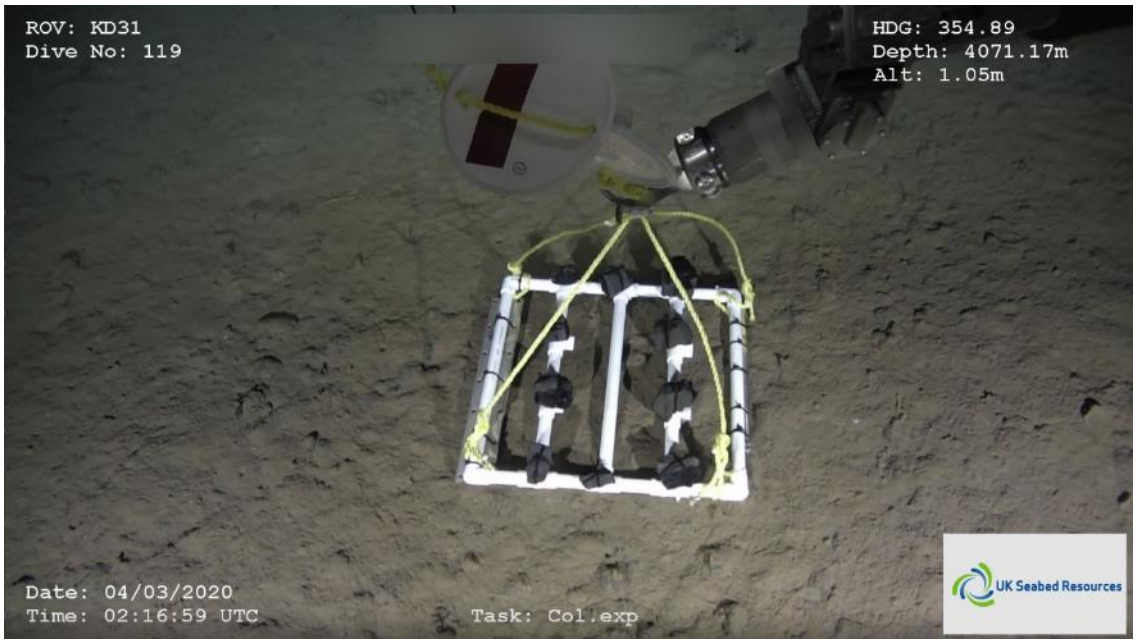

Figure 8: Colonisation Experiment #1 being deployed in OMS portion of AOI-2 survey area.

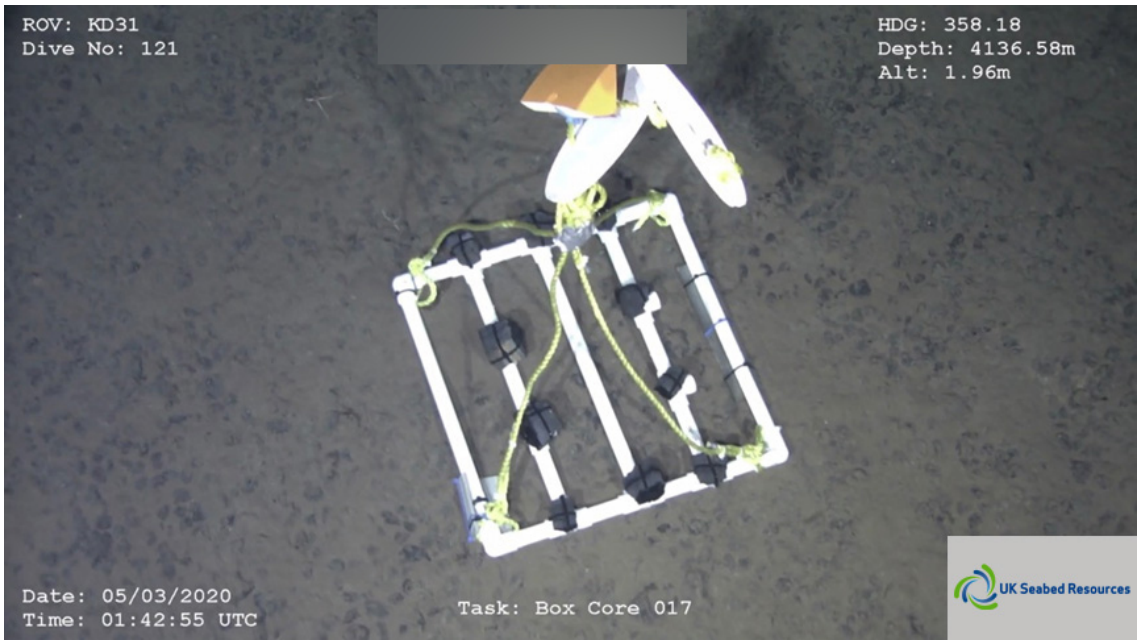

Figure 9: Colonisation Experiment #2 being deployed in UK1 portion of AOI-2 survey area.

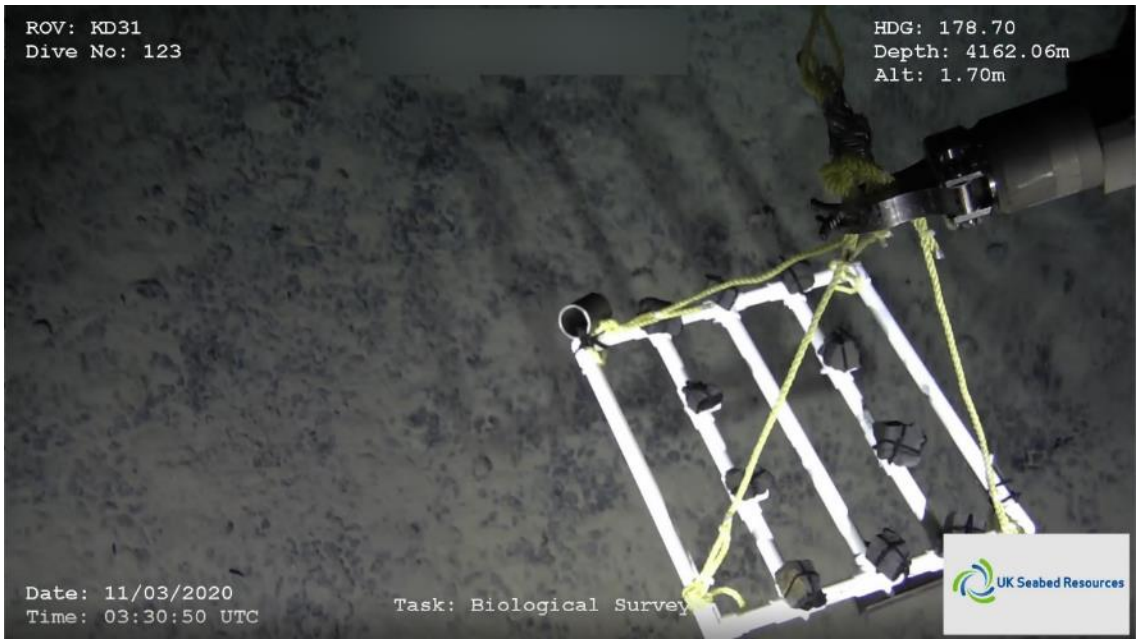

Figure 10: Colonisation Experiment #3 being deployed in UK1 AOI-3 survey area.

Results of Sample Recovery and Data Summary

Boxcore Results

RC01-L1 box core sampling for both SQ and FQ cores was very successful, from the 44 box cores examined (out of n=45, one box failed), 34 were processed for SQ sampling and 10 for FQ sampling (FQ's from the UK1 area only). In total, 1631 samples were collected, the majority of these being individually processed specimens in which a preliminary identification was attempted alongside imagery of key features. A total of 3958 images were taken of these specimens using 24 megapixel (MP) Canon EOS 800D macro 100 mm lens and Leica MZ7.5 also with a BEST scientific Canon EOS mount, Leica photo documentation tube and a second Canon EOS 800D directly mounted on the microscope as shown in Figure 11.

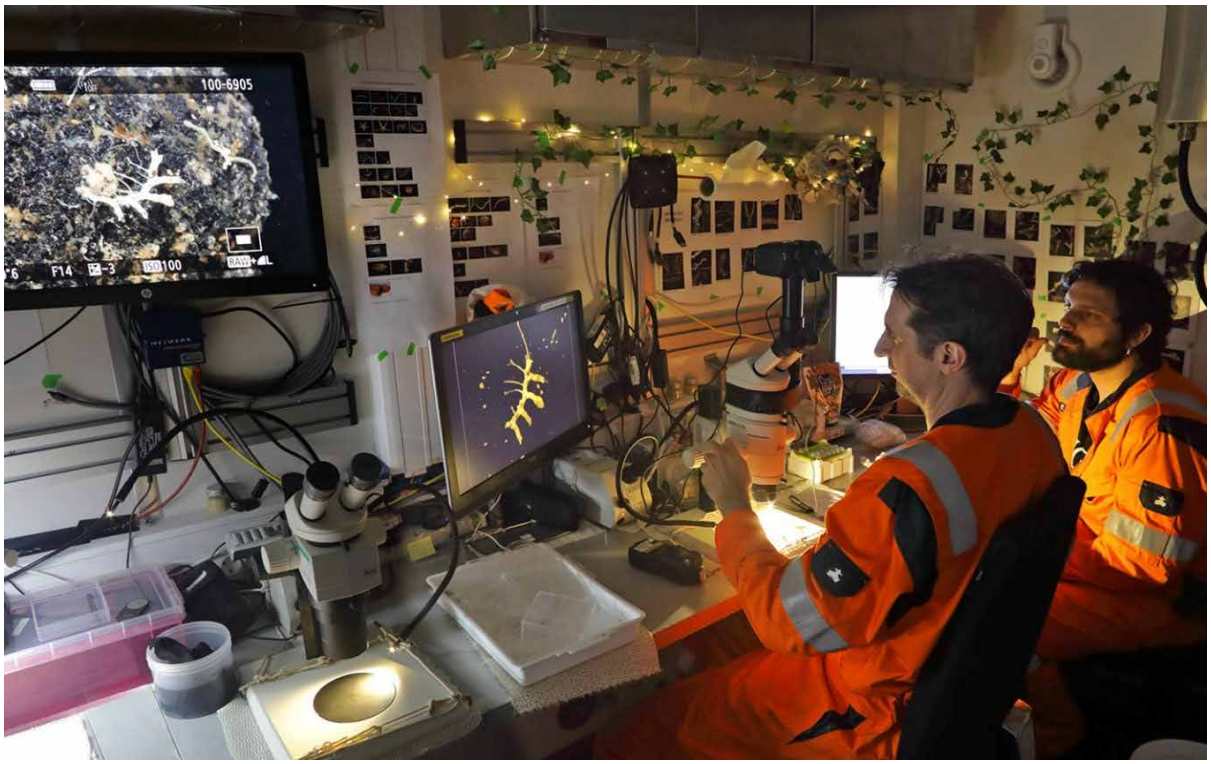

Figure 11. Specimen documentation with Canon EOS camera mounted on microscope.

UK1 box core Deployments

The distributed locations of the RC01-L1 Expeditions 20 box cores recovered on the in UK1 contract area are depicted on the enlarged chart of the AOI-1 survey area in Figure 12. The original plan was to have a distributed array of box core locations throughout the AOI-1 area but due to AUV downtime delays many of the planned box cores were dropped to accommodate remaining schedule. The resultant distribution of samples was reasonably spread in the northern sector of AOI-1 and a cluster in the south near AOI-3 but the mid-section of AOI-1 remains unsampled at this time.

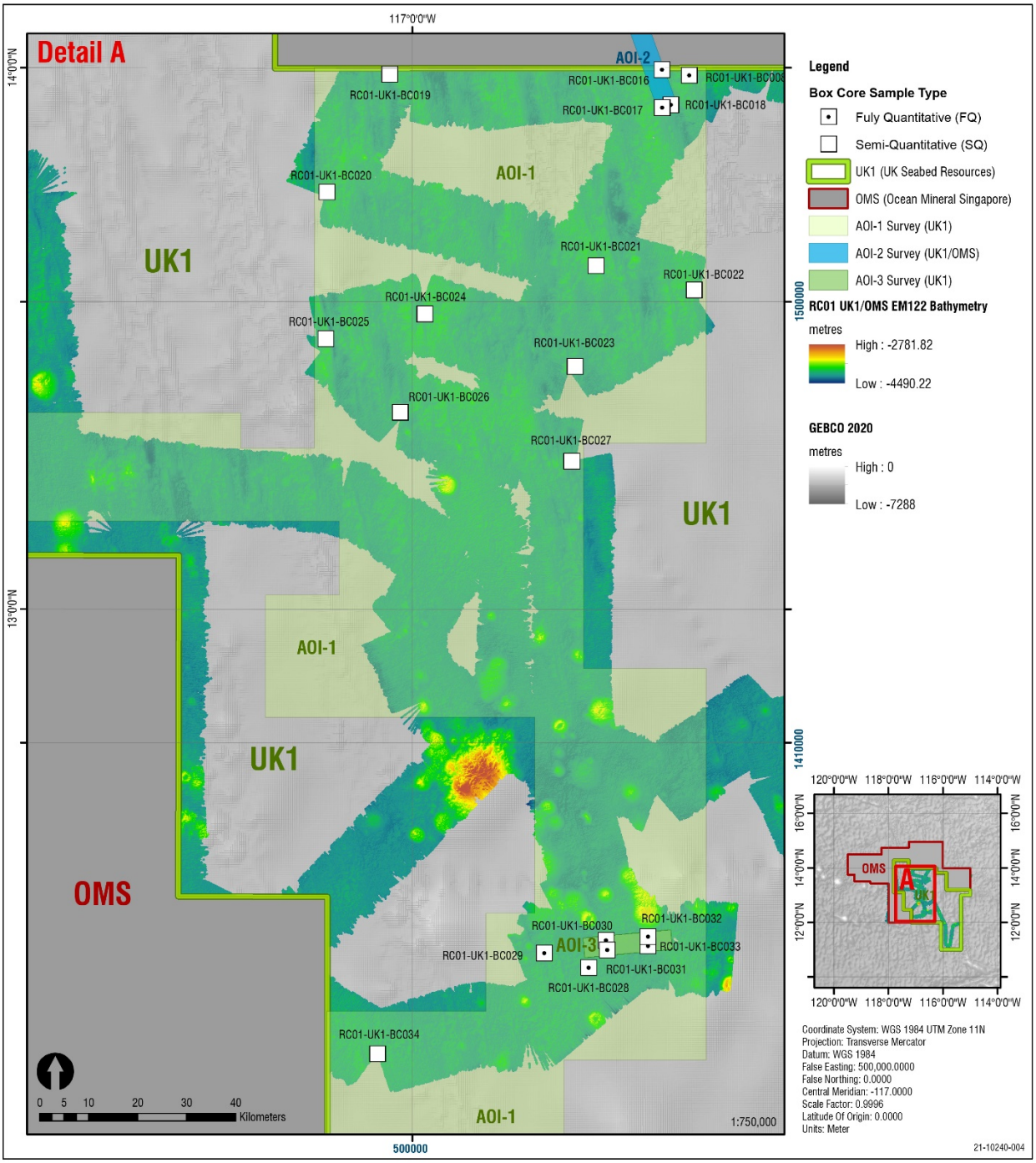

Figure 12. Overview of RC01-L1 box core sample locations and type within UK1 contract area.

Table 3 provides a summary of number of samples, number of images from all samples taken by the UKBio team on RC01-L1 and reside at the natural History Museum (UK). Macro images are 24 megapixel (MP) images of specimens taken with a 100 mm macro lens, Micro images are 24 MP images taken using a photomicroscope, of smaller fauna. This is a lower estimate of the number of images taken, as each specimen was often photographed 2-3 times and the database only records the best image. The light green shaded box core rows in Table 3 below are UK1 contract area box cores, the non-shaded rows are OMS box cores that the UKBio Team processed.

Table 3. Summary of collected samples and images from UKBio Team.

Sampler	# Samples	min. # Macro Images	min. # Micro Images
BC Total	1625	499	1378
BC001	18	12	27
BC002	45	11	40
BC004	37	8	30
BC005	61	25	51
BC006	38	5	37
BC007	34	3	24
BC008	44	25	23
BC009	14	2	14
BC010	38	13	33
BC011	29	3	28
BC012	18		18
BC013	38	4	31
BC014	13	1	16
BC015	41	15	39
BC016	36	17	28
BC017	54	20	24
BC018	27	11	11
BC019	23		22
BC020	42	15	37
BC021	30	12	29
BC022	62	23	54
BC023	33	5	28
BC024	27	4	23
BC025	25	9	25
BC026	52	13	44
BC027	34		30
BC028	64	26	46
BC029	31	19	28
BC030	22	12	16
BC031	37	15	31
BC032	82	30	52
BC033	61	22	47
BC034	54	17	47
BC035	18	5	14
BC036	22	7	22
BC037	18	2	17
BC038	25		25
BC039	48	9	41
BC040	45	11	37
BC041	27	13	25
BC042	24	4	21
BC043	62	15	52
BC044	27	11	24
BC045	45	25	67
Deck Total	3	3	
Deck	3	3	
ROV Total	3	4	
Dive119	1	1	
Dive121	2	3	
Grand Total	**1631**	**506**	**1378**

Preliminary identifications carried out during the live-sorting at sea phase of the SQ box cores (Glover et al 2016) permit at-sea initial analyses of data and community structure (Table 4, Figure 13 and Figure 14). These samples will form the basis of future analyses of biodiversity, biogeography and connectivity, all assessed with modern DNA-based integrative taxonomy (Glover et al 2018).

Table 4 documents the number of individual specimens of different phylogenetic groups collected by the UKBio team in the various areas of interest (AOIs). This sample set is complementary to that collected by the National University of Singapore (NUS) team during the RC01-L1 Expedition.

Table 4. Number of individual specimens of different phylogenetic groups collected by the UKBio team

	OMS				OMS Total	UK1				UK1 Total	Grand Total
Row Labels	**AOI-2**	**AOI-4**	**AOI-6**	**AOI-7**		**AOI-1**	**AOI-2**	**AOI-3**	**AOI-4**		
Annelida	57	53	107	148	365	211	28	113	23	375	**740**
Arthropoda	2	4	7	12	25	8	3	7	2	20	**45**
Brachiopoda			9	4	13	7		3		10	**23**
Bryozoa	22	8	36	39	105	62	29	60	7	158	**263**
Chaetognatha			4	4	8	2		1		3	**11**
Chordata		1	2		3	8	1	2	1	12	**15**
Cnidaria	12	18	49	70	149	44	20	71	5	140	**289**
Echinodermata	13	4	14	23	54	35	3	9	1	48	**102**
Hemichordata				1	1						**1**
Metazoa			3	9	12	4	2		1	7	**19**
Nemertea			1	2	3						**3**
Porifera				5	5		75	31		106	**111**
Priapula				1	1						**1**
Grand Total	**106**	**88**	**232**	**318**	**744**	**381**	**161**	**297**	**40**	**879**	**1623**

Images collected by the UKBio team taken with the Canon 100 mm macrophotography workstation. Image credit: A Glover, H Wiklund, L Bribiesca Contreras, E Simon Lledó

Figure 13. Sample images of the UK Biology Team live-sorted macrofauna and megafauna.

Images collected by the UKBio team taken with the Leica photomicroscopy workstation. Image credit: A Glover, H Wiklund, L Bribiesca Contreras, E Simon Lledó

Figure 14: Sample images of the UK Biology Team live-sorted macrofauna and megafauna.

Ten (10) box cores were processed as FQ samples by the UKBio team, four (4) in AOI-2 and six (6) in AOI-3 (Table 5). These were selected on the basis of proximity to proposed collector test sites. The 4 FQ samples in AOI-2 will be complemented by an additional four (4) samples taken in the same way by the NUS team in the OMS side of AOI-2. Thus, in AOI-2, eight (8) box cores are available now as additional baseline data pre-collector test. In addition, the location of AOI-2 close to the AB01 site UK1 Stratum A from 2013, will provide an additional twelve (12) box cores (Smith et al 2013) of baseline data, albeit it at a different time of year, bringing the total number of potential baseline box cores taken near AOI-2 to twenty (20), to support the upcoming UK NERC SMARTEX project work in the area.

During the RC01-L1 Expedition, the NUS team collected a total of 3849 live-sorted specimens from 44 box core samples from both the OMS and UK1 area, and many of these were preserved in either 96% ethanol or RNAlater and are thus suitable for molecular analyses. These specimens were photographed and sorted into major taxa Figure 15 during the expedition.

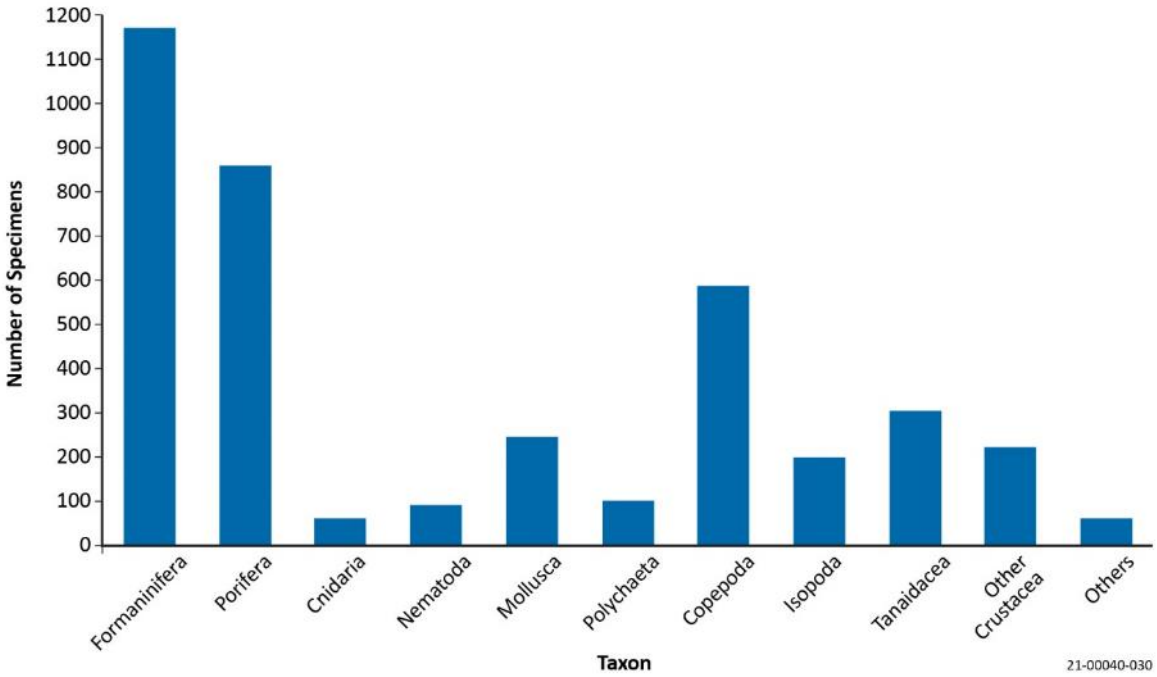

Figure 15. Total numbers of individually preserved specimens of animal groups collected from 44 box core samples

Table 5. Samples taken by the UKBio team for FQ processing.

Note that nodule FQ samples are higher as all nodules were live-sorted and fauna imaged and databased at sea, while the sediment fractions were bulk-preserved in 96% ethanol for DNA.

	UK1				Grand Total
BC/AOI	0-2 cm_FQ	2-5 cm_FQ	5-10 cm_FQ	Nodule_FQ	
BC008/AOI-2	1	1	1	23	**26**
BC016/AOI-2	1	1	1	15	**18**
BC017/AOI-2	2	1	1	18	**22**
BC018/AOI-2	1	1	2	7	**11**
BC028/AOI-3	2	1	1	16	**20**
BC029/AOI-3	2	1	2	14	**19**
BC030/AOI-3	1	1	1	13	**16**
BC031/AOI-3	1	1	1	15	**18**
BC032/AOI-3	1	1	1	16	**19**
BC033/AOI-3	1	1	1	23	**26**
Grand Total	**13**	**10**	**12**	**160**	**195**

ROV Imagery Results

Lead: National Oceanography Centre (UK)

Inspection of the video data obtained enabled the detection of a wide variety of megafauna taxa. From all the fauna observed in imagery, the most remarkable specimens encountered are listed in Table 6, along with the three specimens collected using scoping nets (an Actiniaria, a holothurian, and an ophiuroid). A video snapshot image was generated for each specimen listed in Table 6. The codes provided for each megafaunal morphotype correspond with those in a CCZ standardised megafauna catalogue, a taxonomic atlas previously used in image-based megafauna studies (e.g. Amon et al 2016; Simon-Lledo et al 2019) and in current application for regional synthesis studies (Simon-Lledo et al in prep). Ophiuroidea fauna, brittle-stars, were by far the most abundant taxa observed on the seabed among all dives. In turn, an increased presence of Alcyonacea taxa, soft corals, was observed in Dive 123 near the RC01-UK1-BC032 recovery site inside UK1 AOI-3 survey area. Examples of the diversity of taxa encountered are provided in Figure 16 and Figure 17.

Table 6. Megafaunal specimens observed in ROV HD video.

Observation	Taxonomic ID	Morphotype (CCZ std.)	Dive	Date	Task	Time (UTC)	Easting	Northing	Depth	Sample Image
Polychaete	Fam *Acrocirridae*	ANN_004	117	23/02/2020	Box Core 02	18:00:45	515067	1580034	4123	
Actiniaria (*4730)	undetermined	ACT_002	119	04/03/2020	UTP M13 Recovery	03:04:45	549034	1551620	4070	Figure 16a
Holothuroidea	*Synallactes* sp	HOL_007	119	04/03/2020	UTP M13 Recovery	04:08:40	549036	1551662	4072	
Alcyonacea	Poss. *Keratoisidinae* sp	ALC_035	119	04/03/2020	Box Core 13	01:52:56	549029	1551546	4065	Figure 16f
Ophiuroidea	Poss. *Ophiosphalma glabrum*	OPH_010	119	04/03/2020	Box Core 13	01:59:25	548925	1551362	4067	
Lizard fish	*Bathysaurus mollis*	OST_003	119	04/03/2020	UTP M13 Recovery	03:01:21	549066	1551737	4067	Figure 16n
Octopoda	*Grimpoteuthis* sp	MOL_010	119	04/03/2020	UTP M13 Recovery	02:01:35	549010	1551560	4067	Figure 17i
Holothuroidea	*Synallactes* sp	HOL_007	119	04/03/2020	UTP M13 Recovery	02:04:31	549034	1551598	4070	Figure 16j
Holothuroidea	*Mesothuria* sp	HOL_001	119	04/03/2020	UTP M13 Recovery	02:06:05	549054	1551624	4070	
Pennatulacea	*Umbellula* sp	PEN_003	119	04/03/2020	UTP M13 Recovery	02:07:04	549044	1551647	4070	Figure 16g
Ipnops fish	*Ipnops meadi*	OST_004	119	04/03/2020	UTP M13 Recovery	02:58:57	549036	1551568	4065	Figure 17n
Porifera	*Holascus spinosus*	HEX_014	119	04/03/2020	UTP M13 Recovery	03:00:37	549038	1551575	4066	
Ophiuroidea	*Ophiosphalma glabrum*	OPH_010	120	04/03/2020	Box Core 14	08:51:33	547177	1550909	4129	Figure 17j
Decapoda	*Cerataspis* sp	DEC_001	120	04/03/2020	Box Core 14	08:52:32	547172	1550895	4111	Figure 16i
Hydrozoa (jellyfish)	Fam Rhopalonematidae	HYD_004	121	05/03/2020	Box Core 17	01:25:26	552806	1540307	4135	Figure 17f
Brisingid	*Freyastera* sp	AST_002	121	05/03/2020	Box Core 17	01:44:16	552836	1540336	4153	Figure 17m

Observation	Taxonomic ID	Morphotype (CCZ std.)	Dive	Date	Task	Time (UTC)	Easting	Northing	Depth	Sample Image
Squat lobster	*Munidopsis* sp	DEC_021	121	05/03/2020	Box Core 17	01:45:58	552837	1540354	4136	Figure 16h
Holothuroidea	Fam *Elpidiidae*	HOL_020	121	05/03/2020	UTP M08 Recovery	01:46:18	552837	1540356	4136	
Porifera	*Holascus spinosus*	HEX_014	121	05/03/2020	UTP M08 Recovery	01:48:30	552831	1540367	4135	Figure 17d
Porifera	*Docosaccus maculatus*	HEX_015	121	05/03/2020	UTP M08 Recovery	01:50:59	552872	1540414	4135	Figure 16b
Porifera	Poss. *Hyalonema* sp	HEX_003	121	05/03/2020	UTP M08 Recovery	01:53:22	552838	1540436	4152	
Holothuroidea (*4735)	*Synallactes* sp	HOL_009	121	05/03/2020	UTP M08 Recovery	01:54:59	552821	1540415	4152	Figure 17l
Holothuroidea	*Mesothuria* sp	HOL_001	121	05/03/2020	Misc Sci Exp	01:57:13	552826	1540419	4135	Figure 16l
Squat lobster	*Munidopsis* sp	DEC_021	121	05/03/2020	Misc Sci Exp	01:58:12	552830	1540421	4136	
Actiniaria**	undetermined	undet (too far)	121	05/03/2020	Misc Sci Exp	01:59:03	552824	1540426	4136	
Ophiuroidea (*3700)	*Ophiosphalma glabrum*	OPH_010	121	05/03/2020	Misc Sci Exp	01:59:13	552824	1540426	4136	
Holothuroidea	Poss. *Benthodytes incerta*	HOL_041	122	05/03/2020	Box Core 18	07:26:51	551007	1539804	4084	
Porifera	*Cladorhiza* sp	DES_002	123	11/03/2020	Box Core 30	03:21:04	539586	1369597	4161	Figure 17b
Alcyonacea	Poss. *Keratoisidinae* sp	ALC_035	123	11/03/2020	Box Core 30	03:21:47	539581	1369591	4161	Figure 17g
Ophiuroidea	*Ophiosphalma glabrum*	OPH_010	123	11/03/2020	Box Core 30	03:22:19	539580	1369591	4161	
Antipatharia	*Abyssopathes lyra*	ANT_002	123	11/03/2020	Box Core 30	03:22:20	539580	1369591	4161	Figure 17h
Ophiuroidea	*Ophiuroglypha* sp	OPH_014	123	11/03/2020	Box Core 30	03:23:23	539585	1369577	4177	Figure 16a

Observation	Taxonomic ID	Morphotype (CCZ std.)	Dive	Date	Task	Time (UTC)	Easting	Northing	Depth	Sample Image
Alcyonacea	Poss. *Bathygorgia* sp	ALC_004	123	11/03/2020	Box Core 30	03:23:45	539586	1369576	4161	
Porifera	Poss. *Docosaccus nidulus*	HEX_016	123	11/03/2020	Box Core 30	03:23:57	539585	1369575	4177	Figure 16a
Asteroidea	Fam Pterasteridae	AST_031	123	11/03/2020	Box Core 30	03:24:01	539585	1369575	4177	Figure 16m
Alcyonacea	Poss. *Bathygorgia* sp	ALC_004	123	11/03/2020	Box Core 30	03:24:56	539593	1369557	4161	
Actiniaria	undetermined	ACT_071	123	11/03/2020	Box Core 30	03:25:57	539589	1369541	4161	Figure 17e
Porifera	*Caulophacus* sp	HEX_008	123	11/03/2020	Box Core 30	03:27:07	539584	1369518	4161	Figure 17c
Porifera	undetermined	POR_002	123	11/03/2020	Box Core 30	03:27:19	539583	1369517	4162	Figure 17a
Rat-tail fish	*Coryphaenoides* sp	OST_005	123	11/03/2020	Box Core 30	03:27:49	539578	1369519	4178	Figure 17o
Holothuroidea	*Benthodytes incerta*	HOL_041	123	11/03/2020	Science	08:58:21	539729	1367631	4160	Figure 17k
Holothuroidea	*Synallactes* sp	HOL_008	124	11/03/2020	Science	08:59:35	539704	1367619	4161	Figure 16k
Actiniaria	undetermined	ACT_010	124	11/03/2020	Science	09:06:42	539711	1367603	4160	Figure 16e
Porifera	undetermined	POR_057	124	11/03/2020	Science	09:08:24	539700	1367586	4160	Figure 16c
Actiniaria	undetermined	ACT_004	127	11/03/2020	Biological survey	23:03:22	548079	1370347	4187	

(*) Scooped and successfully recovered; physical sample ID provided. (**) Scooped but not found on deck

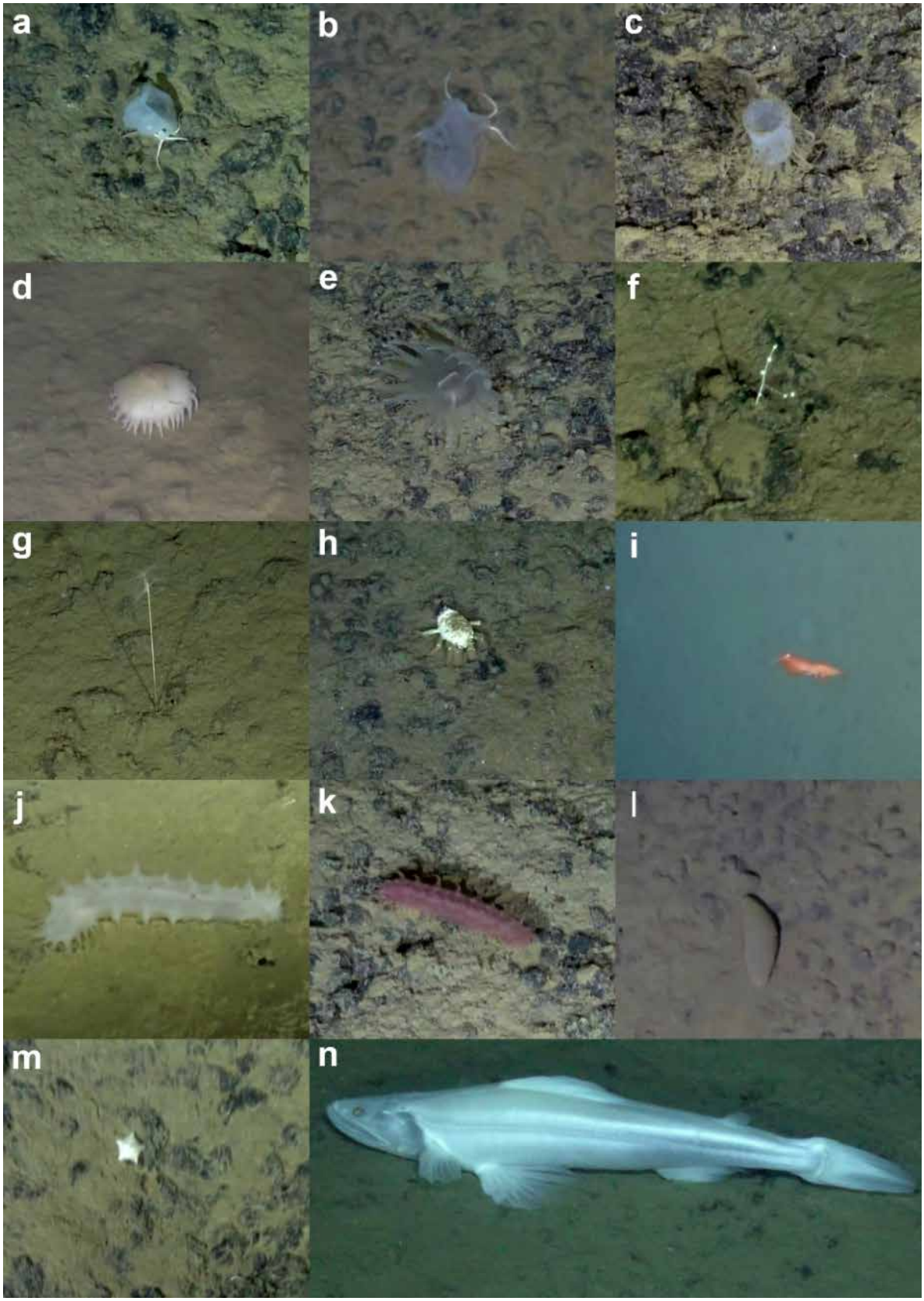

Figure 16. Examples of the megafaunal diversity observed during RC01-L1 opportunistic ROV operations.

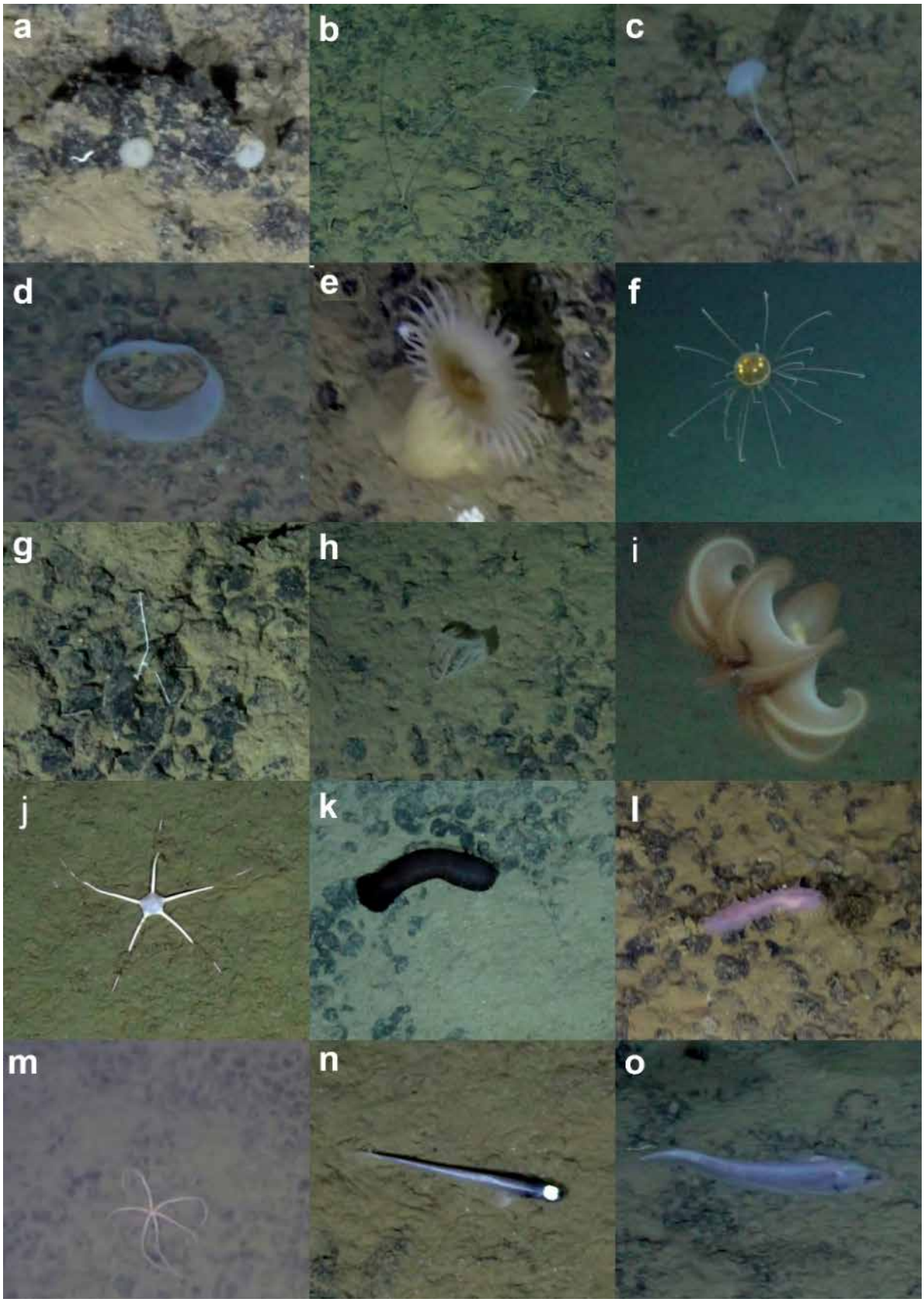

Figure 17. Examples of the megafaunal diversity observed during RC01-L1 opportunistic ROV operations.

Transfer of RC01 Samples to Research Institutions

In March 2020, 40 megafauna specimens from the ABYSSLINE AB01 (2013) and AB02 (2015) survey cruises were packed and shipped from the University of Hawai'i by Dr Diva Amon. This was completed as they were not currently stored in appropriate conditions and require a long-term archive centre. CCZ megafauna specimens are very rare and significant. The samples were received at NHM, the collection was checked, then transfer of the specimens to suitable temporary storage was accomplished just prior to museum lockdown. These specimens have now undergone preliminary curation and are in the process of being formally accessioned into the NHM main spirit collections. A few representative specimens from the collection will potentially be included in an upcoming museum special exhibition. NHM data systems are being updated to link these new specimens to existing records from the previously acquired tissue samples.

Environmental Baseline Status and Recommendations

Physical Oceanography

characteristics of water column and near-bed currents, including current speed and direction, temperatures, turbidity at different water depths, as well as any hydrodynamic modelling

UKSR plans to investigate these requirements as part of the forthcoming SMARTEX programme, with moorings deployed in 2022 and data analysed in 2023.

Chemical Oceanography

characteristics of sea water, including pH value, dissolved oxygen, total alkalinity, nutrient concentrations, dissolved and particulate organic carbon, estimation of mass flux, heavy metals, trace elements and chlorophyll a

UKSR plans to investigate these requirements as part of the forthcoming SMARTEX programme, with expeditions in 2022 and 2023.

Data that have already been partially analysed include vertical profiles using the shipboard CTD rosette at UK1-AB01 (2013) Survey Cruise Stations A, B, and D, which revealed that the water column within the sampling site was typical for this region. Warm (~29ºC) temperatures in the near-surface waters resulted in a thermally-stratified upper ocean, with mixed layer depths restricted to <30 m at all three stations, with a stable deep thermocline extending to ~300 m. Temperatures in the lower mesopelagic waters (300-1000 m) ranged between ~4-10ºC, decreasing steadily with depth, while temperatures in the deep bathypelagic waters (2000-4000 m) decreased from ~4ºC to ~1.5ºC near the sediment boundary. Concentrations of dissolved oxygen were typical for this region, reflecting the westward subduction of low O_2 waters. Below the surface mixed layer where O_2 equilibrates with the atmosphere, concentrations of dissolved oxygen decreased rapidly with depth, reaching concentrations <1 µmol L-1 by 100 m. The anoxic region of the water column extended as deep as ~800 m, below which concentrations sharply increased, exceeding 140 µmol/L in the cold, bathypelagic waters. Water samples were collected from eight depths (5, 150, 300, 500, 1000, 2000, 3000, and ~4100 m).

Biological Communities and Biodiversity Studies

including megafauna, macrofauna, meiofauna, microflora, nodule fauna, demersal scavengers and pelagic communities

Near-bottom Zooplankton

Marine meta-zooplankton (not including meroplankton such as benthic invertebrate larvae) consist of approximately 7,000 species in 15 phyla and can be found in all ocean basins in the entire vertical and horizontal range of the water column. Despite centuries of scientific sampling and programmes, such as the Census of Marine Zooplankton, there are still uncertainties about species identification, biogeography, biodiversity and abundances of zooplankton in certain regions of the world including the deep sea (Bucklin *et al*., 2010). However, knowledge about the structure and health of the abyssal bentho-pelagic zooplankton

community including diversity, abundance, and temporal or spatial distributions is essential prior to anthropogenic disturbances.

At abyssal depths, the bentho-pelagic zooplankton community mainly consists of copepods (50% of total abundance), isopods, chaetognaths and ostracods, and can reach total abundances of 0.021 to 10 individuals m-3 (Christiansen *et al*., 1999; Christiansen *et al*., 2010; Wishner, 1980; Homma and Yamaguchi, 2010; Beckman, 1988). Within the subclass Copepoda, the orders Calanoida and Cyclopoida are the most abundant, with Metridia spp., Clausoclanidae, and Oncaeidae being the most dominant families (Christiansen *et al*., 1999; Christiansen *et al*., 2010; Wishner, 1980; Homma and Yamaguchi, 2010; Beckman, 1988). It has also been suggested that the zooplankton community structure changes with increasing height above bottom. The abyssal bentho-pelagic zooplankton in the Pacific, Indian, and Southern Oceans are particularly poorly studied (Christiansen *et al*., 1999; Christiansen *et al.*, 2010; Wishner, 1980; Homma and Yamaguchi, 2010; Beckman, 1988).

Less is known about benthic invertebrate larvae, which are a part of the bentho-pelagic zooplankton community at abyssal depths. Only a few studies have succeeded in sampling meroplanktonic larvae, such as polychaete or crustacean larvae. Within these studies, they reach densities of zero (none sampled) to 0.0039 m^3 suggesting that larval abundances are 1-2 orders of magnitude lower than overall bentho-pelagic zooplankton abundances (Christiansen *et al*., 1999, Wishner, 1980). More data about the meroplankton are needed because the resiliency of a benthic population to a disturbance partially depends on the ability of larvae to disperse among suitable habitats. Therefore, the presence or absence of benthic invertebrate larvae and ultimately the connectivity of benthic populations determines recolonization abilities of an ecosystem after a disturbance event (Cowen and Sponaugle, 2009).

In general, the abyssal bentho-pelagic zooplankton community in AB01 (2013) consisted of several different taxa, but was highly dominated by copepods, making up 56.19 ± 6.61% of all organisms. Other taxa such as chaetognaths, polychaetes, or gastropods had a relatively low abundance, each reaching not more than 1.5% in relative abundance. Benthic invertebrate larvae were even less abundant (<1%), and only a few polychaete larvae were sampled. In addition, an unexpectedly high proportion of nauplii were collected, which currently are listed in a single operational taxonomic unit (OTU). This OTU reached a relative abundance of 13.34 ± 3.58 %, which is the third highest observed. Difficulties in assigning the nauplii to adult taxa prohibited further classification. The second highest relative abundance observed belonged to the OTU "Molt/Fragment". This category consisted of empty exoskeletons of adult copepods, copepodites, and nauplii. Distinction between molts and fragmented carcasses (dead because of sample method) was not possible and all individuals were categorized for convenience into a single OTU. The copepod community itself was dominated by the two OTUs l and m, which were responsible for 31.78 ± 7.80 % and 24.77 ± 2.74 % of all copepods counted. OTUs a, c, and e, were sub-dominant (relative abundance between 6-15 %), while all other families had very low abundances (<4 %).

Samples from free-vehicle plankton pumps showed little variation in meroplankton diversity and abundance over scales of 30–100 km for time scales of days to weeks. However, sediment-trap samples revealed high temporal variability in vertical flux over weeks to months. Larval abundances and fluxes measured in the abyssal CCZ are ~ 1–2 orders of magnitude lower than observed at deep-sea ridge and hydrothermal-vent habitats. We found significantly higher downward larval fluxes at 11 m above the bottom (mab) than at 146 mab, indicating accumulation or retention of meroplankton within the Benthic Boundary Layer (BBL).

Benthic Communities

Community respiration and nutrient uptake

Benthic lander experiments were conducted during AB01 (2013) and AB02 (2015) surveys. The initial objectives were to measure seafloor respiration (O_2 consumption and CO_2 production) using an autonomous benthic lander. During the AB01 cruise, a total of four lander deployments were carried out in the UK1 Claim Area. Unfortunately, due to experimental problems, no useful data were obtained from the first two deployments. The second two deployments were designed to measure nutrient uptake by injecting algae labelled with heavy stable isotopes (^{13}C) into the surface sediments within benthic chambers pushed into the sediments (termed a

"pulse-chase" experiment). After an incubation time of 36 hours, the chambers were removed from the sediment with the sediments sealed inside, and the lander was recalled to the surface for extensive processing and analysis. Bacteria dominated the short-term uptake of fresh phyto-detritus, consuming 98.8% ± 0.04% (n=4, SE) of the 1.39 ± 0.26 mg C m-2 (n=4, SE) that was assimilated over the ~1.5-day experiment.

Bacterial assimilation of tracer organic matter was clear both in surface and subsurface sediments, highlighting active microbial populations to 5 cm sediment depth. Surprisingly, even though the biomass of bacterial population was only 7% of that at 4,800 m in the abyssal NE Atlantic (Witte et al., 2003), uptake of organic matter by bacteria in the NE Atlantic was 15% of that found by us during the AB01 cruise using the pulse-chase approach. These results clearly show that organic matter entering the seafloor is rapidly assimilated and respired and identify the key role of bacteria in this system.

Bacteria again dominated the short-term uptake of fresh phyto-detritus in the pulse-chase experiments conducted during AB02, consuming 98.5 ± 1.2 % (n=4, SE) of the 0.81 ± 0.14 mg C m-2 (n=4, SE). Thus, the study supports the hypothesis that bacteria dominate the short-term consumption of fresh organic matter at the abyssal seafloor of the CCZ. Because similar responses were observed by the abyssal benthic community at the two study areas that were separated by more than 200 km, it is possible that these results can be generalised over large spatial scales in the central abyssal Pacific Ocean and may possibly be characteristic of other abyssal habitats in the Pacific.

UKSR aims to conduct additional lander experiments, including both measurements of community respiration and nutrient uptake, in completing the environmental baseline survey for UK1. In addition, any changes in benthic ecosystem functioning resulting from mining are likely to alter the ecosystem functions, such as nutrient regeneration, carbon burial (and long-term geological storage), and the dissolution of calcium carbonate, which are all driven by processes such as benthic respiration and carbon turnover (Smith et al., 2008, Wenzhofer et al. 2001). The SMARTEX programme aims to utilise ROV-deployed landers on and adjacent to test miner seabed disturbances (created by Ocean Minerals Company tests in 1978-9) to estimate long-term impacts on these basic ecosystem-function parameters.

The Friday Harbor Workshop (Clark, M., Smith C.R., et al, 2019, Deep CCZ Biodiversity Synthesis Workshop) concluded the following:

- *"In situ POC flux measurements (from sediment traps and/or SCOC) are needed from each APEI and adjacent contract areas to compare particle flux and SCOC in space and time.*
- *Temporal variability of SCOC and other ecosystem functions are needed; most studies encompass measurements made over hours to days.*
- *There are little or no data available for most benthic ecosystem functions (e.g., bioturbation, calcite dissolution) across the CCZ."*

Microbes

Abyssal ocean sediments, comprising 32 x 107 km^2 (>50% of Earth's surface area), constitute some of the largest habitable spaces on the planet. These deep-sea ecosystems are chronically under-sampled, owing to their enormous size and the numerous technical challenges associated with sampling of these remote environments. As a result, our knowledge of biological diversity in these systems is limited. Sediment-dwelling microorganisms constitute the largest component of biomass in the abyssal seabed, with abundances often >5 x 108 cells per gram of sediment (Jørgensen and Boetius, 2007), several orders of 40 magnitude greater than the overlying water. Despite relatively large population sizes, our knowledge of the diversity of deep-sea sediment microbes remains rudimentary.

Although historically considered spatially and temporally homogenous environments, numerous biotic and abiotic factors introduce heterogeneity into these habitats and influence the resulting biodiversity of the seabed. The energetic demands of sediment-dwelling biological communities are ultimately met by material fluxes delivered to the seabed from the overlying water column, thereby linking spatiotemporal variability associated with sediment organisms to the community structure and metabolism of the planktonic community. Respiration by benthic organisms, variation in sediment porosity (a function of grain

morphology and composition), near-bottom currents, and the abundance of burrowing fauna all impact concentrations and diffusion of oxygen into the sediments, resulting in microbially-catalysed vertical gradients in electron donors and acceptors. Moreover, variations in sediment substrate, including the presence of polymetallic nodules, constitute additional sources of physical and chemical complexity and habitat heterogeneity in the abyssal seafloor.

Studies of microbial diversity have benefitted from technological advances in nucleic sequencing, together with improved computational analyses of these sequences. Differentiating microbes based on phenotypic traits has long proved challenging, hindered by poor cultivation successes of these organisms and stemming from the lack of morphological characteristics distinguishing these organisms. Utilisation of DNA as a microbial biomarker has revolutionised our understanding of microbial diversity and function. In particular, the conserved nature of ribosomal RNA genes enables phylogenetic reconstruction of evolutionary relationships among organisms, providing the basis for the classification of three domains of life, Bacteria, Archaea, and Eukarya (Woese and Fox, 1977).

Studies on microbial diversity in deep-sea sediments are relatively sparse. Based on initial cloning and sequencing of relatively few (typically 100s) of PCR amplified 16S rRNA genes from sediments, a few general patterns have emerged regarding bacterial community structure in deep-sea sediments. In particular, for those regions where the sediment receives relatively high organic matter input (typically these are relatively shallow (<1000 m), shelf and slope environments), the upper surficial sediment layers are often dominated by relatively few phyla of presumed organoheterotrophs, including the -Proteobacteria *Pseudoalteromonas*, *Pseudomonas*, and *Oceanospirillum* (Llobet-Brossa *et al*., 1998; Bowman and McCuaig, 2003). The deeper, anoxic strata of the sediments often harbour an increasingly phylogenetically and metabolically diverse group of microorganisms, including those closely related to cultivated sulphur and iron reducing bacteria (Li *et al*., 1999; Shauer *et al*., 2010). While these previous studies have provided an initial framework for evaluating microbial diversity in deep-sea sediments, all were based on analyses of relatively few sequences, and hence provided limited information for assessing microbial biodiversity in the abyssal sea.

The research evaluated microbial diversity within four vertical strata (between 0-10 cm) at 11 discrete sampling locations as part of the AB01 research cruise. At two of the sampling stations, sediment samples were collected from a seabed experiment using a respirometer lander. Using high throughput, next generation sequencing of polymerase chain reaction amplified 16S ribosomal RNA (rRNA) gene fragments, we sought to obtain baseline information on microbial community structure and diversity within the sampling region. The resulting sequence data constitutes one of (if not the) largest inventories of microbial diversity in the abyssal sediments, and provides important new information on spatial structure of microbial communities in the CCZ.

The study obtained more than 15 million sequences from 140 samples, providing a wealth of novel information on microbial community structure of the abyssal ecosystem in this region. Bioinformatic analyses of the resulting sequences revealed a large number of phyla previously described associated with deep-sea sediments, including members of *Thaumarchaea; Proteobacteria; Bacteroidetes*, and *Nitrospirae*. Members of these broad classes and phyla of microorganisms include obligate or facultative chemolithoautotrophs, obtaining energy and reducing power from the oxidation of reduced inorganic substrates (NH_4^+, H_2S, S, and H_2) to catalyse fixation of inorganic carbon (CO_2) into cellular biomass. Such results likely reflect the relatively low organic matter input to the abyssal seabed in this region.

Statistical analysis of the sequences revealed significant differences among sequences collected from the different habitats (sediment, water column, and nodules) within the study site. Water column samples were distinct from the sediment and nodules, supporting the hypothesis that sediment microorganisms are autochthonous to this habitat, rather than being transported to the sediment from the overlying water.

Moreover, the microorganisms varied significantly within the discrete vertical strata sampled within the sediments, with microorganisms dwelling in the upper 5 cm of the sediment differing from those sampled from the lower sediment core (8-10 cm). These analyses further highlighted significant differences between

those microorganisms associated with nodules relative to the surrounding sediments, suggesting unique phyla of microorganisms exploit the chemical or physical environment provided by the nodule.

We examined bacterial communities using high-throughput sequencing of bacterial 16S rRNA gene fragments from samples collected in the water column, sediment, and polymetallic nodules in the Pacific

sampled from each megacorer, except for MC01 (CRS1494) and MC02 (CRS1501), which had one and five cores respectively. In total, they obtained 78 samples for quantitative analysis of meiofauna, 22 samples for qualitative, molecular analysis of crustaceans (two horizons per core), 10 samples for abiotic analysis of sediment parameters and 40 nodule samples. In the laboratory, the meiofauna was extracted from each core and hand-sorted into major taxa under a stereomicroscope. The material was used for genetic, systematics, chorology, and faunistic investigation, such as similarity and diversity analyses.

The meiobenthic taxa present in the samples are common representatives of deep-sea sediments. Average densities in the sediment surface range from 0.01 Ind./10 cm^2 in *Amphipoda* and *Priapulida* to 255 Ind./10 cm^2 in *Nematoda*, which comprise more than 90% of the total meiofauna abundance. Nematodes are the most abundant meiofaunal taxon in deep-sea sediments, followed by harpacticoid copepods, annelids, kinorhynchs, ostracods, gastrotrichs, loriciferans and tardigrads. Preliminary analysis suggested no general pattern for meiofauna abundances at different stations, except for at station C (MC03 or CRS 1506), where mean abundances are conspicuously lower than calculated for the other sampling locations.

A total of 707 complete stained specimens of foraminifera (including 167 that were indeterminate) and 259 stained fragments were found in the MC02 (CRS1501) core; the corresponding numbers for the MC04 (CRS1515) core were 574 complete (233 indeterminate) and 144 fragmentary specimens. The more 'advanced' multichambered taxa represent only about 9–10% of the assemblage and comprise mainly calcareous rotaliids (2–3%) and agglutinated hormosinaceans (4–5%), mainly species in the genera *Reophax* and *Hormosinella*. The most abundant rotaliid was *Nuttallides umbonifera*, a species typical of abyssal depths close to the Carbonate Compensation Depth (CCD) (Mackensen et al., 1995). The assemblages are dominated instead by poorly known monothalamous foraminifera (monothalamids), representatives of 'primitive' groups that branch at the base of the foraminiferal phylogenetic tree, together with the komokiaceans. Among the monothalamids, tubular and other elongate (e.g., spindle-shaped) morphotypes are particularly abundant (~30% of complete specimens in the MC02 sample), but also particularly prone to fragmentation (they represent almost 70% of all fragments in MC02).

The affinities of the komokiaceans (~9% of complete specimens and 25–42% of fragments) are unclear, although this rather poorly-defined group probably belongs with the monothalamids (Gooday et al., 2007). MC02 (CRS1501) and MC04 (CRS1515) both yielded 115 stained species, of which at least some individuals were complete. More than half of the species were represented by 1 or 2 individuals. An additional 14 and 11 species were represented only by fragments to give total species counts of 129 (MC02) and 126 (MC04). When the two samples were combined the number of 'complete' species increased to 181 and the number of 'fragmentary' species increased to 17 to give a total of 198 stained species. Of these, 157 were monothalamids. Other species were represented only by dead specimens – 27 in MC02 and 33 in MC04, combined total 42. It is likely that all the calcareous and many of the multichambered agglutinated species, with the exception of some of the hormosinaceans, have been formally described. The monothalamids and komokiaceans, on the other hand, are poorly known and most of the species are certainly new to science.

Substantial examination of foraminifera (testate protists) was carried out during AB02, including 'live' (Rose Bengal stained) and dead tests, in 5 cores (0–1 cm layer, >150-
megacorer deployments inside a 30 by 30 km seafloor area. In both categories (live and dead) we distinguished between complete and fragmented specimens. The outstanding feature of these assemblages is the overwhelming predominance of monothalamids, a group often ignored in foraminiferal studies. These single-chambered foraminifera, which include agglutinated tubes, spheres and komokiaceans, represented 79% of 3,607 complete tests, 98% of 1,798 fragments and 76% of the 416 morphospecies (live and dead combined) in our samples. Only 3.1% of monothalamid species and 9.8% of all species in the UK1 assemblages are scientifically described and many are rare (29% singletons). The results emphasise how little is known about foraminifera in abyssal areas that may experience major impacts from future mining activities.

Besides foraminifera, other meiofauna, such as nematodes, remain largely under-sampled in the CCZ. The Friday Harbor Workshop noted (p. 14) that:

"There is a major lack of taxonomic work (e.g., species descriptions, barcoding, integration of working species across programs) that would allow comparison of species distributions over wider geographical scales."

Foraminifera attached to nodules

At sea, the ABYSSLINE team recorded around 66 protistan (presumably foraminiferan) morphospecies, based on test characteristics, during the preliminary examination of pristine nodules recovered during boxcorer and megacorer deployments. Following the cruise, they examined six of these nodules in detail and recognised a total of 65 protistan morphospecies. The only obvious metazoans were two sponges. Many of the protistan morphotypes are mat-like formations, clusters of patches, isolated domes, or tubular structures, the latter either lying horizontally on the nodule or extending upwards from the surface. Others are recognisable as komokiaceans. The above-mentioned groups represent 'basal' (i.e. 'primitive') forms. Two komokiacean morphotypes can be assigned to described species (*Chondrodapis hessleri* and *C. integra*), and a few others represent known genera (Baculella, Lana/Reticulum). Other basal morphotypes belong in the genera Saccorhiza, Telammina and Tumidotubus, but the remainder are probably completely undescribed.

More 'advanced' multichambered foraminifera are much less common on nodules. They include the calcareous genus *Cibicides*, the agglutinated genus *Hormosina* and occasional trochamminacean and several orange-coloured agglutinated tests, possibly *Placopsilina*.

Many of the nodule-encrusting morphospecies observed during the cruise also occurred on the nodules that we examined in detail. However, at least 21 of those recognised on the ship were not present on these six nodules. The total number of encrusting protistan species is therefore at least 86, although this minimum estimate will certainly rise as more nodules are subject to detailed scrutiny. An important caveat is that it is often difficult to determine whether the organisms were dead or living when collected. This applies particularly to many of the mat-like formations and systems of anastomosing tubes, which are dark grey in colour and possibly filled with decayed stercomata. The yellow branching tubes (tentatively identified as *Saccorhiza*), present on most nodules, are usually empty and often partly abraded. On the other hand, the calcareous *Cibicides* species is typically represented by living specimens.

Some of the nodule-encrusting forms recognised in the ABYSSLINE material have been found elsewhere. *Telammina* and *Tumidotubus* were first described from the NE Atlantic (Gooday and Haynes, 1983) and *Telammina* has been observed growing on a variety of substrates in the Atlantic, Pacific and Indian Oceans (Aranda da Silva and Gooday, 2009; Gooday, unpublished observations). The yellow *Saccorhiza*-like tubes have been widely reported from Pacific nodules. Others are recognisable as komokiaceans.

Gooday, Goineau and Voltski (2015) reported on encrusting organisms on seven polymetallic nodules from the eastern Clarion Clipperton Fracture Zone (CCFZ, 4070 m water depth, eastern equatorial Pacific). Apart from occasional sponges and a single bryozoan, all the organisms were foraminifera or foraminifera-like protists. A total of 75 morphotypes (presumed to be morphospecies) was recognised, with between 9 and 19 being present on individual nodules. Additional species were observed during shipboard examination of the nodules, bringing the total number of species to 86. The assemblage was dominated by a variety of mat-like formations, clusters of patches, isolated domes, broad trails, anastomosing networks and branched or unbranched tubular structures that either lay flat against the nodule surface or projected away from the surface.

These forms were interpreted as monothalamous foraminifera (monothalamids). Most have mainly agglutinated walls, but a few are predominately organic. Some can be assigned to the Komokiacea (notably the genus *Chondrodapis*) or families such as the Hemisphaeramminidae ('domes'), while others (e.g. many of the mats and patch-like forms) are difficult to place into existing monothalamid groupings. Some of the branching and anastomosing tubes resemble the genus *Rhizammina*. The most easily recognisable morphotypes include Telammina, in which tiny chambers are linked by extremely thin tubes to form a network, and sinuous orange tubes that incorporate sponge spicules and can be assigned to the genus *Saccorhiza* based on the occasional presence of a proloculus.

Polythalamous foraminifera are also fairly common. They include various calcareous species (mainly *Cibicides* spp.), as well as agglutinated forms such as *Hormosina*, *Placopsilina* and trochamminaceans. Similar assemblages, including some morphospecies that are clearly identical to ours, have been described previously from somewhat deeper sites (4500–5000 m) in the CCFZ. In order to explore distributions at a global scale, we compared our Pacific nodule assemblages to foraminifera attached to ice-rafted dropstones from several deep seamounts in the abyssal northeast Atlantic Ocean, mainly on the Porcupine Abyssal Plain (4630–4680 m depth) with additional material from the BIOTRANS area (3796–4351 m). These hosted superficially similar assemblages of mats, tubular forms, komokiaceans and polythalamous calcareous and agglutinated foraminifera. However, apart from the extensive development of Telammina networks, there were no morphospecies in common between the Pacific and Atlantic assemblages. These preliminary observations, based on limited material, suggest that most of the foraminiferal morphospecies encrusting hard substrates are widely distributed at regional scales in the abyssal Pacific but not necessarily at global scales. The study of abyssal encrusting assemblages poses considerable challenges. Priorities for the future include the development of reliable methods for distinguishing living and dead individuals, and molecular approaches to clarifying the taxonomic affinities of novel morphotypes.

***Recommendation**: Further field collections of these fauna might not be necessary to complete the impact analysis; the work completed to date on these groups is top notch, and baseline characterisation might only require incorporation of this work into an examination of existing data from other sources.*

Macrofauna

The macrofauna constitute the size class just below the megafauna; these are animals retained on a 300 to 500 µm sieve can represent a huge diversity of sediment-dwelling taxa in abyssal regions (estimated at many hundreds of species of polychaetes, crustaceans and other invertebrates at a single site; Glover et al., 2002, Smith et al., 2008a and b). The polychaetes dominate macrofaunal standing crop, accounting for about 50-65% of both abundance and biomass in nodule regions (e.g., Borowski and Thiel, 1998; Smith and Demopoulus, 2003).

Seafloor area covered by nodules, varied dramatically between boxcores. Nodule occurance per boxcore varied more than 30-fold (from 8 to 277 nodules), and percent nodule cover varied by more than an order of magnitude (from 3% to 55%). Interestingly, sediment macrofaunal abundance, the abundance of polychaetes, and polychaete family richness per box core (0 – 5 cm level) showed no trends with increasing nodule cover.

Although the sediment macrofaunal data from UK1 Claim Area are still very preliminary and incomplete, we can make some useful comparisons to macrofaunal communities sampled similarly in other areas of the CCZ. During the Kaplan Project, only 32 polychaete families were collected among 485 polychaete individuals sampled by box core at three stations spanning 3000 km of the CCZ (Smith *et al*., 2008b). Thus, at the familial level, the polychaete fauna of the UK1 Claim Area could be relatively diverse compared to the broader CCZ. The relatively high abundance of predator/omnivore polychaete families found in the UK1 box core samples appears to be characteristic of the eastern portion of the CCZ, where overlying waters are relatively productive, and the sinking flux of detrital food material from the euphotic zone is likely to be relatively high (Smith *et al*., 2008a; Wedding *et al*., 2013). Stations further west in the CCZ, underlying less productive ocean waters, appear to have substantially smaller proportion of predator/omnivore polychaete families (Smith *et al.,* 2008b).

A total of 1001 macrofaunal crustaceans, belonging to eight major taxa were isolated, photographed, dissected (if large enough) and preserved in 96% ethanol for further investigation. The first sampling cruise (AB01) was successful with 485 DNA-compatible samples collected and 1482 photomicrographs taken. 138 species have been determined based on initial high-resolution imagery and taxonomic classification. Investigation of these by DNA analysis is ongoing, but for specimens for which DNA has been extracted, we have over 90% success rate in DNA sequencing.

Biodiversity based on preliminary statistical extrapolation methods is estimated between 170-270 species of macrofauna, this may be an underestimate as the DNA analysis may subsequently demonstrate that

morphologically similar species are in fact separate genetically (cryptic species). In addition, statistical analysis shows that the UK1 claim area is still under-sampled.

***Recommendation**: Further field collections of these fauna also might not be necessary to complete the initial impact analysis; the work completed to date on these groups, outlined above, is extensive, and baseline characterization might only require incorporation of this work into an examination of existing data from other sources.*

Megafauna (Surface deposit and filter feeders)

The largest faunal size class in benthic ecosystems is the megafauna; these are animals large enough to be recognised in bottom photographs and range from ~2 cm to >100 cm in length. Previous studies have suggested that some megafaunal species, in particular surface deposit feeders, may be particularly sensitive to the impacts of polymetallic-nodule mining (Bluhm *et al*., 1995; Bluhm, 2001), though these studies were conducted in the Peru Basin off the west coast of South America, a very different habitat than the CCZ exploration claim area.

The most diverse phylum thus far is the Cnidaria. These included 2 primnoid octocorals, 2 isidid octocorals, 1 anemone, 4 hydroids (including 2 pelagic siphonophores accidentally caught) and a scyphozoan jellyfish (in the benthic stage of the life cycle). Two taxa matched previously published genetic sequences (pelagic siphonophores), two taxa matched published morphological descriptions (abyssal primnoids described from the same locality in 2015) and the remaining 6 taxa are potentially new species.

This is followed by Echinodermata (41 morphotypes), Porifera (30 morphotypes), Xenophyophora (17 morphotypes), Arthropoda (12 morphotypes), Chordata and Annelida (9 and 8 morphotypes respectively), Bryozoa (4 morphotypes) and Mollusca (1 morphotype). There are also 11 morphotypes that could not be assigned to a phylum and thus were labelled 'Unknown'.

Gastropods identified included 21 species from 42 records identified by a combination of morphological and genetic data, including molecular phylogenetic analyses. These included 3 heterodont bivalves, 5 protobranch bivalves, 4 pteriomorph bivalves, 1 caudofoveate, 1 monoplacophoran, 1 polyplacophoran, 4 scaphopods and 2 solenogastres. Echinoderms included 17 species (4 Asteroidea, 4 Crinoidea, 2 Holothuroidea and 7 Ophiuroidea) identified by a combination of morphological and genetic data. No taxa matched previously published genetic sequences, but 8 taxa could be assigned to previously described species based on morphology.

Xenophyophores are large foraminifera that constitute a major component of the megafauna in some parts of the deep sea. During the AB01 cruise, we recognised 14 xenophyophore species, 12 belonging to the order *Psamminida* and 2 to the order *Stannomida*. Half of the psamminid species were attached to manganese nodules recovered in box cores. Most were represented by single specimens but a new species of *Aschemonella* with a test in the form of a large, irregularly segmented tube composed of micro-nodules, was fairly common. Other psamminids were assigned to the genera *Aschemonella, Galatheammina, Homogammina, Occultammina*, and *Psammina* with a greater or lesser degree of confidence.

Two species of *Stannophyllum*, a stannomid genus that is common in the eastern Pacific, are each represented by single specimens collected by the Brenke epibenthic sled. *Stannophyllum* sp. 1 is a small, possibly juvenile specimen with a light-coloured test composed largely of radiolarians. *Stannophyllum* sp. 2 has a larger, flaccid, dark-brown test with weakly-developed zonation. Neither can be identified with confidence, although *Stannophyllum* sp. 2 may be *S. zonarium*, the most frequently reported species of the genus (Tendal, 1972). A remarkable species that forms an extensive system of small, rounded, triangular or star-shaped chambers joined together by stolons to create a delicate 3-dimensional lattice, occurs in two samples. It may also be a xenophyophore, possibly related to the genus *Aschemonella.*

In summary, we distinguished 170 morphotypes within the UK1 contract area but species-richness estimators suggest this could be as high as 229. Megafaunal abundance averaged 1.48 individuals/m^2. Seven of 12 collected metazoan species were new to science, and four belonged to new genera. Approximately half of the morphotypes occurred only on polymetallic nodules. There were weak, but

statistically significant, positive correlations between megafaunal and nodule abundance. Eastern-CCZ megafaunal diversity is high relative to two abyssal datasets from other regions. There appear to be at least 55 distinct morphospecies (8 Annelida, 12 Arthropoda, 4 Bryozoa, 22 Chordata, 5 Ctenophora, and 4 Mollusca) identified mostly by morphology but also using molecular barcoding for a limited number of animals that were collected.

The 2019 Friday Harbor Workshop concluded as follows:

> *"Evaluation of the relationships between* [particulate organic carbon] *POC flux and megafaunal community parameters is hindered by lack of direct measures of POC flux or sediment community oxygen consumption for most sites (forcing use of coarse resolution (in space and time) modelled data)."*

Recommendation: *Studies carried out with specimen collections and photographic surveys conducted in AB01 and AB02 may be sufficient for impact analysis, when augmented by the ongoing studies of the archival OMCO photo surveys and incorporation of external literature reviews and database entries.*

Utilization as an "indicator species" of the newly discovered species of sponge (Plenaster craigi) is likely to be limited to the assessment of impacts to areas near to mined areas that have not been mined, and further limited by our ability to assess sublethal impacts to the sponge itself. Because this sponge may be associated exclusively with the nodules themselves, it would be removed by mining operations and not useful as an "indicator" in the usual sense. It will be very important to determine whether or not the sponge and perhaps other species designated as "indicator species" occur on other hard substrates within the mining areas, such as hard-rock outcrops, which could maintain the species viability after mining has removed the nodules. This does not appear to be included in the SMARTEX programme.

Megafauna (top predators)

In abyssal regions most of the top predators, including large mobile fishes, shrimps and amphipods, are also scavengers, and are commonly studied using baiting techniques (Bailey *et al*., 2007). Top trophic level animals can have important influences on communities and ecosystems, controlling prey biomass or abundance, exerting selective pressures, and altering the behavior and habitat choices of potential prey (Micheli, 1999; Pace *et al*., 1999; Myers *et al*., 2007; Polovina *et al*., 2009; Estes *et al*., 2011). Top predators are also susceptible to anthropogenic changes, particularly habitat alteration, because many have large habitat requirements (Fosså *et al*., 2002; National Research Council, 2002). Thus, it is important to understand the potential consequences of nodule mining on the top predator and scavenger assemblage. This community in the Pacific abyss is poorly studied and no information exists for the proposed mining areas.

In the AB01 surveys, the camera documented four species of fishes (*Coryphaenoides armatus/yaquinae* (these two are not easily distinguishable in photographs), *Pachycara* cf. *bulbiceps*, *Histiobranchus bathybius*, *Bassozetus* sp.), two species of shrimps (*Benthiscymus*? spp. and *Hymenopeneaus nereus*), and many small amphipods. Despite the loss of the baited camera, we were also able to conduct a bait experiment on the last ROV dive. We observed three species of fish (*C. armatus/yaquinae*, *P.* cf. *bulbiceps*, and an unknown cusk eel). There were also several species of scavenging fauna observed during ROV transects. The baited trap was also very successful. In six deployments, over 1000 small amphipods, seven shrimp (*H. nereus*), 12 rattail fish (3 *C. armatus* and nine *C. yaquinae*), two eelpout fish (*P.* cf. *bulbiceps*), and two giant amphipods (*Eurythenes* spp. - one of which may be a new species) were collected. The specimens of *H. nereus* are the first to be collected since the Albatross expedition in 1891 (Mary Wicksten, Texas A&M, pers. comm.) from the same region.

We can begin to address whether the UK1 claim area fauna is broadly distributed or endemic. The fishes observed are known from widely spaced locations across the Pacific at abyssal depths. The abyssal shrimps observed are much less well known but represent morphotypes similar to those found elsewhere. Studies in the oligotrophic Pacific abyss have identified an assemblage with more cusk eels and large Aristeid shrimp (Yeh and Drazen, 2009) than the rattail-dominated scavenger fauna in eutrophic sites off California (Isaacs and Schwartzlose, 1975; Priede *et al*., 1994; Yeh and Drazen, 2011) or near the subtropical chlorophyll front

(Armstrong *et al*., 1991). The fauna observed in the UK1 claim area included a combination of rattails (*C. armatus* and *C. yaquinae*), which were more common than the cusk eels (*Bassozetus* sp. and unidentified species) and this may reflect the sites productivity being intermediate to California and Hawai`i. We are currently working with colleagues to compile abyssal baited-camera deployments around the world in an effort to understand scavenger biogeography and the influence of the productivity regime on community composition. This exercise could provide us with some ability to predict changes in the ichthyofauna and scavengers across the CCZ region.

The baited camera was used in AB02 (2015) to obtain high-quality imagery of the ichthyofauna and invertebrate scavenging fauna. All thirteen deployments were successful during cruise AB02; six within the OMS study area, six within the UK1 AB02 area, and one within the ISA APEI-6 study area. During the AB02 cruise, the baited trap was used to sample ichthyofauna and invertebrate scavenging fauna. All thirteen deployments were successful during AB02; six within the OMS study area, six within the AB02 UK1 area, and one within the APEI-6 study area.

The scientists completed molecular analysis of 800 scavenging amphipods (400 from UK1 and 400 from OMS). sequences belonging to ten putative species (excluding potential cryptic species). Six scavenging amphipod families (Alicellidae, Cyclocaridae, Eurytheneidae, Scopelocheiridae, Uristidae, Valettiopsidae) were identified, based on combined DNA and morphology data. The eelpouts, *Pachycara* n. sp., collected during both AB01 and AB02 are genetically identical to each other but different than the few other species for which sequence data is available. Morphologically they are also a new species.

In summary, the scavenger community composition is intermediate between that typical of the Californian margin and Hawai`i, generally dominated by rattail fishes, dendrobranchiate shrimp, and zoarcid and ophidiid fishes. Additionally, the western and eastern ends of the CCZ seem distinct, with the western region characterised by decreased dominance of rattails and small shrimps and increased dominance of ophidiids (especially *Bassozetus* sp. and *Barathrites iris*) and large shrimps.

The 2019 Friday Harbor Workshop concluded that standardisation of baited-trap and baited-camera data collections would greatly help the effort to apply the relatively well-studied areas in the east (AB01 and AB02) with future work needed in the central CCZ.

Recommendation: *Impacts to mobile predators are of particular concern for any environmental impact assessment, since these groups represent the top of the food web and can be more sensitive to impacts than many other groups. Results from the AB01 (2013) cruise suggest that, except for some crustaceans, these top predators occur in many deep benthic environments and may not be significantly impacted by mining. Specimen collections and photographs of animals were more successful for AB02 (2015) and are outlined below. Additional studies of these top predators should be a high priority for future UKSR field efforts. The SMARTEX programme will include extensive studies of these megafauna at Sites N and A using ROV and AUV photographic surveys. It will be important also to conduct baited camera and trap surveys at Site N prior to and after test mining.*

DNA taxonomy and biodiversity

The focus of the work in 2020 has been the taxonomy, biodiversity and connectivity of the polychaetes. A major paper on the nereidiform annelids living on the nodules has been prepared and submitted (Drennan et al in review currently), with additional papers now in advanced stages on the Spioniformia (a large and complex annelid paper including 24 new species with 3 formally described) (Neal et al in prep), a major paper in preparation on the biodiversity, biogeography and connectivity of the annelids (Stewart et al in preparation) and a data paper to support this latter publication on the annelids so far undocumented (Wiklund et al in prep). Given that the NHM/NORCE goal to publish taxonomic papers on several polychaete groups will take a number of years to complete, this new approach will allow the team to publish a significant proportion of UKSR specimen records, 526 in total, making the data available for additional work, both by ourselves and other groups. As such, this paper will support future publications currently in preparation or planning stage.

Analysis of the taxonomic data has continued using the combined morphological and molecular approach outlined in Glover et al. (2015), and the species determinations reported in the ISA template follow a newly developed convention to provide working taxonomic names based on the voucher specimen used to describe that taxon. For example, in Dahlgren et al. (2016) the cnidarian coral Mopseinae sp. 'NHM_330' is the working taxonomic name for a species for which there are several individuals recovered (e.g NHM_002, NHM_019 etc) but in which specimen NHM_330 was the best available to describe the species (e.g in optimum morphological condition), with the highest-quality DNA sequences. This is similar to the 'type specimen' concept used in full species description and using a clear and consistent naming system based on openly accessible voucher and DNA materials will allow for full species description in the future.

Describing formally, with new names, all of the species collected is currently beyond the scope of this project but it is an entirely reasonable and significant advance to make all data available both genetic and morphological on the working species units, making it possible for others to build on this knowledge (Glover et al. 2018).

All of the new analytical work carried in 2020 has focused on the Annelida (polychaetes) which are the most biodiverse component of the fauna. Current estimates are that there are over 400 new species of annelids in samples collected from the ABYSSLINE cruises in 2013 and 2015 alone, not even including additional material from complementary cruise programmes such as RC01, MIDAS and JPI. In order to make progress on the vast task of making these data fully available through descriptions, genetic data and imagery, NHM are breaking the operation down into manageable portions of work that involve the description of at most 20-30 species in a paper, which is still by taxonomic standards a very significant body of work. In addition, NHM scientist are working on a larger data paper on all of the unpublished annelid species hypotheses (Wiklund et al., in prep) to increase the rate of progress to this goal.

Ecosystem Functioning

such as measures of bioturbation, stable isotopes and sediment community oxygen consumption

Functional assessment of microorganisms associated with polymetallic nodules in the Clarion-Clipperton Zone was provided in an interim report to the ISA summarising research activity during the 2017 reporting period focused on assessing metabolic potential and phylogenetic diversity of microorganisms associated with polymetallic nodules collected as part of the UKSR survey efforts. Three peer reviewed papers were published by the research team:

Shulse, C.N., Maillot, B., Smith, C.R., and Church, M.J. **2017**. Polymetallic nodules, sediments, and deep waters in the equatorial North Pacific exhibit highly diverse and distinct bacterial, archaeal, and microeukaryotic communities. Microbiology Open, **6**: e428, doi:10.1002/mbo3.428

Lindh, M.V., Maillot, B., Shulse, C.N., Gooday, A.J., Amon, D., Smith, C.R., and Church, M.J. **2017**. From the Surface to the Deep-Sea: Bacterial Distributions across Polymetallic Nodule Fields in the Clarion-Clipperton Zone of the Pacific Ocean. Frontiers in Microbiology, **8**, doi: 10.3389/fmicb.2017.01696.

Lindh, M.V., Maillot, B., Smith, C.R., and Church, M.J. **2017**. Epi- and mesopelagic bacterial communities above polymetallic nodule fields and sediments in the Pacific Ocean at the Clarion-Clipperton fracture zone are structured by habitat filtering. *In Review. Environmental Microbiology Reports.*

Comparison of environmental results in similar areas to understand species ranges and dispersal on the scale of ocean basins.

The comparison of environmental results in the UKSR Licence Areas to that of similar ISA licenced areas is ongoing, and is documented (to the extent that such comparisons are currently possible) in many of the published papers listed in Appendix A. Some species have been found to be common across the two UK1 30 km x 30 km areas sampled thus far, those in the German area, Singapore area and also common with those in other oceans. Important progress has been made in assessing species connectivity and dispersal across the CCZ, with preliminary analysis suggesting meaningful species connectivity and gene flow across large distances, at least among megafauna.

One key study released in 2019, (Toboada et al), *Implications of population connectivity studies for the design of marine protected areas in the deep sea: An example of a demosponge from the Clarion-Clipperton*

Zone, looked at which has been found to be a very abundant encrusting sponge on nodules, understanding its genetic diversity and connectivity could provide important insights into extinction risks and design of marine protected areas. The science team's main aim was to assess the effectiveness of the Area of Particular Environmental Interest 6 (APEI- 6) as a potential genetic reservoir for three adjacent mining exploration contract areas (UK1A, UK1B and OMS-A). As in many other sponges, showed extremely low variability even for samples ~900 km apart. Conversely, the 168 individuals of *P. craigi*, genotyped for 11 microsatellite markers, provided strong genetic structure at large geographical scales not explained by isolation by distance (IBD). Interestingly, the science team detected molecular affinities between samples from APEI- 6 and UK1A, despite being separated ~800 km. Although the migration analysis inferred very little progeny dispersal of individuals between areas, the major differentiation of OMS-1A from the other areas might be explained by the occurrence of predominantly north-easterly transport predicted by the HYOM hydrodynamic model. The study suggests that although APEI- 6 does serve a conservation role, with species connectivity to the exploration areas, it is on its own inadequate as a propagule source for for the entire eastern portion of the CCZ. Our new data suggest that an APEI located to the east and/or the south of the UK1, OMS, BGR, TOML and NORI areas would be highly valuable.

Regional scale biodiversity, biogeography and connectivity

Abyssal plains are hypothesised to harbour limited temporal and spatial habitat heterogeneity (Etter et al., 2005). With no obvious barriers to dispersal and a "stable" environment, genetic diversity is hypothesised to be low, but actual data on populations is extremely limited for macrofauna species at abyssal plains covering 54% of the earth surface. This deficiency in data from a major part of the planet was highlighted in a recent review (Taylor & Roterman, 2017), indicating that less than a handful of organisms have been studied.

Current ideas in community ecology emphasise that multiple facets of biodiversity, e.g. taxonomic, functional, and phylogenetic diversity, should be considered when describing communities (Heino and Tolonen, 2017). Phylogenetic diversity in particular is often suggested to be an important consideration for conservation targets, as it can be related to essential processes such as ecosystem functioning, extinction, and be used as a proxy for functional diversity. Few studies have focused on patterns of phylogenetic diversity within the CCZ, but work has revealed unprecedented levels of abyssal phylogenetic diversity including at least three ancient (>70 Ma) ophiuroid clades (Christodoulou et al., 2019), and high phylogenetic rarity in nematode assemblages (Macheriotou et al., 2020). While this data is useful for setting conservation priorities, it also allows for the evaluation of ecological and evolutionary dynamics of communities in an ecosystem where the mechanisms supporting high diversity are still incompletely understood.

Analyses

The focus of work in 2020 was on the regional scale of assessment of biodiversity, biogeography and connectivity working towards a major publication (Stewart et al in prep) in 2021 that includes all UKSR/OMS data in collaboration with new data on the target taxa from the JPI-Oceans, MIDAS and DeepCCZ projects, as well as previously published genetic datasets from other licence areas. This manuscript is in advanced stages, and includes a number of new phylogenetic analyses that have not been conducted on deep-sea data before. This work contributes to evaluations of the effectiveness of the current spatial conservation strategy at conserving both genetic and taxonomic diversity, improving the understanding of potential losses in biodiversity due to nodule mining. We provide here a preview of the major findings.

A total of 979 16S sequences and 1120 COI sequences have been included in analyses. A range of different molecular species delimitation analyses have been conducted with these datasets to allocate samples into molecular operational taxonomic units (species). The results of all delimitations were compared against morphological species identifications (undertaken on each sample upon collection) to check for consensus. Based on these comparisons, the Automatic barcode gap discovery (ABGD) delimitation was deemed to be the most congruent with morphological-based identifications. Based on AGBD delimitation, the 16S dataset was found to contain 289 species, while the COI dataset contained 280 species. Non-parametric species

richness estimators have been calculated for each area to estimate the total regional polychaete biodiversity. These estimators predict polychaete diversity across the eastern CCZ to be in the range of 584 – 787 species. This represents a 117 – 158% increase on previously published estimates of polychaete species diversity in the region (Bonifacio et al., 2020).

At a regional-scale, analyses have been conducted to study the total biodiversity of the UKSR/OMS areas, while comparing this to previously published data on other CCZ licence areas and the degree of species range overlaps within and between UKSR/OMS and the neighbouring regions. These analyses are a forthcoming

Assessment of statistical robustness/power

taking into account sample sizes, sample number and, for biological communities, the abundance of individual species (with evidence for statistical significance)

Please refer to the discussion from each research group in the above Sections and to the relevant peer reviewed scientific journal publications for details regarding statistical robustness/power with respect to the studies of each of the organism classes and metrics in the summary matrices of Table 1Table 1 By documenting its findings through peer reviewed publications, the scientific team sponsored by UKSR holds itself to the highest scientific standards and will publish only findings which are underpinned by data with sufficient statistical robustness, and that make clear the degree of statistical certainty that the data sets represent.

Gap analysis and future strategy to achieve the goals of the five-year programme of activities and the requirements contained in ISBA/19/LTC/8

UKSR's integrated environmental baseline programme for UK1 and UK2 contract areas was designed at inception to meet the detailed requirements set out in the ISA's guidance to contractors. A preliminary Gap Analysis was commissioned by UKSR in 2017 to take stock of the state of knowledge across the CCZ and in UK1 in particular. A planning workshop in early 2018 reviewed this analysis and defined a strategy to achieve the requirements contained in ISBA/19/LTC/8. This gap analysis was again revisited in 2020 and updated to incorporate the revised requirement from ISBA/25/LTC/6 Rev 1.

Evaluation of the advantages and disadvantages of different sampling and analysis methods, including quality control

The scientific team that UKSR sponsors employs the highest quality, practical sampling and analysis methods known to the international scientific community. The sampling apparatus and analysis methods that have been used to collect and analyse samples from the AB01 and AB02 cruises are published in:

> Glover, A. G., Dahlgren, T. G., Wiklund, H., Mohrbeck, I., & Smith, C. R. (2016a). An End-to-End DNA Taxonomy Methodology for Benthic Biodiversity Survey in the Clarion-Clipperton Zone, Central Pacific Abyss. Journal of Marine Science and Engineering, 4(1), 2. doi: 10.3390/jmse4010002

This document is publicly available online.

For RC01, the principal upgrade to these sampling methodologies was switch from a 0.5m boxcore to a 0.75m boxcore – a larger unit of the same basic design. This instrument proved highly reliable, with the additional weight providing stability in the water column and elevated triggering forces for the boxcore doors. In addition to the operational advantages, the larger sampled area and volume provided significantly more data (2.25 times the sample surface area), contributing greater statistical power to overall population analysis. However the larger sample size does take more researcher-hours to sort and analyse, creating a

trade off between deck time between deployments vs robust quantitative data capture. In addition, new statistical methodologies had to be developed and tested to enable quantitative comparison and eliminate sampling biases between the 0.75m boxcore data from RC01 and the 0.5m boxcore data from AB01 and AB02. In summary, UKSR found the advantages of the 0.75m boxcore to outweigh the disadvantages, particularly for resource assessment purposes, and will generally prefer to utilise this equipment in future.

Other than the boxcore, the same sampling techniques and analysis are deemed as current best practice and were employed once again on the UK1 RC01-L1 (2020) expedition samples. The same methodology is planned to be utilised for all the UK2 exploration programme as well.

Data reporting

All samples used as the basis for the environmental baseline work described in this document have been reported to UKSR by the scientific team it sponsors. UKSR has previously submitted environmental reporting in Microsoft Excel format annually consistent with the specified Annex IV template from the science teams into a uniform format. The UKSR environmental template submittal to the ISA for 2020 contains data on 1,982 samples from UK1 analysed by the Natural History Museum and Norwegian Research Centre (NORCE) from AB01 (2013) and AB02 (2015). This updated template includes species-level determinations to date as of January 2021 and is inclusive of the new data from the 2020 reporting period. Specifically, the updates are as follows:

- 203 new records representing the identified nodule fauna
- 1038 record edits (taxonomic name refinements)
- 34 taxonomic name updates on existing records

NHM/NORCE have recommended to UKSR and the ISA that these new data overwrite (rather than add to) the existing data submitted previously to ISA in order to maintain quality and iterative improvement of taxonomic knowledge. Further data submitted by the National University of Singapore (NUS) provide initial accounting of the 865 samples collected from the 2020 RC01-L1 survey work in the UK1 contract area. Additional analysis is expected to take place on theses samples in 2021.

Geographical details of the sampling areas are illustrated in Figure 18. UK1 Science team environmental sample updates from AB01 (2013), AB02 (2015) and RC01(2020) Surveys submitted for 2020 reporting period on PMN Environmental reporting template.

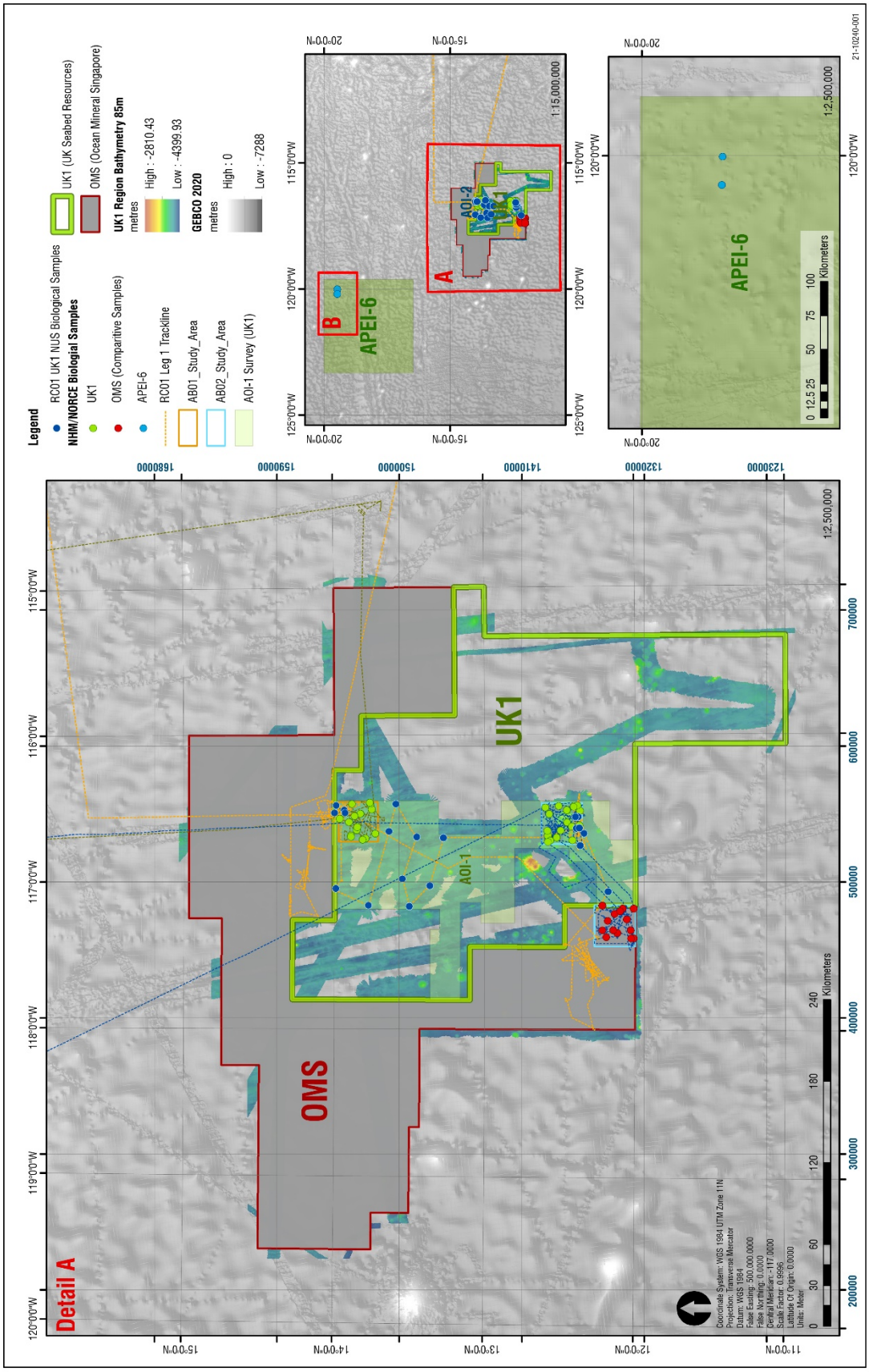

Figure 18. UK1 Science team environmental sample updates from AB01 (2013), AB02 (2015) and RC01(2020) Surveys submitted for 2020 reporting period on PMN Environmental reporting template. OMS comparative sample locations for reference only.

Environmental Impact of Exploration Activities

Including information on a monitoring programme before, during and after specific activities with the potential for causing serious harm

UKSR, per its approved plan of work, has not embarked on any exploration activities with the potential for causing serious harm in the UK1 contract areas or which required an environmental impact assessment.

UKSR has carried out exploration activities in the area during the during the 2020 reporting period in the UK1 contract areas. No incidents occurred which would trigger reporting thresholds under existing protocols or regulations, or approach thresholds requiring an environmental impact assessment under the ISA's revised guidelines for exploration.

UKSR has not carried out any test mining activities during the initial 5-year plan of work or the 2020 reporting period in the UK1 contract areas.

Examination of the recovery over time of seabed communities following disturbance experiments conducted on the sea floor

UKSR has not conducted an examination of recovery over time within the period of reporting in the UK1 or UK2 contract areas. However, the Seabed Mining And Resilience To EXperimental impact (SMARTEX) project intends to develop important information related to this recovery time. The first half of this project will survey the site of Lockheed's (as part of the Ocean Minerals Company (OMCO) Consortium) 1979 pilot test mining system (Figure 19) to examine the residual disturbance and recovery over the intervening 42 years. This test site lies approximately mid-way between UK1 and UK2, so a survey of this area will be the first long-term recovery study of a representative disturbance in the CCZ, and will yield important species distribution and connectivity data as well as an important indication of long-term recoveries in both UKSR licence areas (and the wider Eastern CCZ),

UKSR supported the planning and development of a bid co-led by the National Oceanography Centre (NOC) and The National History Museum (NHM) for $5.98m USD (£4.5m) towards the SMARTEX project, a 21-page proposal submitted in March 2019. In September 2019 they were fully awarded the project by The Natural Environment Research Council (NERC) with inter-institutional project agreements put in place as well as exchange of award letters from NERC, completed in November 2019. NERC requested confirmation of UKSR's ongoing material commitment to the SMARTEX project, which UKSR was able to provide.

The first full meeting of the UK-based project team, including UKSR, occurred in May 2020, virtually due to ongoing restrictions presented by the COVID-19 pandemic. The project is due to commence in July 2021 with 4 years of funding and 2 NERC cruises planned; One to the former OMCO pilot test collector sites in the central CCZ (Figure 20 through Figure 21) and the other to the UK1 area. The SMARTEX project opens up a major new environmental science programme in the CCZ funded by the UK Government, led by NOC and NHM and supported by UKSR, which will contribute fundamental scientific knowledge in the region. It will also contribute to the REMP process being developed and expanded by the ISA, e.g. the selection and refinement of APEIs. Finally, it will provide the fundamental scientific observational data for UKSR's Environmental Impact Assessment in both UK1 and UK2 licence areas.

The RC01-L1 data will provide additional baseline sampling points from an exploration contract point of view, as well as delivering additional data and samples to integrate into the SMARTEX programme. Part of the RC01-L2 (2020) survey provided an opportunity to pre-survey a 70m multibeam grid over the OMCO pilot test site to provide detail planning information for the SMARTEX programme as shown in Figure 20 below.

In addition to SMARTEX development, NHM, NOC and NORCE commenced planning in 2019 of the UKSR/OMS led 'RC01-L1 (2020)' resource assessment expedition, which took in Feb-Mar 2020. Together, NHM, NORCE and NOC have made available 4 persons to participate in the cruise, as well as provide leadership of the biological sampling, integration with previous ABYSSLINE cruises AB01(2013) and AB02(2015), as well as integration into the future SMARTEX programme. Extensive advice, meetings and discussion was provided on all aspects of the biological sampling programme on RC01-L1 (2020) expedition completed in March 2020.

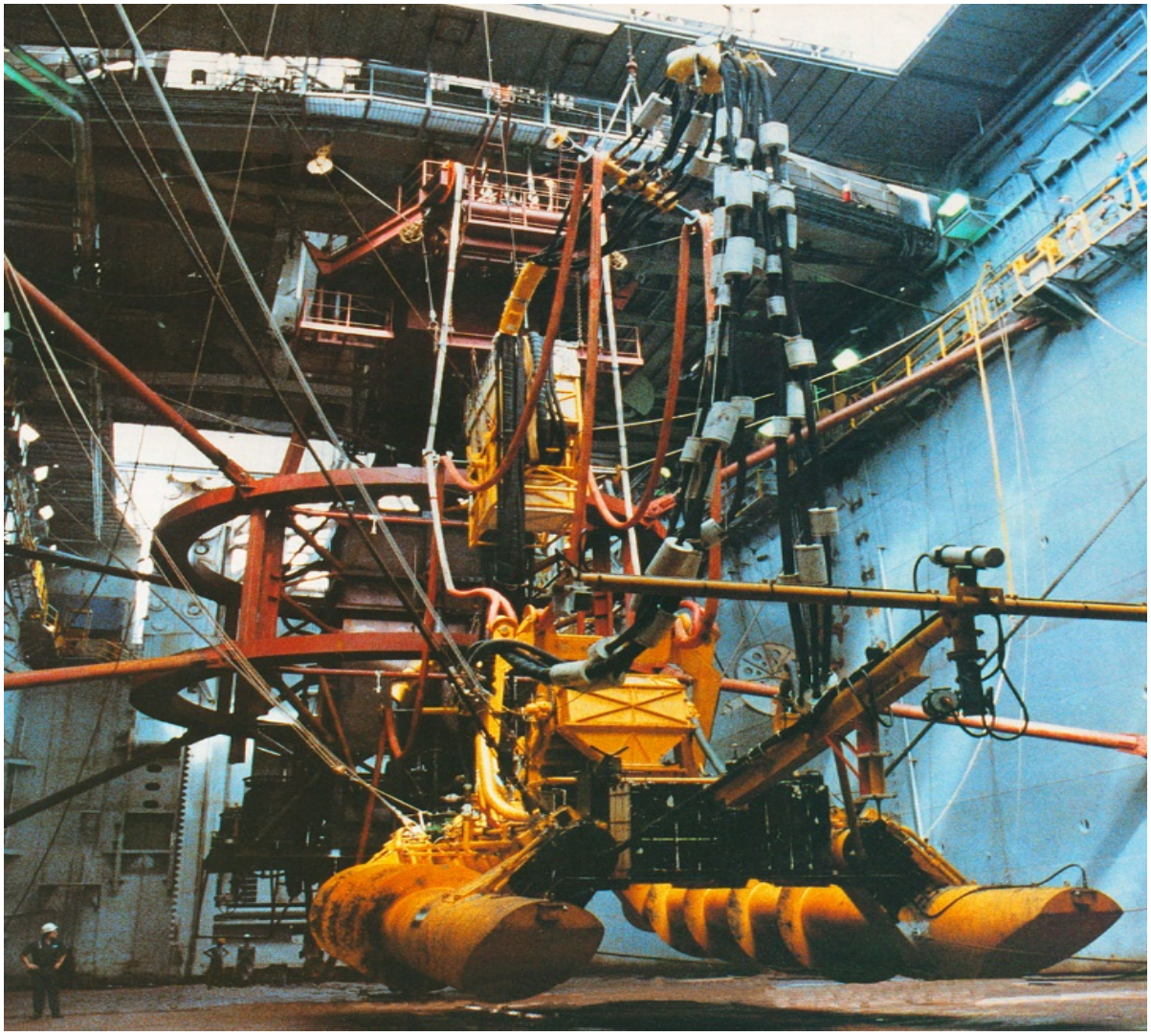

Figure 19. OMCO 1000 tonne per day self-propelled pilot test miner and slurry buffer assembly in Hughes Glomar Explorer well deck prior to deployment in CCZ. (Source: Ocean Minerals Company/Lockheed Martin Archive)

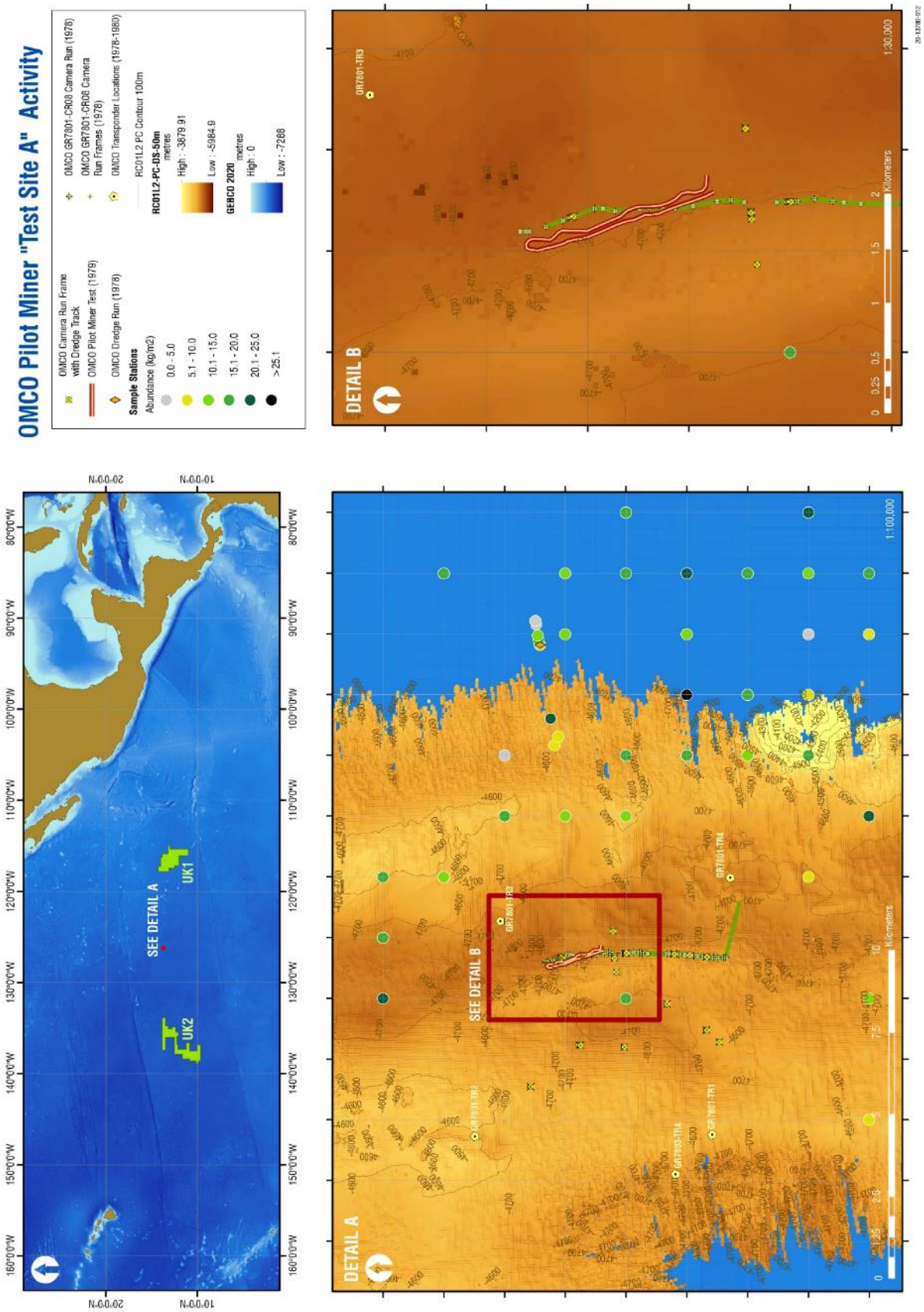

Figure 20. OMCO Pilot Miner "Test Site A" and pre-UNCLOS sampling activity in the general vicinity with newly collected RC01-L2 bathymetry of site.

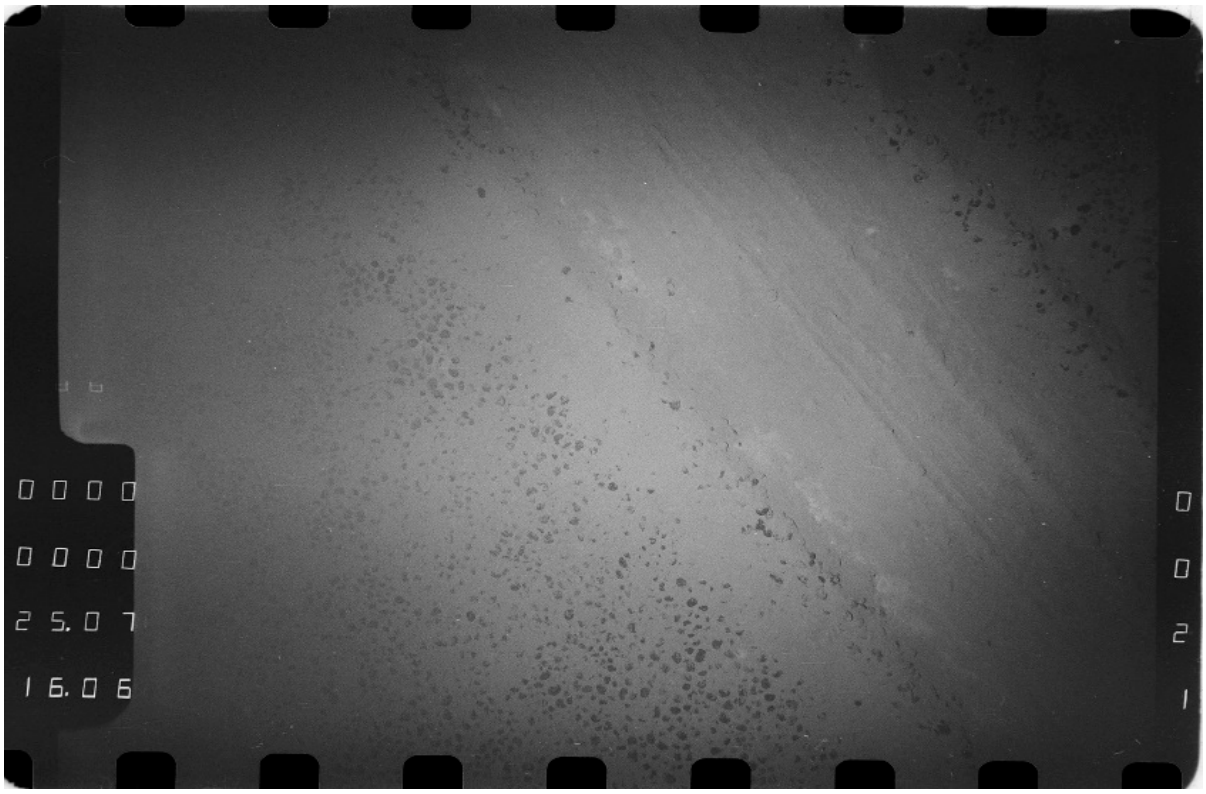

Figure 21. Polymetallic nodule dredge tracks imaged by OMCO benthic towed camera in 1978 in the close proximity of the later site of 1979 OMCO pilot test. (Source: Ocean Minerals Company / Lockheed Martin Archive).

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Appendix A: relevant publications in peer-reviewed journals published during the 2020 reporting year

UKSR is providing a list below of peer-reviewed publications and other references arising from ongoing work in the UK1 contract area which is providing the foundational elements of a comparative environmental baseline and informing the planning for future baseline development work in the UK2 contract area.

2020 Publications Arising from UK1 AB01 (2013) and AB02 (2015) Datasets

McQuaid K.A., Attrill M.J., Clark M.R., Cobley A., Glover A.G., Smith C.R. and Howell K.L. (2020) Using Habitat Classification to Assess Representativity of a Protected Area Network in a Large, Data-Poor Area Targeted for Deep-Sea Mining. Front. Mar. Sci. 7:558860. doi: 10.3389/fmars.2020.558860

Abstract: Extractive activities in the ocean are expanding into the vast, poorly studied deep sea, with the consequence that environmental management decisions must be made for data-poor seafloor regions. Habitat classification can support marine spatial planning and inform decision-making processes in such areas. We present a regional, top–down, broad-scale, seafloor-habitat classification for the Clarion-Clipperton Fracture Zone (CCZ), an area targeted for future polymetallic nodule mining in abyssal waters in the equatorial Pacific Ocean. Our classification uses non-hierarchical, k-medoids clustering to combine environmental correlates of faunal distributions in the region. The classification uses topographic variables, particulate organic carbon flux to the seafloor, and is the first to use nodule abundance as a habitat variable. Twenty-four habitat classes are identified, with large expanses of abyssal plain and smaller classes with varying topography, food supply, and substrata. We then assess habitat representativity of the current network of protected areas (called Areas of Particular Environmental Interest) in the CCZ. Several habitat classes with high nodule abundance are common in mining exploration claims, but currently receive little to no protection in APEIs. There are several large unmanaged areas containing high nodule abundance on the periphery of the CCZ, as well as smaller unmanaged areas within the central CCZ, that could be considered for protection from mining to improve habitat representativity and safeguard regional biodiversity.

McQuaid K.A., (2020) Ecological Studies of an Abyssal Nodule Province to Inform the Management of Deep-sea Mining. PhD Thesis, University of Plymouth, https://pearl.plymouth.ac.uk/handle/10026.1/16676

Abstract: The studies carried out in this thesis aimed to contribute to our understanding of the epibenthic megafauna communities in areas targeted for mining, and to support the environmental management of mining activities through informing recommendations on environmental survey design and spatial planning. Imagery from nine transects of 800 m^2 in the central Clarion Clipperton Fracture Zone (CCZ) were analysed to describe the epibenthic megafaunal communities at both regional (>1000 km) and local (2 km) scales. The relationship between biological data derived from image analysis and modelled environmental data was examined to determine potential drivers of community composition and diversity, and rarefaction and extrapolation curves were used to assess levels of sampling required to establish baseline faunal assessments. Finally, clustering algorithms were used to classify broad-scale, modelled environmental data into different habitat types, to assess the effectiveness of the existing protected area network in the CCZ.

Megafauna morphotypes most vulnerable to mining, including rare, nodule-specific, suspension feeding, and sessile organisms, formed a large proportion of the CCZ epibenthic megafauna. Several dominant morphotypes were homogenous over large scales, but there was high turnover of rare morphotypes at regional and local scales. In addition, broad-scale bathymetric position index was identified as an important driver of both megafauna and metazoan diversity

at regional scales. To characterise the community at 99% sample coverage, sampling units of ~2 800 - 4 600 m^2, or 780 - 960 individuals, were required, with 26 - 27 x 800 m^2 replicate transects. This sampling effort was much greater than is generally used in the deep sea. Finally, a top-down, broad-scale habitat classification of the CCZ identified 46 habitat types and revealed that many of these were underrepresented in the current protected area network, with several occurring almost exclusively in mining areas.

The body of research contained in this thesis suggests that 1) those morphotypes most vulnerable to mining form a substantial proportion of the megafauna communities in the CCZ, 2) greater sampling effort is required to fully characterise baseline environmental conditions of the CCZ, and 3) the current protected area network established in the CCZ does not adequately capture the range of habitats present. This thesis advocates for the use of Regional Environmental Assessment to address some of the pressing issues preventing progress in environmental management of deep-sea mining.

Hestetun, J. T., Bye-Ingebrigtsen, E., Nilsson, R. H., Glover, A. G., Johansen, P. O., & Dahlgren, T. G. (2020). Significant taxon sampling gaps in DNA databases limit the operational use of marine macrofauna metabarcoding. Marine Biodiversity, 50(5), 1-9. doi:10.1007/s12526-020-01093-5, https://doi.org/10.1007/s12526-020-01093-5

Abstract: Significant effort is spent on monitoring of benthic ecosystems through government funding or indirectly as a cost of business, and metabarcoding of environmental DNA samples has been suggested as a possible complement or alternative to current morphological methods to assess biodiversity. In metabarcoding, a public sequence database is typically used to match barcodes to species identity, but these databases are naturally incomplete. The North Sea oil and gas industry conducts large-scale environmental monitoring programs in one of the most heavily sampled marine areas worldwide and could therefore be considered a "best-case scenario" for macrofaunal metabarcoding. As a test case, we investigated the database coverage of two common metabarcoding markers, mitochondrial COI and the ribosomal rRNA 18S gene, for a complete list of 1802 macrofauna taxa reported from the North Sea monitoring region IV. For COI, species level barcode coverage was 50.4% in GenBank and 42.4% for public sequences in BOLD. For 18S, species level coverage was 36.4% in GenBank and 27.1% in SILVA. To see whether rare species were underrepresented, we investigated the most commonly reported species as a separate dataset but found only minor coverage increases. We conclude that compared to global figures, barcode coverage is high for this area, but that a significant effort remains to fill barcode databases to levels that would make metabarcoding operational as a taxonomic tool, including for the most common macrofaunal taxa.

Clark, M., Smith C.R., et al, Deep CCZ Biodiversity Synthesis Workshop, Friday Harbor, Washington, USA, 1-4 October 2019, International Seabed Authority, https://www.isa.org.jm/files/files/documents/Deep%20CCZ%20Biodiversity%20Synthesis%20Workshop%20Report%20-%20Final-for%20posting-clean-1.pdf

Summary of NHM/NORCE publications in peer-reviewed literature and broader impact:

A total of 16 publications in peer-reviewed literature have now been fully published by the NHM/NORCE group on UKSR baseline studies since the end of 2015. Analysis of impact of these publications has been undertaken, Table 7 below, showing that the NHM/NORCE team papers have been cited 341 times and viewed over 63,000 times (cumulative data) since publication highlighting UKSR baseline work to a wide audience of scientists and stakeholders. All papers published have been made fully open-access. Interviews have also led to quotes in science articles on deep-sea mining for New Scientist and for Nature magazine. The report from the 2019 CCZ biodiversity workshop organized by ISA with NHM/NORCE contributions was published/made available on the ISA website in March 2020.

One additional manuscript (not listed here) is in the review stage (Drennan et al) and two additional manuscripts in advanced preparation stage (Neal et al, and Stewart et al).

List of peer-reviewed papers published in temporal order cited in the above statistics. Those in reporting year 2020 are highlighted in bold:

1. Amon, D. J., Ziegler, A. F., Dahlgren, T. G., Glover, A. G., Goineau, A., Gooday, A. J.,... Smith, C. R. (2016). Insights into the abundance and diversity of abyssal megafauna in a polymetallic-nodule region in the eastern Clarion-Clipperton Zone. Scientific Reports, 6:30492. doi:10.1038/srep30492

2. Dahlgren, T. G., Wiklund, H., Rabone, M., Amon, D. J., Ikebe, C., Watling, L., . . . Glover, A. G. (2016). Abyssal fauna of the UK-1 polymetallic nodule exploration area, Clarion-Clipperton Zone, central Pacific Ocean: Cnidaria. Biodivers Data J, 4: e9277 (30 June 2016). doi:10.3897/BDJ.4.e9277

3. Glover, A. G., Dahlgren, T. G., Wiklund, H., Mohrbeck, I., & Smith, C. R. (2016a). An End-to-End DNA Taxonomy Methodology for Benthic Biodiversity Survey in the Clarion-Clipperton Zone, Central Pacific Abyss. Journal of Marine Science and Engineering, 4(1), 2. doi:10.3390/jmse4010002

4. Glover, A. G., Wiklund, H., Rabone, M., Amon, D. J., Smith, C. R., O'Hara, T., . . . Dahlgren, T. G. (2016b). Abyssal fauna of the UK-1 polymetallic nodule exploration claim, Clarion-Clipperton Zone, central Pacific Ocean: Echinodermata. Biodivers Data J, e7251. doi:10.3897/BDJ.4.e7251

5. Glover, A. G., Paterson, G. L. J., Wiklund, H., Taboada, S., Waeschenbach, A., Cobley, A., . . . Amon, D. J. (2016c). The London Workshop on the Biogeography and Connectivity of the Clarion-Clipperton Zone. Research Ideas and Outcomes, 2: e10528. doi:10.3897/rio.2.e10528

6. Lim, S.-C., Wiklund, H., Glover, A. G., Dahlgren, T. G., & Tan, K.-S. (2017). A new genus and species of abyssal sponge commonly encrusting polymetallic nodules in the Clarion-Clipperton Zone, East Pacific Ocean. Systematics and Biodiversity, 15(6), 507-519. doi:10.1080/14772000.2017.1358218

7. Taboada, S., Kenny, N. J., Riesgo, A., Wiklund, H., Paterson, G. L. J., Dahlgren, T. G., & Glover, A. G. (2017). Mitochondrial genome and polymorphic microsatellite markers from the abyssal sponge Plenaster craigi Lim & Wiklund, 2017: tools for understanding the impact of deep-sea mining. Marine Biodiversity, 1-10. doi:10.1007/s12526-017-0786-0

8. Wiklund, H., Taylor, J. D., Dahlgren, T. G., Todt, C., Ikebe, C., Rabone, M., & Glover, A. G. (2017). Abyssal fauna of the UK-1 polymetallic nodule exploration area, Clarion-Clipperton Zone, central Pacific Ocean: Mollusca. Zookeys (707), 1. doi:10.3897/zookeys.707.13042

9. Taboada, S., Riesgo, A., Wiklund, H., Paterson, G.L., Koutsouveli, V., Santodomingo, N., Dale, A.C., Smith, C.R., Jones, D.O., Dahlgren, T.G. and Glover, A.G. (2018). Implications of population connectivity studies for the design of marine protected areas in the deep sea: An example of a demosponge from the Clarion- Clipperton Zone. Molecular ecology. doi:10.1111/mec.14888

10. Glover, A.G., Wiklund, H., Chen, C. and Dahlgren, T.G. (2018). Point of View: Managing a sustainable deep-sea 'blue economy'requires knowledge of what actually lives there. eLife, 7, p.e41319. doi:10.7554/eLife.41319

11. Gooday, A.J., Sykes, D., Góral, T., Zubkov, M.V. and Glover, A.G., (2018). Micro-CT 3D imaging reveals the internal structure of three abyssal xenophyophore species (Protista, Foraminifera) from the eastern equatorial Pacific Ocean. Scientific reports, 8(1), p.12103. doi: 10.1038/s41598-018-30186-2

12. Wiklund, H., Neal, L., Glover, A.G., Drennan, R., Rabone, M., Dahlgren, T.G. (2019). Abyssal fauna of polymetallic nodule exploration areas, eastern Clarion-Clipperton Zone, central Pacific Ocean: Annelida: Capitellidae, Opheliidae, Scalibregmatidae and Travisiidae. ZooKeys 883: 1-82. doi:10.3897/zookeys.883.36193

13. Rabone, M., Harden-Davies, H, Collins, J.E., Zajderman, S, Appeltans, W., Droege, G., Brandt, A., Pardo-Lopez, L., Dahlgren, T.G., Glover A.G., Horton, T. (2019) Access to Marine Genetic Resources (MGR): Raising Awareness of Best-Practice Through a New Agreement for Biodiversity Beyond National Jurisdiction. Frontiers in Marine Science. 6:520. doi:10.3389/fmars.2019.00520

14. Guggolz T, Meißner K, Schwentner M, Dahlgren TG, Wiklund H, Bonifacio P, Brandt A (2020) High diversity and pan-oceanic distribution of deep-sea polychaetes: Prionospio and Aurospio (Annelida: Spionidae) in the Atlantic and Pacific Ocean. Organism, Diversity and Evolution 109:138–19 doi:10.1007/s13127-020-00430-7

15. Hestetun, J. T., Bye-Ingebrigtsen, E., Nilsson, R. H., Glover, A. G., Johansen, P. O., & Dahlgren, T. G. (2020). Significant taxon sampling gaps in DNA databases limit the operational use of marine macrofauna metabarcoding. Marine Biodiversity, 50(5), 1-9. doi:10.1007/s12526-020-01093-5

16. McQuaid KA, Attrill MJ, Clark MR, Cobley A, Glover AG, Smith CR, Howell KL. Using Habitat Classification to Assess Representativity of a Protected Area Network in a Large, Data-Poor Area Targeted for Deep-Sea Mining. Frontiers in Marine Science. 2020 Dec 9;7:1066.

Table 7. Publication impact of UKSR Environmental Baseline Studies from the Natural History Museum and NORCE group. Data sources: Altmetric, Scopus / Web of Science, Google Scholar and individual journal publishers (for views data).

Paper	Citations	Altmetric	News Outlets	Views
Amon et al. 2016 (megafauna)	110	274	23	2968
Dahlgren et al. 2016 (corals)	26	12	-	5045
Glover et al. 2016a (methods)	55	18	-	6784
Glover et al. 2016b (echinoderms)	30	33	1	7516
Glover et al. 2016c (workshop)	6	13	-	8850
Lim et al. 2017 (sponges)	27	96	11	2764
Taboada et al. 2017 (genome)	3	7	-	1596
Wiklund et al. 2017 (molluscs)	16	70	5	6188
Taboada et al. 2018 (connectivity)	15	29	1	1398
Glover et al. 2018 (point of view)	16	101	2	1874
Gooday et al. 2018 (micro-CT)	11	34	1	1497
Wiklund et al. 2019 (annelids)	8	71	1	3675
Rabone et al. 2019 (review)	13	96	6	9341
Guggolz et al. 2020 (annelids)	3	-	-	965
Hestetun et al. 2020 (methods)	2	-	-	1300
McQuaid et al. 2020 (REMP)	-	4	-	2027
Total	**341**	**880**	**51**	**63488**