National Oceanography Centre

Cruise Report

RRS *James Cook* Cruise JC241

4 February – 26 March 2023

Puerto Caldera to Puerto Caldera, Costa Rica

Seabed Mining And Resilience To EXperimental impact

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2023

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JC241 Cruise Report

Contents

1. Scientific Personnel

Daniel Jones	Principal Scientist	National Oceanography Centre, OBG
Adrian Glover	Co-Principal Scientist	Natural History Museum, London
Tim Le Bas	Mapping	National Oceanography Centre, OBG
Catherine Wardell	Mapping	National Oceanography Centre, OBG
Pierre Josso	Geochemistry	British Geological Survey
Hannah Grant	Geochemistry	British Geological Survey
Louisa Norman	Biogeochemistry	University of Liverpool
Erik Simon Lledó	Megafauna	National Oceanography Centre, OBG
Loïc Van Audenhaege	Megafauna	National Oceanography Centre, OBG
Bethany Fleming	Megafauna	University of Southampton
Susan Evans	eDNA RoCSI	National Oceanography Centre, OTE
Bryan O'Malley	Foraminiferal Ecology	University of South Florida, St. Petersburg
Lupita Bribiesca Contreras	Biology Team	Natural History Museum, London
Eva Stewart	Biology Team	University of Southampton
Belen Arias	Biology Team	Natural History Museum, London
Lucas King	Biology Team	Natural History Museum, London
Eleanor Mortimer	Film maker	LunaFilms
Andrew Sweetman	Ecosystem Functioning	Scottish Association of Marine Sciences
Mark Hartl	Scavenger Ecotoxicology	Heriot-Watt University, Edinburgh

2. Ship and Technical Personnel

James Gwinnell	Master		
Philip Gauld	C/O	Sean Angus	ERPO
Declan Morrow	2/O	Christopher Keighley	Head Chef
Charlotte Astbury	3/O	Coleen Hayward-Macleod	Chef
Christopher Uttley	C/E	Jane Bradbury	Stwd
Michael Murray	2/E	Miranda Hall	A/Stwd
Edin Silajdzic	3/E	Antonio Calado	Tech
Gary Slater	3/E	William Handley	Tech
Conrad Laversuch	ETO	Howard King	Tech
Paul Lucas	PCO	Russell Locke	Tech
Martin Harrison	CPOS	Stephen Mcdonagh	Tech
Nathaniel Gregory	CPOD	Emre Mutlu	Tech
Iain Forbes	POS	Eoin O'Hobain	Tech
John Allen	POD	Richard Phipps	Tech
Brian Burton	SG1A	Billy Platt	Tech
Neil Channing	SG1A	Josue Rivero	Tech
Paul Marshall	SG1A	Martin Yeomans	Tech

3. Itinerary

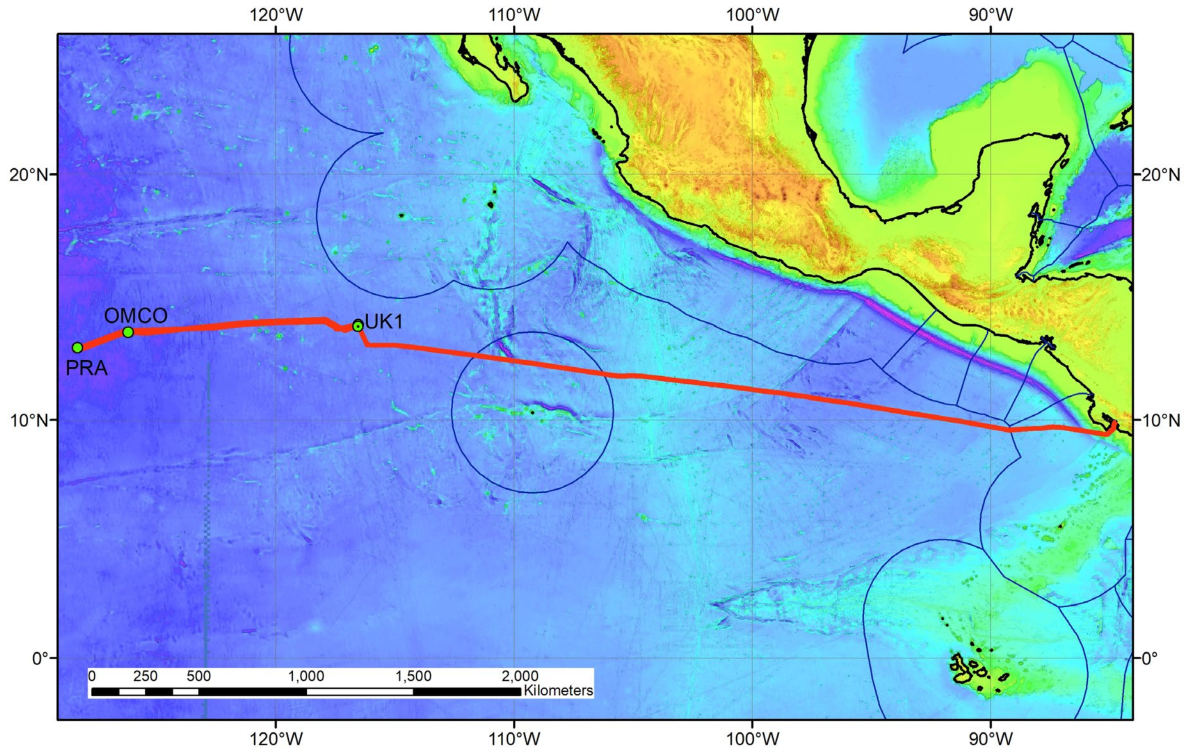
Port of mobilisation of equipment: Puerto Caldera, Costa Rica

Departure: Puerto Caldera, Costa Rica, 4 February 2023

Arrival: Puerto Caldera, Costa Rica, 26 March 2023

Number of days: 50

4. Track Chart



5. Cruise Photo

Cruise Photograph taken 21 March 2023 on rear deck of James Cook in front of ROV ISIS.

Back row (Photo Left to Right): Martin Yeomans, Russell Locke, William Handley, Adrian Glover, Pierre Josso, Loïc Van Audenhaege, Charlotte Astbury, Christopher Uttley, Miranda Hall, Andrew Sweetman, Lucas King, Erik Simon Lledó.

Middle Row (Left to Right): Mark Hartl, Emre Mutlu, Bethany Fleming, Hannah Grant, Bryan O'Malley, Tim Le Bas, Eva Stewart, Eleanor Mortimer, Susan Evans

Front row (left to right): Lupita Bribiesca Contreras, Stephen Mcdonagh, Josue Rivero, Daniel Jones, Catherine Wardell, Belen Arias, Louisa Norman, Billy Platt, Eoin O'Hobain

6. Background

This cruise is part of the Seabed Mining And Resilience To EXperimental impact (SMARTEX) project funded by the UK Natural Environment Research Council (Grant Reference NE/T003537/1).

This is the second UK cruise to the Clarion Clipperton Zone (CCZ) in the northern equatorial Pacific, an area likely to be targeted for deep-sea mining for polymetallic nodules. The first (JC120) was carried out in 2015. This is the first cruise for the SMARTEX project (of two planned).

Scientific aims

1. Understand the remaining impact of a realistic mining test on the benthic environment and its fauna after 44 years
2. Assess natural temporal change in macrofaunal communities between 1989 and 2023 (at PRA site)
3. Provide the first characterisation of megafaunal species and communities present at APEI-13 (linked outreach and policy interaction aim)
4. Characterise the environment and ecology of the UK-1 area in advance of a planned nodule collector test

Scientific objectives by site

OMCO 1979 experimental site

- Map impacts of 1979 test on seabed habitats
- Assess geochemical changes caused by test
- Assess biological changes caused by test (mega, macro, meiofauna + eDNA)
- Assess changes in ecosystem functioning caused by track (nutrient uptake / foodwebs)

PRA

- Assess natural temporal change in macrofaunal communities between 1989 and 2023

APEI-13 (near PRA) – Not achieved

- Collect specimens and images (video and still) of megafauna using the ROV for biodiversity assessment
- Characterise megafaunal community patterns in a representative sample of seafloor photographs collected by AUV

UK-1

- Understand seasonal changes in seafloor currents and benthic boundary layer processes at UK-1
- Produce a map of seabed habitats in area of planned collector test
- Understand spatial variability in sedimentary geochemistry and biogeochemistry
- Assess the scale dependency of megafaunal communities and eDNA at scales of 1 – 10s of km at UK-1
- Assess seasonal changes (over 1-year) in benthic megafaunal and nodule fauna presence and activity (time-lapse camera study)
- Extend our biodiversity assessment of the UK-1 area (from ABYSSLINE) particularly in benthic megafauna, scavenging fishes, and amphipods and macrofauna (sediment and nodule-dwelling)

Multi-site

- Extend our biodiversity assessment of the CCZ particularly in benthic megafauna, scavenging fishes, and amphipods and macrofauna (sediment and nodule-dwelling)
- Improve our understanding of connectivity of mega and macrofauna
- Obtain baseline measurements of exposure biomarkers in fish blood and tissue samples (OMCO and UK-1)

7. Equipment Summary

Gear	Details	Deployments
Shipboard Multibeam	Kongsberg EM122	Transit and surveys at OMCO, PRA and UK1
CTD	Seabird 911	6 Deployments
Boxcore	USNEL / SMBA	43 Deployments
Gravity core	NOC system with 3 and 5 m barrels	8 Deployments
Megacore	Bowers and Connelly	9 Deployments
Remotely Operated Vehicle	ISIS ROV	15 Dives
Moorings	Oceanographic - See Section below	3 Moorings
Time-lapse camera	Bathysnap	3 Deployments
Fish trap	Fish Trap Lander with internal amphipod trap units	4 Deployments

8. Narrative

Day-by-day account of activities written by chief scientist, Daniel Jones.

Tue 31 Jan 2023

Science party started to arrive in Costa Rica – accommodated in San Jose

Wed 1 Feb 2023

First members of science party (DJ, EM) and two of the ROV team (WH, AC) arrived on ship via boat transfer. RRS *James Cook* moored around 1km offshore Puerto Caldera (while other vessels loading) 9°55.4'N 084°43.7W.

Thu 2 Feb 2023

Majority of science party flew to Costa Rica

Ship moved to dock. All fast at 14:30 (GMT-6).

Fri 3 Feb 2023

ISIS and final supplies loaded. Bunkering.

Full complement of scientists arrived on ship.

Sat 4 Feb 2023

Ship alongside. Bunkering.

Noon. Clear skies, 32°C air temperature. 23.4°C sea temperature. Force 2 wind.

Ship sailed at 21:44 Costa Rica Time (GMT-6)

Sun 5 Feb 2023

In transit. Position at noon 9°41.9'N 087°17.6W.

Noon. 28.9°C air temperature. 22°C sea temperature. Low swell. Clear. Force 4 wind.

Ship induction, safety meeting and tour

Full ship boat drill 16:00 (GMT-6)

Mon 6 Feb 2023

In transit. Position at noon 10°06.6'N 092°11.4'W.

Noon. 28.5°C Air temperature, 20.6°C sea temperature. Clear skies, low swell. Force 2 winds.

08:30 (GMT-6) first meeting with captain and crew

9:30 (GMT-6) started marine mammal observations prior to acoustic work.

11:20 (GMT-6) reached edge of Costa Rica EEZ and turned on **multibeam** (sample **JC241_001**) and **underway instruments** (ADCP; **JC241_002**)

Tue 7 Feb 2023

In transit. Position at noon 10°51.4'N 097°04.6'W.

Noon. 29.3°C Air temperature, 22.3°C sea temperature. Clear skies, low swell. Force 3/4 winds.

18:30 (GMT-6) science presentations by each team

Wed 8 Feb 2023

In transit. Position at 11:00 (GMT-6) 11°27.3'N 101°34.8'W.

8:30 (GMT-6) Captain and watch keepers meeting

Noon. 29.4°C air temperature. 22.3°C Sea temperature. 3/8 Cloud, calm sea with building NE swell. Force 2/3 winds.

19:00 (GMT-6) Talk for crew on project and deep-sea mining

Switched ship time zone to GMT-7 at midnight. Most scientists started getting onto their shift patterns. Most people were doing a 12 – 12 shift.

Thu 9 Feb 2023

3:17 (GMT-7) arrived at edge of eddy propagating westwards and carried out full depth **CTD01 (JC241_003)**. Eddy clearly visible as strong currents in underway ADCP data. Oxygen minimum zone observed in CTD data.

8:30 (GMT-7) Captain and watch keepers meeting

8:59 (GMT-7) CTD01 complete and on deck, transit 100 km westward to center of eddy

10:54 (GMT-7) Arrive at center of eddy for **CTD02 (JC241_004)**. Full depth CTD with Bathysnap mooring releases to test.

Position at noon. 11°49.3'N 105°38.3'W.

Noon. 30.1°C Air temperature. 23.4°C Sea temperature. 6/8 cloud cover. Slight sea with NE swell. Force 3 wind.

13:20 (GMT-7) CTD02 complete and on deck, release test successful. Transit towards UK-1

15:32 (GMT-7) entered EEZ of Clipperton Island (France) and turned off multibeam and underway instruments.

Fri 10 Feb 2023

Transit to UK-1. Noon position 12°28.1'N 110°10.0'W.

Noon. 29°C Air temperature, 22.6°C sea temperature. 6/8 cloud cover, fair, low swell. Force 1/2 wind.

8:30 (GMT-7) Captain and watch keepers meeting

10:30 (GMT-7) Fire Drill

12:30 (GMT-7) Science Meeting, followed by Boxcore training followed by ROV induction

17:00 (GMT-7) Marine Mammal Watch until sunset

19:23 (GMT-7) Reached the western edge of the Clarion Island (France) Exclusive Economic Zone and turned on underway ship systems and multibeam (**JC241_005** and **JC241_006**). Continued transit to UK-1.

Sat 11 Feb 2023

Transit to UK-1 and reached UK-1.

Noon position 13° 04.6'N 114°52.5'W.

Noon. 27°C Air Temperature. 21.6°C sea temperature. Weather overcast, slight sea, fair. Force 3 wind.

8:30 (GMT-7) Captain and watch keepers meeting

12:30 (GMT-7) Science Meeting

18:00 (GMT-7) approx.. Arrived at UK-1 and followed a course to obtain multibeam data missing from UK-1 map.

23:52 (GMT-7) Deployed Bathysnap time-lapse camera to the south west of the area of interest for the future collector test. 13°57.611'N 116°33.122'W. Station **JC241_007**

Transit towards OMCO site using a route that filled in gaps in our available multibeam map. Because we stopped logging for the Bathysnap deployment we restarted the multibeam station number **JC241_008**.

Ships clocks moved to GMT-8 at midnight

Sun 12 Feb 2023

Transit towards OMCO.

8:30 (GMT-8) Captain and watch keepers meeting

Noon position 14°06.8'N 118°54.4'W.

Noon. 27.5°C air temperature. 20.9°C sea temperature. Force 2/3 winds. Low swell, partly cloudy.

12:30 (GMT-8) Science Meeting

Mon 13 Feb 2023

Transit towards OMCO.

8:30 (GMT-8) Captain and watch keepers meeting

Noon position 13°47.4'N 123°30.3'W.

Noon. Air temperature 25.5°C. Sea temperature 19.9°C. Force 3/4 wind. Moderate swell. Overcast.

12:30 (GMT-8) Science Meeting

14:00 (GMT-8) deployed ROV umbilical for wire stream to reduce wire twist (did not collect data so no station number)

20:00 Proceed transit to OMCO site

Tue 14 Feb 2023

8:30 (GMT-8) Captain and watch keepers meeting

Noon position: 13°43.7’N 126°14.5’W

Noon. Air temperature 25°C. Sea temperature 19.8°C. Cloudy, moderate swell. Force 4/5 wind.

12:30 (GMT-8) Science Meeting

12:30 (GMT-8) Arrive at OMCO site

12:38 (GMT-8) CTD (3) deployed over expected track location **JC241_009.** CTD reached seabed at 14:06 (GMT-8). On the way up it collected water samples for Louisa (biogeochemistry) and Susan (eDNA). On deck at 15:59 (GMT-8).

Moved to CASIUS location (13°42.949’N 126°14.197’W) and deployed CASIUS mooring at 16:52 (GMT-8) station **JC241_010**. CASIUS (Calibration of Attitude Sensors in USBL Systems) primarily calculates the misalignment between the transceiver and the ships motion reference units and improves accuracy of the USBL (Ultra-short Baseline Navigation) system.

Wed 15th Feb 2023

3:28 (GMT-8) Completed CASIUS calibration.

04:19 (GMT-8) Gravity core off deck for station **JC241_011** at location 13°44.268’N 126°12.172’W. This was the first gravity core of the cruise. This core was deployed at the area we designated (using RC01 shipboard bathymetry) as a control area, >1km to the east of the expected track location.

07:05 (GMT-8) Gravity core on deck. Some over penetration but full core. Changed over to box coring.

07:40 (GMT-8) Box core off deck for station **JC241_012**. We moved the ship around 50 m away from the gravity core location. This was boxcore 1.

8:30 (GMT-8) Captain and watch keepers meeting

11:26 (GMT-8) Box core returned (not triggered) wire was wrapped around box core head.

Noon. Air temperature 25.5°. Sea temperature 19.1°C. Force 4. Slight swell. Partly overcast.

12:30 (GMT-8) Science Meeting

12:25 (GMT-8) ROV off deck for first ROV dive **JC241_013** (ISIS Dive 399) around 1km to the east of the expected track location 13°43.846’N 126°12.746’W. The aim of this dive was to try and locate the tracks and the extent of the tracks.

16:17 (GMT-8) ROV reached the seafloor 4729m depth. Transited due west (270°). Located possible epibenthic sled track running approximately NE – SW (220°) at 18:00 (GMT-8). Followed these for approx 50m so we could see the track in the navigation data. Turned back to transit moving to the west. Found OMCO tracks 18:32 (GMT-8). Extremely distinctive with deep furrows where Archimedes screw propulsion system had propelled the collector along. We followed the tracks to the north. The central area, between the screw depressions, had nodules in places and had removed nodules in other places. There was no evidence of any plume impact to the edge of the tracks. There were a few large sponges observed in the central area of the track but only where nodules were also present. The Archimedes screw tracks appeared to be acting as sediment traps and looked like they had considerably greater densities of large sessile animals (like holothurians and rolling anemones), with several in view at one time on occasion.

We found an area where the second track was visible in sonar 19:32 (GMT-8) and we moved to go along that track (also north). The second track had fewer nodules in the center. As we travelled along the track we found an area of churned up sediment, where it looked like they had done a 3-point turn at 13°44.019'N 126°13.227'W. There was another similar area, possibly associated with a maneuverability test at 13°44.0378'N 126°13.2298'W. We found what looked like an epibenthic sled track crossing the collector track at 19:57 (GMT-8) at 13°44.0404'N 126°12.2395'W. The epibenthic sled track was going approximately SE / NW direction. There was an area with clearly reduced nodule numbers starting at 20:20 (GMT-8) at 13°44.109'N 126°12.234'W. The screw tracks started to get very deep (approx. 1 meter – they had been approx. 50cm before) at 20:22 (GMT-8) at 13°44.121'N 126'12.231'W. Further to the north the two sets of collector tracks crossed at 20:26 (GMT-8) at 13°44.133'N 126° 13.231'W.

We found an area where the collector seemed to be working really well, the central area was completely devoid of nodules. We stopped there at 21:48 (GMT-8) at 13°44.199'N 126°13.234'W and started push coring in the central area of the track. We obtained two BGS cores (one drilled and one not), three cores for University of Liverpool, one core for foraminiferal assessment and one core for biodiscovery (NHM). The sediment appeared to have a more unconsolidated, soft surface layer. We then (22:30 GMT-8) moved to about 1km outside the outer edge of the Archimedes screw track to sample an area that would have been impacted by the plume (although it did not appear any different to the normal nodule areas). At this area we obtained two cores for BGS (one drilled for porewater analysis and one not drilled), a core for foraminiferal analysis and one for biodiscovery – finishing this operation at 23:29 (GMT-8). The cores were difficult to obtain in this area because of the high density of nodules and we had two cores that did not look successful. The sediment appeared more compacted without the soft surface. There were two holothurians in view at this location, which we collected with the manipulator arms and put in the forward biobox. The large purple holothurian had a polynoid scale worm on its dorsal surface. We then transited south 50 m and repeated the coring, obtaining two cores in the track and two outside for BGS. One of these cores did not look successful. This was all of our 18 push cores.

We transited north again to try and find the end of the tracks (expecting a turn in the track). The ROV was losing pressure in the hydraulic oil so we knew time was running out for this dive. Before leaving the seabed we obtained samples of an urchin and brisingid sea star as well as a galatheid crustacean (in suction sampler).

Thursday 16th Feb 2023

The ROV dive was finished without reaching the northern end of the track at 02:12 (GMT-8) at 13°44.303'N 126°13.235'W and the ROV left the seabed.

8:30 (GMT-8) Captain and watch keepers meeting

8:36 (GMT-8) ROV on deck.

9:18 (GMT-8) Boxcore off deck for **JC241_014**, a repeat of the earlier failed boxcore. This deployment (boxcore 2) was also unsuccessful and arrived on deck at 12:59 (GMT-8) not having triggered. The box core was looked over and the slider bar was greased.

Noon. 26°C air temperature. 19.8°C sea temperature. Partly cloudy. Force 4/5 winds. Slight swell.

12:30 (GMT-8) Science Meeting

13:37 (GMT-8) Fish trap (with internal amphipod traps (x2)) was released for **JC241_015** at 13°44.1588'N 126°12.0389'W.

13:59 (GMT-8) Box core (deployment 3) deployed for **JC241_016** at 13°44.212'N 126°12.209'W. Obtained a good sample.

18:37 (GMT-8) ROV off deck for ISIS Dive 400 **JC241_017.** This is a multibeam dive to map the tracks. ROV Reached near the seabed at 23:23 (GMT-8) at 13°44.4'N 126°13.2'W. It took about 3 hours for the team to establish a connection with the Reson multibeam. Collected around 50 minutes of multibeam data to north of expected track location.

Friday 17th Feb 2023

Loss of ROV communications at 03:52 (GMT-8). Recovery of dead vehicle required. ROV on surface at 10:39 (GMT-8). The problem was tension in the cable that resulted in the fibre optic connection to the ROV becoming twisted and preventing information transfer. This represented 15 hours 12 minutes of lost time.

8:30 (GMT-8) Captain and watch keepers meeting

11:17 (GMT-8) deployed Boxcore 4 for **JC241_018** at 13°42.950'N 126°12.250'W. This boxcore was a success and came up with clear top water. The boxcore deployment approach that has worked for us is deploying at 45m/min, slowing to 20m/min until it hits the seafloor, paying out a further 10 m of wire, then recovering at 20m/min until we see a pull out tension. The rate can then be increased to 60m/min for the rest of the ascent. Boxcore on deck at 14:41 (GMT-8).

Noon. 26°C air temperature. 19.7°C sea temperature. Sunny with broken cloud, slight seas. Force 4 wind.

12:30 (GMT-8) Science Meeting

17:04 (GMT-8) Fish trap arrived on surface (**JC241_015**). It has been released about 14:30. Fish trap recovered. Contained two fish specimens (*Coryphenoides sp* and *Barathrites sp*). Specimens were immediately transferred to the CT room and processed. Fish length was measured, and photos taken for later identification. Blood samples were taken and processed for Comet assay on board and also frozen (-80ºC) in 10%DMSO/HBSS for analysis in Edinburgh. Gill samples were taken and processed for on board Comet assay. Gill samples were also taken, shock frozen in liquid nitrogen and stored at -80ºC for later oxidative stress assessment. Further tissue samples taken were: liver samples preserved in 4% formalin for histological assessment; muscle samples were taken and frozen for stable isotope assessment; the stomach was frozen for later microplastic content assessment. The amphipod traps were successful and caught several hundred small (all < 2cm) amphipods.

18:19 (GMT-8) deployed the first megacore at station **JC241_019** at 13°43.949'N 126°12.250'W. This was recovered at 22:28 (GMT-8) but was not a successful core.

23:13 (GMT-8) deployed the fifth boxcore for **JC241_020** at 13°44.301'N 126°12.339'W.

Saturday 18th Feb 2023

02:37 (GMT-8) recovered boxcore. This had a part missing and was processed for qualitative assessment but rejected for quantitative assessment.

03:27 (GMT-8) deployed 4m-long gravity core (GC2) for **JC241_021** at 13°44.301'N 126°12.340'W. This was recovered at 06:18 (GMT-8). The gravity cores are quicker than the other cores because they are lowered at 90 m/min. The core was good and 3.5m of core was recovered.

6:59 (GMT-8) deployed the sixth boxcore of the trip for **JC241_022** at 13°44.249'N 126°12.289'W. This was recovered at 10:26 (GMT-8). It was a very good sample with clear top water.

8:30 (GMT-8) Captain and watch keepers meeting

Noon. Air temperature 27.0°C. Sea temp 19.9°C. Low swell, slight sea. Force 4 wind. Partly cloudy.

12:30 (GMT-8) Science Meeting

11:14 (GMT-8) deployed the ROV for ISIS Dive 401 station **JC241_023** at 13°44'539'N 126°13.260'W. The aim of this dive was to collect multibeam bathymetry data over the mining tracks using the Reson system on the ROV. The ROV got to the target depth, 50 m above the seafloor at 15:37 (GMT-8). The ROV turned to start the survey and lost communications. This meant carrying out a dead sub recovery, which was considerably slower than normal. The sub reached the surface at 20:06 (GMT-8). This represented 8 hours 52 minutes of lost time. Total lost time for ROV operations so far 24 hours 4 minutes. This does not include the time required for getting between positions. The RoCSI was working successfully and obtained a single eDNA sample before the ROV cut out.

20:41 (GMT-8) deployed megacore (megacore2) for **JC241_024** at 13°44.248'N 126°12.288'W.

Sunday 19th Feb 2023

00:36 (GMT-8) Megacore core was recovered to deck with all 8 cores being full and used for science.

01:21 (GMT-8) Boxcore (BC7) deployed for **JC241_025** at 13°44.114'N 126°12.324'W. The boxcore was recovered at 04:50 (GMT-8). It failed, we think it hit something and did not trip the crescent moon.

05:13 (GMT-8) Boxcore (BC8) deployed again for **JC241_026** at 13°44.248'N 126°12.343'W. This is an area 100 m west of the previous sample. The boxcore was recovered at 8:57 (GMT-8) and was successful.

08:30 (GMT-8) Captain and watch keepers meeting

09:38 (GMT-8) Fish trap (deployment 2) with internal amphipod traps (x2) was released for **JC241_027** at 13º 44.151 N 126º 12.091 W.

09:50 (GMT-8) began ROV wire stream to try and remove any tension in the wire. This was aborted at 10:41 (GMT-8) because a rope had caught on the cable causing damage to it and requiring another re-termination.

11:14 (GMT-8) the CASIUS beacon was released from the seafloor. It arrived at the surface at 12:33 (GMT-8) and was recovered 12:40 (GMT-8).

Noon. Air temperature 24.5°C. Sea temp 19.9°C. Low swell, slight sea. Force 5 wind. Partly overcast.

12:30 (GMT-8) Science Meeting

13:09 (GMT-8) deployed 5 m Gravity core (GC3) for **JC241_028** at 13°43.82'N 126°12.27'W. The core was back on deck at 15:46 (GMT-8). This was a very good core with 3.75 m of penetration and a relatively undisturbed surface.

16:41 (GMT-8) deployed a boxcore (BC9) for **JC241_029** at 13°43.856'N 126°12.247'W. This came up at 20:07 (GMT-8). It was a successful core and the fifth good core at the OMCO control area. This was the required number of good boxcores in the control area.

20:50 (GMT-8) Start ROV wire stream.

Monday 20th February 2023

06:41 (GMT-8) Completed wire stream. Total of 10 hours 34 minutes.

08:30 (GMT-8) Captain and watch keepers meeting

15:00 deployed Megacore (megacore3) for **JC241_030** at 13°43.856'N 126°12.247'W. On deck at 10:56. Good core with only 1 of 8 tubes rejected.

11:33 deployed CTD (CTD4) for **JC241_031** at 13°44.251'N 126°12.299'W. This obtained water samples for eDNA and nutrient analysis (University of Liverpool). CTD on deck at 14:43 (GMT-8).

Noon. Air temperature 25.2°C. Sea temperature 20.0°C. Overcast, slight sea, showers. Force 5/6 wind.

12:30 (GMT-8) Science Meeting

15:18 (GMT-8) deployed ISIS ROV for **JC241_032** at 13°44.538'N 126°13.281'W for a multibeam dive. We started the multibeam survey at 19:26 (GMT-8) to the north of the track lines we had seen during the first ROV dive and headed approximately south at an altitude of 50m above the seabed. The collector test tracks were clearly visible in the multibeam. We got to just under half way down the track and the ROV lost communications at 21:58 (GMT-8), requiring a dead sub recovery.

Tuesday 21st February 2023

The ROV was on deck at 01:50 on 21 Feb. Aside from the 2h32m multibeam acquisition (incomplete) we lost a total of 8 hours 35 minutes. The multibeam data were good and the tracks were clearly visible. We captured the northern loop in the tracks, several cross overs of the tracks. We could also see three epibenthic sled tracks that crossed the location of the OMCO collector tracks.

02:24 (GMT-8) deployed boxcore for **JC241_033** at 13°44.1003'N 126°13.2377'W on the OMCO track. We had carefully selected the location based on the video from ROV dive 1 and picked an area with high disturbance. The boxcore wire had a USBL beacon on it to try and accurately position the core over the track. After the core was taken at 4:33 (GMT-8) the winch broke on recovery (at 100 m above the seabed). The winch was fixed and the core was recovered to deck at 08:00 (GMT-8).

08:30 (GMT-8) Captain and watch keepers meeting

The fish trap was released at 7:15 (GMT-8) and arrived on the surface at 9:48 (GMT-8). The fish trap contained 4 rat tail fish (3 on hooks outside the trap and one inside the trap), which were processed for ecotoxicology work by Mark Hartl and Andrew Sweetman. The two amphipod traps inside caught two *Eurythenes* sp. and hundreds of smaller amphipods (*Paralicella* sp. and some *Abyssorchomene* sp.).

10:49 (GMT-8) deployed 5m long gravity core for **JC241_034** at 13°44.1003'N 126°13.2377'W on the OMCO track. This was recovered at 13:22 (GMT-8). It was an excellent core including a level sediment surface. Core recovery was 3.83m. No USBL was attached to verify position over track. No nodules in gravity core but seemed similar to other control sites.

Noon. Air temperature 24.1°C. Sea temperature 19.9°C. Moderate swell. Force 6 wind. Overcast with showers.

12:30 (GMT-8) Science Meeting

The ROV team needed some time to fix the ROV so I decided to move to the PRA site.

The PRA site was first studied by the Biological Evaluation of a Preservation Reserve Area (BEPRA I) cruise on the RV *Moana Wave* (University of Hawaii) from 22 September to 14 October 1989. During this expedition the science team, led by George D. F. Wilson, sampled 16 boxcores in the Preservation Reserve Area (PRA), which was defined by the National Oceanic and Atmospheric Administration (see Wilson, 1990[1]) as be a stable reference area that would be unaffected by manganese nodule mining activities. These sites were thought similar to the mining sites so they could serve as controls. We wanted to repeat the same box core locations to assess change in the 33 years since this work.

We left for the PRA site at 13:54 (GMT-8) using a slightly indirect route to obtain good multibeam of the OMCO area and the PRA site. This survey **JC241_035** continued until we arrived at the PRA site at 3:59 (GMT-8) on 22 Feb 2023.

Wednesday 22 February 2023

We arrived at the PRA site at PRA Station 5 (following the Wilson, 1990, naming convention). We decided to sample PRA 5 first and then go round the stations in a clockwise direction to minimize transit times.

4:10 (GMT-8) deployed boxcore for **JC241_036** at 12°56.81'N 128°20.64'W. This came up at 7:50 (GMT-8). It was a good core. Not many nodules and those that were there were small, spherical and spikey.

08:30 (GMT-8) Captain and watch keepers meeting

8:35 (GMT-8) deployed boxcore for **JC241_037** at 12°58.313'N 128°21.964'W or PRA station 17. This came up at 11:59. The core washed out on arriving at the surface and was not used for quantitative assessment. It was used for a qualitative live sort of fauna.

12:30 (GMT-8) Science Meeting

12:20 (GMT-8) deployed boxcore for **JC241_038** to repeat the sample at 12°58.313'N 128°21.964'W or PRA station 17. The boxcore was on the surface at 15:26. It was a good sample.

16:14 (GMT-8) deployed boxcore for **JC241_039** at 12°58.436'N 128°21.002'W or PRA station 20. The boxcore was on the surface at 19:40. This was a good sample with only a few small nodules

[1] Wilson, G. 1990. Biological evaluation of a preservational reserve area (BEPRA I) cruise report and interim report on laboratory analysis. Prepared under National Oceanic and Atmospheric Administration Ocean Minerals and Energy Office contract number 50-DSNC-9-00108. November 1990. SIO Reference Series Number 90-37. University of California and Scripps Institute of Oceanography.

visible. This sample contained a surface-dwelling enteropneust (possibly *Tergivelum* sp.) which was a very lucky catch.

20:30 (GMT-8) deployed boxcore for **JC241_040** at 12°58.116'N 128°19.631'W or PRA station 14. Boxcore on deck at 23:55. This was a failed core as it dewatered as spade had not shut fully. A sample was taken for qualitative work.

Thursday 23 February 2023

00:00 (GMT-8) deployed boxcore for **JC241_041** at 12°58.116'N 128°19.631'W or PRA station 14. This was a repeat of the last core. The boxcore was on deck at 03:33 (GMT-8). Successful core. It only had two nodules in the core.

04:05 (GMT-8) deployed boxcore for **JC241_042** at 12°58.643'N 128°18.989'W or PRA station 21. Boxcore on deck at 07:33 (GMT-8). Successful core.

08:30 (GMT-8) Captain and watch keepers meeting

Noon. 24.9°C Air temperature, 20.0°C sea temperature. Low/medium swell. Force 5/6 wind. Overcast.

12:30 (GMT-8) Science Meeting and watched video of OMCO mining operation

08:35 (GMT-8) commenced ROV wire stream operation. For the wire stream the ROV team had added a current meter to the wire. They hoped that by using the data logged from the internal compass they could calculate the number of turns the wire was making as it was lowered to the seafloor. Each wire stream took over 6 hours. The data from the first deployment showed that the wire made 27 turns on its way down and the same in reverse on the way up. During the second wire stream the wire made 17 turns in a consistent way to the first. The turns started at approximately 2000 m depth.

The wire stream was complete at 22:49 (GMT-8). This represented 14 hours 14 minutes of lost time.

22:57 (GMT-8) deployed boxcore for **JC241_043** at PRA Station 18 at 12°58.63'N 128°17.84'W. This was recovered at 02:20 (GMT-8). This boxcore lost its topwater and was only sampled for qualitative taxonomy.

Friday 24th February 2023

02:25 (GMT-8) deployed boxcore for **JC241_044** to repeat PRA Station 18 at 12°58.63'N 128°17.84'W. This was recovered at 06:00 (GMT-8). This boxcore also lost its topwater and was only sampled for qualitative taxonomy.

06:20 (GMT-8) deployed boxcore for **JC241_045** at PRA Station 22 at 12°59.92'N 128°17.75'W. This was recovered at 10:10 (GMT-8). This was a good core. It had a large asteroid (Porcellanster) on the surface of the sediment.

08:30 (GMT-8) Captain and watch keepers meeting

Noon. Air temperature 26.2°C. Sea temperature 20.1°C. Fine. Low swell. Force 4/5 winds.

12:30 (GMT-8) Science Meeting

10:23 (GMT-8) commenced third ROV wire stream with current meter. This was completed at 16:59 (GMT-8). The number of turns of the wire decreased again to 13 turns but followed a similar pattern to the other streams. The ROV team spoke to Dave Turner at NOC and together agreed to turn the

ROV 8 times from 2000 m depth to the seabed to try to reduce the torsion on the fibre-optic cables at the vehicle connection. This represented 6 hours 38 minutes of lost time.

17:13 (GMT-8) deployed boxcore for **JC241_046** at PRA Station 13 at 12°55.075'N 128°16.958'W. Core on deck at 20:42 (GMT-8). This core lost its topwater and was only used for qualitative analysis.

21:23 (GMT-8) deployed boxcore for **JC241_047** at PRA Station 13 at 12°55.075'N 128°16.958'W. Core on deck at 00:54 (GMT-8). This core lost its topwater and was only used for qualitative analysis.

Saturday 25th February 2023

01:34 (GMT-8) deployed boxcore for **JC241_048** at PRA Station 16 at 12°55.83'N 128°18.90'W. Core on deck at 05:06 (GMT-8). This core lost its topwater and was only used for qualitative analysis.

05:55 (GMT-8) deployed boxcore for **JC241_049** to repeat PRA Station 16 at 12°55.83'N 128°18.90'W. Core on deck at 09:26 (GMT-8). Good core. The three failed cores had been obtained with a different boxcore to this one – it seems that one corer works better than the other.

08:30 (GMT-8) Captain and watch keepers meeting

10:14 (GMT-8) deployed boxcore for **JC241_050** at PRA Station 15 at 12°55.498'N 128°19.23'W. Core on deck at 13:50 (GMT-8). Good core.

Noon. 26.1°C Air temperature. 20.1°C sea temperature. Overcast with showers. Force 5 winds. Moderate swell.

12:30 (GMT-8) Science Meeting

14:54 (GMT-8) Deployed boxcore for **JC241_051** at PRA Station 4 at 12°55.48'N 128°19.60'W. Recovered core at 18:18 (GMT-8). Good core.

19:17 (GMT-8) deployed boxcore for **JC241_052** at PRA Station 8 at 12°54.568'N 128°20.678'W. Recovered core at 22:48 (GMT-8). Good core.

23:38 (GMT-8) deployed boxcore for **JC241_053** at PRA station 6 at 12°55.27'N 128°21.53'W. Recovered core at 03:07 (GMT-8). Good core.

Sunday 26th February 2023

03:55 (GMT-8) deployed boxcore for **JC241_054** at PRA Station 2 at 12°56.06'N 128°21.941'W. This was the twelfth and last station we would investigate at the PRA site. Recovered core 07:20 (GMT-8). Good core.

07:50 (GMT-8) left PRA site for transit to OMCO area. We offset our course so we obtained multibeam over a parallel track (**JC241_055**) to the one we had come in on. The data appeared fairly good despite generally worse multibeam quality heading east than west.

08:30 (GMT-8) Captain and watch keepers meeting

Noon. Air temperature 25.1°C. Sea temperature 20.1°C. Overcast. Slight seas. Force 4 winds.

12:30 (GMT-8) Science Meeting

22:43 (GMT-8) arrived at OMCO and deployed fishtrap at 23:02 (GMT-8) for **JC241_056** on the top of a small hill at 13°43.08'N 126°10.98'W.

23:46 (GMT-8) deployed megacore for **JC241_057** at 13°44.165'N 126°13.233'W on the mapped track location.

Monday 27th February 2023

03:52 (GMT-8) recovered megacore. Of the 8 cores on the megacore 7 had sediment but only one was usable.

05:02 (GMT-8) deployed 5m gravity core for **JC241_058** at the same location as the megacore on the mapped track. Core recovered at 8:11 (GMT-8). Obtained a great 4.85m sediment core with the top intact.

08:30 (GMT-8) Captain and watch keepers meeting

Noon. Air temperature 26.1°C. Sea temperature 20.1°C. Weather partly overcast. Moderate swell. Force 5 wind.

12:30 (GMT-8) Science Meeting

09:23 (GMT-8) ROV launched for photography dive 403 station **JC241_059** at the northern edge of the mapped tracks. ROV arrived at the seabed at 13:40 (GMT-8) to the west of the start location. Spent about an hour testing and setting up the AUV (AESA) stills camera system and the ROV Scorpio stills and video system. Both were pointing downwards in the tool tray of ISIS. The Scorpio camera had a parallel red laser system mounted that projected two red dots 100 mm apart on the seafloor – these were visible in both camera systems. We transited across to the start of the first track transect and during the transit we came across a mooring (15:28 GMT-8), which looked old and was presumably from 1979. It had a metal cube weight tied to a yellow polypropylene rope extending to a cylindrical instrument, which may be been an acoustic beacon. It had another length of polypropylene rope to a Benthos glass sphere float, which was still intact and effective. The whole mooring was around 20m tall. It had some corrosion on the instrument and a relatively large anemone growing on the rope. We gave the mooring a wide berth and went to the track. We set the AUV camera up to image the central part of the track (between the two Archimedes screw tracks), this meant that the wider angle Scorpio camera imaged the central portion of the track and some of the right-hand Archimedes screw track. We set the science camera to image the Archimedes screw track. The camera transect started at 16:08 (GMT-8) and we went down the western side of the western track passing over several crosses. We saw a *Psychropotes* sp. holothurian swimming using its long tail as a sail at 19:47 (GMT-8). At the end there was a patch where the collector vehicle had moved sideways (19:56 GMT-8). We followed this east for a few tens of meters. We then went back to the west and picked up a track line. We thought it was the same one we had come down before, but it later transpired that it was the other track line. We followed this track to the north and then went back down the first track (the westernmost). After this we transited around 1.5km over to the east to the control area. We passed several yellow machinery parts that looked like part of the collector vehicle. At the control area we got to the southern edge of the westernmost line.

Tuesday 28th February 2023

We started the first control line at 10:49 (GMT-8) on 28 Feb and proceeded northwards along this line. Half way along the 2km planned line the hydraulic oil on the ROV reached the minimum and we had to recover the vehicle, leaving the seabed at 13:47 (GMT-8).

08:30 (GMT-8) Captain and watch keepers meeting

Noon. Air temperature 26.5°C. Sea temperature 20.0°C. Partly overcast. Low swell. Force 5/6 wind.

12:30 (GMT-8) Science Meeting

16:30 (GMT-8) release fish trap deployed as **JC241_056**

17:34 (GMT-8) ROV on deck

19:08 (GMT-8) fish trap on deck. Caught two fish (Rat tail and ophiid) and a small catch of amphipods. The bait outside the amphipod traps reduced the numbers that were inside. The outside baits were skeletonized whereas the baits inside the traps were minimally eaten.

20:50 (GMT-8) ROV launched for Dive 404 station **JC241_060** at the control site. The ROV was launched half way through the first control line at 13°44.103'N 126°12.350'W.

Wednesday 1st March 2023

01:37 (GMT-8) ROV reached the seabed and started imaging on the control line.

05:29 (GMT-8) ROV hydraulic oil had run out and the dive was aborted after completing the second half of the first control line during this dive.

08:30 (GMT-8) Captain and watch keepers meeting

09:59 (GMT-8) ROV back on deck. Without the science part, this resulted in 9 hours 1 minute lost time (we did not want to come up and down)

10:32 (GMT-8) Boxcore deployed for **JC241_061** at 13°44.165'N 126°13.233'W on the OMCO track. Boxcore was recovered at 14:05 (GMT-8). Good core that had a soft sloping part of the surface and fairly deeply buried nodules. Appeared to have hit the track or at least a disturbed area near the track.

Noon. Air temperature 26.0°C. Sea temperature 20.0°C. Partly overcast. Moderate swell. Force 5/6 wind.

12:30 (GMT-8) Science Meeting

15:04 (GMT-8) ROV launched for Dive 405 for **JC241_062** at the start of the second control line at 13°44.664'N 126°12.395'W. Reached seabed at 19:21 (GMT-8). The ROV team started the dive practicing using the hydraulic cutter to cut a length of cable (same type as umbilical). This test was complete at 19:59 (GMT-8). Transect 2 in the control site was commenced at 20:00 (GMT-8) and the ROV transited south down the line.

Thursday 2 March 2023

08:30 (GMT-8) Captain and watch keepers meeting

01:38 (GMT-8) ROV completed control imaging transect 2 (transect 1 had been completed over the two previous dives).

01:55 (GMT-8) ROV started control imaging transect 3, which was completed at 07:49 (GMT-8). Imaging transect 4 was started at 08:26 and completed at 12:23 (GMT-8). The ROV left the seabed at 12:37 (GMT-8) and was on deck at 16:30 (GMT-8). This ROV dive was completed at the choice of the science party so we could recover the vehicle and turn around for a later dive. Unfortunately, the ROV tether was round to be damaged on the surface, with twists near the ROV floats. This required a termination. With this termination there are only two available wire potting kits for the cruise.

Noon. 25.5°C Air temperature. 19.9°C Sea temperature. Overcast. Slight to moderate seas. Force 6/7 wind.

12:30 (GMT-8) Science Meeting

17:11 (GMT-8) deployed megacore for **JC241_063** on the OMCO tracks at 13°44.164'N 126°13.232'W. The core was recovered at 21:22 (GMT-8) and 7 of 8 cores were used.

17:55 (GMT-8) fish trap lander deployed for **JC241_064** at 13°44.842'N 126°13.211'W. Lander targeted a deeper area of seabed to the north of the OMCO tracks.

22:22 (GMT-8) deployed boxcore for **JC241_064** at the OMCO track at 13°44.1102'N 126°13.23'W.

Friday 3 March 2023

01:52 (GMT-8) Boxcore recovered. The core hit the track – the sloped sediment and disturbed sediment was clearly visible on the core.

02:43 (GMT-8) 5m gravity core deployed for **JC241_066** at the OMCO control at 13°44.114'N 126°12.324'W. Gravity core recovered at 05:33 (GMT-8). The core was good and 3.8m of sediment was recovered.

06:11 (GMT-8) Boxcore deployed for **JC241_067** at the OMCO track site at 13°44.01'N 126°13.206'W. The core was recovered at 9:56 (GMT-8). It was a good core. It hadn't obviously hit the tracks as it had nodules and a normal sediment type. It had a large spiny urchin in the center of the core.

08:30 (GMT-8) Captain and watch keepers meeting

10:20 (GMT-8) started ROV wire stream operations.

Noon. Air temperature 25.0°C. Sea temperature 19.9°C. Overcast. Low swell. Force 4 wind.

12:30 (GMT-8) Science Meeting

18:49 (GMT-8) complete wire stream operations. Represents 8 hours 29 minutes lost time.

19:11 (GMT-8) deploy 5m gravity core for station **JC241_068** at the control site at 13°44.048'N 126°12.017'W. The core was back on deck at 21:38 (GMT-8). It was a successful core.

20:27 (GMT-8) In position for ROV dive. Took some time for the ROV team to complete operations and do pre-dive checks.

Saturday 4 March 2023

00:14 (GMT-8) ROV in water for Dive 406 at station **JC241_069** at the OMCO track site at 13°44.175'N 126°13.248'W. ROV reached the seafloor at 04:14 (GMT-8). The ROV deployed the four cube experiments, two in the track and two outside the track about 20 m apart. The cubes were used to inject isotopically labelled algae onto the seafloor. They will be recovered subsequently and cored to assess the uptake of the algae by the fauna. They do not make any respiration measurements. The ROV then collected 12 pushcores in and out of the track. Finally some megafaunal specimens were collected including 4 *Psychronaetes hanseni* holothurians and spikey deimatidae holothurian a *Hymenaster* sp. sea star and a hexatinellid sponge collected on the centre between the two Archimedes screw tracks (which broke off and was lost during recovery). ROV on deck at 18:33 (GMT-8).

08:30 (GMT-8) Captain and watch keepers meeting

Noon. Air temperature 26.5°C, Sea temperature 20.0°C. Fair. Calm Seas. Force 3/4 wind.

12:30 (GMT-8) Science Meeting

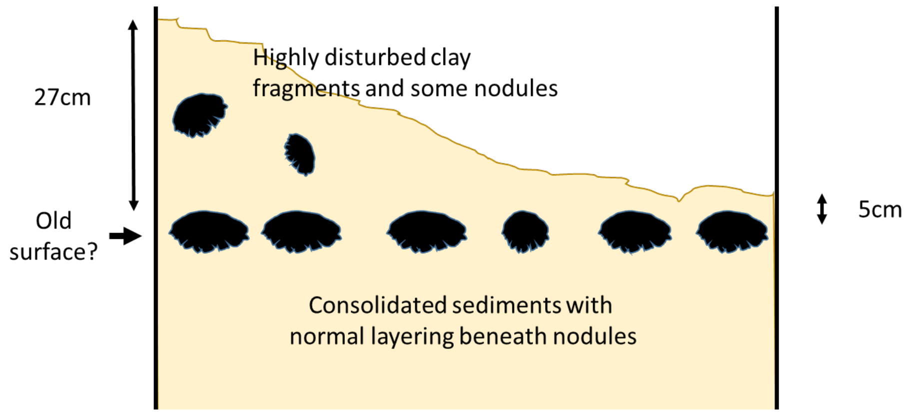
20:45 (GMT-8) deployed boxcore for station **JC241_070** at the OMCO track site at 13°44.14'N 126°13.24'W.

Sunday 5 March 2023

00:34 (GMT-8) boxcore on deck. Good sample. Had hit tracks.

01:16 (GMT-8) deployed megacore for station **JC241_071** in the control area at 13°44.115'N 126°12.322'W. Core recovered to deck at 05:15 (GMT-8). Successful core with 5 of 8 recovered.

05:53 (GMT-8) Deployed boxcore on station **JC241_072** on track at 13°44.064'N 126°13.218'W. Core recovered at 09:26 (GMT-8). Core successful and hit one of the berms in the centre of the track. The surface sediment was sloped and consisted of poorly consolidated <10mm balls of clay. There was an almost complete coverage layer of large ~10cm length nodules at the base of the disturbed sediment. This ranged from 27cm below the sediment surface to around 5 cm on the different sides of the core.



Schematic of boxcore JC241_072

08:30 (GMT-8) Captain and watch keepers meeting

Noon. Air temperature 26.1°C. Sea temperature 20.0°C. low swell. Fair. Force 3 / 4 wind.

12:30 (GMT-8) Science Meeting

12:16 (GMT-8) ROV Deployed for Dive 407 at Station **JC241_073** at 13°44.189'N 126°13.225'W. ROV was on seafloor at 16:36 (GMT-8). We transited over to the track locations quite near the cube experiments, which were clearly visible in the ROV sonar. We collected some push cores in the tracks and outside. Inside the tracks the only thing that looked like it had grown there since the tracks were made were some xenophyophores, probably *Reticulammina* spp.. We collected several of these in push cores. We completed collection of 10 push cores in the track for biogeochemical assessment, foraminiferal analysis and biodiscovery. We then went looking for animals to collect for a range of biological studies. This was very successful, with a range of collections including numerous holothurians, asteroids, a comatulid crinoid, barnacles, anemones, ophiuroids and a xenophyophore (*Psammina* sp.). After midnight the watch collected pushcores outside the tracks and more specimens including large tunicates, antipatharians, asteroids, holothurians and a sponge.

Monday 6 March 2023

07:21 (GMT-8) ROV left seabed and was on deck at 11:13 (GMT-8).

08:30 (GMT-8) Captain and watch keepers meeting

11:55 (GMT-8) Megacore deployed at control site for **JC241_074** at 13°44.05'N 126°12.016'W. Megacore recovered at 15:50 (GMT-8). Good core.

Noon. Air temperature 26.5°C. Sea temperature 20.3°C. Partly cloudy. Low swell. Force 4 wind.

12:30 (GMT-8) Science Meeting

16:24 (GMT-8) Megacore deployed at track site for **JC241_075** at 13°44.122'N 126°12.239'W. Core arrived back on deck at 20:35 (GMT-8). Good cores.

21:15 (GMT-8) ROV in water for ISIS Dive 408 station **JC241_076** at OMCO track, start position 13°44.205'N 126°13.233'W. This dive was for collection and sampling of the cube experiments for Andrew Sweetman. The cubes add ^{13}C enriched algae to the seafloor.

Tuesday 7 March 2023

01:03 (GMT-8) ROV arrived at the seafloor

03:44 (GMT-8) All cube experiments collected and 3 push cores taken in the area of the seafloor under each cube. The rest of the dive was spent collecting animals. These included *Plesiodiadema globulosum* (urchin; easiest to collect with the manipulator arms), stalked sponge with anemone, *Psychropotes* spp. *Dytaster* sp., the huge sea cucumber *Psychronaetes hanseni*, *Amperima* sp. and an ophiuroid. We also collected a ~30cm length of painted yellow metal pipe, which was from the 1979 test and had some colonisers.

07:31 (GMT-8) ROV left the seabed coming towards the surface.

08:00 (GMT-8) Daniel Jones flew DJI Mini 2 drone to collect still and video footage of ship. The drone was flown from the lifeboat deck (forward of the bridge). Wind 12-15 knots, which was close to the practical limit for the drone to get good images. Some reasonable images and video of the ship in the light. Used one battery – around 25 mins.

08:30 (GMT-8) Captain and watch keepers meeting

Noon. Air temperature 25.5°C. Sea temperature 20.1°C. Partly cloudy. Low swell. Force 4 wind.

12:30 (GMT-8) Science Meeting

13:39 (GMT-8) ROV on deck**.**

14:02 (GMT-8) Boxcore off deck for station **JC241_077** at OMCO track position approx. 13°44.08'N 126°13.23'W. Boxcore on deck at 17:31 (GMT-8). Good core.

18:42 (GMT-8) Boxcore off deck for station **JC241_078** at OMCO track position 13°44.031'N 126°13.226'W. Boxcore on deck at 22:11 (GMT-8). Good core.

23:07 (GMT-8) Boxcore off deck for station **JC241_079** at OMCO track position 13°44.139'N 126°13.239'W. Boxcore on deck at 02:27 (GMT-8). Good core.

Wednesday 8 March 2023

02:39 (GMT-8) transit south collecting multibeam for JC241_080. We had done the minimum number of boxcores and wanted to investigate another control site to ensure representability. We selected a control site around 10km south of the OMCO track area, with a very similar geomorphology (on a slight slope dipping to the east) and similar depth to the tracks. This site was very similar except for the proximity of a relatively large knoll (around 400 m tall) to the east rather than the small hill to the east of the track site.

03:47 (GMT-8) Boxcore off deck for station **JC241_081** at the new control site at 13°38.121'N 126°11.981'W. Boxcore on deck at 07:14 (GMT-8). We did record multibeam on the way back to the OMCO site (at a ~2mile offset to the other line) but this was not given a station number.

06:00 (GMT-8) Daniel Jones flew DJI Mini 2 drone to collect still and video footage of ship. Wind 11-12 knots, which was ideal for drone flying. Took off in the dark and collected photographs and video as the sun rose until it was light. Used three batteries one after another. Last photograph was taken at 07:13 (GMT-8). Some excellent images and video including images over 1 km away from ship (there was no other ships in the area).

08:30 (GMT-8) Captain and watch keepers meeting

09:53 (GMT-8) ROV in water for ISIS dive 409 station **JC241_082** at the OMCO tracks start position 13°44.363'N 126°13.244'W. This was set up as an imaging dive with both the AESA (AUV) camera and the Scorpio camera point downwards. It also had RoCSI obtaining eDNA samples.

Noon. Air temperature 27.1°C. Sea temperature 20.1°C. Fair. Low swell. Force 4 wind.

12:30 (GMT-8) Science Meeting

The ROV arrived at the seabed at 13:52 (GMT-8). During this dive we completed 2 transects at 5 m distance from the tracks, 2 transects at 10 m distance from the tracks and the final transect along the tracks.

Thursday 9 March 2023

08:30 (GMT-8) Captain and watch keepers meeting

Noon. Air temperature 26.6°C. Sea temperature 19.9°C. Partly cloudy and fair. Low swell. Force 4 wind.

12:30 (GMT-8) Science Meeting

15:00 (GMT-8) Completed the ROV imaging dive. The ROV was on deck at 19:08 (GMT-8).

18:40 (GMT-8) Daniel Jones flew DJI Mini 2 drone to collect still and video footage of the ROV recovery. The wind was 15+ knots and it was dark, which made for difficult flying. Obtained some reasonable images and video of the ROV recovery, although a low battery meant it had to be flown back and the battery changed just after the ROV surfaced. Used two batteries. Last image obtained at 19:07 (GMT-8).

19:34 (GMT-8) Boxcore off deck for station **JC241_083** at OMCO tracks at 13°44.139'N 126°13.239'W. Boxcore on deck at 23:02 (GMT-8). USBL was not working. Good core but did not hit tracks.

Friday 10 March 2023

00:11 (GMT-8) Boxcore off deck for station **JC241_084** at OMCO tracks at 13°44.1432'N 126°13.2294'W. Boxcore on deck at 03:38 (GMT-8). Good core.

04:43 (GMT-8) Boxcore off deck for station **JC241_085** at OMCO tracks at 13°44.058'N 126°13.218'W. Boxcore on deck at 8:13 (GMT-8). Good core.

08:30 (GMT-8) Captain and watch keepers meeting

09:02 (GMT-8) ROV launched for multibeam dive ISIS Dive 410 at station **JC241_086** at 13°43.982'N 126°13.179'W. ROV was in water at 09:15 (GMT-8).

Noon. Air temperature 23.9°C. Sea temperature 19.9°C. Slight sea, overcast, fine. Force 4 wind.

12:30 (GMT-8) Science Meeting

12:48 (GMT-8) ROV arrived at the seafloor at the edge of the previous bathymetry (over track) and commenced survey at 50 m altitude and speed of 0.15 m/s using Reson multibeam.

Saturday 11 March 2023

07:00 (GMT-8) ROV completed dive around 1/3 of the way along the second control line.

08:30 (GMT-8) Captain and watch keepers meeting

10:30 (GMT-8) ROV recovered to deck after successful dive.

10:59 (GMT-8) boxcore deployed for **JC241_087** at OMCO Track position 13°44.017'N 126°12.215'W. On deck at 14:23 (GMT-8). Good core but didn't appear to hit track.

Noon. Air temperature 26.1°C. Sea temperature 19.8°C. Partly cloudy, low sea. Force 4/5 wind.

12:30 (GMT-8) Science Meeting

15:23 (GMT-8) boxcore deployed for **JC241_088** at OMCO control position 13°44.151'N 126° 12.150'W. On deck at 18:50 (GMT-8). Good core but USBL failed.

20:05 (GMT-8) ROV deployed for ISIS Dive 411 station **JC241_089**, which was a collection and non-quantitative photography dive at the OMCO control area start position 13°43.610'N 126°12.250'W. ROV was in water at 20:14 (GMT-8) and reached the seafloor at 00:04 (GMT-8).

Sunday 12 March

During this dive we put the Scorpio still and video camera on the ROV toolbar looking forward. This was very effective, allowing us to get much better quality images of the fauna than before and adjust the lighting so it was more atmospheric. The ROV dive start was the position of a large stalked crinoid. We had not expected to be able to locate it again but we found it fairly quickly. We transited northwards making some collections. We were aiming to assess some of core locations to see if we could locate their position then we targeted a feature visible on the multibeam. The multibeam feature turned out to be a mooring from 1978, with a large cuboid battery, linked to an instrument with an electrical cable and a rope. There were four floats above the instrument. Several anemones were living on the box. We obtained nine normal push core samples. We also took a single sample using the large (~10cm diameter and 60cm tall) pushcore. This was successful and got a core like a megacore. In terms of biological samples, we obtained two large stalked crinoids, sponges (tall, tulip, *Axoniderma* sp.), several holothurians (Elpidiidae, *Amperima* sp., *Psychronaetes hanseni* and *Psychropotes* sp.), asteroids (Pterasteridae, Brisingid) and urchins (*Plesiodiadema globulosum*). The ROV left the seabed at 11:07 (GMT-8) and arrived back on deck at 14:45 (GMT-8).

08:30 (GMT-8) Captain and watch keepers meeting

Noon. Air temperature 26.1°C. Sea temperature 20.0°C. Partly cloudy, low sea. Force 4 wind.

12:30 (GMT-8) Science Meeting

15:13 (GMT-8) CTD deployed for station **JC241_090** at OMCO Control position 13°43.804'N 126°12.269'W. We obtained water from the Niskin bottles near the seafloor to use for the sediment traps. The CTD was on deck at 18:16 (GMT-8).

18:32 (GMT-8) left OMCO site and transited to UK-1 following line to optimize our multibeam station **JC241_091**. We passed a very high seamount on the way, rising from the abyss to less than 700 m.

Monday 13 March

In transit

08:30 (GMT-8) Captain and watch keepers meeting

Noon. Air temperature 26.2°C. Sea temperature 19.8°C. Cloudy, slight sea, force 5 wind.

Noon Position 13°50.7'N 123°13.6'W

12:30 (GMT-8) Science Meeting

16:00 (GMT-8) Fire drill

Tuesday 14 March

In transit

08:30 (GMT-8) Captain and watch keepers meeting

Noon. Air temperature 26.3°C. Sea temperature 20.8°C. 3 / 8 cloud cover, slight sea, force 4 wind.

Noon Position 14.01'N 119°07.1'W

12:30 (GMT-8) Science Meeting

I spent most of the day preparing the sediment traps for deployment tomorrow with Andrew Sweetman.

Wednesday 15 March

03:40 (GMT-8) Arrived at UK-1

03:54 (GMT-8) Billy sent the release command to the Bathysnap lander.

03:59 (GMT-8) lander surfacing at around 60 m /min

05:11 (GMT-8) Bathysnap on surface and on deck at 05:32 (GMT-8). Unfortunately a fault with the computer meant that we had only recorded a few days of photographs (151 images). In addition to this, the gain setting had not been checked and meant that the images were too dark (gain = 0).

After Bathysnap was recovered, work began on the moorings. The first mooring deployed was mooring 2. This was a short (100 m long) mooring, deployed at the northern site.

07:51 (GMT-8) Mooring 2 released at station **JC241_092** at 13°56.519'N 116°30.339'W.

We then repositioned and started preparations for Mooring 1 (the long, 1000m, mooring)

08:30 (GMT-8) Captain and watch keepers meeting

09:39 (GMT-8) The first floats were in the water for mooring 1.

11:30 (GMT-8) mooring anchor weight dropped and the mooring sank to the seafloor. Station **JC241_093** at 13°53.405'N 116°29.384'W.

Noon. Air temperature 26.2°C. Sea temperature 22.3°C. 1/8 cloud cover. Fine. Calm sea. Force 4 wind.

There was a problem with the crane hydraulics, which resulted in an approximately 1 hour delay.

12:30 (GMT-8) Science Meeting

13:40 (GMT-8) The first float was in the water for mooring 3 (short – 100m long).

14:39 (GMT-8) The mooring was released at station **JC241_094** at position 13°52.453'N 116°32.592'W.

15:43 (GMT-8) The ROV was off deck for ISIS Dive 412, a specimen collection dive. Station **JC241_095** start point 13°57.798'N 116°33.217'W. ROV reached the seabed at 19:34 (GMT-8). The start point of this dive was planned to be a lander weight deployed on the Abyssline cruise AB01 in 2013. We wanted to see if there was any evidence of growth on the weight after nearly 10 years. We did not succeed in finding the weight. We picked up several holothurians ,including *Benthodytes* sp.? and another species, and a sea urchin that may have been *Echinocrepis* sp. This sea urchin, when lit from the back, was supporting a community of small decapods? living around the spines. We then transited to the location of a lander that had been wrecked by imploding buoyancy spheres on the same Abyssline AB01 cruise. The lander was a baited timelapse camera lander owned by Jeff Drazen from University of Hawaii. We succeeded in finding this lander. The damage caused by the implosion was very obvious. The lander was lying upside down in the sediment and there were no floats with any buoyancy. At it was fairly safe we approached the lander and took lots of photographs and video to assess recolonization. There were several mobile organisms on and around the lander including comatulid crinods and ophiuroids. The aluminum frame was covered in white patches, which we first thought were sponges, but proved to be strange corrosion features (also visible on the structures from 1978 at OMCO). We sampled an aluminium bar from the lander. During the remainder of this collections dive we sampled hexactinellid sponges, several sea cucumbers, octocorals (c.f. *Calyptrophora* sp. and *Abyssoprimnoa* sp.), a Brisingid sea star. We collected 12 push cores for Bryan's foramineral studies, for Louisa at University of Liverpool and for Susan for eDNA analysis.

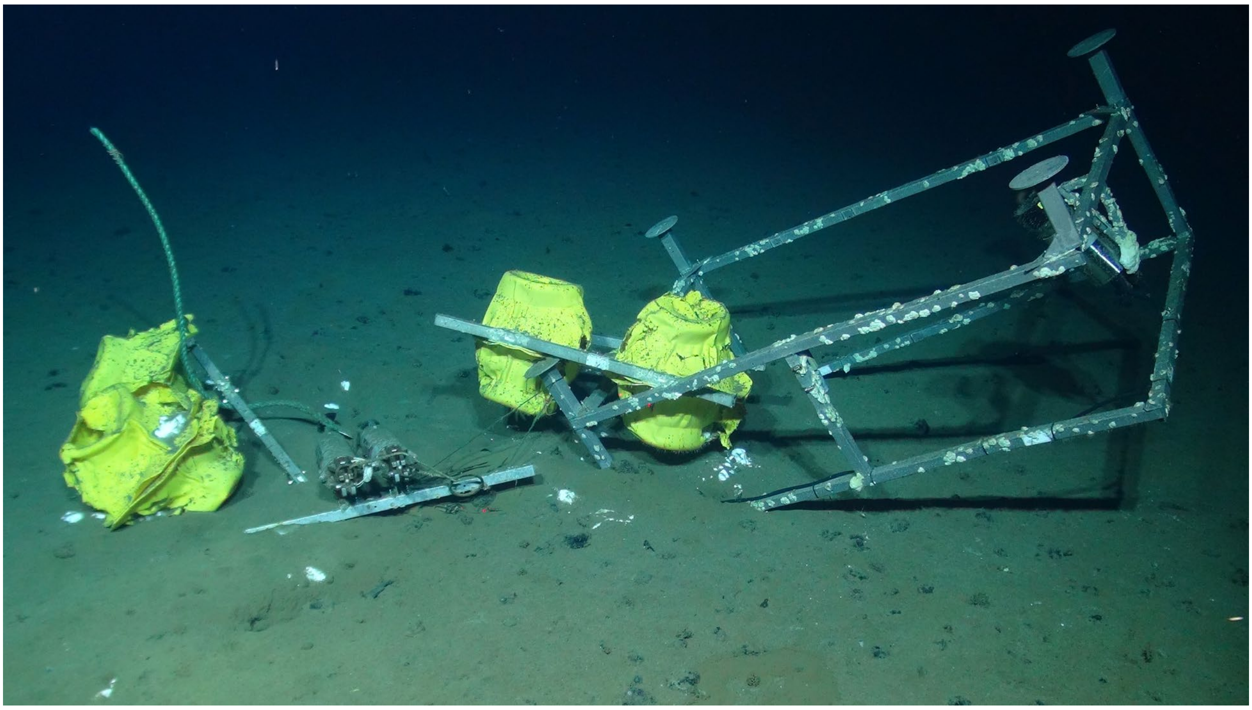


Figure 8.1: The University of Hawaii lander

Thursday 16 March

07:55 (GMT-8) The ROV left the seabed

08:30 (GMT-8) Captain and watch keepers meeting

11:15 (GMT-8) ROV back on surface.

Noon. Air temperature 26.1°C. Sea temperature 22.3°C. Broken cloud, slight seas, low swell, Force 3 wind.

12:25 (GMT-8) CTD off deck for **JC241_096** at position 13°54.839'N 116°29.639'W. Full depth cast to validate moorings. Back on deck at 15:19 (GMT-8)

12:30 (GMT-8) Science Meeting

16:16 (GMT-8) Gravity core (5m) off deck for station **JC241_097** at position 13°58.398'N 116°32.403'W. On deck at 18:42 (GMT-8). Good core with recovery of 3.28m of sediment.

16:26 (GMT-8) ROV in water for ISIS Dive 413 at station **JC241_098**. The dive started at 13°55.942'N 116°30.660'W at the location of a colonization experiment that was deployed on the UK Seabed Resources RC01 cruise in March 2020. The colonization experiment had basalt blocks on a 50 x 50 cm plastic frame. We were able to obtain very good downward-facing imagery of the blocks. They had some abundant mobile organisms, including an urchin (*Plesiodiadema globulosum*) and shrimp. There may have been an anemone growing on the blocks. There were some long worms that appeared to be benthic chaetognaths. This dive also had the RoCSI eDNA sampler onboard, so we fired the big Niskin bottle to get a water sample to cross validate the RoCSI data. RoCSI can collect up to 24 samples and was set to sample once an hour. After this had been done we set off to carry out 5 x 2km straight line transects in a zig-zag pattern for quantitative megafaunal assessment. The ROV had the Scorpio camera with parallel red lasers (10cm apart) downward facing (taking video and stills every 10 seconds) and the AUV AESA camera (taking images every 3 seconds). Each 2km transect took approximately 4 hours.

Saturday 18 March 2023

A total of 6.5 transects were done. The ROV left the seafloor at 09:09 (GMT-8).

08:30 (GMT-8) Captain and watch keepers meeting

Noon. Air temperature 29.1°C. Sea temperature 22.4°C. Fair, low seas, Force 3 wind.

12:41 (GMT-8) ROV on deck.

14:00 (GMT-8) Bathsnap was deployed for station **JC241_099** at location 13°54.86'N 116°31.26'W. I filmed the deployment with a GoPro camera on a pole.

14:48 (GMT-8) the second Bathysnap was deployed for station JC241_100 at location 13°53.25'N 116°30.59'W. I also filmed this deployment with the GoPro. There were large numbers of salps in the surface waters, which were visible in the underwater video.

15:25 (GMT-8) the megacore was deployed for station **JC241_101** at location 13°58.40'N 116°32.41'W. The core was recovered at 19:02 (GMT-8) and was a good sample. This was the last seabed sample of JC241.

19:15 (GMT-8) start transit back to Caldera. We planned a route to collect multibeam bathymetry (station **JC241_102**) on the way back along a parallel line to the route we had taken here.

Clocks went forward to GMT-7

Sunday 19 March 2023

00:43 (GMT-7) Entered EEZ of France (Clipperton Island) and turned off data logging.

In transit

Noon position: 13°03.0'N 113°59.0'W

Monday 20 March 2023

In transit

10:30 (GMT-7) Safety Meeting

Noon position: 12°22.4'N 109°26.3'W

Tuesday 21 March

Sunrise Marine Mammal Watch

07:33 (GMT-7) Left EEZ of France (Clipperton Island) and resumed data logging

In transit

Noon position: 11°43.3'N 105°04.9'W

12:30 (GMT-7) Cruise Photograph

Wednesday 22 March

In transit

Noon position: 11°17.7’N 100°51.1’W

12:30 (GMT-7) Science meeting

Ship’s clocks changed to GMT-6 (Costa Rica Time)

Thursday 23 March

In transit

Noon position: 10°44.3’N 096°51.1’W

12:30 (GMT-6) Science talks with highlights of expedition findings from each group.

Friday 24 March

In transit

Noon position: 10°08.7’N 093°06.4’W

19:00 (GMT-6) Did talk for officers and crew about the science findings and highlights of the trip. Also showed a compilation video of material obtained on the cruise.

Saturday 25 March

In transit

05:10 (GMT-6) Entered Costa Rican EEZ and turned off data logging.

Noon position: 09°35.2’N 089°00.00’W

12:30 (GMT-6) Final Science meeting

Sunday 26 March 2023

05:58 – 07:47 (GMT-6) *James Cook* was accompanied by two Rigid Inflatable Boats and the M.V. *Arctic Sunrise* operated by Greenpeace. They put two people in the water immediately in front of the *James Cook* on at least four occasions and one person boarded the vessel and attached themselves to the port side scupper with a rope. The captain was in regular contact with the *Arctic Sunrise* as soon as their actions became apparent to try to prevent further interactions (without success).

12:00 (GMT-6) Arrived in Caldera

Table of lost science time caused by malfunctions or non-science ship operations. The operations labelled with NI are not included in the total as they are deemed to be standard operations. This does not include the 8 boxcores that collected sediment but that washed out on the surface.

Date	**Time lost**	**Reason**
14 Feb 2023	10 hours 36 minutes	CASIUS deployment – NI
19 Feb 2023	1 hour 59 minutes	CASIUS Recovery – NI
13 Feb 2023	6 hours	ROV wire stream – NI
15 Feb 2023	4 hours 21 minutes	Failed Boxcore – wire around boxcore head
16 Feb 2023	4 hours 14 minutes	Failed Boxcore
17 Feb 2023	15 hours 12 minutes	ROV failure 1 – dead sub recovery
18 Feb 2023	8 hours 52 minutes	ROV failure 2 – dead sub recovery
19 Feb 2023	4 hours 14 minutes	Failed Boxcore
19 Feb 2023	51 minutes	Aborted wire stream – cable damage
20 Feb 2023	10 hours 34 minutes	ROV wire stream
22 Feb 2023	8 hours 35 minutes	ROV failure 3 – dead sub recovery
23 Feb 2023	14 hours 4 minutes	ROV wire stream x 2 with current meter
24 Feb 2023	6 hours 36 minutes	ROV wire stream with current meter
1 Mar 2023	9 hours 1 minute	Oil failure on ROV Dive 404 mean extra dive needed – wasted time to and from seafloor
3 Mar 2023	8 hours 29 minutes	ROV wire stream
Sub Totals	82 hours 14 minutes	ROV operations
	12 hours 49 minutes	Boxcoring operations (complete failure)
Total	**95 hours 3 minutes**	All operations

9. Physical Oceanography

CTD Deployments

Daniel Jones and Billy Platt

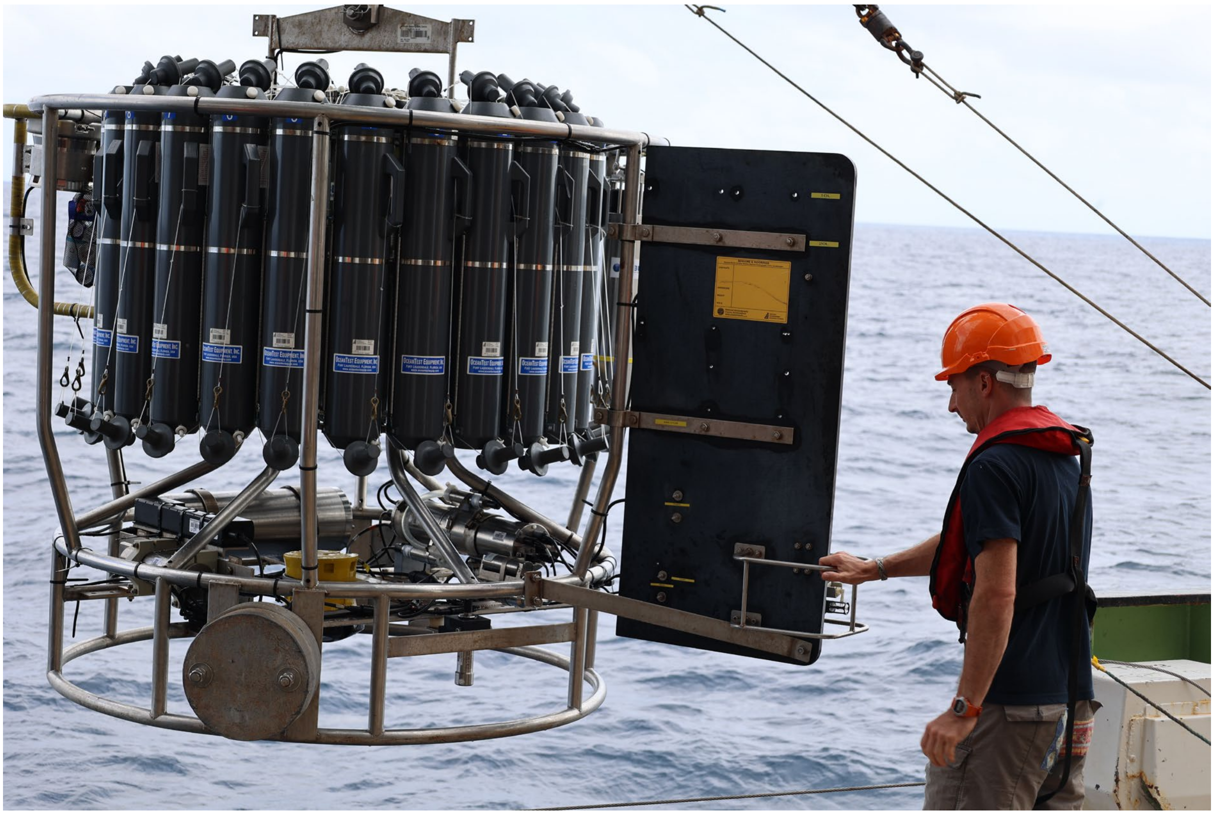


Figure 9.1: CTD being deployed on JC241.

The CTD was deployed 6 times on JC241. The first two CTDs targeted an Eddy Feature for the Scottish Association of Marine Sciences team. Three CTDs were deployed at the OMCO area to provide a sound speed profile for the acoustic work and to collect water for eDNA and nutrient analysis. The final CTD was done at UK-1 in a position between three deployed moorings to act as a cross validation for the instruments onboard.

The eddy feature was observed tracking westwards from the central American coast. These features are caused by the 'Tehuantepecer', the mountain gap winds that form this time of year and impact the E Pacific. The eddy feature was tracked using windy.com (https://www.windy.com/-Currents-currents?currents,14.435,-103.645,5,m:dZjadiX), which uses satellite data from Copernicus to display surface ocean currents (Figure 9.2). The eddy system was propagating westward at a speed of 9.9 nautical miles per day (0.766 km/h). The eddy's shape is elliptical with radius lengths between approximately 65 and 100 km. The eddy system was clearly visible in the underway ADCP data (Figure 9.3). We obtained two CTDs, 1) one full depth CTD profile at the Eddy edge – corresponding with maximum orbital speed ~100 km SE & NW from the centre; 2) one full-depth CTD cast in the eddy centre (~ 10-20 m above seabed for safety. We would have liked to get additional CTD data from the eddy (at the western edge and on the return journey to Caldera) but the eddy system had propagated into the EEZ of Clipperton Island (France).

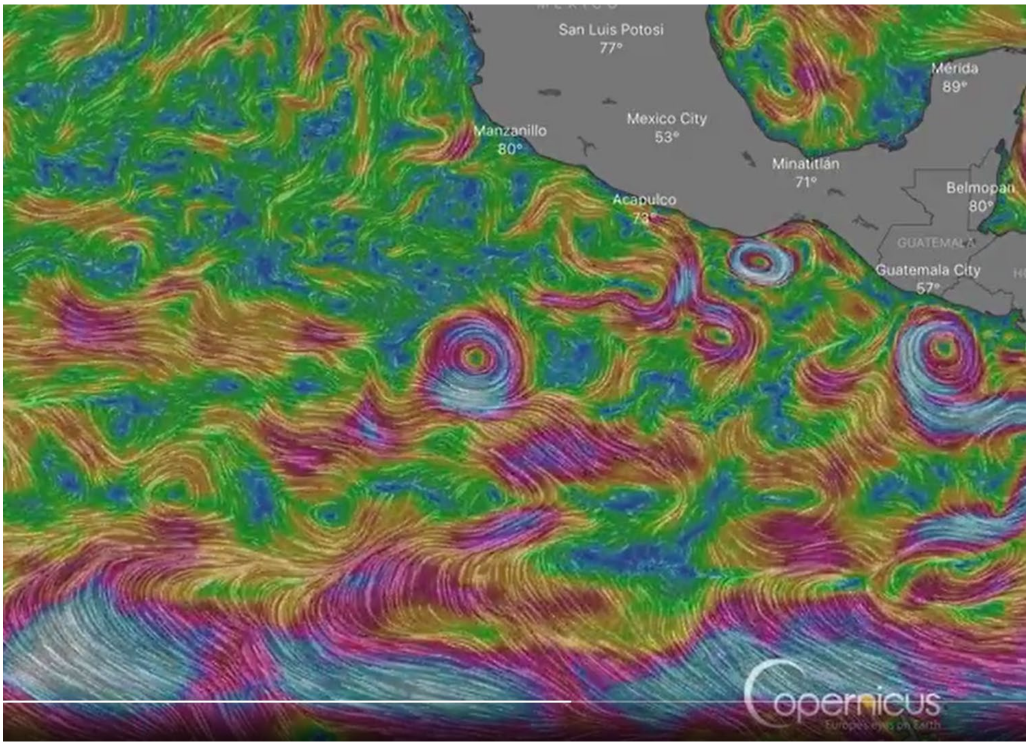


Figure 9.2: Eddy system (centre) that was the target of CTD investigations.

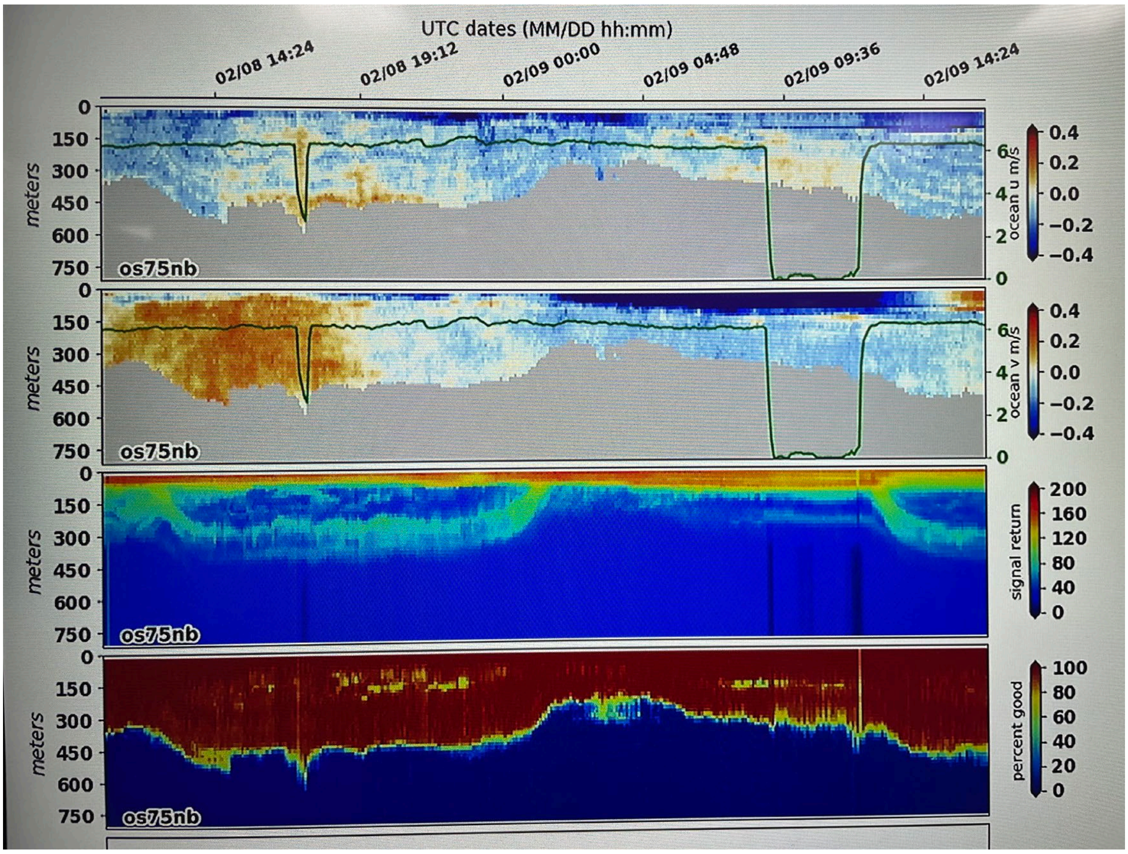


Figure 9.3: Underway ADCP records showing transition into Eddy system. The CTD location is shown then the vessel speed (green line) slowed for around 4 hours. The first brief dip in vessel speed was required to avoid another ship.

Table 9.1: CTD deployments

Station	Name	Location	Date	Time at Seabed	Latitude Deg (N)	Latitude Min	Longitude Deg (W)	Longitude Min	Depth, m
JC241_003	CTD01	Eddy Edge	09/02/2023	10:50:00	11	49.0660	104	42.6050	3180
JC241_004	CTD02	Eddy Centre	09/02/2023	18:04:00	11	49.2798	105	38.2972	3368
JC241_009	CTD03	OMCO track	14/02/2023	22:06:00	13	43.8748	126	13.3597	4694
JC241_031	CTD04	OMCO control	20/02/2023	21:03:00	13	44.2500	126	12.3000	4675
JC241_090	CTD05	OMCO tracks	13/03/2023	00:43:00	13	43.8043	126	12.2685	4692
JC241_096	CTD06	UK-1	16/03/2023	21:50:00	13	54.0379	116	29.6377	4224

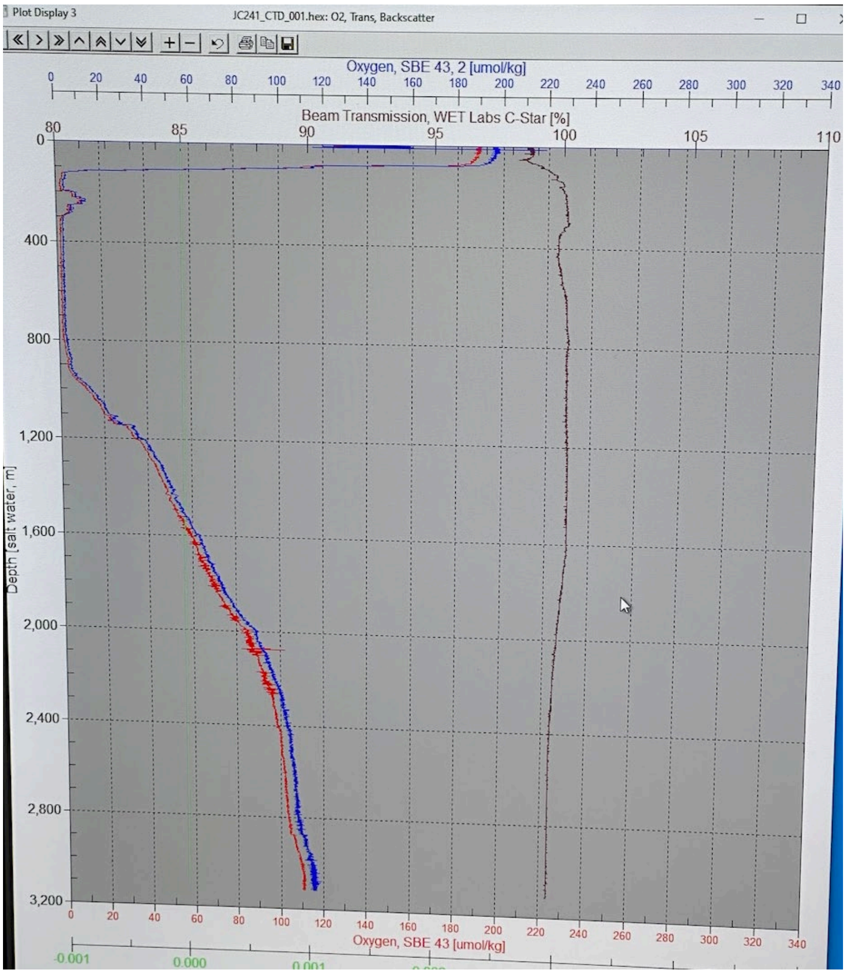


Figure 9.4. Screenshot of CTD01 deployment showing clear oxygen minimum zone between ~100 and 900 m depth.

Table 9.2: CTD instruments and setup details from Billy Platt

Instrument / Sensor	Manufacturer/ Model	Serial Number	Channel	Casts Used
Primary CTD deck unit	SBE 11plus	11P-19817-0495	n/a	All casts
CTD Underwater Unit	SBE 9plus	09P-39607-0803	n/a	All casts
Stainless steel 24-way CTD frame	NOCS	SBE CTD8	n/a	All casts
Primary Temperature Sensor	SBE 3P	03P-2729	F0	All casts
Primary Conductivity Sensor	SBE 4C	04C-2164	F1	All casts
Digiquartz Pressure sensor	Paroscientific	93896	F2	All casts
Secondary Temperature Sensor	SBE 3P	03P-2674	F3	All casts
Secondary Conductivity Sensor	SBE 4C	04C-2450	F4	All casts
Primary Pump	SBE 5T	05T-7517	n/a	All casts
Secondary Pump	SBE 5T	05T-7516	n/a	All casts
24-way Carousel	SBE 32	32-19817-0243	n/a	All casts
Primary Dissolved Oxygen Sensor	SBE 43	43-0363	V0	All casts
Secondary Dissolved Oxygen Sensor	SBE 43	43-0709	V1	All casts
BBRTD	WetLabs ECO-AFL	5466	V2	All casts
Flourometer	Chelsea Aqua 3	88-2615-124	V3	Casts 4-6
Transmissometer	WetLabs C-Star	CST-2150DR	V4	All casts
Altimeter	Valeport VA500	81630	V5	All casts
LADCP Down looking	RDI 300KHz	4275	n/a	All casts
LADCP uplooking	RDI 300KHz	10607	n/a	All casts
10L Water Samplers	Ocean Test Equipment	Set D	n/a	All casts
Titanium EM CTD Swivel	MDS V2_2	1253-2	n/a	All casts

Moorings

Daniel Jones and Billy Platt

(on behalf of Andrew Dale and Dmitry Aleynik – Scottish Association of Marine Sciences)

Three moorings were deployed at UK-1 to the south of Area of interest 2 (AOI-2). The design is an L-shape with the long mooring at the angle and two perpendicular directions to the two shorter moorings. Each leg of the L is 6 km, one along a bathymetric trough, and the perpendicular leg to the summit of a ridge. The idea is to allow us to compare the spectral properties of the flow from ridge to trough versus along a trough. The northern-most mooring is close but not within AOI-2.

The moorings were equipped with Sediment traps (see section below), Nortek single point current meters (sampling every 5 minutes (300 seconds)), Sea-Bird SBE37 CTDs (sampling every 10 minutes (600 seconds)) and RBRsolo and StarOdi thermistors (sampling every 1 second). The location and serial numbers of these instruments are listed on the mooring diagrams (Figures 9.6 - 9.9).

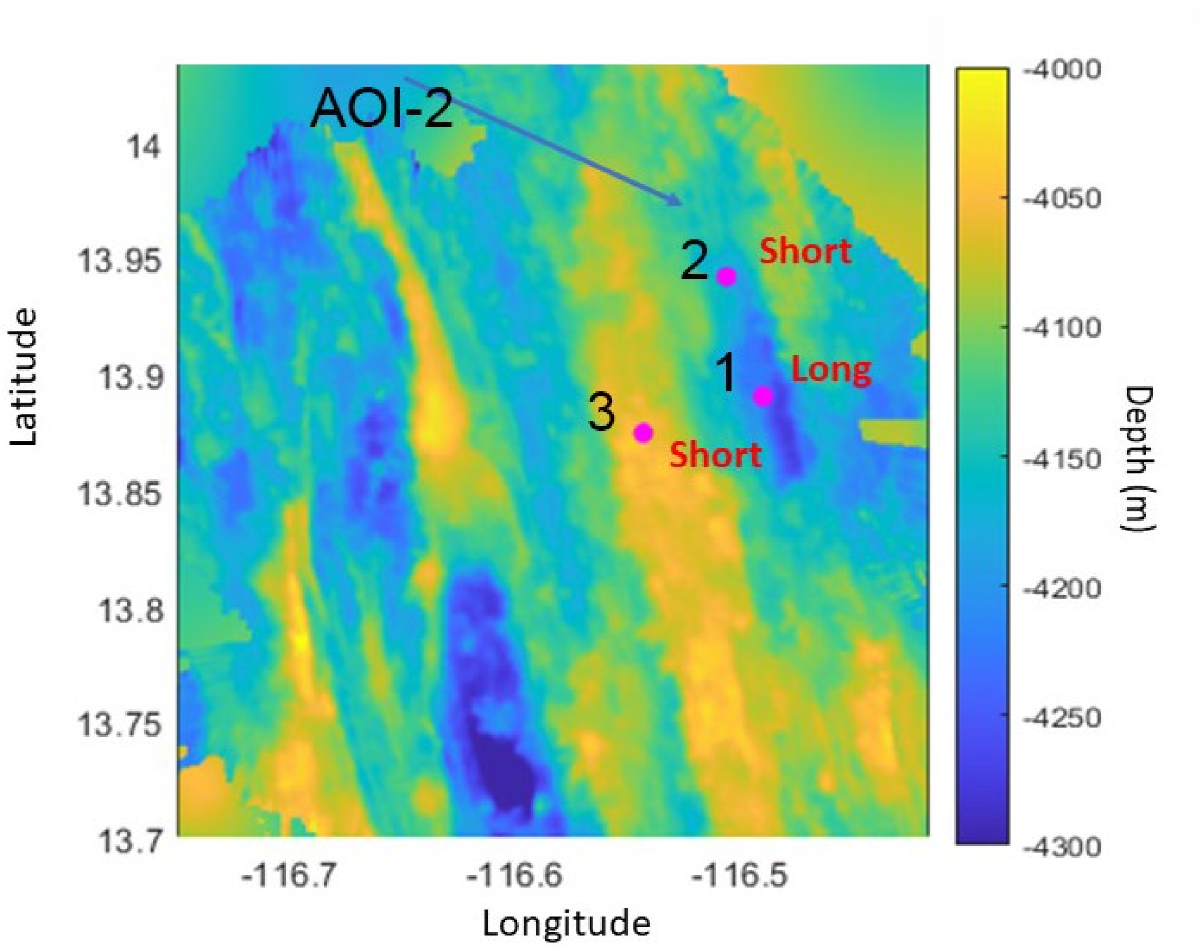


Figure 9.5: Map of mooring locations in UK-1

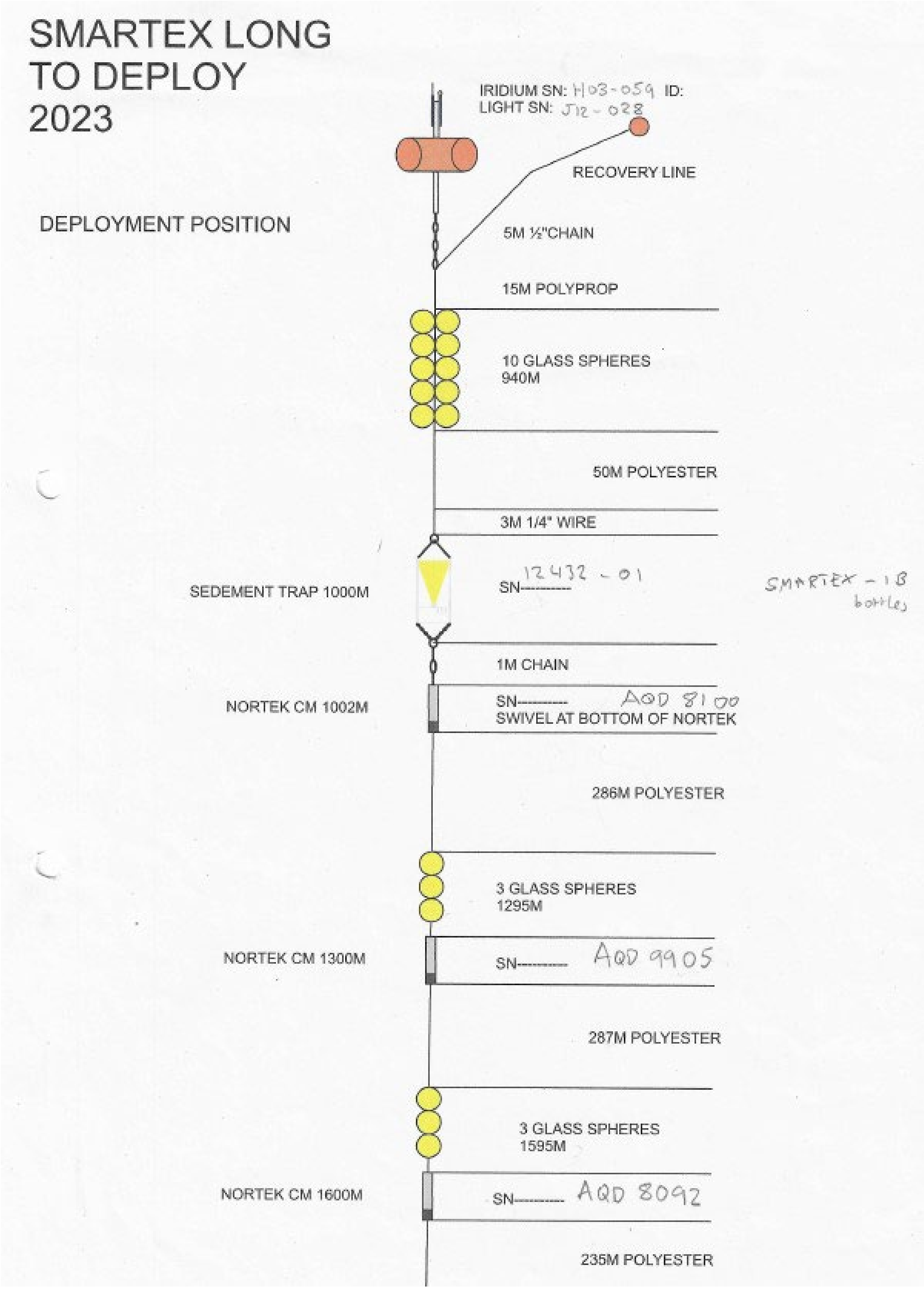


Figure 9.6: Diagram of Mooring 1 (long) top half

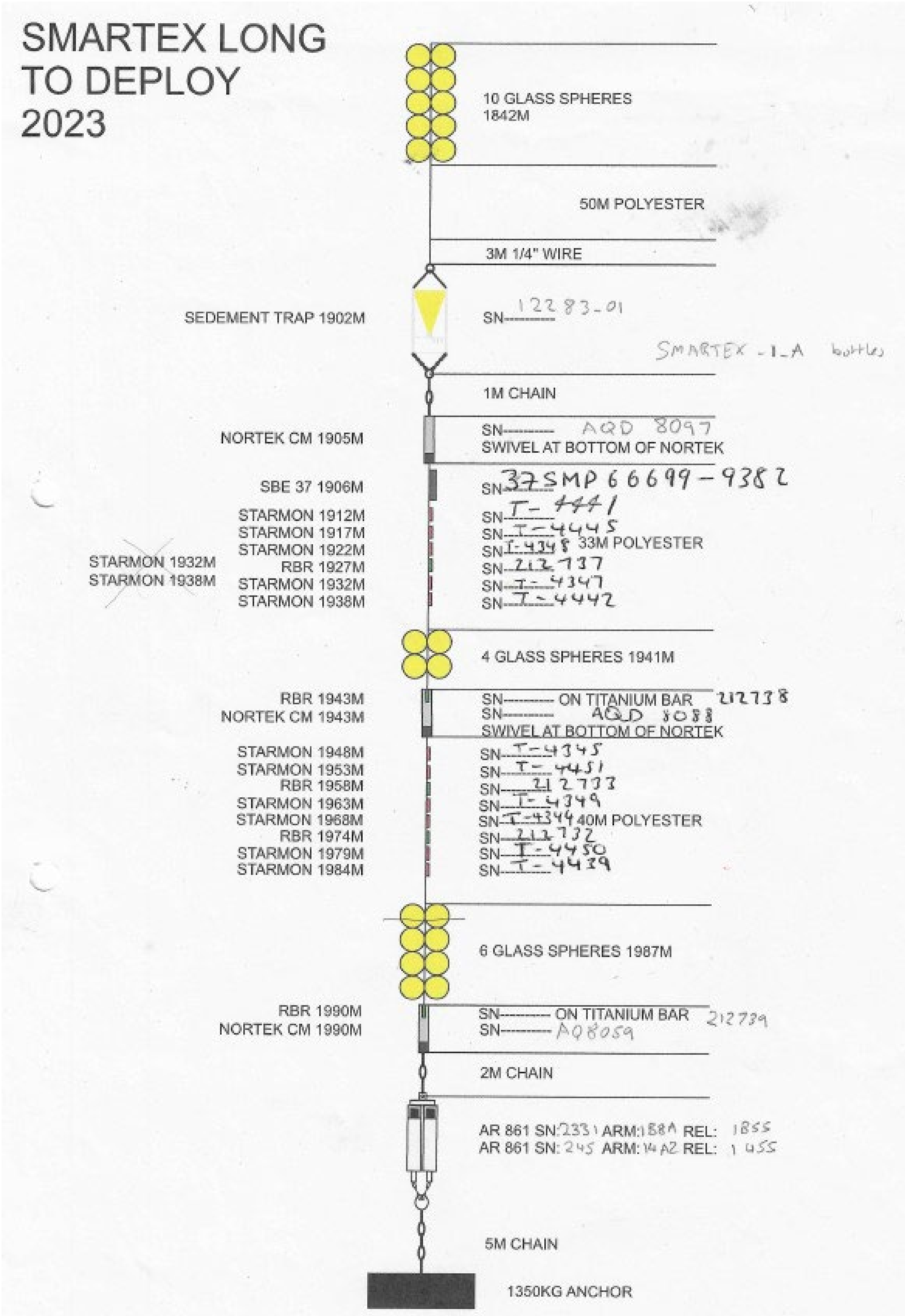


Figure 9.7: Diagram of Mooring 1 (long) bottom half

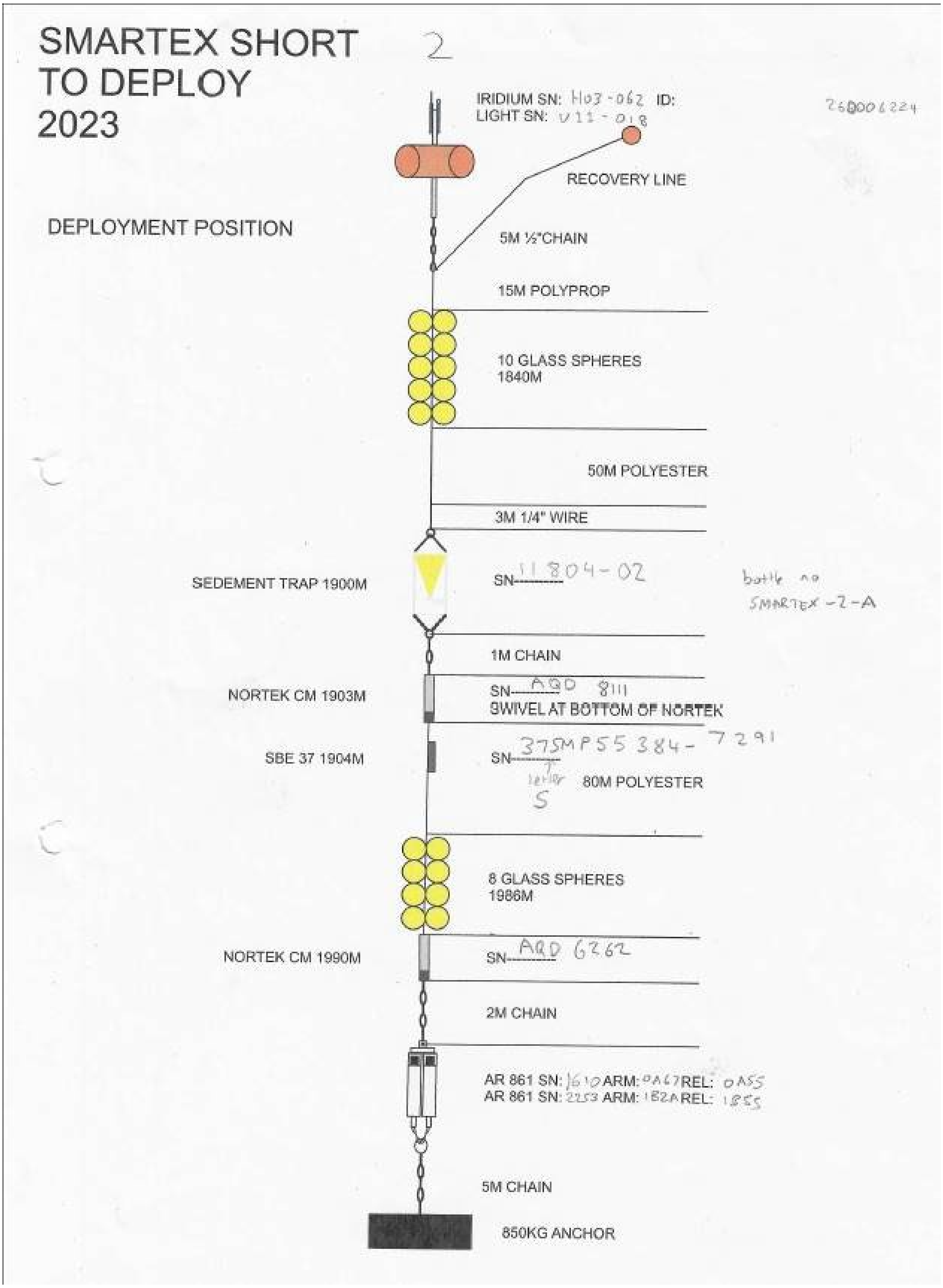


Figure 9.8: Diagram of Mooring 2 (short)

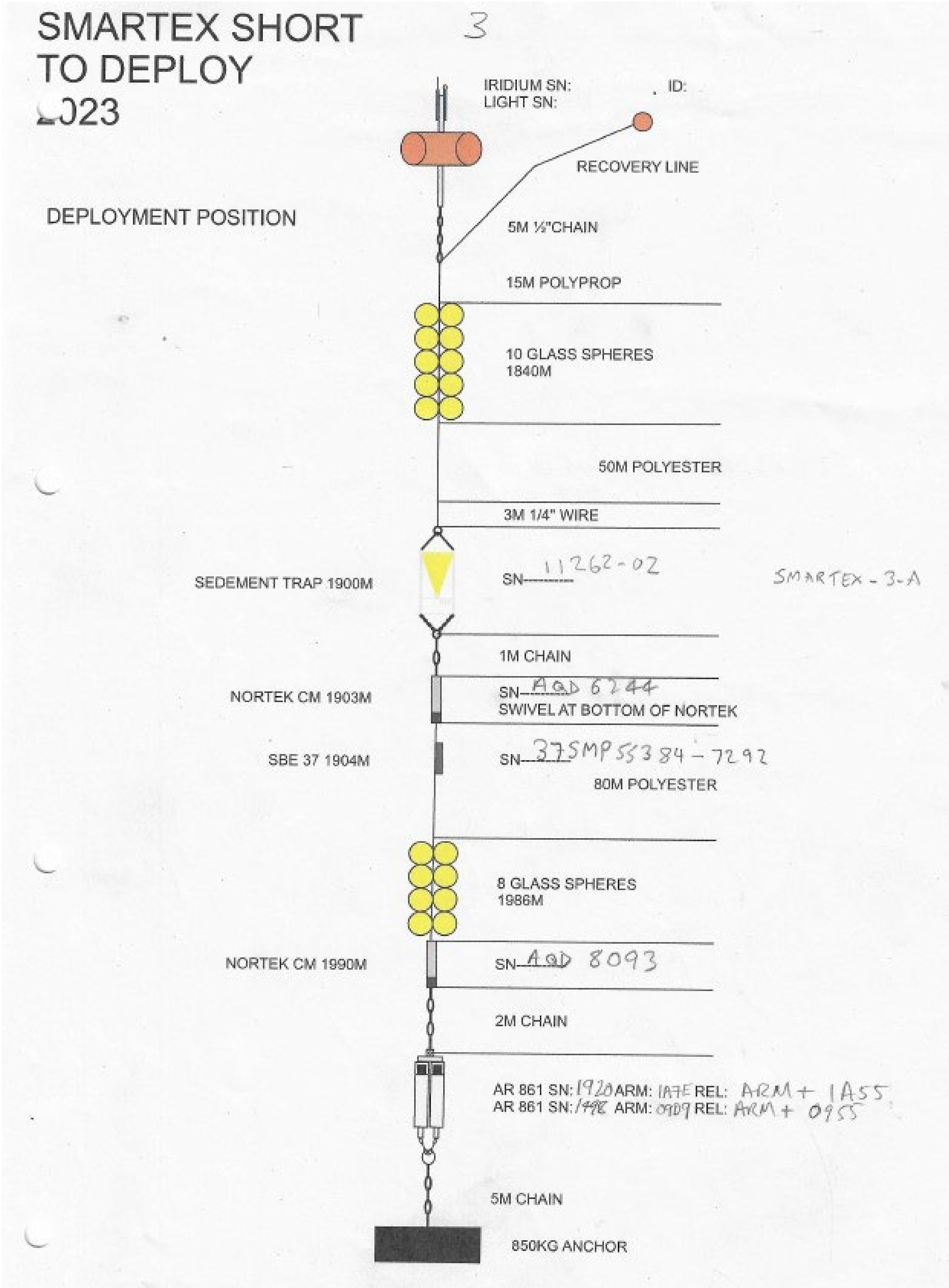


Figure 9.9: Diagram of Mooring 3 (short)

Sediment traps

Table 9.3: Setup details for four sediment traps on JC241

Trap	Description	Position (decimal degrees)	Serial	Bottle Labels
Trap A	100m off seabed - mooring 1 (long)	116.49000 W 13.89000 N	12283-01	SMARTEX-1-A
Trap B	1000m off seabed - mooring 1 (long)	116.49000 W 13.89000 N	12432-01	SMARTEX-1-B
Trap C	100m off seabed - mooring 2 (short)	116.50626 W 13.94164 N	11804-02	SMARTEX-2-A
Trap D	100m off seabed - mooring 3 (short)	116.54320 W 13.87421 N	11262-02	SMARTEX-3-A

The sediment traps were programmed to start on 20 March 2023 at 12:00 (GMT) with 17 day interval. They were filled with bottom water (collected by CTD near the seabed) with 50ml per litre of seawater borax-buffered 37% formaldehyde and 5g per litre of seawater sodium chloride added.

Table 9.4: Deployment details for all sediment traps on JC241. Trap A used as example for numbering system

Number	Open Date at 1200h (GMT)
smartex-A-1	20/03/23
smartex-A-2	06/04/23
smartex-A-3	23/04/23
smartex-A-4	10/05/23
smartex-A-5	27/05/23
smartex-A-6	13/06/23
smartex-A-7	30/06/23
smartex-A-8	17/07/23
smartex-A-9	03/08/23
smartex-A-10	20/08/23
smartex-A-11	06/09/23
smartex-A-12	23/09/23
smartex-A-13	10/10/23
smartex-A-14	27/10/23
smartex-A-15	13/11/23
smartex-A-16	30/11/23
smartex-A-17	17/12/23
smartex-A-18	03/01/24
smartex-A-19	20/01/24
smartex-A-20	06/02/24
smartex-A-21	23/02/24
Final move to open hole	11/03/24

10. Mapping

Shipboard Multibeam EM122

The ship is equipped with two hull-mounted multibeam systems. It was the Kongsberg EM122 system that was solely used for the survey. It is a 12kHz system with 512 beams and was used with a 150° swath width and equidistant beam sampling. Datafiles were divided into 2 hour passes. The lever arm values and patch test values used were:

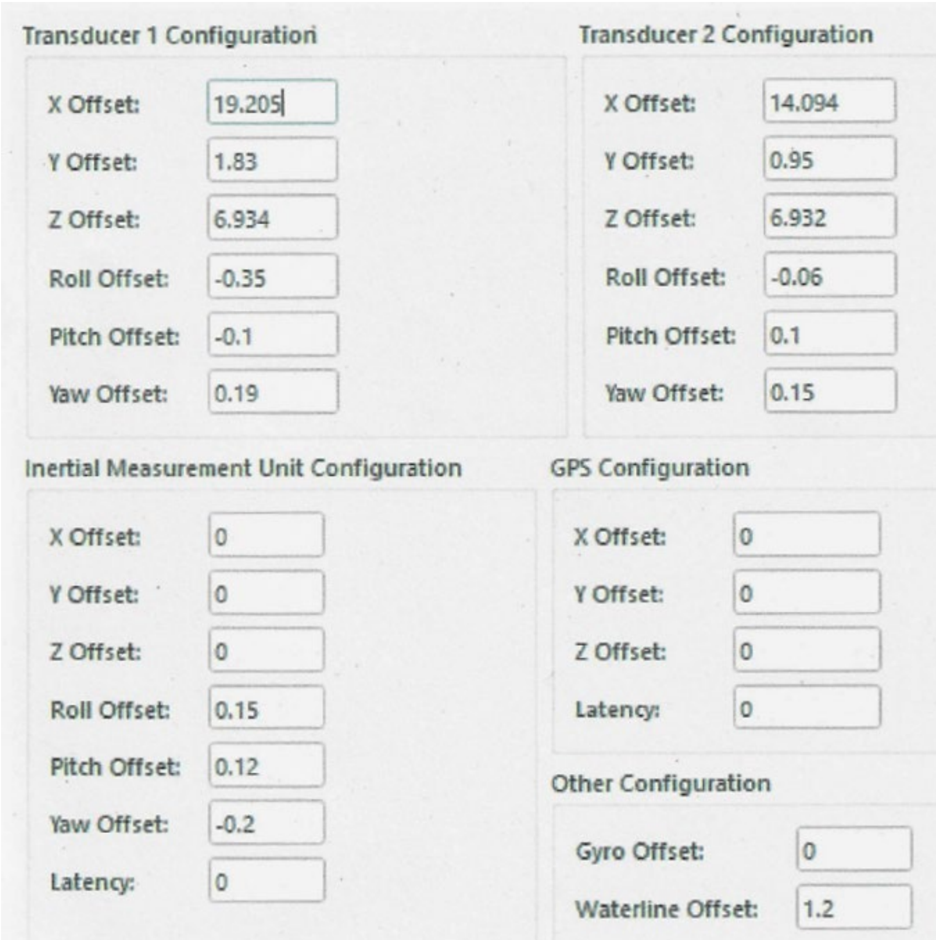


Figure 10.1. Kongsberg EM122 Configuration

Data was not collected in Costa Rica's EEZ or in French waters around Clipperton Island EEZ. Before each switching on of the multibeam system, a marine mammal observation (MMO) watch was done for an hour before a slow ramp up of the ping strength over 20 minutes (see separate section of this report). The system was left running all the time whilst on the various sites but not logging when the ship's speed was less than 1.5 knots.

Good data was recorded on the passage days travelling west, even at full speed of 11knots. Occasionally when the weather was rougher the quality of the data deteriorated. Poor data was also seen when travelling north near the OMCO site.

Table 10.1. Summary of EM122 MBES data collection

Pass No.	Start & End Date &Time	Approximate Start & End Latitude & Longitude	Description
0 - 37	20230206_172557 20230209_222005	10° 40' 25" N 92° 05' 02" W 11° 53' 59" N 106° 11' 09" W	Costa Rica EEZ to French EEZ
38 - 79	20230211_022209 20230214_201206	12° 40' 26" N 111° 39' 26" W 13° 43' 52" N 126° 13' 25" W	French EEZ via UK1 to OMCO
80 - 82	Small files	13° 43' 52" N 126° 13' 25" W	On OMCO site – poor quality data
83 - 90	20230221_215316 20230222_115948	13° 45' 48" N 126° 08' 41" W 12° 54' 23" N 128° 27' 23" W	OMCO to PRA
91- 93	Small files	-	On PRA site – poor quality data
94 - 102	20230226_155004 20230227_063534	12o 52' 24" N 128° 19' 10" W 13° 42' 39" N 126° 11' 17" W	PRA to OMCO
103 - 105	Small files	13° 44' 23" N 126° 13' 54" W 13° 38' 08" N 126° 12' 44" W	On OMCO site – poor quality data
106 - 135	20230313_023201 20230315_115730	13° 41' 40" N 126° 10' 35" W 13° 54' 39" N 116° 33' 10" W	OMCO to UK1
136 - 151	20230318_210106 20230320_074312	13° 58' 17" N 116° 32' 11" W 12° 45' 35" N 111° 39' 01" W	UK1 to French EEZ
152-202	20230321_140333 20230325_111024	11° 44' 47" N 105° 48' 21" W 9° 35' 38" N 89° 10' 6" W	French EEZ to Costa Rica EEZ

The data was recorded in the Kongsberg ".all" format and processing was done in both CARIS HIPS & SIPS v10.4 and Qimera v2.4.3 to compare processing results.

ROV Multibeam Reson7125

The ISIS multibeam bathymetry system is a Reson7125 working at 300-400kHz. It is fixed near the base of the ROV and attached via a camera feed line into the ROV. Four Dives were done with the multibeam system attached. Unfortunately, the first three dives were aborted due to communication issues to the ISIS vehicle and subsequently the Reson system.

Table 10.2. Summary of ROV ISIS dives

Dive No.	Date & Time of start of data	Date & Time of end of data	Comment
400	17/02/23 10:45	17/02/23 11:49	One line north – ROV failed on turning
401	18/02/23 23:37	18/02/23 23:53	Manoeuvring – ROV failed on turning
402	21/02/23 03:26	21/02/23 06:02	One line South – ROV failed midway
410	10/03/23 21:10	11/03/23 15:00	Seven Lines – Successful survey

The Reson 7125 system was set to:

210dB Power
36dB Gain
40µs Pulse length
125m Range
120° Swath angle
10 p/s Max Pulse Rate

72dB/km Absorption
1535.4m/s Sound Velocity
35.0ppm Salinity
-1.85° Roll (determined during post processing)
0.0° (assumed) Pitch
0.0° (assumed) Yaw

The ROV was driven at between 50-55m altitude above the seafloor at speeds of 0.2-0.3 knots.

The Reson PDS2000 software was used to record the multibeam data and recorded the data in .pds format in 500Mb chunks. Software Configuration was done by copying the 2022 (JC237) wall survey configuration.

Table 10.3. Summary of the 9 Reson MBES lines acquired during ISIS dives in the OMCO area.

Dive No. / Line No.	SOL time GMT	EOL time GMT	Length (m)	Average Speed (m/s)	Course over ground	Comments
400/1	10:45	11:49	507	0.13	352	Trial line and settings
402/1	03:26	06:02	1129	0.12	172	OMCO tracks mapping
410/1	21:10	23:26	1124	0.14	172	
410/2	23:31	23:38	64	0.15	262	
410/3	23:41	04:48	1918	0.10	352	Strong current against
410/4	05:05	08:39	1734	0.14	82	
410/5	08:50	12:11	1711	0.14	172	
410/6	12:34	13:00	73	0.05	82	Cable management
410/7	13:04	15:00	614	0.09	352	Strong current against

Figure 10.2. Image of the Reson MBES acquisition in the ROV ISIS van

It was found that the .pds files were not immediately able to be converted to other format such as .s7k or .xtf. A workaround was made, creating a .sub file.

<u>Conversion of .pds files to .xtf and .s7k (if not in fileset)</u>

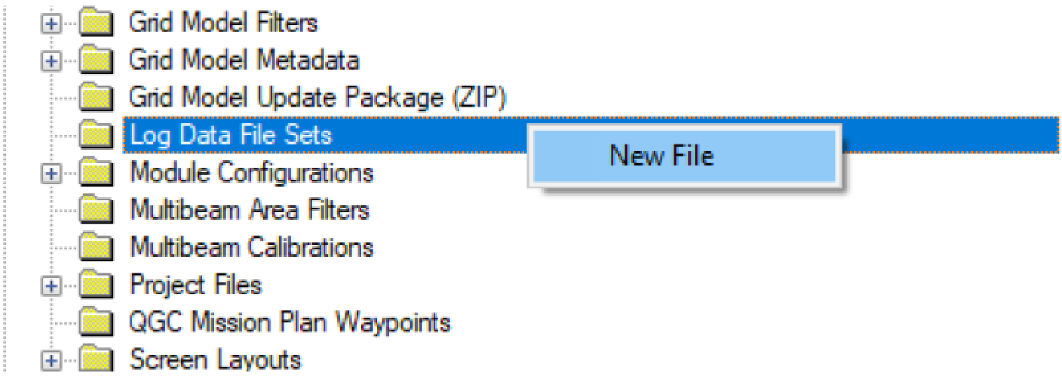


Figure 10.3. Location of New File creation in PDS2000

Create a new file for the Log Data File Sets in PDS2000 (above). Give it a name (it will be empty) and select OK.

This will open a .sub file in an editor and the user can enter the list of .pds files (in the format as shown below) to create the file selector to open the .pds log files. Make sure the file numbers start at 0.

```
[Files]
File(0) = LogData\rov[Multivessel Survey]_JC241_Smartex_OMCO_Dive410-20230310-210955
File(1) = LogData\rov[Multivessel Survey]_JC241_Smartex_OMCO_Dive410-20230310-211522
File(2) = LogData\rov[Multivessel Survey]_JC241_Smartex_OMCO_Dive410-20230310-212054
File(3) = LogData\rov[Multivessel Survey]_JC241_Smartex_OMCO_Dive410-20230310-212622
File(4) = LogData\rov[Multivessel Survey]_JC241_Smartex_OMCO_Dive410-20230310-213151
File(5) = LogData\rov[Multivessel Survey]_JC241_Smartex_OMCO_Dive410-20230310-213722
File(6) = LogData\rov[Multivessel Survey]_JC241_Smartex_OMCO_Dive410-20230310-214250
```

Figure 10.4. Example of .pds file structure

The next step is to export the fileset (Tools … Export) to create .s7k files.

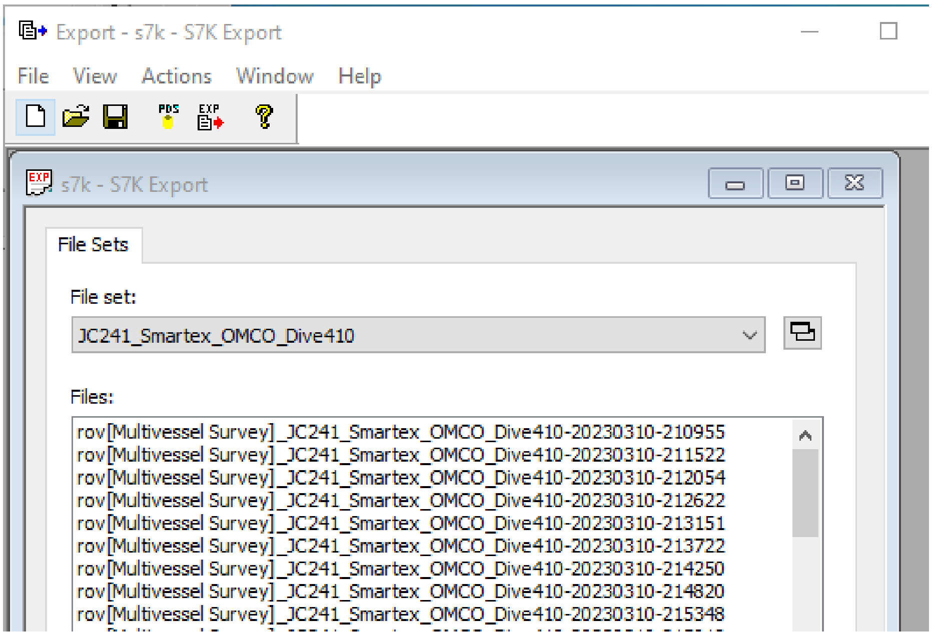


Figure 10.5. Example of exporting .S7K files from PDS2000

The lines were then entered into Qimera (v2.4.3) using the .s7k format and later exported from Qimera into .gsf format. This allowed the pair of .s7k and .gsf files to be used in FMGT (v7.10.1) for backscatter mapping. It was found that the .s7k datafiles had similar filename beginnings. To do multiple filename changes in Windows explorer:

1) Select multiple files to change names. Hold CTRL down while clicking the files.
2) Press F2
3) Type the new name and press ENTER

The process of hydrographic processes for deep ocean ROV (& AUV) multibeam data involves several extra steps compared with surface systems. This is due to the uncertainty of the navigation. Two navigation systems were logged during the surveys: USBL and DVL. The DVL system was reset at the beginning of each line to match the latest and “best” USBL position.

Steps for multibeam processing:

1) Import of .s7k files into Qimera
2) Import of Navigation data (from DVL log). These data included Date and Time, Latitude and Longitude, and Depth of the ROV vehicle.
3) Import Tide and clean pings

4) Creation of a dynamic surface at about 50cm resolution
5) A patch test had not been acquired and so two overlapping swath were compared and a value of -1.85° for roll was measured and then applied to the data. As the navigation had not been corrected at this time, nor a tidal correction added, the two overlapping surfaces were separated by about a meter but this was considered acceptable at this stage.
6) Export the gridded surface from Qimera and Import into ArcMap
7) Hill-shade maps were created for each line with an azimuth direction perpendicular to the track to minimise ping to ping differences, in this case either 82° or 172°. This showed the pilot miner tracks very visibly on the lines.
8) Whilst looking at the hill-shade imagery, the start location of each line was used as a fixed point, and the last ping swath to match the last and "best" USBL position, a linear navigational adjustment was calculated (and numerics saved) to georectify the hill-shade imagery. Comparison of the imagery in the hill-shading allowed good matching of the miner tracks so confirm the navigational adjustment calculations. This worked well for lines of Dive402, Dive410 Lines 1 and 3.
9) Once navigational adjustment calculations had been saved for these lines, the actual bathymetry were georeferenced using a simple stretch between points as it was assumed that the DVL was mostly accurate except for a small drift.
10) Lines 4, 5, and 7 had no track lines to follow and relied solely on the start location of each line as a fixed point, and the last ping swath to match the last and "best" USBL position. Navigational adjustments were saved and used on the bathymetry data.
11) Contours were created at 1m resolution.

Table 10.4. Summary of Reson MBES files acquired during ROV ISIS dives in the OMCO area

Dive No. / Line No.	SOL time GMT	EOL time GMT	Files in Line
400/1	10:45	11:49	19700101_000000_21_rov.s7k - 19700101_000000_35_rov.s7k
402/1	03:26	06:02	19700101_000000_1_rov.s7k - 19700101_000000_26_rov.s7k
410/1	21:10	23:26	Dive410_0_rov(1).s7k - Dive410_0_rov(24).s7k
410/2	23:31	23:38	Dive410_0_rov(25).s7k - Dive410_0_rov(26).s7k
410/3	23:41	04:48	Dive410_0_rov(27).s7k - Dive410_0_rov(82).s7k
410/4	05:05	08:39	Dive410_0_rov(83).s7k - Dive410_0_rov(121).s7k
410/5	08:50	12:11	Dive410_0_rov(122).s7k - Dive410_0_rov(158).s7k
410/6	12:34	13:00	Dive410_0_rov(159).s7k - Dive410_0_rov(163).s7k
410/7	13:04	15:00	Dive410_0_rov(164).s7k - Dive410_0_rov(184).s7k

Table 10.5. Navigational adjustment coordinates (In UTM WGS84 Zone 9) for each survey line.

Navigational Adjustments	From X	From Y	To X	To Y
Dive402	800693.0	1519918.1	800698.1	1519888.0
Dive402	800613.5	1520020.9	800614.9	1519999.1
Dive402	800558.5	1520631.4	800563.7	1520621.1
Line 1 Point 1	800777.2	1518951.2	800782.8	1518932.6
Line 1 Point 2	800682.5	1519896.5	800688.0	1519893.8
Line 3 Point 1	800761.5	1518803.8	800761.5	1518803.8
Line 3 Point 2	800515.3	1520653.1	800510.4	1520698.6
Line 4 Point 1	802105.0	1520903.8	802149.2	1520911.4
Line 4 Point 2	800548.7	1520700.2	800558.1	1520708.8
Line 5 Point 1	802181.2	1520914.5	802181.2	1520914.5
Line 5 Point 2	802402.1	1519302.1	802403.6	1519258.1
Line 7 Point 1	802338.2	1519831.3	802321.4	1519851.0
Line 7 Point 2	802451.3	1519264.4	802441.8	1519263.9

It was noted that there was a discrepancy of depths of overlapping lines of varying amounts from -2m to +2m. This was attributed to tidal variation. However, as no tide data was available the difference of heights were measured for differing times. 6 points were measured.

Table 10.6. Tidal differences measured between Reson MBES lines

Date	Time	Line1 Depth	Date	Time	Line3 Depth	Difference
10/03/2023	21:20	-4703.76	11/03/2023	02:40	-4705.07	1.31
10/03/2023	21:41	-4701.75	11/03/2023	02:17	-4703.13	1.38
10/03/2023	22:11	-4701.18	11/03/2023	01:43	-4702.42	1.24
10/03/2023	22:34	-4700.73	11/03/2023	01:14	-4701.78	1.05
10/03/2023	23:01	-4696.57	11/03/2023	00:19	-4697.27	0.70
10/03/2023	23:22	-4695.24	10/03/2023	23:46	-4695.76	0.52

If T_x = Tide Height at time *x* and T_y = Tide Height at time *y*, the difference in height is known. If it is assumed that at time A the tide is at height M and it is a sinusoidal curve with a wavelength of 12.3 hours:

$T_x = M \times cos\left(\frac{(A-x)\times 24}{12.3} \times 2\pi\right)$ and $T_y = M \times cos\left(\frac{(A-y)\times 24}{12.3} \times 2\pi\right)$

Therefore T_x - T_y = Difference = $M \times \left(cos\left(\frac{(A-x)\times 24}{12.3} \times 2\pi\right) - cos\left(\frac{(A-y)\times 24}{12.3} \times 2\pi\right)\right)$

This has two unknowns A and M and can be solved using any two of the difference values. Approximations for M was found to equal 2.32m and A = 10/03/23 18:30. All times were measured in decimal time of day from 0 to 1.

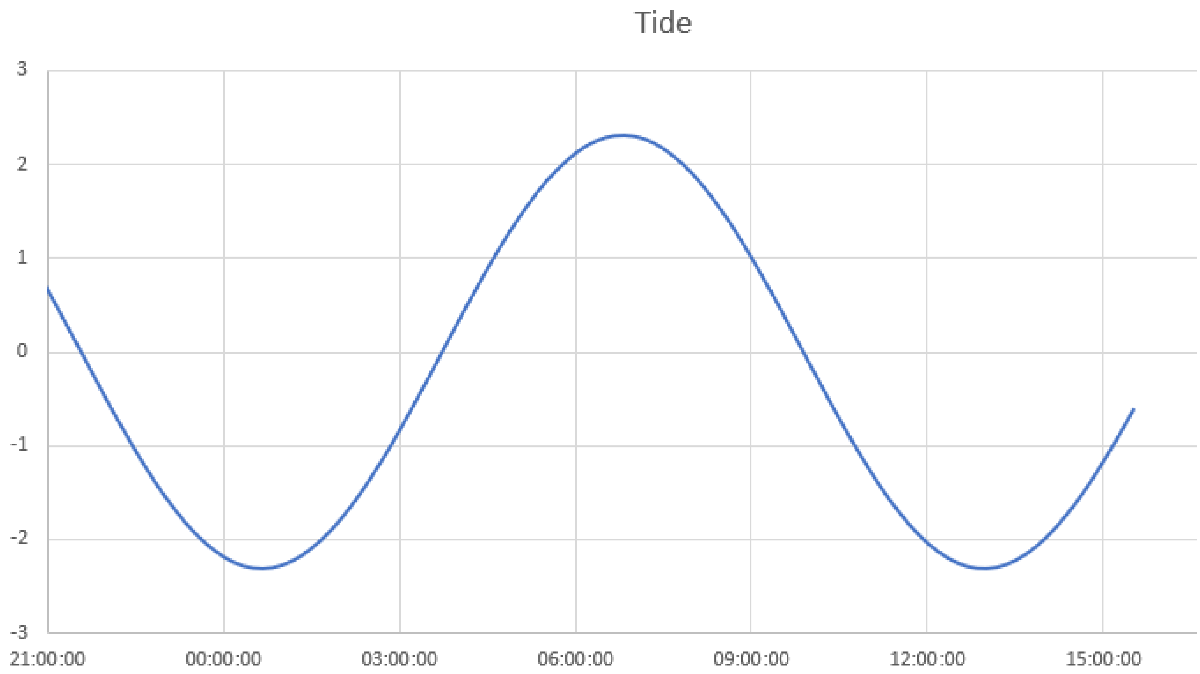


Figure 10.6. Tidal curve calculated for ROV ISIS dives

As this tide was created after the initial Qimera processing the tide was added back into the Qimera data processing (with positive downwards) and new results created. A small tidal linear correction was also made for data from Dive 402 from 2.0m to 0.0m.

Final results were tested with creation of 1m contours and the lines merged into a single bathymetry grid.

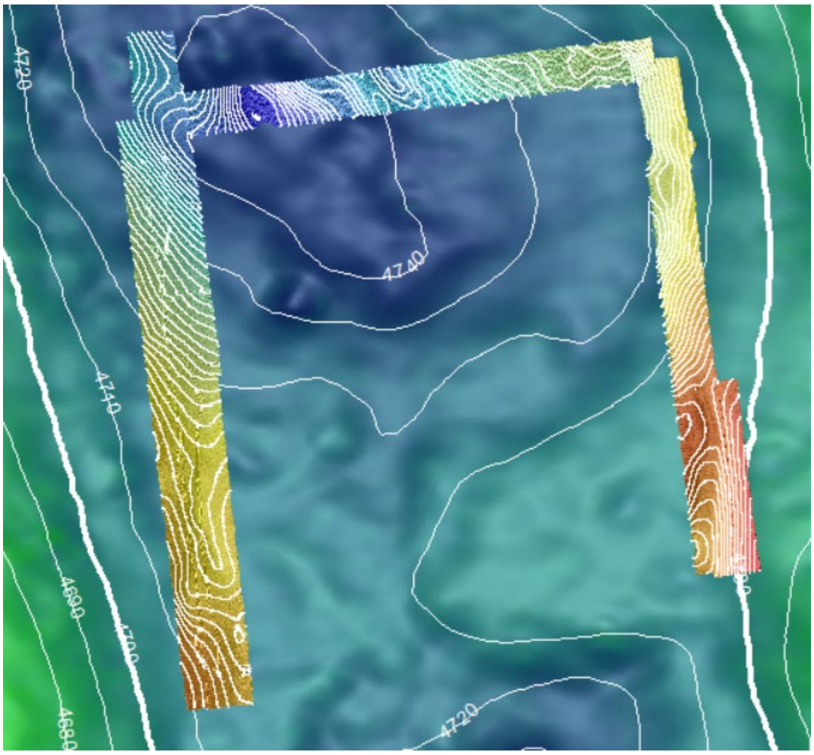


Figure 10.7. Reson 7125 bathymetry grid with 1m contours laid over ship multibeam data with 10m contours.

To create the backscatter the cleaned bathymetry data was exported to gsf format. This was then imported in FMGT v7.10.1. Each survey line was processed individually and then the navigational adjustments determined previously were applied, to its correct navigational place and then merged together into a final grid.

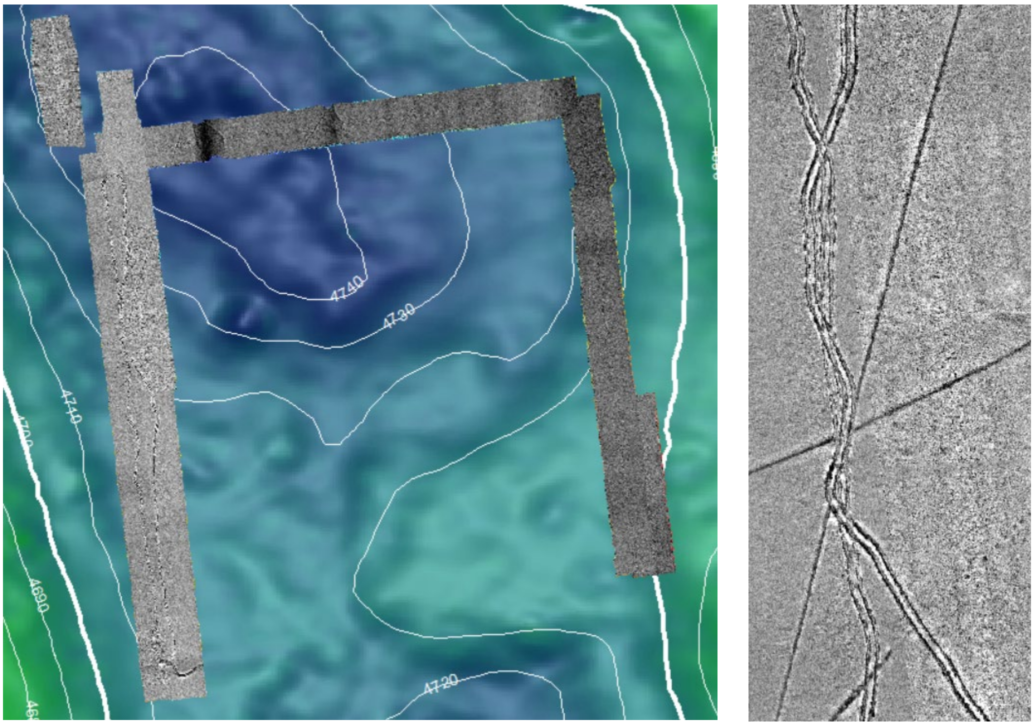


Figure 10.8. Backscatter Mosaic from Reson 7125. Closeup shows miner tracks and sledge trails. Black is low backscatter, white is strong backscatter.

Marine Mammal Observations

Marine mammal observations were conducted prior to starting the multibeam to protect marine life from acoustic disturbance. As per the Marine Environment Interaction Policy, the mitigation zone was 500m and watches were conducted for 60 minutes prior to soft starting the equipment. If a mammal was spotted, the observation watch was paused until 20 minutes after the last sighting of the mammal in the mitigation zone. Details of mammal sightings and watches are listed below.

Table 10.7. Details of MMO watches conducted during JC241

Date	Visual watch or PAM (v/ p)	Observer's/ operator's name(s)	Time of start of section of watch (UTC, 24hr clock)	Time of end of section of watch (UTC, 24hr clock)	Source activity (f/ s/ r/ n/ v)	Start position (latitude and longitude)	Depth at start (m)	End position (latitude and longitude)	Depth at end (m)	Speed of vessel (knots)	Wind dir'n	Wind force (B'fort scale)	Sea state (g/ s/ c/ r)	Swell (o/ m/ l)	Vis. (visual watch only) (p/ m/ g)	Sun glare (visual watch only) (n/ wf/ sf/ vf/ wb/ sb/ vb)	Precip. (n/ l/ m/ h/ s)
6/2/2023	v	Catherine Wardell	15:30	16:30	n	10 ° 0.10'N 91° 43.90'W	3660	10 ° 2.64'N 91 °54.44'W	3660	11	E	2	s	o	g	wb	N
6/2/2023	v	Catherine Wardell	16:30	16:59	n	10 ° 2.64'N 91 °54.44'W	3660	10 ° 3.19'N 91 °58.36'W	3660	11	E	2	s	o	g	wb	N
11/2/2023	v	Catherine Wardell	00:00	01:00	n	12 ° 36.64'N 111 °10.82'W	3455	12 ° 38.22'N 111°22.78'W	3800	12.1	ne	4	s	o	g	vf	N
11/2/2023	v	Catherine Wardell	01:00	02:00	n	12 ° 38.22'N 111°22.78'W	3800	12 ° 39.98'N 111 °34.86'W	4060	12.1	ne	4	s	o	g	vf	N
11/2/2023	v	Catherine Wardell	02:00	02:18	n	12 ° 39.98'N 111 °34.86'W	4060	12 ° 40.34'N 111 °38.53'W	4060	12.1	ne	4	s	o	g	vf	N
21/03/2023	v	Catherine Wardell	13:00	14:00	n	11 ° 46.36'N 105 °59.70'W	3464	11 ° 44.66'N 105 °48.01'W	3500	11	ne	3	s	o	g	wf	n

Visual watch or PAM: v = visual watch; p = PAM

Source activity: **f = full power; s = soft start; r = reduced power (not soft start); n = not active; v = variable (e.g. tests)**

Sea state: **g = glassy (like mirror); s = slight (no/ few white caps); c = choppy (many white caps); r = rough (big waves, foam, spray)**

Swell: **o = low (< 2 m); m = medium (2-4 m); l = large (> 4 m)**

Visibility: **p = poor (< 1 km); m = moderate (1-5 km); g = good (> 5 km)**

Sun glare: **n = none; wf = weak forward; sf = strong forward; vf = variable forward; wb = weak behind; sb = strong behind; vb = variable behind**

Precipitation: **n = none; l = light rain; m = moderate rain; h = heavy rain; s = snow**

MARINE MAMMAL RECORDING FORM - SIGHTINGS

Regulatory reference number (e.g. DECC no., BOEM permit no., OCS lease no., etc.)	Ship/ platform name RRS James Cook	Sighting number **(start at 1 for first sighting of survey)** 1	Acoustic detection number **(start at 500 for first detection of survey)**
Date 06/02/2023		**Time at start of encounter** (UTC, 24hr clock) 15:57	**Time at end of encounter** (UTC, 24hr clock) 15:57
Were animals detected visually and/ or acoustically? ☐ ✓ visual ☐ acoustic ☐ both	**How were the animals first detected?** ☐ ✓ visually detected by observer keeping a continuous watch ☐ visually spotted incidentally by observer or someone else ☐ acoustically detected by PAM ☐ both visually and acoustically before operators/ observers informed each other		
Observer's/ operator's name Catherine Wardell	**Position** (latitude and longitude) 10° 1.68'N 91° 48.2'W		Water depth **(metres)** 3660
Species/ species group Dolphin sp.		**Description** (include features such as overall size; shape of head; colour and pattern; size, shape and position of dorsal fin; height, direction and shape of blow; characteristics of whistles/ clicks) >1.5m length, at least 2 dolphins	
Bearing to animal **(when first seen or heard) (bearing from true north)** 260	**Range to animal** (when first seen or heard) (metres) **50**		

Total number 2	**Number of adults** (visual sightings only) 2	**Number of juveniles** (visual sightings only) 0	**Number of calves** (visual sightings only) 0	**Photograph taken** ☐ yes ✓ no
Behaviour (visual sightings only) Travelling past at speed, only surfaced o				

Direction of travel (relative to ship) ☐ towards ship ☐ away from ship ☐ parallel to ship in same direction as ship ✓ parallel to opposite direction to ship ☐ crossing perpendicular ahead of ship ☐ variable ☐ milling ☐ stationary ☐ other ☐ unknown	**Direction of travel** (compass points) ☐ N ☐ NE ✓ E ☐ SE ☐ S ☐ SW ☐ W ☐ NW ☐ variable ☐ stationary ☐ unknown

Airgun (or other source) activity when animals first detected ☐ full power ☐ ✓ not firing ☐ soft start ☐ reduced power (other than soft start)	**Airgun (or other source) activity when animals last detected** ☐ full power ☐ ✓ not firing ☐ soft start ☐ reduced power (other than soft start)	**Time animals entered mitigation/ exclusion zone** (UTC, 24hr clock) 15:57 **Closest distance of animals from airguns (or other source)** (metres) **50**	**Time animals left mitigation/ exclusion zone** (UTC, 24hr clock) 1557 **Time of closest approach** (UTC, 24hr clock) **15:57**
If seen during soft start give: *First distance* *Closest distance* *Last distance* during soft start (metres)	**What action was taken?** (according to requirements of guidelines/ regulations in country concerned) ☐ none required ☐ ✓ delay start of firing ☐ shut-down of active source ☐ power-down of active source ☐ power-down then shut-down of active source	**Length of power-down and/ or shut-down** (if relevant) (length of time until subsequent soft start, in minutes) 58	**Estimated loss of production (if relevant) due to mitigating actions** (km) -

MARINE MAMMAL RECORDING FORM - SIGHTINGS

Regulatory reference number (e.g. DECC no., BOEM permit no., OCS lease no., etc.)	Ship/ platform name RRS James Cook	Sighting number **(start at 1 for first sighting of survey)** 2	Acoustic detection number **(start at 500 for first detection of survey)**
Date 06/02/2023		**Time at start of encounter** (UTC, 24hr clock) 16:41	**Time at end of encounter** (UTC, 24hr clock) 16:44

Were animals detected visually and/ or acoustically?
- ☐ ✓ visual
- ☐ acoustic
- ☐ both

How were the animals first detected?
- ☐ ✓ visually detected by observer keeping a continuous watch
- ☐ visually spotted incidentally by observer or someone else
- ☐ acoustically detected by PAM
- ☐ both visually and acoustically before operators/ observers informed each other

Observer's/ operator's name Catherine Wardell	**Position** (latitude and longitude) 10º 2.94’N 91 º 56.67’W	Water depth **(metres)** 3660

Species/ species group
Sperm Whale

Bearing to animal **(when first seen or heard) (bearing from true north)** 255	**Range to animal** (when first seen or heard) (metres) **1000**

Description (include features such as overall size; shape of head; colour and pattern; size, shape and position of dorsal fin; height, direction and shape of blow; characteristics of whistles/ clicks)

Pale belly, spouting, long

Total number 1	**Number of adults** (visual sightings only) 1	**Number of juveniles** (visual sightings only) 0	**Number of calves** (visual sightings only) 0	**Photograph taken** ☐ yes ✓ no

Behaviour (visual sightings only)
Occasionally surfacing, spouting

Direction of travel (relative to ship)
- ☐ towards ship
- ☐ away from ship
- ☐ parallel to ship in same direction as ship
- ✓ parallel to opposite direction to ship
- ☐ crossing perpendicular ahead of ship
- ☐ variable
- ☐ milling
- ☐ stationary
- ☐ other
- ☐ unknown

Direction of travel (compass points)
- ☐ N
- ☐ NE
- ✓ E
- ☐ SE
- ☐ S
- ☐ SW
- ☐ W
- ☐ NW
- ☐ variable
- ☐ stationary
- ☐ unknown

Airgun (or other source) activity when animals first detected
- ☐ full power
- ☐ ✓ not firing
- ☐ soft start
- ☐ reduced power (other than soft start)

Airgun (or other source) activity when animals last detected
- ☐ full power
- ☐ ✓ not firing
- ☐ soft start
- ☐ reduced power (other than soft start)

Time animals entered mitigation/ exclusion zone (UTC, 24hr clock)	**Time animals left mitigation/ exclusion zone** (UTC, 24hr clock) -
Closest distance of animals from airguns (or other source) (metres) **1000**	**Time of closest approach** (UTC, 24hr clock) **16:44**

If seen during soft start give:
First distance
Closest distance
Last distance
during soft start (metres)

What action was taken? (according to requirements of guidelines/ regulations in country concerned)
- ☐ ✓ none required
- ☐ delay start of firing
- ☐ shut-down of active source
- ☐ power-down of active source
- ☐ power-down then shut-down of active source

Length of power-down and/ or shut-down (if relevant) (length of time until subsequent soft start, in minutes)
-

Estimated loss of production (if relevant) due to mitigating actions (km)
-

11. Geochemistry

SMARTEX Geochemistry cruise report

Pierre Josso, Hannah Grant, Louisa Norman

Coring deployment methodology (general science protocols)

Gravity core

Seven gravity core deployments for geochemistry were carried out at the OMCO site, three in the vicinity of the mining tracks, and five at a control site approximately 1 nautical mile to the east (Figure 11.1). One gravity core deployment was undertaken at the UK-1 sampling site. The gravity corer had excellent recovery versus total core barrel size at all sites, including a 4.85 m core with in-situ seafloor surface recovery from a 5-m barrel deployment at station 058. The seafloor surface was recovered in all gravity cores except the deployment at station 011; this station was considered a rejected core due to the large overshoot and was also compromised by later drilling of holes for measuring dissolved oxygen (dO_2) and porewater extraction after the core was recovered (Table 11.1). For deployments after station 011, three-meter plastic liners were pre-drilled at 5 cm intervals with a 4 mm drill head, tightly taped before deployment and the liners inserted into the gravity core apparatus. Following load and handling tests by the crew, the length of deployed gravity cores increased from 3 to 5 m barrels over the first three deployments (Figure 11.2).

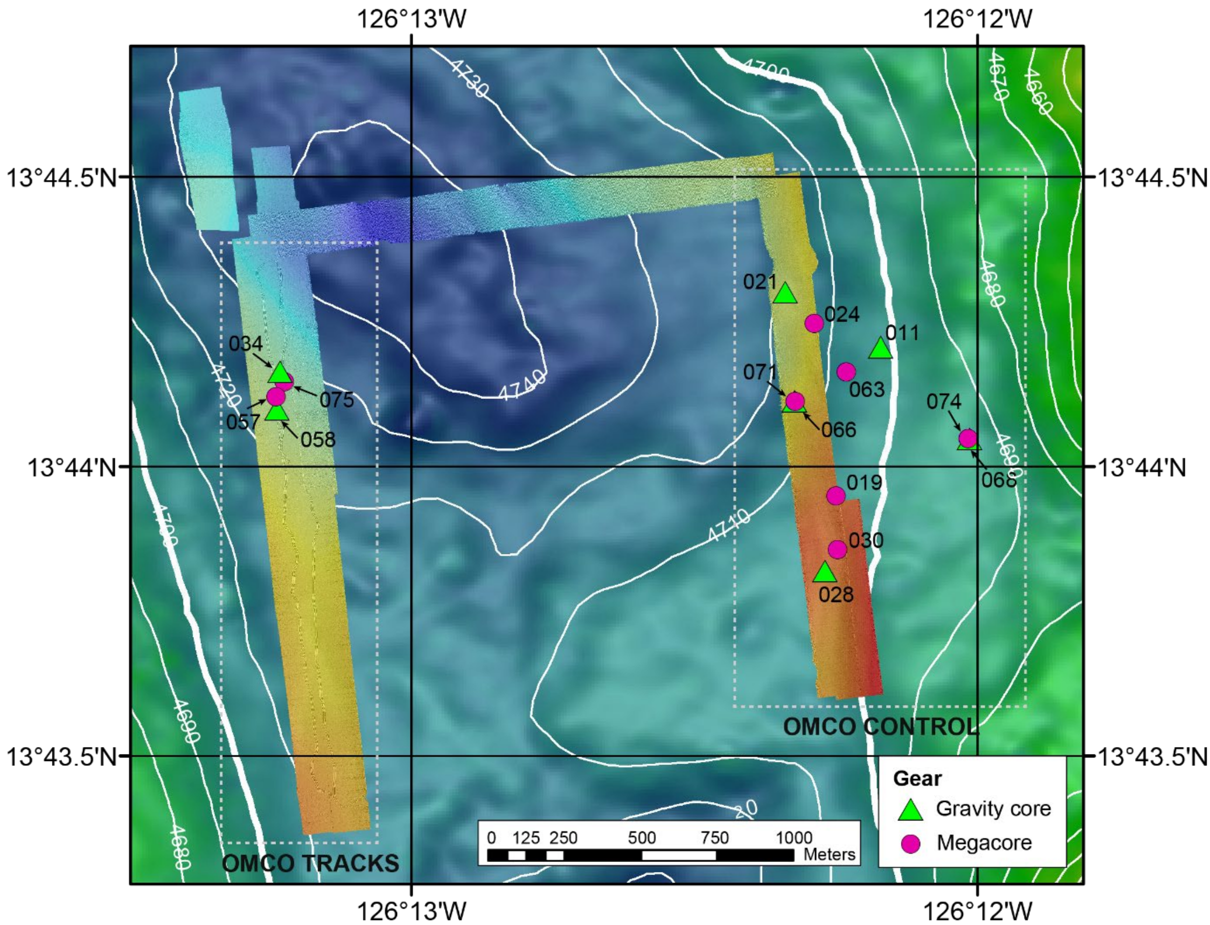


Figure 11.1: Locations of gravity cores and megacore sites by station number for the OMCO sampling site. Push core locations are detailed in individual scientific chapters.

Descent and ascent of the gravity cores were generally conducted at 70-90 m/min with a speed reduction to 30 m/min over the last 100 m above the seabed for the first trial deployment (Station 011), and 15 m/min for all gravity core deployments thereafter. Assessment of the gravity core penetration in the seabed and extraction from the seabed was conducted via monitoring of the cable tension. Pull-out of the apparatus was initiated immediately after seabed penetration was observed. Maximum cable tension on pull out and the cable winch speed were also noted. Station data were recorded for the moment of bottom contact: time (UTC), ship's position, estimated gear position if attached (USBL), sounding, and metres of wire deployed. Despite reducing speed of penetration and increasing barrel length whilst maintaining a load of 200 kg, over-penetration of the core was observed at all recoveries with mud staining the load 40 to 90 cm above the top of the barrel (head weight). Despite this, the barrels never overfilled with sediments.

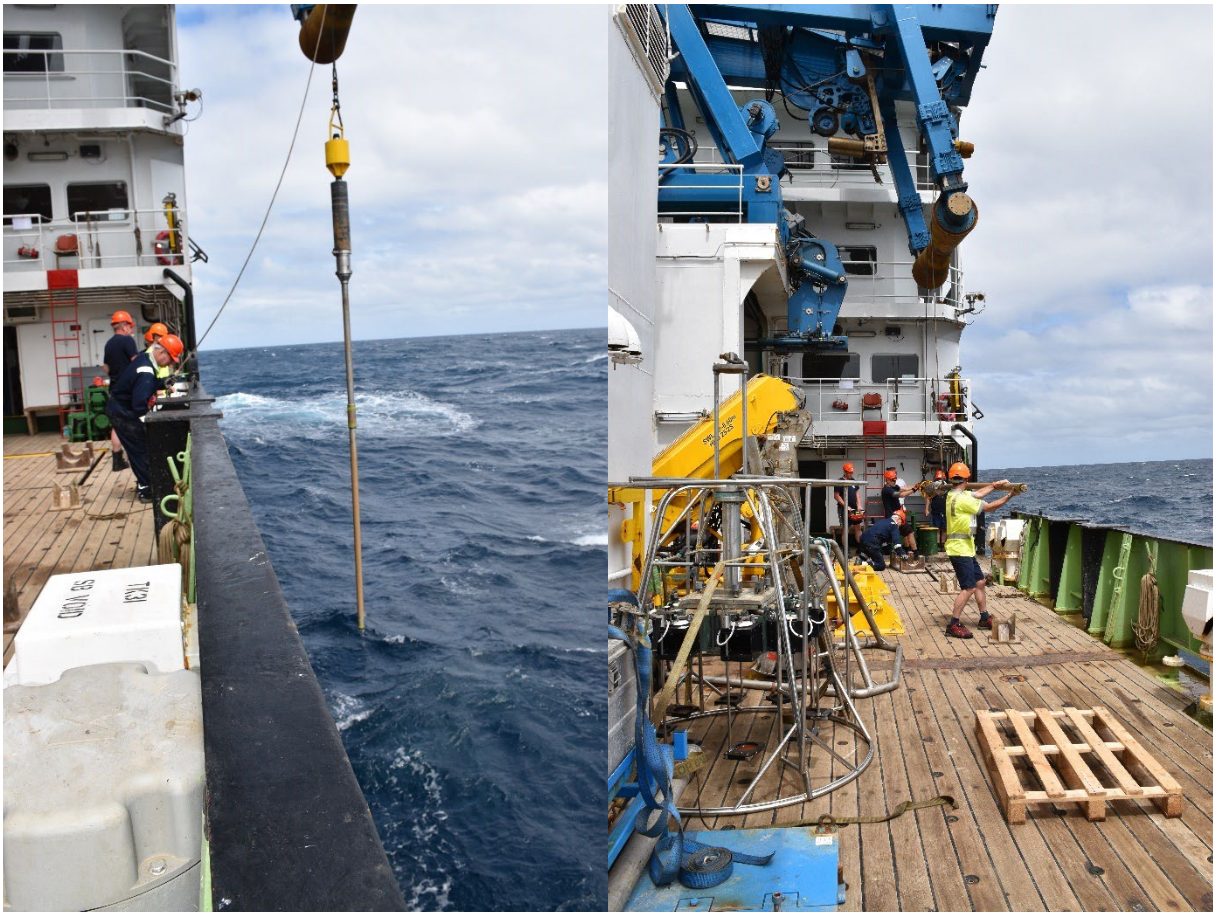


Figure 11.2: Gravity core apparatus and recovery during the JC241 cruise.

Station	Location	Water depth (m)	Latitude		Longitude		Core comments
			deg (N)	minutes	deg (W)	minutes	
011	OMCO control	4686	13	44.2070	126	12.1710	Compromised
021	OMCO control	4705	13	44.3007	126	12.3402	
028	OMCO control	4696	13	43.8200	126	12.2700	
034	OMCO tracks	4707	13	44.1000	126	13.2380	
058	OMCO tracks	4739	13	44.1650	126	13.2330	
066	OMCO tracks	4701	13	44.1140	126	12.3240	
068	OMCO control	4666	13	44.0485	126	12.0168	
097	UK-1	4118	13	58.3977	116	32.4031	

Table 11.1: Gravity cores recovered on the JC241 cruise. See the Geochemistry section for more information.
Once on deck, cores were sliced into 1 m sections, capped, taped, and labelled. Sections were labelled from A-E, with section A removed from the core barrel first and corresponding to the base of the core, then section B, section C and the uppermost stratigraphic section (and surface) corresponding to section D or E depending on amount of core recovery. Where required, high density foam was used to plug gaps at the top and base of the gravity core prior to capping and taping. Opportunistic microbiology sub-samples for eDNA analysis were recovered from section ends after sectioning, but prior to sections being capped at stations 058, 068 and 097. Core sections were then transferred to the controlled environment temperature laboratory (CET lab).

Megacore

The deep seas group Megacorer was employed during JC241 (Figure 11.3). For each deployment, it was fitted with eight 10 cm internal diameter coring units and its standard ballast load. A variable amount of the core tubes were variably pre-drilled, or bleached depending on scientific needs.

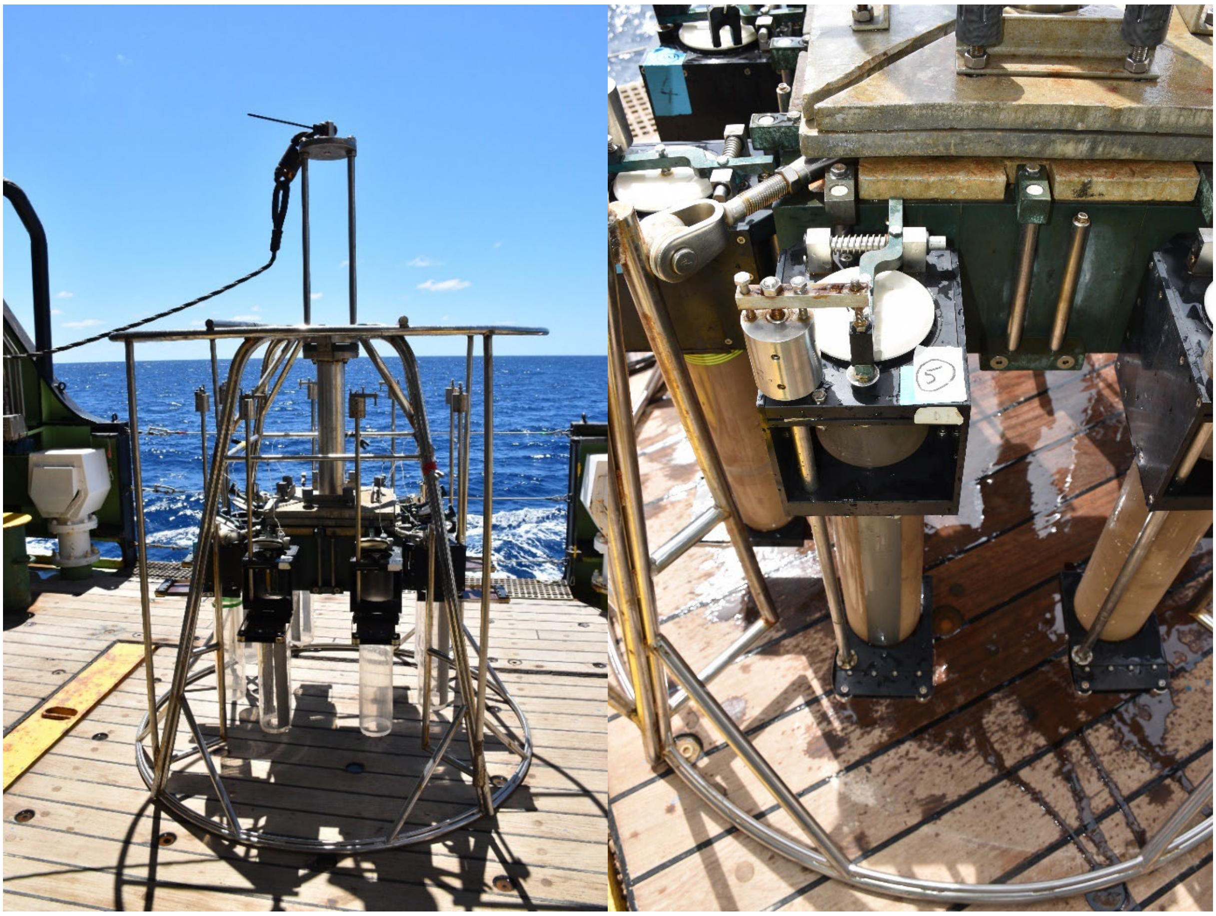


Figure 11.3: Megacorer apparatus used during the JC241 cruise.

In total, the Megacorer was deployed nine times for the sampling of sediments for biology, eDNA, and geochemistry analysis and performed well except for the first deployment in the OMCO control area (station 019) where the corer did not penetrate the seabed well and recovered 1 out of 8 core barrels of acceptable core (Table 11.2). Despite nodules exceeding 10-15 cm in some sample areas, recovered core length was excellent overall with an average successful core recovery size of 38.6 cm, and 56 successful cores were recovered from the eight coring units over the nine deployments. This equates to 78% recovery, or 86% recovery if station 019 is excluded. Station 074 from the OMCO track and station 075 in the OMCO control area were the most successful with all cores recovered and an average core length of 36 and 40 cm, respectively. The primary reasons for cores being rejected upon recovery were due to extreme nodule drag heavily disturbing the stratigraphy, or cracks and air bubbles likely compromising the solid and porewater phases. Three station deployments were monitored with an NMFSS-supplied 1-second 10kHz pinger and an USBL beacon mounted on the wire 50 m above the gear. The USBL system appeared to work well throughout and data was recovered for more precise locations of seabed penetration.

Descent and ascent of the megacore apparatus was conducted at 40-50 m/min with a speed reduction to 20 m/min over the last 100 m above seabed. Station data were recorded for the moment of bottom contact: time (UTC), ship's position, estimated gear position (USBL), sounding, and metres of wire deployed. Assessment of megacore penetration in the seabed and extraction from the seabed was conducted via monitoring of the cable tension. Maximum cable tension on pull out and the cable winch speed were also noted. Once on-deck, cores were photographed, quality assessed using criteria listed on a core assessment sheet produced by Bryan O'Malley, and assigned to scientists depending on specific requirements. Cores were then transferred to the CET lab.

Acceptance or rejection of cores were based on answers (ANS) to these criteria:

- Surface nodules present - ANS: yes or no
- Sed/water interface disturbed – ANS: No, Slight, Moderate, Extreme
- Nodule drag - ANS: None, Slight, Moderate, Extreme
- Undisturbed stratigraphy for sectioning - ANS: Yes, No
- Cracks, gaps, air bubbles - ANS: Yes, No
- Top water intact - ANS: Yes, No
- Top water clarity acceptable- ANS: Yes, No
- Core slippage: ANS: Yes, No
- Recovery of core in cm in 0.5 cm increments – ANS: XX cm
- Accept or Reject – ANS: A, R
- Sample designation – ANS: BGS, eDNA, Liverpool, Forams, NHM, Biodiscovery, Sweetman (etc)

Station	Location	Water depth (m)	Latitude		Longitude		Core comments
			deg (N)	minutes	deg (W)	minutes	
019	OMCO control	4682	13	43.9500	126	12.2500	1 accepted
024	OMCO control	4697	13	44.2480	126	12.2880	7 accepted
030	OMCO control	4694	13	43.8560	126	12.2470	7 accepted
057	OMCO tracks	4741	13	44.1700	126	13.2300	5 accepted
063	OMCO tracks	4715	13	44.1650	126	13.2323	6 accepted
071	OMCO control	4696	13	44.1140	126	12.3230	6 accepted
074	OMCO control	4668	13	44.0497	126	12.1202	8 accepted
075	OMCO tracks	4724	13	44.1215	126	13.2387	8 accepted
101	UK-1	4118	13	58.3990	116	32.4030	7 accepted

Table 11.2. Megacore station deployments during the JC241 cruise.

ROV push cores

Stations 013, 069, 073 and 089 were ROV collection dives partially encompassing ROV push core collection. Each ROV push core collection dive had at least three 30 cm ('standard') push core tubes (5.5 cm Ø) bleached and/or pre-drilled coring tubes depending on the sample assignment. Multiple pre-drilled and/or bleached tubes optimised potential for recovery of at least one successful pre-drilled push core suitable for geochemical or biological analysis.

Each ROV sampling site was chosen in live time. Individual push cores were identified by unique coloured tape markings on the T-handle and allocations were assigned according to the relevant ROV Dive plan. Sample assignment after collection was noted on both the ROV sample logs and a schematic sample map.

At station 013 (ROV Dive 399), 16 standard push cores were collected directly in the centre of the OMCO collection track, and 2 standard push cores approximately one meter away from the outside extent of the sediment ridge, off-track into the nodule field. At station 069 (ROV Dive 406), 6 standard push cores were taken on track and 6 standard push cores within a meter off-track. At station 073 (ROV Dive 407), 10 standard push cores were taken on track and 14 off-track, approximately a meter away from the outside extent of the sediment ridge. On the final ROV collections dive at the OMCO control site (station 089; ROV Dive 411), 6 standard push cores were taken, as well as a 60 cm large push core (8 cm Ø). This larger push core was deployed for testing recovery of characteristic CCZ deep-sea sediment using a larger coring tube. The high cohesion and shear resistance of the deep-sea sediments made this deployment test a success with the recovery of a 39 cm core which had very similar characteristics and recovery to megacores recovered from this area. Details of specific push core locations are detailed in this cruise report by scientific discipline.

Even though push cores were taken in live time under direction from scientific teams, it was not possible to fully assess a core quality until examined on surface in the CET lab. Acceptance or rejection of cores were based on answers to the assessment criteria listed under the general scientific megacore protocol above.

Ground truthing of coring location

Ground truthing of some coring locations were made using observations recorded during ROV dives. The DVL and/or USBL position and the time of observation in the video or Scorpio photo footage were cross checked in ArcGIS against the list of stations for megacores, gravity cores and boxcores in order to identify the likely station number associated with the observation (Table 11.4). Distance from the OMCO track was also noted where relevant. Not all observations were cross checked and ground truthed as there were either uncertainties in the type of coring apparatus causing the seafloor disturbance or the observation recorded in the OFOP (or a waypoint) was not found in any video or image. Multiple observations of the same coring site were made for one megacore at station 030, one gravity core (station 058) and one boxcore (station 061).

Both gravity core deployments in the OMCO track area observed during ROV dives have precise locations relative to the tracks noted (stations 034 and 058; Table 11.4). One gravity core sample was within a meter of the tracks (station 034) and the other approximately 10 meters away (station 058; Figure 11.4).

One megacore deployment was ground truthed at the OMCO control site (station 061) and two at the OMCO tracks (stations 057 and 075). Both megacores deployed in the OMCO track sampling area sampled the seafloor within one meter of the edge of the sediment ridge caused by the disturbance. An observation of a megacore imprint on the seafloor from station 061 at the OMCO control site is shown in Figure 11.4.

Eight boxcore deployments were ground truthed, all at the OMCO track site (Table 11.4). The boxcore from station 084 sampled over the sediment ridge and into the furrow of the collector disturbance. One boxcore sample was within 1 m of the tracks (station 033), and five other boxcores were within 1 to 2 meters of the track (Table 11.4). One boxcore was within 4 meters of the track (station 067). A boxcore imprint on the seafloor from station 061 is shown in Figure 11.4.

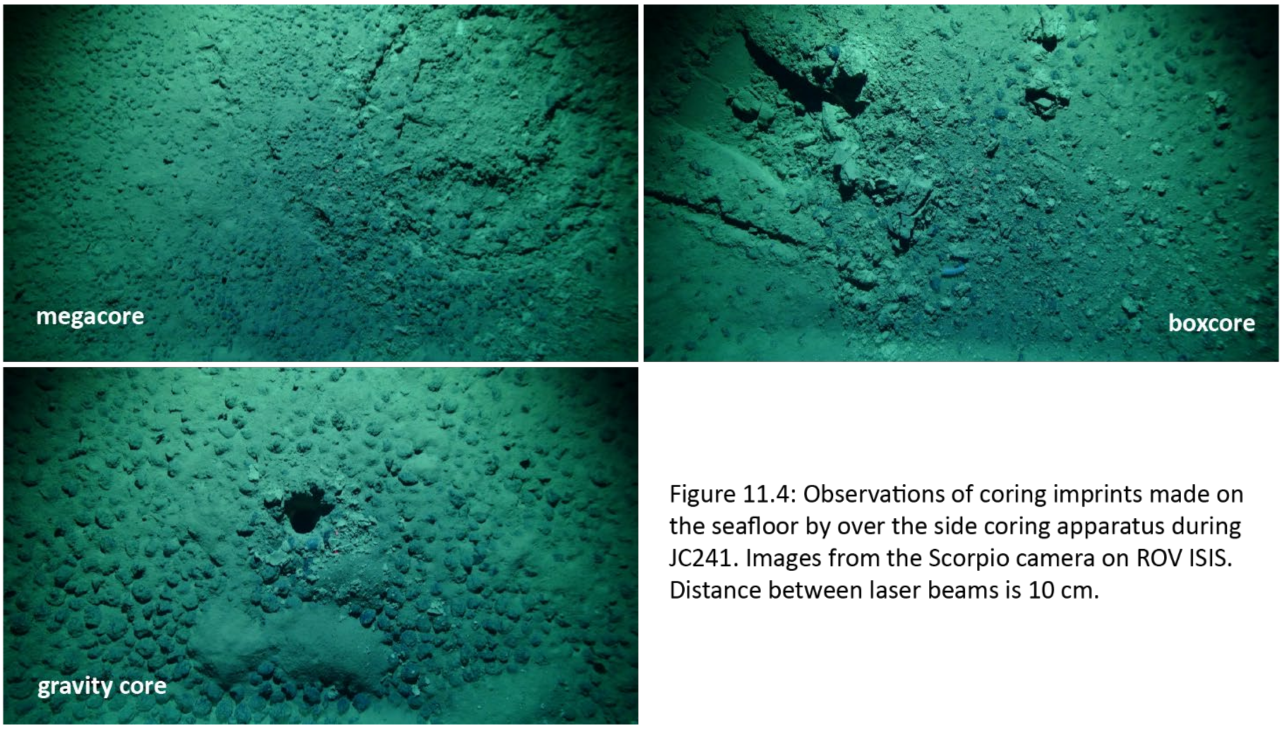


Figure 11.4: Observations of coring imprints made on the seafloor by over the side coring apparatus during JC241. Images from the Scorpio camera on ROV ISIS. Distance between laser beams is 10 cm.

Station	Gear	ROV Dive	Latitude	Longitude	Comment in OFOP or waypoint	Distance relative to OMCO tracks
030	MC	405	13.731030	-126.203922	Possible multi-core	N/A - control site
030	MC	405	13.730096	-126.203568	Multi-core trace	N/A - control site
061	BC	406	13.735504	-126.220287	Boxcore track	Within 2 m
061	BC	406	13.735512	-126.220288	Box core track	Within 2 m
058	GC	407	13.735270	-126.220431	Gravity core spotted	5-10 m distance
067	BC	407	13.732804	-126.219961	Box core hole observed outside track	Within 4 m
058	GC	409	13.735036	-126.220054	gravity core site	~10 m distance
085	BC	409	13.733477	-126.219991	boxcore	Within 2 m
057	MC	409	13.735645	-126.219652	Megacore imprint	Within 1 m
084	BC	409	13.735532	-126.219652	Boxcore _hit_track	Hit sediment ridge, track furrow
079	BC	409	13.734992	-126.219696	Boxcore _miss	Within 1.5 m
075	MC	409	13.734659	-126.219812	megacore_miss	Within 1 m
033	BC	409	13.734311	-126.219840	boxcore_offtrack	Within 1 m
034	GC	409	13.734204	-126.219852	gravity core off track	Within 1 m
072	BC	409	13.733636	-126.219852	boxcore off track	Within 2 m
087	BC	409	13.732679	-126.219846	possibly another off track boxcore	Approximately 2 m

Table 11.4: Ground truthing of over 13 side coring locations using video and photo observations cross checked with DVL and/or USBL navigation data from ROV ISIS.

Overview of Geochemistry objective

This objective assesses the behaviour of interlinked sub-surface geochemical components and, in particular, the contribution of the pore-water, surface and sub-surface nodules, and sediment components to large-scale trace element fluxes and ecosystem-dependant biogeochemical pathways. Disruption of steady-state trace element geochemical fluxes and biogeochemical pathways essential to abyssal ecosystems could occur from the uppermost sediment layer to far below the surface as the extent of the biogeochemical response to the removal of overlying oxic sediment is not known.

Sampling strategy

With the end objective of producing a model of geochemical cycling occurring within the sediment pile of the disturbed OMCO polymetallic nodule exploitation area, a range of coring deployments were conducted. Various spatial and depth resolutions were achieved combining gravity cores, megacores, and push cores recovered at various scales in and out of the OMCO disturbance track, and further away within a control zone at approximately 1 km distance to the east (Figure 11.1), considered representative of the background environment and untouched by the OMCO nodule collection programme. A paired gravity core and megacore deployment was also carried out at UK-1 to obtain a small dataset to be used as a trial for the biogeochemical modelling.

At the OMCO area, three environments were targeted: (i) the disturbed track, (ii) in the immediate vicinity of the track potentially receiving fall-out products from the disturbance, and (iii) the control site. For each environment, the sampling strategy was aimed at recovering paired records of the pore water and solid phase at two (or more) sites to quantify natural variability and explore variance between environments. For each sampling site, a paired gravity core and megacore were deployed to obtain complementary shallow and deep sediment and pore water records. Even though USBL transponders were attached to the majority of deployments within the OMCO track area, the spatial precision of deployment from the vessel more than 4500 m above the seabed is limited. To quantify at a high spatial resolution, paired samples within, and in the immediate vicinity of the disturbed tracks, these environments were sampled directly with push cores from the ROV ISIS.

Geochemistry sampling methodology

Gravity cores

Once the cores were labelled on deck, the cores were moved into the CET lab and photographed and described. The dissolved oxygen (dO_2) content of the core was then measured in 5 cm intervals avoiding the first and last 5 cm of each section to minimise air contamination introduced from the sectioning on deck. A larger interval of up 8 cm at section tops and bases were not analysed for dO_2 or pore water extracted where microbiology samples were taken. Dissolved oxygen was not measured at stations 066 and 068 (see Dissolved oxygen section). Pore water was generally extracted every 20 cm in the top 2 m and at a 30 cm resolution below with some flexibility to adjust for core sections ends. Stratigraphic sections with interesting features were sampled in higher resolution (10 cm). This strategy was repeated for ammonium (when recovered) with a 10 cm offset to the pore water extraction intervals. The liner was then drilled with 20 mm diameter holes using a cylindrical hollow head drill for solid phase extraction. Sampling intervals were every 20 cm in the top 200 cm and then every 30 cm below with opportunistic or regular samples taken at interesting intervals. After station 011, the gravity core barrels were pre-drilled every 5 cm for dO_2 analysis and taped as drilling into the core liner after the first gravity core recovery (station 011) very clearly compromised the dO_2 and resulted in erratic measurements.

Figure 11.5: Example of a full gravity core from station 066.

Megacores and push cores

For each megacore and push core deployment, the sampling strategy required a pair of complimentary cores. One core was utilised for dO_2 measurements, followed by pore water extraction, and the second core for solid phase sampling. Each ROV push core collection dive contained two to three pre-drilled 30 cm push core tubes (5.5 cm Ø) for geochemistry to allow for dO_2 measurements and pore water extraction. Multiple pre-drilled tubes also optimised potential for recovery of at least one pre-drilled push core suitable for dO_2 analysis and pore water extraction. Each ROV sampling site was chosen in live time and sampling was observed by a member of the Geochemistry team as per the relevant ROV Dive plan. On the final ROV dive at the OMCO control site (station 089; ROV Dive 411), a 60 cm large push core (8 cm Ø) was deployed for testing recovery of characteristic CCZ deep-sea sediment (Figure 11.6). Precise positioning data for ROV push cores from stations 013, 069, 073 and 089 (ROV Dives 399, 406, 407 and 411) are available from ROV USBL data (Table 11.5). The push core sampling locations are also spatially referenced relative to the OMCO tracks. Once the ROV push cores were recovered from the vehicle at surface, the cores were taken directly to the CET lab.

Prior to megacore deployment, 60 cm megacore tubes were pre-drilled at 1 cm intervals on two sides of the tube for geochemistry. Two to three pre-drilled core tubes were part of each deployment to optimise potential for recovery of at least one pre-drilled megacore suitable for dO_2 measurements and pore water extraction (Figure 11.3).

Figure 11.6: Real-time sampling view of the large ROV push core at the OMCO control site (station 089).

On arrival of cores into the CT lab, all cores were photographed and described and assessed for quality as per the core assessment criteria listed under the general megacore protocol above. For the megacore or push core assigned to geochemical pore water analysis, dO_2 was performed every 1 cm through the pre-drilled holes. Dissolved oxygen for megacores and push cores was not measured at stations 069, 071, 073, 075 and 089 (see Dissolved oxygen section). Following dO_2 measurements every cm, staggered pore water sampling alternating between tube sides enabled (i) minimising the drawn pore water cone of influence as much as possible, and (ii) an easier manipulation of Rhizons and syringes. For megacores, pore water was drawn every cm between 1-10 cm and then every 2 cm below. Due to the smaller diameter of the push cores (5.5 cm) and overlap of pore water cone of extraction at 1 cm intervals, pore water was drawn every 2 cm for push cores. Where possible, a duplicate sample of the overlying seawater was taken at each station after pore water extraction. Solid phase sampling from megacores and push cores was conducted at 1 cm intervals from 0 to 10 cm below surface, and then every 2 cm below 10 cm to the end of each core. In the event of an oblique surface, the top of the sediment was considered as the reference point for the 0 cm mark. The last slice of each core remaining on the extruder was not sampled due to higher potential for contamination. The push core sampled in ROV Dive 407 on the edge of the disturbed ridge sediment potentially overlying buried ‘undisturbed’ seafloor was sampled every 1 cm for the solid phase to enable higher resolution analysis of this unusual and disturbed stratigraphy (station 073).

Station	Gear	Location	Water depth (m)	Core length (cm)	Latitude		Longitude		Comments
					deg (N)	minutes	deg (W)	minutes	
011	GC	OMCO control	4686	279	13	44.2070	126	12.1710	Compromised core
021	GC	OMCO control	4705	325	13	44.3007	126	12.3402	
028	GC	OMCO control	4696	375	13	43.8200	126	12.2700	
034	GC	OMCO tracks	4707	383	13	44.1000	126	13.2380	
058	GC	OMCO tracks	4739	485	13	44.1650	126	13.2330	
066	GC	OMCO tracks	4701	370	13	44.1140	126	12.3240	
068	GC	OMCO control	4666	335	13	44.0485	126	12.0168	
097	GC	UK-1	4118	328	13	58.3977	116	32.4031	
019	MC	OMCO control	4682	-	13	43.9500	126	12.2500	Only 1 core recovered
024	MC	OMCO control	4697	43, 44	13	44.2480	126	12.2880	
030	MC	OMCO control	4694	32, 40, 42	13	43.8560	126	12.2470	
057	MC	OMCO tracks	4741	38, 42	13	44.1700	126	13.2300	
063	MC	OMCO tracks	4715	37, 37	13	44.1650	126	13.2323	
071	MC	OMCO control	4696	34, 22	13	44.1140	126	12.3230	
074	MC	OMCO control	4668	38, 37	13	44.0497	126	12.1202	
075	MC	OMCO tracks	4724	38, 39	13	44.1215	126	13.2387	
101	MC	UK-1	4118	40, 40, 41, 40	13	58.3990	116	32.4030	
013	PC	OMCO tracks (middle)	4712	25	13	44.1981	126	13.2204	
013	PC	OMCO tracks (middle)	4712	24.5	13	44.1981	126	13.2204	
013	PC	OMCO tracks (outside)	4712	29.5	13	44.1968	126	13.2202	
013	PC	OMCO tracks (outside)	4712	-	13	44.1968	126	13.2202	Failed core
013	PC	OMCO tracks (outside)	4712	28.5	13	44.1968	126	13.2202	
013	PC	OMCO tracks	4727	29	13	44.1719	126	13.2341	
013	PC	OMCO tracks	4727	-	13	44.1719	126	13.2341	Failed core
013	PC	OMCO tracks	4727	-	13	44.1753	126	13.2214	Failed core
013	PC	OMCO tracks	4727	23-27	13	44.1753	126	13.2214	
069	PC	OMCO tracks (outside)	4726	22	13	44.1787	126	13.2108	
069	PC	OMCO tracks (middle)	4712	17	13	44.1840	126	13.2270	
073	PC	OMCO tracks (edge of track)	4706	21	13	43.9630	126	13.1810	
089	PC	OMCO control	4690	39	13	43.8218	126	12.2679	Large ROV push core
089	PC	OMCO control	4688	22	13	43.9661	126	12.2261	

Table 11.5: Overview of Geochemistry sampling locations for gravity cores (GC), megacores (MC) and ROV push cores (PC).

Dissolved oxygen (dO_2)

The dissolved oxygen content of sediment pore water and the overlying seawater (when preserved) was measured using Pyroscience OXB230 optodes, needle-type fibre-optic oxygen mini-sensors connected to a FireSting O2 external connector logging onto a dedicated computer using the Pyro Oxygen Logger software (Figure 11.7). All dO_2 measurements were paired with a temperature reading taken in an appropriate medium, e.g., calibration solutions or equivalent core material when measuring core samples. The paired temperature measurement was used to compensate for the effects of temperature on each dO_2 reading. Calibration of the probes was made using a two-point reference system with 100% dO_2 saturation measured in well shaken oxic seawater, whilst the anoxic reference was obtained by measuring dO_2 in seawater admixed with a small amount of sodium dithionite ($Na_2S_2O_4$). Calibration of the probes were conducted before receipt of samples from each station. The accuracy of the sensors was excellent with regards to dO_2 saturation calibration. However, it was observed that the probe sensitivity decreased throughout each core measurement series as a result of the abrasive contact of sediments on the fibre optic. The main impact was an increase in detection limit at low anoxic levels requiring regular monitoring of the probe reading in the anoxic reference solution every 2-5 measurements. When the probe sensitivity decreased too much, zeroing was required. Readings for the dO_2 saturated reference solution remained within 2% of the calibrated value.

To avoid measuring any oxygen diffusing from the outside into the sediment during recovery and storage, at each sample site, the oxygen sensor was slowly driven towards the centre of the core until the signal was stable. Due to the continually abrasive nature of the sediments on the sensors, the signal intensity of all six available optodes decreased below acceptable levels after station 063 and calibration values were not stable despite multiple calibration attempts. The decision was made to discontinue dO_2 measurements at the OMCO sampling sites as the remaining operational optodes were required for later respiration experiments. Following the respiration experiments, working optodes were used to measure dO_2 for a gravity core and megacore pair at the UK-1 sampling site (station 097 and 101).

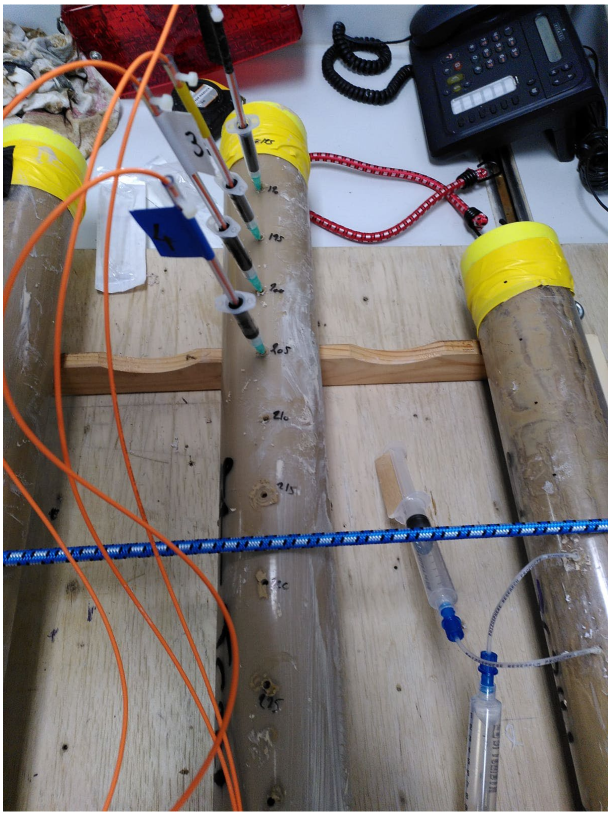


Figure 11.7: Example of dissolved oxygen measurements on a gravity core.

Pore water extraction, processing, and storage

Pore water was directly extracted from sediments in all core types using Rhizons (Rhizon CSS flat tip moisture sampler: length 5 cm, pore diameter 0.15 µm, PVC/PE tubing 12 cm, female luer; Rhizosphere Research Products, Wageningen, Netherlands) inserted though the pre-drilled holes in the core liners and connected to a 10 ml syringe on which a small under-pressure was applied (Figure 11.8). The pore water was split into three aliquots for onshore analysis of alkalinity (2 ml), trace metals (3 ml), and nutrients (5 ml) at the British Geological Survey (BGS). Additional opportunistic pore water samples were taken (10 ml) for onshore analysis of ammonium. Some pore water samples taken close to the top of cores noticeably drew in the overlying seawater. The level of overlying seawater was monitored in cores and where the level dropped this was noted as a potentially compromised pore water sample.

- 2ml Alkalinity glass vials were filled up leaving as little air as possible, immediately poisoned with 5 µl of a saturated $HgCl_2$ solution to prevent bacterial activity, covered with parafilm and preserved at 2°C for later onshore analysis at the BGS.
- The trace metal aliquot was transferred into 8 ml HDPE bottles and acidified with 10 µl of ultra-pure concentrated nitric acid (Fisher Optima Grade HNO_3) and preserved at 2°C for later analysis by ICP-MS at the BGS.
- The nutrient aliquot was transferred into 15 ml HDPE vials and frozen at -20°C for later analysis by ion chromatography at the BGS.
- The ammonium aliquot was transferred into 15 ml HDPE vials and immediately frozen at -80°C for later analysis. A suitable laboratory will be sourced for the analysis of ammonium pending a funding review.

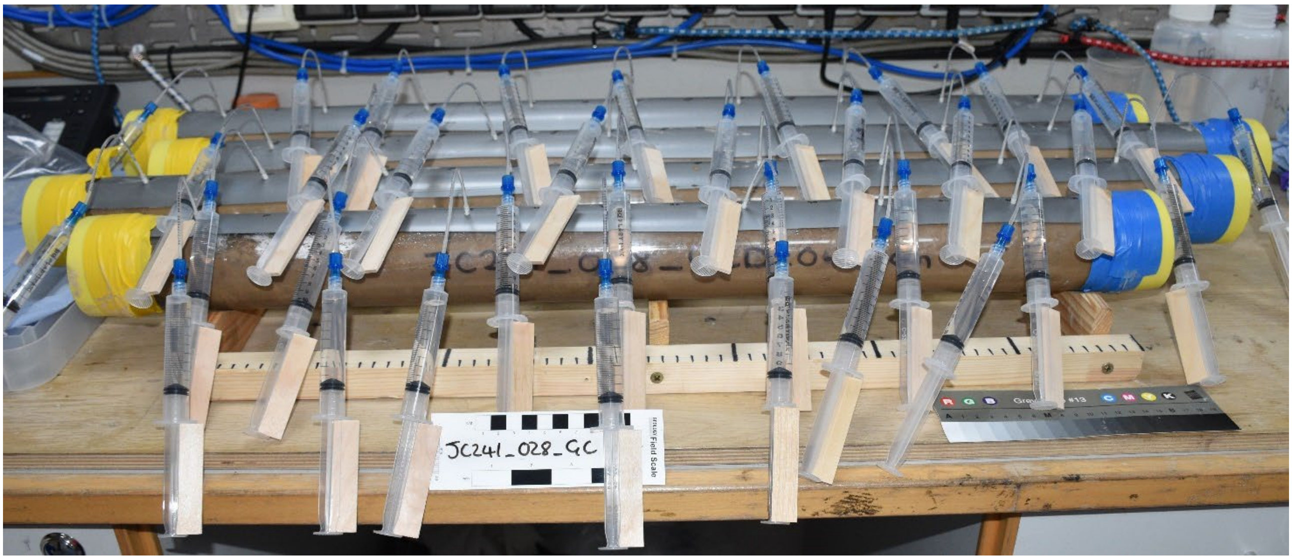


Figure 11.8: Pore water extraction example from a gravity core.

Solid phase extraction, processing, and storage

For gravity core sediment sampling, a pre-cleaned 10 ml syringe with a pre-cut tip was inserted into the drilled 20 mm hole in the liner, and extraction made of a plug of sediment (~8 ml) by applying a small amount of under-pressure (Figure 11.9). This sample was split between an aliquot for bulk geochemical analysis for later analysis at the BGS, and an archive aliquot. Solid phase sampling from megacores and push cores was conducted by direct core extrusion and recovery of individual slices. Each slice was split into a bulk geochemistry aliquot, and an archive sample. An aliquot for potential carbon, nitrogen, and sulfur isotope analysis was also taken for megacores. All solid phase samples were frozen at -20°C for later analysis or long-term storage of archive samples. A subset of samples will be taken onshore from the archive samples across all core types for 1) grain-size analysis and 2) XRD mineralogy, both for analysis at the BGS. One whole megacore from the UK-1 sampling site (station 101) was frozen at -20 degrees for later analysis of physical properties at the BGS.

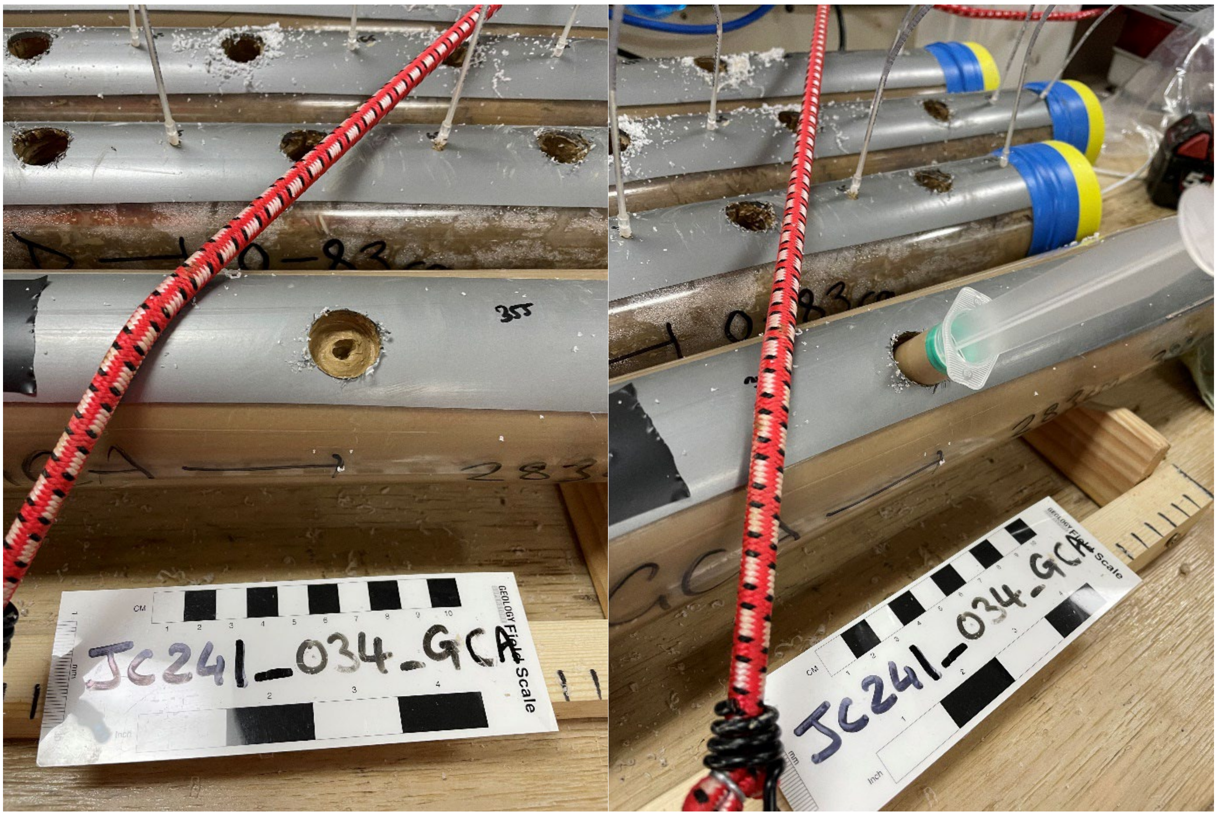


Figure 11.9: Example of sediment plug sampling from a gravity core.

Mn-nodule sampling and storage

Pierre Josso, Hannah Grant, Daniel Jones

After being examined for any biological feature, nodules recovered from boxcores were cleaned, dried, measured and photographed by station. For each nodule the long axis (L) and perpendicular second axis (W) of the equatorial plane, as well as the height (h) were measured, noting when nodules were intact or broken. Summary statistics of nodule sizes are available in Table 11.6. A preliminary assessment of nodule dry density on the seafloor from boxcore data (0.25 m^2) was calculated assuming ideal nodule ellipsoidal volumes ($V = 4/3 * \pi * L/2 * W/2 * h/2$) and a dry density of 1.13 g/cm^3 (Wijk and de Hoog, 2020). When nodule dimensions were estimated using ImageJ software, solely L and W could be measured. Hence, for missing height data, ‘h’ was estimated based on the linear relationship observed on data collected for whole nodules.

Nodules were photographed in trays with white background by station with appropriate tag and scale for reference. Nodules recovered from boxcores from the OMCO area were finally bagged (Hubco Sentry Geological sample bags) and tagged with serial numbered zip ties to be sent to UKSR for further geochemical analysis.

Representative nodules collected from boxcores from OMCO, APEI-13, PRA and megacores (where available) were desalinated in fresh water, allowed to air dry and bagged by station for more detailed onshore geochemical and isotopic analyses (e.g. Figure 11.10).

Station	Location	n	Length (avg ± 1σ)	Width (avg ± 1σ)	Height (avg ± 1σ)	kg/m2
JC241_016	OMCO Ctrl	26	45 ± 37	38 ± 32	24 ± 17	9.0
JC241_018	OMCO Ctrl	40	48 ± 40	41 ± 34	25 ± 18	16
JC241_020	OMCO Ctrl	45	47 ± 27	39 ± 21	25 ± 12	11
JC241_022	OMCO Ctrl	43	39 ± 25	35 ± 21	21 ± 11	6.6
JC241_026	OMCO Ctrl	36	39 ± 23	32 ± 20	22 ± 14	5.2
JC241_029	OMCO Ctrl	90	35 ± 22	29 ± 19	21 ± 14	11
JC241_033	OMCO track	49	65 ± 26	50 ± 21	33 ± 12	17
JC241_065	OMCO track	5	32 ± 10	25 ± 10	10 ± 4	0.1
JC241_067	OMCO track	53	69 ± 19	52 ± 15	36 ± 12	20
JC241_068	OMCO track	32	82 ± 19	64 ± 14	42 ± 10	19
JC241_070	OMCO track	28	90 ± 17	70 ± 14	47 ± 10	21
JC241_072	OMCO track	78	64 ± 23	50 ± 20	36 ± 13	29
JC241_077	OMCO track	34	82 ± 19	63 ± 14	39 ± 10	18
JC241_078	OMCO track	35	76 ± 23	57 ± 17	38 ± 10	16
JC241_079	OMCO track	37	76 ± 20	57 ± 17	39 ± 10	17
JC241_083	OMCO track	38	79 ± 18	63 ± 18	40 ± 12	20
JC241_084	OMCO track	39	69 ± 25	54 ± 22	33 ± 11	15
JC241_085	OMCO track	32	75 ± 20	57 ± 19	38 ± 9	14
JC241_087	OMCO track	44	73 ± 20	55 ± 15	38 ± 11	19
JC241_088	OMCO track	38	57 ± 41	43 ± 30	31 ± 27	21
JC241_081	OMCO Ctrl 2	307	23 ± 12	18 ± 10	13 ± 7	8.4
JC241_036	PRA	14	35 ± 6	27 ± 2	22 ± 3	0.7
JC241_038	PRA	10	26 ± 12	22 ± 10	16 ± 6	0.3
JC241_039	PRA	7	37 ± 8	31 ± 8	20 ± 4	0.4
JC241_041	PRA	6	31 ± 13	26 ± 10	19 ± 5	0.3
JC241_042	PRA	2	28 ± 1	23 ± 1	19 ± 2	0.1
JC241_045	PRA	7	35 ± 6	26 ± 5	20 ± 3	0.3
JC241_048	PRA	19	84 ± 23	61 ± 17	42 ± 10	11
JC241_049	PRA	22	82 ± 29	66 ± 25	41 ± 13	15
JC241_050	PRA	19	75 ± 61	63 ± 52	38 ± 28	25
JC241_051	PRA	6	80 ± 54	63 ± 41	40 ± 25	6.1
JC241_053	PRA	3	53 ± 6	38 ± 4	28 ± 3	0.4
JC241_054	PRA	12	26 ± 8	24 ± 8	16 ± 4	0.3

Table 11.6: Summary table of nodule statistics from the OMCO and PRA sampling sites.

Preliminary observations and sampling outcome

A total of 8 gravity cores were deployed (1 rejected on examination on deck; Table 11.1) averaging a recovery of 370 ± 51 cm (1σ, n = 7) recovery for deployment with 3- 5 m barrels. The consistent overpenetration observed by the mud staining is unlikely to have been detrimental to the coring activity and integrity of retrieved cores as the surface was observed preserved in all cores but one.

The 9 megacore deployments resulted in an average of 38 ± 5 cm (1σ, n = 9) per core for those used by the geochemistry team (n = 21) and were processed for dO_2, pore water and solid phase extraction.

Figure 11.10. Photograph of nodules recovered from a boxcore deployment at station 033.

Successful ROV push core recovery for the geochemistry team averaged 24 ± 4 cm (1σ, n = 10), excluding the large push core which recovered 39 cm.

In total, 1098 pore water samples were recovered representing a total volume of 4.5 litres, whilst 1476 solid phase subsamples were bagged during processing in the CET lab. Summary statistics for the subsampling of all cores from the geochemistry team is presented in Table 11.7.

Sample type	Pore water (n)	Solid phase (n)
Alkalinity (2 ml)	322	-
Trace elements (3 ml)	322	-
Nutrients (4 ml)	322	-
Ammonia (10 ml)	132	-
Archive	-	529
Trace elements	-	537
C N S isotopes	-	410
Total	1098	1476

Table 11.7: Break down of pore water and solid phase subsamples recovered by the geochemistry team. Note that pore water volumes are minimal volume required for analysis and that a larger volume was taken when possible.

A total of 435 dissolved oxygen measurements were performed at the OMCO sampling sites and UK-1, producing 12 complete profiles for megacores, push cores and gravity cores. The data requires further processing and cross calibration to ensure measurements are comparable across stations. Overall, profiles are consistent with previously published data showing a rapid decrease of dO_2 from seawater values (160 µmol/l) to 50 µmol/l below the first 15-30 cm, with further decrease to values 0.1-5 µmol/l generally observed below 2 m in gravity cores.

Megacores and push cores had a comparable stratigraphy at the OMCO sampling site showing a top unit of darker mud over the top 10 to 15 cm of cores. Fresh traces of bioturbation were frequently preserved at the sediment-seawater interface and are likely responsible for the homogenous texture of the top 10-15 cm. The next unit extended to the end of push cores and megacores presenting a mixed or mottled texture mostly comprised from mixing of lighter and darker brown sediments (often lightening to the base) and this was also observed in gravity cores to 1.5 to 2 m depth (Figure 11.11). Micronodules were common in many megacores, especially in the top 5 cm (e.g. Figure 11.12), and were also observed in gravity cores as deep as 2-3 m.

Figure 11.11: Examples of mixing texture seen in both gravity cores (left) and megacores (right). The gravity core plug is from station 066 (OMCO tracks) and the extruded sediment slice is from station 101 at UK-1.

Gravity core stratigraphy was more varied but was also more complex to evaluate due to partial visibility and smearing along the liners. A darker unit was commonly observed in the top part of the core (Figure 11.5), similar in nature and depth to the bioturbated horizon of megacores, giving confidence in the quality of the stratigraphic recovery and that the top of the gravity cores did recover the sediment-seawater interface. Consistent stratigraphic horizons were observed between multiple cores through visual observation but also in the dO_2 data. Such excursions have been observed before but never commented in published literature and could form a particular point of observation as a reference stratigraphic horizon for the CCZ. Sediment plug subsamples gave limited but valuable in-situ observation of the mineralogical texture of cores, highlighting that the mottled and mixed texture observed in the lower part of push cores and megacores is

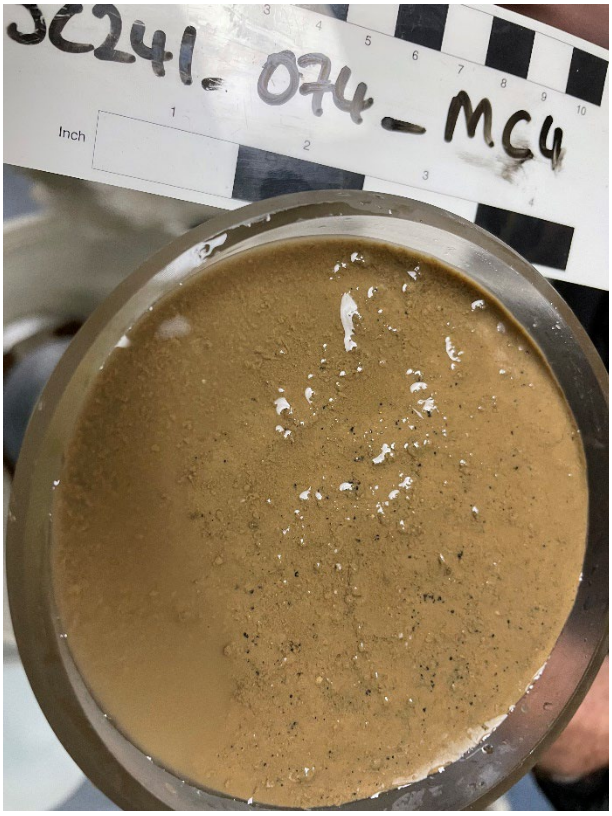


Figure 11.12: Example of micronodules (black specks) on the surface of a megacore recovered from station 074 at the OMCO control site. Similar micronodules were observed in multiple gravity cores.

likely to be a major feature of this area extending as far below surface as 3 m. Commonly, sediment stratigraphy below 2 m was darker and more homogeneous. At UK-1, the megacore and gravity core pair showed a substantial difference to the OMCO site. Overall, the mud was of much darker brown colour and more water saturated and soupy, especially in the top 5 cm of the megacore. This may represent the higher sedimentation rate of the eastern CCZ versus the OMCO site, and therefore may proportionally lower compaction of the top stratigraphic sections. A similar 10-15 cm bioturbated top section was observed in the megacores from UK-1 when compared to the OMCO sampling sites, followed by a contrasting lighter brown 5 cm horizon with strong mottled and mixed texture transitioning into a mixed and mottled texture of intermediate brown shade (Figure 11.11). These limited JC241 observations at UK-1 will be complemented by access to data generated during the JC120 MIDAS expedition, although the area sampled was not visited during the 2015 JC120 expedition.

REFERENCE: J.M. van Wijk, and E. de Hoog, (2020) Size reduction of CCZ polymetallic nodules under repeated impact fragmentation. Results in Engineering, vol. 7, 100154. https://doi.org/10.1016/j.rineng.2020.100154.

Radioisotope Sampling

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Samples for Pb-210 (^{210}Pb) were taken from cores in the tracks, near the tracks, and in a control area at the OMCO site. Sedimentary short-lived radioisotope activities will be measured to provide bioturbation proxies and identification of surface sediment dynamics (passive transport, inventories). Sediment core samples will be analyzed for short-lived radioisotopes by gamma spectrometry on Series HPGe (high-purity Germanium) Coaxial Planar Photon Detectors for total ^{210}Pb (46.5 keV), ^{214}Pb (295 keV and 351 keV), ^{214}Bi (609 keV), ^{234}Th (63 keV). Activities will be expressed as disintegrations per minute per gram of sediment (dpm/g) using methodology described by Brooks et al. (2015). An inventory approach will also be utilized to assess sediment focusing (Brooks et al., 2015; Schwing et al., 2017). These measurements will provide a robust baseline to which the post OMCO collector test measurements can be compared to determine the extent and magnitude of the collector sediment plume, any sediment focusing areas and potentially any alteration to bioturbation intensity.

Radioisotopes

- n (MUC cores): 3
- n (PUC cores): 3
- Sampling Increments: 0-0.5 cm, 0.5-1 cm, 1-2 cm, 2-3 cm, 3-5 cm, 5-7 cm, 7-9 cm
- n samples (MUC cores): 21
- n samples (PUC cores): 21

For each core:

1. Place tube on extruder and slide the bottom bung out.
2. Slide core tube until 5-10 cm overlying water is accessible (avoid resuspension as much as possible. If resuspension occurs, collect all overlying water that may be cloudy).
3. Siphon off water and discard.
4. Remove nodules with clean forceps and rinse with a milli-q squirt bottle catching the sediment in the 0-1 cm bag.
5. Place nodules in a designated whirl-pak bag.
6. Place slicing ring onto core tube.
7. Push tube down so that the desired sediment (0.5 cm intervals for top 1cm; 1 cm intervals from 1-3 cm; 2 cm intervals from 3-9 cm; 0-9 cm total) slice sits within the slicing ring.
8. Using an aluminium plate or putty knife, slice the mud in between the core tube and the slicing ring. Using a smooth movement and with downward pressure, slide the plate off the tube, removing the slicing ring and mud with it. If you pull the plate off in an upward movement, mud may stick to the bottom of the slicing plate and pull the mud (what remains in the core tube) out of the tube, resulting in inaccurate sediment horizons.

Figure 11.13 Steps 7 & 8 of the Th-234 protocol

9. On a secure and clean surface area of the washing table or regular table, place the plate, with one edge on the table and the other edge off the surface.
10. Using the designated circular "stamp", stamp the middle of the sediment horizon and discard the "rind".

Figure 11.14 Steps 10 & 11 of the radioisotope protocol

11. Using the putty knives, slice chunks of mud and place them in labelled, designated whirl-pak bags. If sediment is soupy, then use a plastic funnel to funnel the slurry into the bag.
12. Label the bag appropriately, ensuring unique identifier or code (including cruise, date, station, gear, event number, number of MUC/PUC tube , number of the deployment, and slice depth in centimeters).

Figure 11.15 Step 12 of the radioisotope protocol

13. Repeat step 7-15 until finished.
14. Consolidate samples in a larger Ziploc bag and store at ambient temperature away from sunlight. Freezing (-20^{o}) or refrigerating (4^{o}) subsamples is acceptable, but not necessary.
15. Freeze-dry surface samples if possible.

Notes on radioisotope sampling:

- Sample Resolution: Nods, 0–0.5 cm and 0.5–1.0 cm; 1–2 and 2–3 cm; and 3–5, 5–7 and 7–9 cm.

Sample Storage Radioisotopes

- All samples were stored in Ziploc bags.
- Stored in a cardboard “cream crackers” box in the reefer van with other NHM deepsea samples.
- All samples will be transported to the Natural History Museum in London, UK.

References:

Brooks, G. R., Larson, R. A., Schwing, P. T., Romero, I., Moore, C., Reichart, G. J., ... & Hollander, D. (2015). Sedimentation pulse in the NE Gulf of Mexico following the 2010 DWH blowout. *PloS one*, *10*(7), e0132341.

Schwing, P. T., Brooks, G. R., Larson, R. A., Holmes, C. W., O’Malley, B. J., & Hollander, D. J. (2017). Constraining the spatial extent of marine oil snow sedimentation and flocculent accumulation following the Deepwater Horizon event using an excess 210Pb flux approach. *Environmental Science & Technology*, *51*(11), 5962-5968.

12. Biogeochemistry: Stable isotopes, amino acids and lipid biochemistry

Louisa Norman and Rachel Jeffreys, University of Liverpool.

Megacore and ROV pushcore sediment sampling.

Background and objectives.

As with other anthropogenic disturbances, deep-sea mining is expected to change the distribution of particulate organic matter at the sediment surface (Pusceddu et al., 2014). To investigate this, sediment samples were collected in the OMCO track area and at a control site located 1000 m from the tracks, using megacores and ROV pushcores to characterise the sedimentary organic matter by way of changes in total carbon and nitrogen. As the sediments act as a food source for a variety of fauna, stable isotopes of C and N, and δ15N and δ13C amino acids within the sediments and faunal tissue (see section 15.5, Food webs) will be used to quantify characteristics of the food webs including, trophic position, food chain length, and trophic niche width. Lipid biochemistry and biopolymeric carbon will also be analysed if there are sufficient funds available.

In addition to the OMCO cores, a small number of cores were also collected at the UK1 site to provide preliminary data regarding the sedimentary organic matter characteristics of this site in readiness for the 2024 SMARTEX expedition.

Sampling strategy.

Given the dimensions of the tracks at OMCO it was determined that the megacorer was unlikely to hit the tracks with sufficient accuracy to be certain of what sediment had been recovered. The use of ROV ISIS to collect pushcores would provide complete accuracy as to where the cores were collected and so this method of collection was used.

To obtain adequate representation of the natural variability of sediments at the OMCO site, three replicate collections of ROV pushcores were undertaken at locations in the tracks where nodules were mostly absent and just outside of the tracks where the sediment was likely to have been disturbed. The megacorer was deployed at a control location situated approximately 1000 m away from the track area where nodule coverage had not been disturbed. In addition, the megacorer was deployed over the tracks with a USBL attached to record the exact location. Ground truthing from observations during subsequent ROV dives indicated that these deployments were very close to the tracks (see section 11, Geochemistry, for information). At each collection or deployment three cores were recovered to provide sufficient material for all planned analysis. In addition to the cores recovered at OMCO, two pushcores and two megacores were collected at two locations at the UK1 site.

ROV pushcores.

ROV pushcores were collected at OMCO during two ROV dives (dives 399 and 407). The 'in-track' collection area was selected as it was clearly a disturbed and, mostly, nodule free region within the tracks. Triplicate pushcores were collected at three locations between 30 m and 50 m apart within this nodule-free area. To collect the 'off-track' cores the ROV moved a few metres away from the tracks and a similar sampling strategy was employed. Where possible the pushcores were taken between nodules to avoid surface sediment disturbance and nodule drag. Of the 18 pushcores recovered, only one core was not recovered sufficiently to be used and was noted as a failed core.

The two pushcores recovered at UK1 were collected during ROV dive 412. As with the off-track OMCO cores, these were taken between nodules to avoid surface sediment disturbance. Pushcore locations can be found in fig. 12.1.1 and 12.1.2

Megacores.

Megacores were collected from five deployments in the OMCO control area, two deployments in the OMCO track area and one deployment at UK1. A core recovery assessment sheet was completed prior to the cores being removed from the megacorer and photographs taken to assess the condition of the cores in terms of surface sediment disturbance, top water clarity, presence of nodules and nodule drag, cracks and breaks etc. and to determine whether, or not, the cores were usable (See section 11, Geochemistry, for details). The first megacore deployment (JC241-019) yielded just one suitable core and the deployment was deemed a failure. Sediment type and nodule distribution may have contributed to this. All other deployments were generally good and the requested three cores were recovered at all but one control deployment and one track-area deployment. In these instances, two cores were recovered. As with pushcore collections, the megacore deployment locations aimed to provide representative samples of natural variability. Megacore locations can be found in fig. 12.1.1

Core processing methods.

Once removed from ROV ISIS or the megacorer the cores were taken to the temperature controlled (CT) laboratory (set at 4˚C) for processing. The same procedure was used for both pushcores and megacores. The overlying water was removed from the core tubes using a plastic tube and a top-down picture was taken. The core tubes were then transferred onto a core extruder, carefully removing the rubber bung to avoid sediment loss. The sediment was manually pushed up on the extruder and the core sliced using a measured mark coring ring, stainless steel plates and wearing nitrile gloves to avoid contamination. The plates and coring ring were washed with freshwater and rinsed with Milli-Q ultrapure water between slices. Slicing intervals were; 5 mm from 0 to 20 mm, 10 mm from 20 to 50 mm and 20 mm slices, thereafter. Surface nodules, if present were removed prior to slicing and subsurface nodules removed and recorded when encountered. Of the triplicate cores, one was sliced to the full depth of the core, and the remaining two cores to 50 mm. Core slices ≥ 10 mm were placed into foil-lined (combusted at 400 ˚C for 4 h) petri dishes, whereas the 20 mm slices were wrapped in combusted foil and then placed in bags. The processed cores were labelled with location, station number, megacore or pushcore number, tube number or ID and depth interval and then stored at - 20˚C. All analysis will be conducted at the home laboratory at the University of Liverpool. Details of station, latitude, longitude, location, cores taken and length, and analysis to be undertaken are provided in table 12.1.1 for OMCO pushcores and 12.1.2 for OMCO megacores and 12.1.3 for UK1 cores.

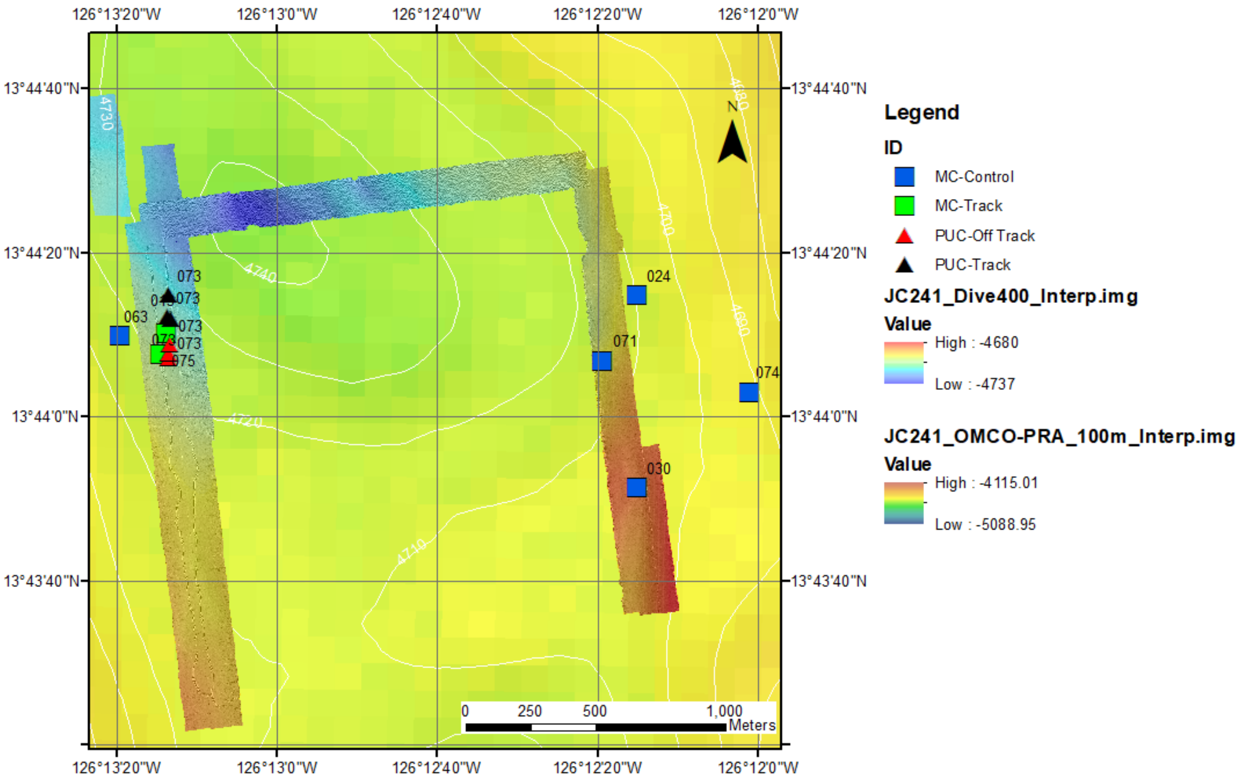


Fig.12.1.1 Locations of megacore deployments at OMCO control (blue square) and OMCO track (green square) together with ROV ISIS pushcore recovery at OMCO track (black triangle) and off track (red triangle). Triplicate cores recovered at each location, except for stn 57 and 71 where two cores were recovered. Map produced by Catherine Wardell, NOC.

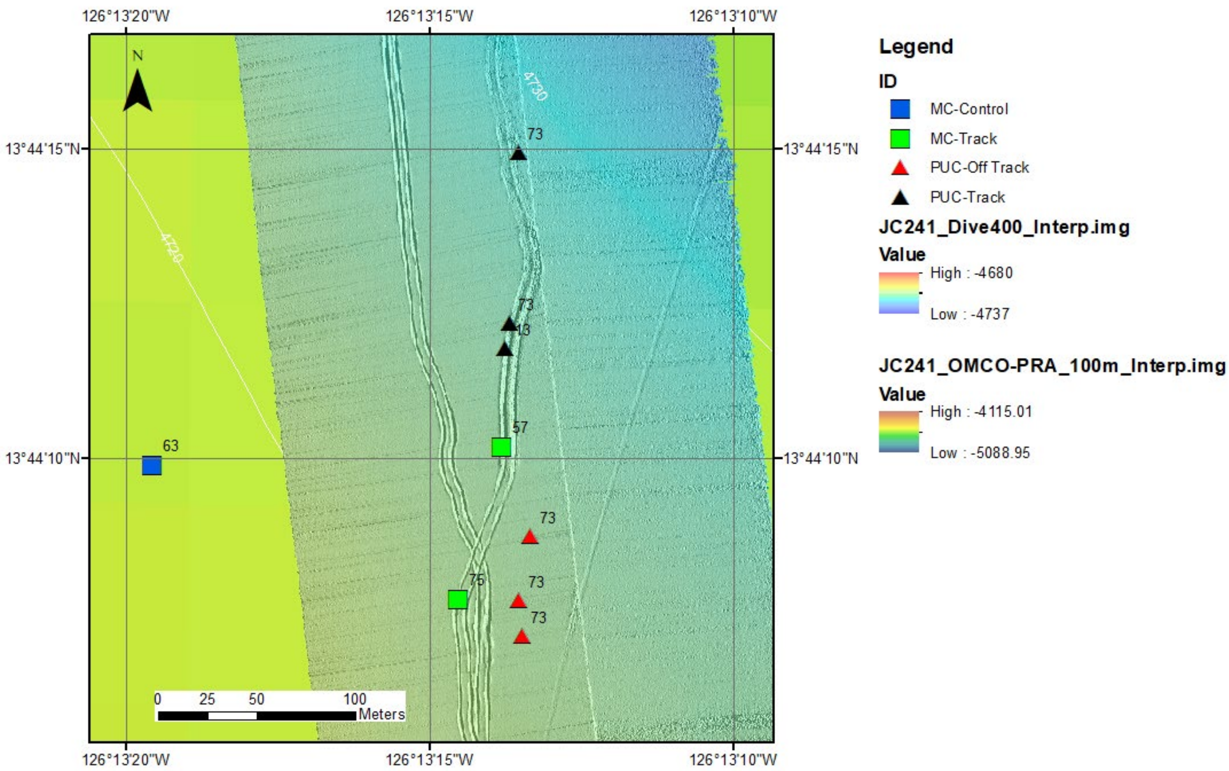


Fig.12.1.2 Zoomed in view of OMCO track locations of megacore deployments (green square) and ROV ISIS pushcore recovery (black triangle = track, red triangle = off track). Triplicate cores recovered at each location, except for stn 57 where two cores were recovered. Map produced by Catherine Wardell, NOC.

Table 12.1.1 Details of pushcores taken by ROV ISIS at OMCO including station, latitude, longitude, location, water depth, cores taken and length, and analysis to be undertaken.

Station number	Latitude N	Longitude W	Location & water depth	Liverpool core no. (tube ID.)	Cores taken	Analysis at Liverpool
JC241_013 ROV 399	13˚44.198	-126˚13.2204	OMCO – In track 4712 m	PC1 (S3) PC2 (G2) PC3 (G3)	Core length: 220 mm; sliced to 200mm Core length: 170 mm; sliced to 50 mm Core length: 190 mm; sliced to 50 mm	Quantification of C and N. Bulk stable isotopes of C and N and d15N & d13C amino acids. Lipid biochemistry. Biopolymeric carbon (if material available).
JC241_073 ROV 407	13˚44.249	-126˚13.2255	OMCO – In track 4713 m	PC9 (B1) PC7 (R3) PC8 (R2)	Core length: 210 mm; sliced to 200mm Core length: 150 mm; sliced to 50 mm Core length: 175 mm; sliced to 50 mm	Quantification of C and N. Bulk stable isotopes of C and N, and d15N & d13C amino acids. Lipid biochemistry. Biopolymeric carbon (if material available).
JC241_073 ROV 407	13˚44.203	-126˚13.2280	OMCO – In track 4713 m	PC12 (S3) PC10 (G3) PC11 (S2)	Core length: 120 mm; sliced to 100mm Core length: 110 mm; sliced to 50 mm Core length: 120 mm; sliced to 50 mm	Quantification of C and N. Bulk stable isotopes of C and N and d15N & d13C amino acids. Lipid biochemistry. Biopolymeric carbon (if material available).
JC241_073 ROV 407	13˚44.1193	-126˚13.2245	OMCO – Off track 4709 m	PC5 (S1) PC6 (Y2) PC4 (Y3)	Core length: 215 mm; sliced to 200mm Core length: 190 mm; sliced to 50 mm Failed recovery	Quantification of C and N. Bulk stable isotopes of C and N, and d15N & d13C amino acids. Lipid biochemistry (if material available).
JC241_073 ROV 407	13˚44.1286	-126˚13.2253	OMCO – Off track 4723 m	PC15 (YY1) PC13 (YY3) PC14 (YY2)	Core length: 205 mm; sliced to 180mm* Core length: 180 mm; sliced to 50 mm Core length: 110 mm; sliced to 50 mm	Quantification of C and N. Bulk stable isotopes of C and N, and d15N & d13C amino acids. Lipid biochemistry. Biopolymeric carbon (if material available).
JC241_073 ROV 407	13˚44.1459	-126˚13.2222	OMCO – Off track 4711 m	PC16 (YYR3) PC17 (YYR2) PC18 (YY2)	Core length: 205 mm; sliced to 200mm Core length: 155 mm; sliced to 50 mm Core length: 150 mm; sliced to 50 mm	Quantification of C and N. Bulk stable isotopes of C and N and d15N & d13C amino acids. Lipid biochemistry. Biopolymeric carbon (if material available).

Table 12.1.2 Details of megacores taken at OMCO including station, latitude, longitude, location, water depth, cores taken and length, and analysis to be undertaken.

Station	Latitude N	Longitude W	Location & water depth	Liverpool core no. (tube no.)	Cores taken	Analysis at Liverpool
JC241_019	13˚43.9490	-126˚12.2500	OMCO – Control 4682 m		None – Failed deployment	
JC241_024	13˚44.2480	-126˚12.2880	OMCO – Control 4697 m	MC1 (2)	Core length: 350 mm; sliced full depth	Quantification of C and N, *bulk stable isotopes of C and N, and **d15N & d13C amino acids.
				MC2 (3)	Core length: 370 mm; sliced to 50 mm	Lipid biochemistry.
				MC3 (8)	Core length: 360 mm; sliced to 50 mm	Biopolymeric carbon
JC241_030	13˚43.8560	-126˚12.2470	OMCO – Control 4694 m	MC4 (8)	Core length: 440 mm; sliced full depth	Quantification of C and N, *bulk stable isotopes of C and N, and ** d15N & d13C amino acids.
				MC5 (3)	Core length: 400 mm; sliced to 50 mm	Lipid biochemistry.
				MC6 (1)	Core length: 420 mm; sliced to 50 mm	Biopolymeric carbon
JC241_057	13˚44.1700	-126˚13.2300	OMCO - Track area 4711 m	MC7 (3) MC8 (4)	Core length: 390 mm; sliced fill depth Core length: 380 mm; sliced to 50 mm	Quantification of C and N, *bulk stable isotopes of C and N, and ** d15N & d13C amino acids. Lipid analysis; Biopolymeric carbon (if material available)
JC241_063	13˚44.1650	-126˚13.3230	OMCO - Control 4715 m	MC9 (4)	Core length: 370 mm; sliced full depth	Quantification of C and N, *bulk stable isotopes of C and N, and ** d15N & d13C amino acids.
				MC10 (2)	Core length: 410 mm; sliced to 50 mm	Lipid biochemistry.
				MC11 (8)	Core length: 450 mm; sliced to 50 mm	Biopolymeric carbon
JC241_071	13˚44.1140	-126˚12.3230	OMCO – Control 4696 m	MC12 (1)	Core length: 360 mm; sliced to 340 mm	Quantification of C and N, *bulk stable isotopes of C and N, and ** d15N & d13C amino acids.
				MC13 (8)	Core length: 350 mm; sliced to 50 mm	Lipid biochemistry. Biopolymeric carbon (if material available).
JC241_074	13˚44.0509	-126˚12.0163	OMCO – Control 4670 m	MC14 (8)	Core length: 340 mm; sliced to 320 mm	Quantification of C and N, *bulk stable isotopes of C and N, and ** d15N & d13C amino acids.
					Core length: 330 mm; sliced to 50 mm	Lipid biochemistry.
				MC15 (1)	Core length: 370 mm; sliced to 50 mm	Biopolymeric carbon
				MC16 (5)		
JC241_075	13˚44.1200	-126˚13.2400	OMCO - Track area 4730 m	MC17 (6)	Core length: 380 mm; sliced full depth	Quantification of C and N, *bulk stable isotopes of C and N, and ** d15N & d13C amino acids.
				MC18 (1)	Core length: 390 mm; sliced to 50 mm	Lipid biochemistry.
				MC19 (4)	Core length: 400 mm; sliced to 50 mm	Biopolymeric carbon

Table 12.1.3 Details of megacores and pushcores taken at OMCO including station, latitude, longitude, location, water depth, cores taken and length, and analysis to be undertaken.

Station	Latitude N	Longitude W	Location & water depth	Liverpool core no. (tube ID.)	Cores taken	Analysis at Liverpool
Megacores						
JC241_101	13˚58.4000	-116˚32.4100	UK1 7118 m	MC20 (C1) MC21 (C5)	Core length: 420 mm; full core sliced Core length: 400 mm; sliced to 50 mm	Quantification of C and N, *bulk stable isotopes of C and N, and ** d15N & d13C amino acids. Lipid analysis; Biopolymeric carbon (if material available).
Pushcores						
JC241_095	13˚53.8388	-116˚29.6388	UK1	PC19 (Y1) PC20 (W1)	Core length: 190 mm; sliced to 180mm Core length: 170 mm; sliced to 50 mm	Quantification of C and N. Lipid biochemistry (if material available). Bulk stable isotopes of C and N, and d15N & d13C amino acids.

Water sampling from the CTD: Macro nutrients and $\delta^{15}N\text{-}NO_3$

Background and objectives.

Seawater samples were taken for the determination of macronutrients (nitrate+nitrite, silicate, phosphate) and δ15N-NO3 to provide additional and complimentary data to the sediment cores detailed above. Nitrate measurements are required in the analysis of δ15N-NO3 which is a useful water mass tracer and baseline provider. In addition, the samples were taken at the same depths as those taken for eDNA (Susan Evans, NOC, section 19, Microbial and eDNA) and the data will be available should these be useful to that project.

Sampling strategy and sample processing.

Seawater was collected at the OMCO track site, OMCO control and UK1. Five sampling depths were targeted, which were; as close to the bottom as possible (generally 5 m), 10 m above this, depth of oxygen minimum, chlorophyll maximum and 5 m below the surface. The oxygen minimum and chlorophyll maximum were located on the downcast using the fluorescence and oxygen sensors fitted to the CTD. At each depth four Niskin bottles were fired, one for nutrients and δ15N-NO3 and three for eDNA. Upon recovery of the CTD, nutrient and δ15N-NO3 samples were collected directly from the Niskin bottles using acid-clean tubing attached to a PALL Acropak 500 capsule filter. Starting with the deepest sample, water was allowed to flow through the Acropak to fill and rinse the capsule, after which 2 x 125 mL acid cleaned HDPE bottles were rinsed three times with the sample and the filled to just below the shoulder. This process was repeated for all samples. Bottles were labelled with station number, Niskin number and sample type and then frozen at - 20˚C for storage. Analysis for nutrients will be conducted at the home laboratory at the University of Liverpool. δ15N-NO3 will be conducted by colleagues at SAMS should there be available funds to do so. Details of station number, latitude, longitude, location, samples taken, depths and analyses to be conducted are provided in table 12.4

References

Pusceddu et al. (2014) Proc Nat. Acad Sci USA 111:8861-8866

Table 12.2.1 Details of seawater samples taken from CTD casts at OMCO track, OMCO control and UK1 including station number, latitude, longitude, location, water depth, samples taken and analysis to be conducted.

Station	Latitude N	Longitude W	Location & water depth (m)	Samples taken	Analysis at Liverpool
JC241_009	13˚43.8760	-126˚13.3600	OMCO – Track area 4717 m	2 x 125 mL at depths; 7 m, 68 m, 698.5 m, 4683 m, 4694 m.	Nutrients: Nitrate+nitrite, nitrite, silicate, phosphate. ($d^{15}N-NO_3$ – TBC)
JC241_031	13˚44.2510	-126˚12.2990	OMCO – Control 4688 m	2 x 125 mL at depths; 7 m, 70 m, 653 m, 4693 m, 4704 m.	Nutrients: Nitrate+nitrite, nitrite, silicate, phosphate. ($d^{15}N-NO_3$ – TBC)
JC241_096	13˚54.8388	-116˚29.6388	UK1 4223 m	2 x 125 mL at depths; 6.5 m, 56 m, 602 m, 4201 m, 4211 m.	Nutrients: Nitrate+nitrite, nitrite, silicate, phosphate. ($d^{15}N-NO_3$ – TBC)

13. Ecosystem Functioning

Benthic cube studies

Andrew Sweetman, Scottish Association for Marine Sciences

To measure benthic ecosystem function, specifically the rate of C-processing by the benthos in impacted and non-impacted locations, stable-isotope pulse chase experiments were undertaken during ROV dive JC241_69 in the OMCO tracks and to the east of the OMCO tracks (Table 1). Four benthic incubation CUBES were deployed on the 4th of March 2023. Two CUBES were deployed in the OMCO tracks approximately 15-20 m from one another (termed "on track"), while two CUBES were deployed to the east approximately 20m away from the OMCO tracks (termed "off-track) (Table 1). These two off-track CUBES were separated by approximately 15-20m.

Table 13.1: Description of depths and position of CUBE deployments during JC241.

Sample station	AKS #	Cube #	Position	Depth (m)	Latitude	Longitude	Time deployed (UTC)	Time sampled (UTC)
JC241_69	343	1	On track	4726	13° 44.17678N	126° 13.22639W	13:17	10:05
JC241_69	343	2	Off track	4726	13° 44.18736N	126° 13.21625W	14:15	11:10
JC241_69	343	3	Off track	4727	13° 44.18188N	126° 13.21997W	14:37	10:41
JC241_69	343	4	On track	4727	13° 44.19466N	126° 13.22721W	13:45	09:35

The CUBES contained 6 syringes previously filled with filtered seawater and a total of 0.2g of isotope-labelled *Phaeodactylum tricornutum* (grown in media with 25% ^{13}C and ^{15}N). Immediately after the CUBES were placed and pushed into the sediment, a safety pin was removed and the syringe depressed by the ROV manipulator.

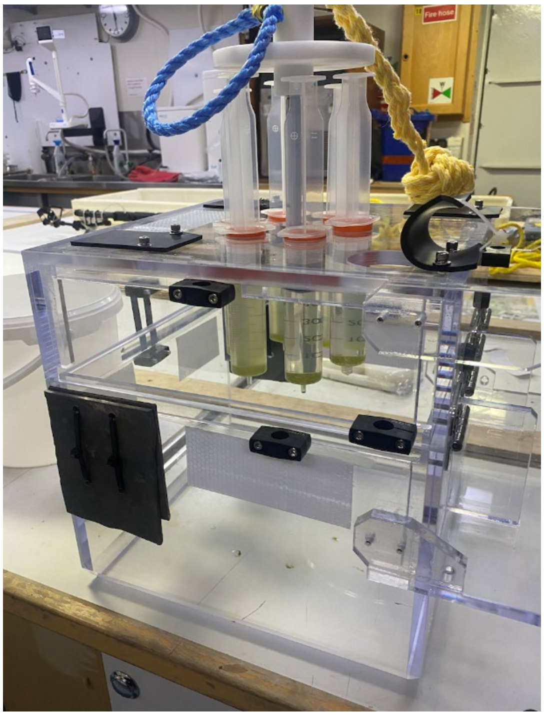


Figure 13.1: A benthic incubation CUBE with the 6 syringes filled with hydrated stable isotope labelled P. tricornutum (green colour at the bottom of the syringes).

The CUBES were sampled on the morning of the 7th of March 2023 (Fig. 2, Table 1). To sample the CUBES, the ROV manipulator was used to carefully lift the CUBE out of the sediment and place back on the ROV. Three push-cores were then collected from each CUBE inside the imprint of the cube and placed in the quivers.

Figure 13.2 Approaching one of the CUBES that was deployed in the off-track site.

Following the ROV dive, the push cores were placed in a bucket with ice and transferred to the cold room. Here, each push core was extruded at 0-2, 2-5 and 5-10cm depth for the following samples (Table 2): microbial genomics (5-10ml sediment sample^{-1}), microbial meta-transcriptomics (1-2ml sediment sample^{-1}), bacterial fatty acids (20-45ml sediment sample^{-1}), meiofauna (entire core slice) and macrofauna (entire core slice). The microbial genomic and fatty acid samples were then placed in the freezer at -80ºC, while the transcriptomic sample was flash frozen in liquid nitrogen and placed at -80ºC. The fauna samples were preserved in 4% buffered formaldehyde for later processing.

Table 13.2 List of samples collected from each CUBE.

AKS #	Cube #	Microbial genomics samples	Microbial transciptomics samples	Microbial fatty acids	Meiofauna	Macrofauna
343	1	0-2cm, 2-5cm 5-10cm	0-2cm, 2-5cm 5-10cm	0-2cm, 2-5cm 5-10cm	0-2cm, 2-5cm 5-10cm	0-2cm, 2-5cm 5-10cm
343	2	0-2cm, 2-5cm 5-10cm	0-2cm, 2-5cm 5-10cm	0-2cm, 2-5cm 5-10cm	0-2cm, 2-5cm 5-10cm	0-2cm, 2-5cm 5-10cm
343	3	0-2cm, 2-5cm 5-10cm	0-2cm, 2-5cm 5-10cm	0-2cm, 2-5cm 5-10cm	0-2cm, 2-5cm 5-10cm	0-2cm, 2-5cm 5-10cm
343	4	0-2cm, 2-5cm 5-10cm	0-2cm, 2-5cm 5-10cm	0-2cm, 2-5cm 5-10cm	Core lost	0-2cm, 2-5cm 5-10cm

14. Megafaunal Imaging

Loïc Van Audenhaege, Bethany Fleming, Erik Simon Lledó and Daniel Jones

Exploration of the OMCO tracks

Dive narrative

Prior to this cruise, the OMCO track position was estimated by the Lockheed Martin team based on data from 1979. SMARTEX were provided with a hand-drawn contour map that depicted the location of the mining test, which was digitised (Figure 14.1). The track was oriented north south and formed a loop, connected at the northern side. As the accuracy of the OMCO tracks location was uncertain, the dive D399 of the ROV *Isis* was planned to locate their actual positions as well as the connection between the two tracks, which was then determined as the starting point for multibeam and imaging ROV surveys. In addition, bioboxes, 18 pushcores, Niskin bottles and slurp chambers were prepared for sampling (see relevant sections for further details on those sample collections).

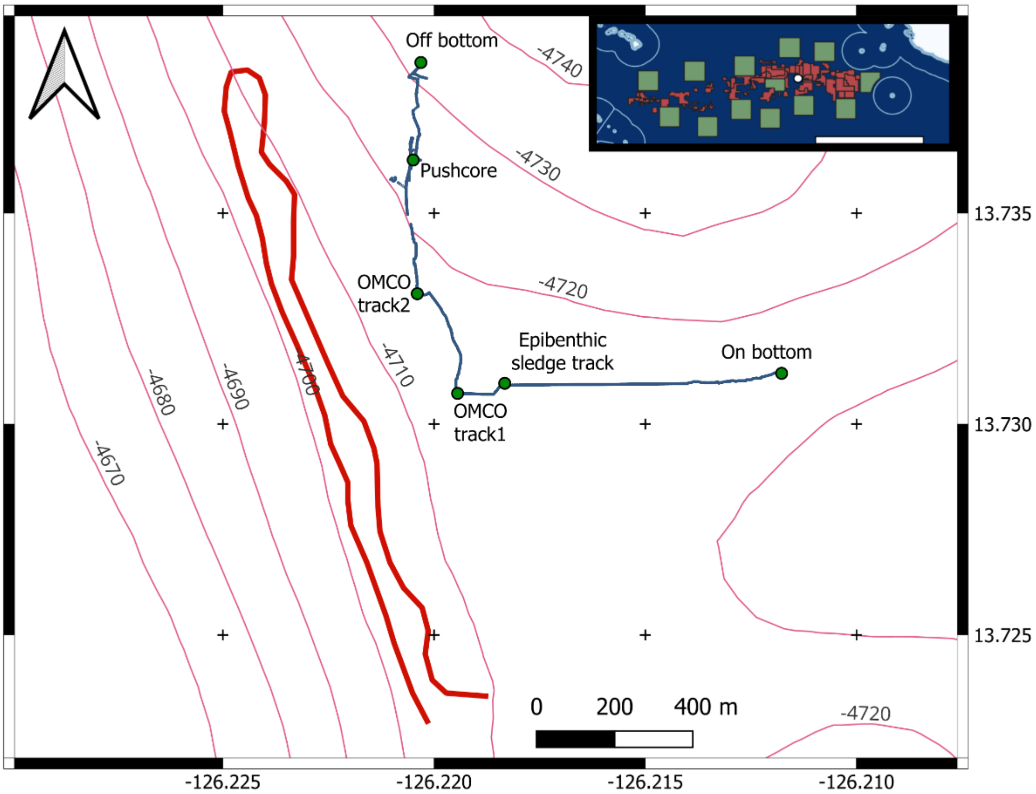


Figure 14.1 – Bathymetric contour map (pink line) of estimated location of the OMCO tracks (red line). Location of waypoints (green circles) is plotted over the DVL navigation of D399 (blue line). The upper right inset positions the OMCO tracks (white circle) over a map of the Clarion-Clipperton Zone delimited with APEIs (green), contractor areas (red) and Exclusive Economic Zone (EEZ). Coordinate reference system: WGS84.

Isis started her descent at 20:39 15/02/2023. She arrived at the bottom at 00:13 the 16/02/2023 (13˚43.87222'N, -126˚12.70972'W, -4699 m), ~1000 m eastern from the expected OMCO track locations (Figure 14.1). *Isis* was then flown with a heading of ~270˚ (alt. 1.6 m, speed 0.15 m/s), perpendicular to the track orientation, in order to cross them (Figure 14.1). All cameras were recording (Pilot, HSCI [Science] and SCORPIO). Despite crossing of an epibenthic sledge track (13˚43.8561'N, -126˚13.1016'W; Figure 14.1A), the wider and deeper OMCO tracks were in sight at 02:32, ~240 metres away from their estimated position (Figure 14.1).

Observations

Tracks consisted of two parallel 'troughs' created by the Archimedes screws of the nodule collector. The area between those tracks was the location where nodule recovery took place. As we expected tracks to elongate north to south, we followed it northward (heading of 332˚). Decision was made at 03:33 to go further west in order to locate the second track, which become in sight 5 min later. We kept going up north until the surveyed track displayed no nodule, possibly indicative of effective retrieval of nodules of the OMCO collector. As a result of the absence of nodules in tracks, push cores were performed at 05:55 in the section between the tracks. Push coring was performed in and out the tracks. We kept going north, until 10:12 (13˚44.303'N, -126˚13.246'W, -4700 m) when *Isis* started the ascent because of oil problem. *Isis* was recovered on deck at 16:36.

Photogrammetry reconstruction

In order to characterise the topography of the tracks with high resolution, photogrammetry reconstructions were performed using the SCORPIO footage to better apprehend the morphology across the tracks (Table 14.1). The SCORPIO camera was front mounted on the ROV (angle), and the zoom level was maintained at the widest angle.

Table 14.1 – Optical details of the Super-SCORPIO camera mounted on the ROV Isis.

Model	HDR-CX560V	
Focus range	203 mm (8 inches) to Infinity	
Aperture	F1.8-3.4	
Lens Focal Length		
Video	3.8 mm	38 mm
Photo	26.3 mm	263 mm
Angle of view in water	Wide angle, 3.8 mm/26.3 mm	Telephoto, 38 mm/263 mm
Diagonal	115˚	11˚
Horizontal	103˚	9˚
Vertical	87˚	7˚
Output		
Photo	12.3 MP (4672 x 2628; 16:9); .jpg; Exif Ver. 2.3 compatible	
Video	Approx. 6140K pixels (16:9); MPEG4-AVC/H.264 AVCHD format compatible (1080/60p:original format); STD: MPEG2-PS	

To carry out photogrammetry reconstruction, we considered using only the two videos of transit before push core activities occurred (i.e. for transect 1, for transect 2). Images were extracted every three seconds from video sequences to ensure at least 70% of overlap between images (MATISSE software, Ifremer). Those .jpg images were cropped to remove objects that could undermine photogrammetric outputs: i) the darkness of the image upper horizontal edge and ii) the ROV basket and push cores at the lower horizontal edge, using the Irfan view batch processing. After underwater colour correction, photogrammetry reconstruction was carried out using the open source Meshroom software, using a combination of sift and Akaze feature-matching algorithms). Since this dive was not planned for photogrammetry purpose, lack of overlap resulted in three portions of tracks instead of one single 3D model (Figure 14.1, Table 14.2). The dark edges of the models were removed manually in the open-source software MeshLab.

Table 14.2 – Dimensions of the 3D photogrammetry models reconstructed from video sequences acquired when exploring the OMCO tracks during D399.

	Length [m]	Width [m]	Number of images
Track 1 – portion 1	~ 100	~ 6 to 11 (depending on ROV altitude)	642
Track 1 – portion 2	~ 180		1187
Track 2	~ 250		1298

Those tracks were first scaled and repositioned manually using the software CloudCompare, based on the DVL navigation of the ROV. Note that bathymetry acquisition during dive D402 allowed a more accurate repositioning of 3D models, since holding a more accurate georeferencing than the drifting DVL navigation. Those 3D models will be useful for further measurements of the morphology of the mining tracks.

Table 14.3 – Details of SCORPIO videos acquired by Isis during D399

Dive	Name of videos	Start (UTC)	Size on disk [GB]
D399	JC241_SCO_DIVE399_1	15/02/2023 20:16	92
	JC241_SCO_DIVE399_2	22:19	78
	JC241_SCO_DIVE399_3	23:47	107
	JC241_SCO_DIVE399_4	16/02/2023 01:49	93
	JC241_SCO_DIVE399_5	03:34	79
	JC241_SCO_DIVE399_6	05:44	98
	JC241_SCO_DIVE399_7	07:36	115
	JC241_SCO_DIVE399_8	09:53	109
	JC241_SCO_DIVE399_9	11:52	108
	JC241_SCO_DIVE399_10	13:36	101
	JC241_SCO_DIVE399_11	15:53	36
		End at 16:34	Total = 968

*Table 14.4 – Details of HDSCI videos acquired by Isis during D399. *The camera repeatedly stopped recording and split the output from the 5th to the 13th video.*

Dive	Name of videos	Start (UTC)	Size on disk [GB]
D399	JC241_HDSCI_DIVE399_1	15/02/2023 20:16	107
	JC241_HDSCI_DIVE399_2	22:19	79
	JC241_HDSCI_DIVE399_3	23:47	110
	JC241_HDSCI_DIVE399_4	16/02/2023 01:49	92

	Ends at 03:33	
JC241_HDSCI_DIVE399_5	04:13	4
JC241_HDSCI_DIVE399_6	*	> 1
JC241_HDSCI_DIVE399_7	*	3
JC241_HDSCI_DIVE399_8	*	> 1
JC241_HDSCI_DIVE399_9	*	2
JC241_HDSCI_DIVE399_10	*	6
JC241_HDSCI_DIVE399_11	*	5
JC241_HDSCI_DIVE399_12	*	2
JC241_HDSCI_DIVE399_13	*	> 1
JC241_HDSCI_DIVE399_14	05:44	100
JC241_HDSCI_DIVE399_15	07:36	117
JC241_HDSCI_DIVE399_16	09:53	109
JC241_HDSCI_DIVE399_17	11:52	114
JC241_HDSCI_DIVE399_18	13:36	110
JC241_HDSCI_DIVE399_19	15:53	37
	End at 16:34	Total = 949

Imaging survey of the effect of mining disturbance on megafaunal benthic communities

Material and set-up

The survey was planned to test if significant differences occur in the composition of megafaunal benthic community (i.e., organisms > cm) after 44 years, since the disturbance induced by the OMCO nodule collection. To do so, we essentially used the SCORPIO and AESA camera. They were set up in a downward-looking configuration (Figure 14.4; Supplementary Figure S14.1). Pre-dive tests were performed on-deck to check the AESA camera (Tables 14.5 & 14.6) was working and evaluate image imprint and design the survey based on track morphology of observed in D399. Two lasers were downward mounted, positioned 10 cm apart on the SCORPIO camera (Figure 14.4B; Table 14.7). The flash of the AESA camera and the light of the SCORPIO camera were positioned at the back of *Isis* near the pressure gauges 238 cm apart, pointing obliquely towards the seabed (~45˚, Figure 14.4C). In addition, *Isis* was equipped with 4 CATHX Aphos-16 lights and 2 Multi-SeaLite Matrix lights oriented frontward as well as 2 downward SeaLite Lumos, being the 'standard' light setup for any *Isis* dive.

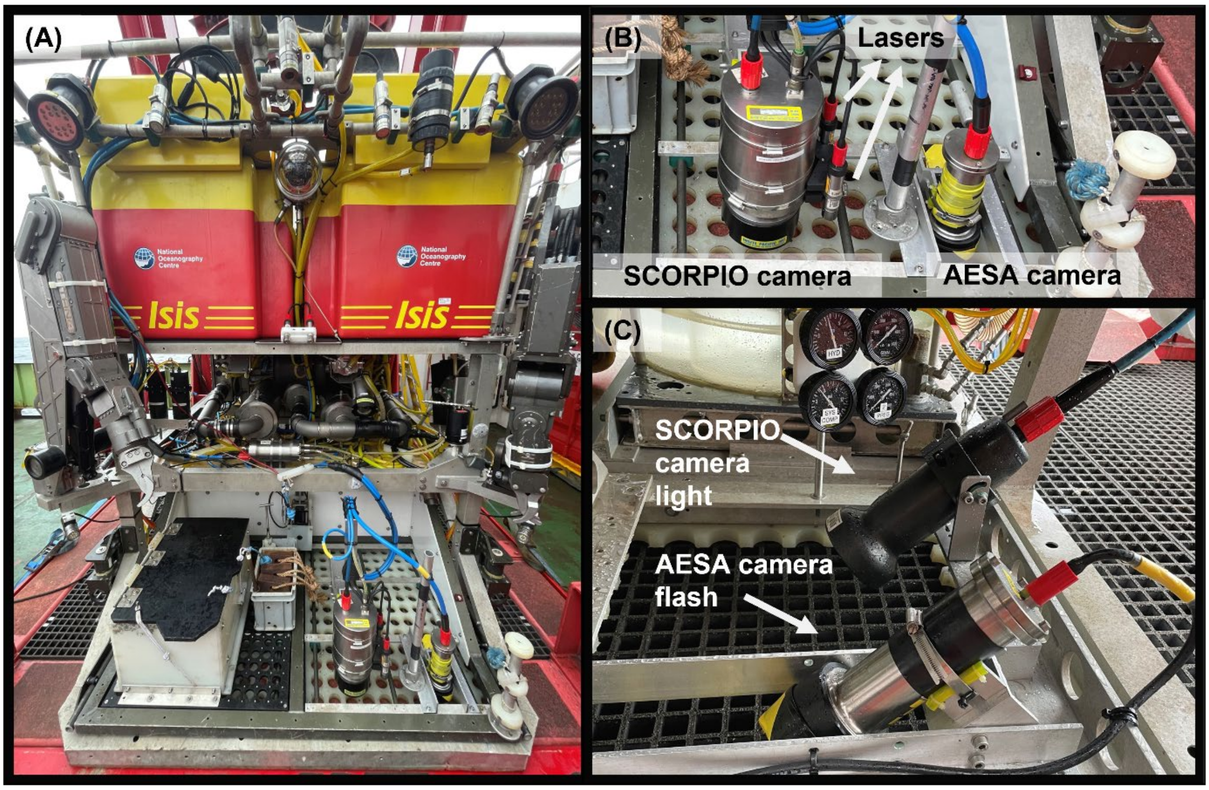


Figure 14.4 – (A) Isis configuration for D403, 404, 405 and 409. (B) Positioning of the two lasers and the SCORPIO and AESA cameras. (C) Position of the AESA camera flash and SCORPIO camera light. See Supplementary Figure S14.1 for offset measurement.

Table 14.5 – Optical details of the AESA camera

Serial number	11370385
Camera model	Grasshopper2 GS2-GE-50S5C
Sensor	Sony ICX625AQ (2/3" 2448x2048 CC)
Resolution	2448x2048
Image pixel format	RAW8

Table 14.6 – Optical details of the AESA lens

Lens model	Fujifilm HF12.5SA-1
Focus range	100 mm to Infinity
Aperture	F1.8-3.4
Lens Focal Length	12.5 mm
Angle of view	2/3''
Horizontal	38˚
Vertical	29˚
Focal range	2.28m

Table 14.7 – Optical details of the Super-SCORPIO camera mounted on the ROV Isis.

Model	HDR-CX560V	
Focus range	203 mm (8 inches) to Infinity	
Aperture	F1.8-3.4	
Lens Focal Length		
Video	3.8 mm	38 mm
Photo	26.3 mm	263 mm
Angle of view in water	Wide angle, 3.8 mm/26.3 mm	Telephoto, 38 mm/263 mm
Diagonal	115˚	11˚
Horizontal	103˚	9˚
Vertical	87˚	7˚
Output		
Photo	12.3 MP (4672 x 2628; 16:9); .jpg; Exif Ver. 2.3 compatible	
Video	Approx. 6140K pixels (16:9); MPEG4-AVC/H.264 AVCHD format compatible (1080/60p:original format); STD: MPEG2-PS	

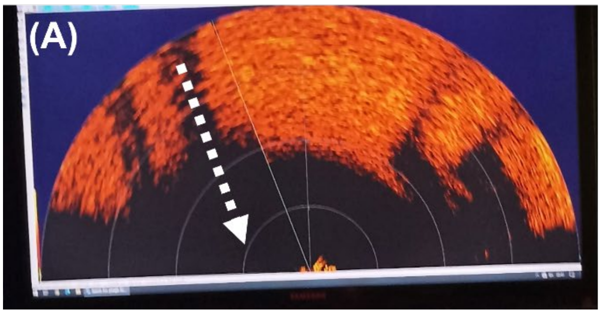
At the seabed, the AESA and SCORPIO camera were set to acquire one image every 3 and 10 seconds respectively. The AESA camera was calibrated at the seabed and the flash was triggered with a lag of 0.014ms (Supplementary Figures S14.2 & S14.3).

Survey design

We performed a total of 12 imaging transects to investigate two types of disturbance and a control community over 4 dives:

i. Physical disturbance of mining tracks was evaluated using a total of four transects collected inside the tracks (hereafter 'within tracks'). Two transects were conducted in each 'leg' of the tracks, one following the left furrow and the other following the right to ensure full coverage of the tracks. The HDSCI camera (pan-and-tilt Kongsberg Eyeball camera of *Isis*) was oriented to help maintain the ROV position within the tracks.
ii. Proximal plume disturbance was assessed outside of tracks (hereafter 'outside tracks'), with two transects performed on each side of the tracks by maintaining *Isis* 5 m and 10 m away from the tracks by the help of the ROV sonar (Figure 14.5).
iii. No disturbance (hereafter 'control') with four parallel tracks 2 km long, oriented 6˚W and equispaced 50 m apart from each other (Figure 14.6)

For each transect, *Isis* was flown at a target altitude of 3.0 m over the seabed (as measured by the altimeter. Offset from altimeter to cameras = – 0.51 m; Supplementary Figure S14.1), at a speed ranging from 0.05 to 0.20 m/s. The DVL was systematically reset at the start of each transect.



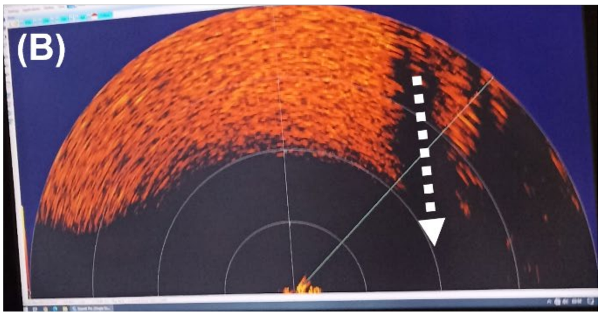


Figure 14.5 – Pictures of the sonar for transects during which Isis was flown (A) 5 m and (B) 10 metres away from the mining tracks. Each semicircle increments 5 metres of distance from the ROV.

Dive narratives

Initial survey design aimed for 3 transect replicates within tracks and in the control area at minimum, and 4 replicates within tracks and in the control area at best. Four dives were necessary to carry out the transects (Table 14.8).

D403 allowed us to set up the settings of the AESA camera, mounted on *Isis* for the first time, as well as establishing the field of view of the SCORPIO camera to complement AESA images. Three transect replicates within the track were carried out (A1-2-3 in Table 14.8). As the minimum number of replicates was collected (i.e., 3), we transited 1.5 km East to the control area (Figure 14.6; 28/02/2023, 16:08 to 18:53). However, only 1 km was covered (C1.1 in Table 14.9). The dive was cut short owing to a hydraulic oil shortage which forced *Isis*' ascent.

D404 aimed to finish imaging transects in the control area. The transect started where D403 stopped (Figure 14.6). As we completed the first control transect (C1.2 in Table 14.9), a lack of hydraulic oil forced the ascent of *Isis*.

D405 aimed to finish the three remaining imaging transects in the control area (Figure 14.6). The control transects were completed and no major issue was reported.

D409 planned to finish the 4^{th} within-track transect as well as conducting 2 pairs of replicates of outside-track transects for 'plume disturbance assessment' (Figure 14.6). This dive was extended for 2 hours. All transects were completed and no major issue was reported.

In general, we observed that the altitude of the vehicle was steady when flying southward (3.0 ± ~ 0.2 m, pers. obs.), while the altitude was bouncier when going northward (3.0 ± ~ 0.6 m). Furthermore, episodes of rapidly changing altitude occurred when the position of the ship was unsuitable, which resulted in the tether pulling the ROV and unsteady images (Figure 14.7). The USBL navigation remained noisy, jumping often more than 20 m between records, while the DVL navigation drifted more than 10 m away (pers. obs.).

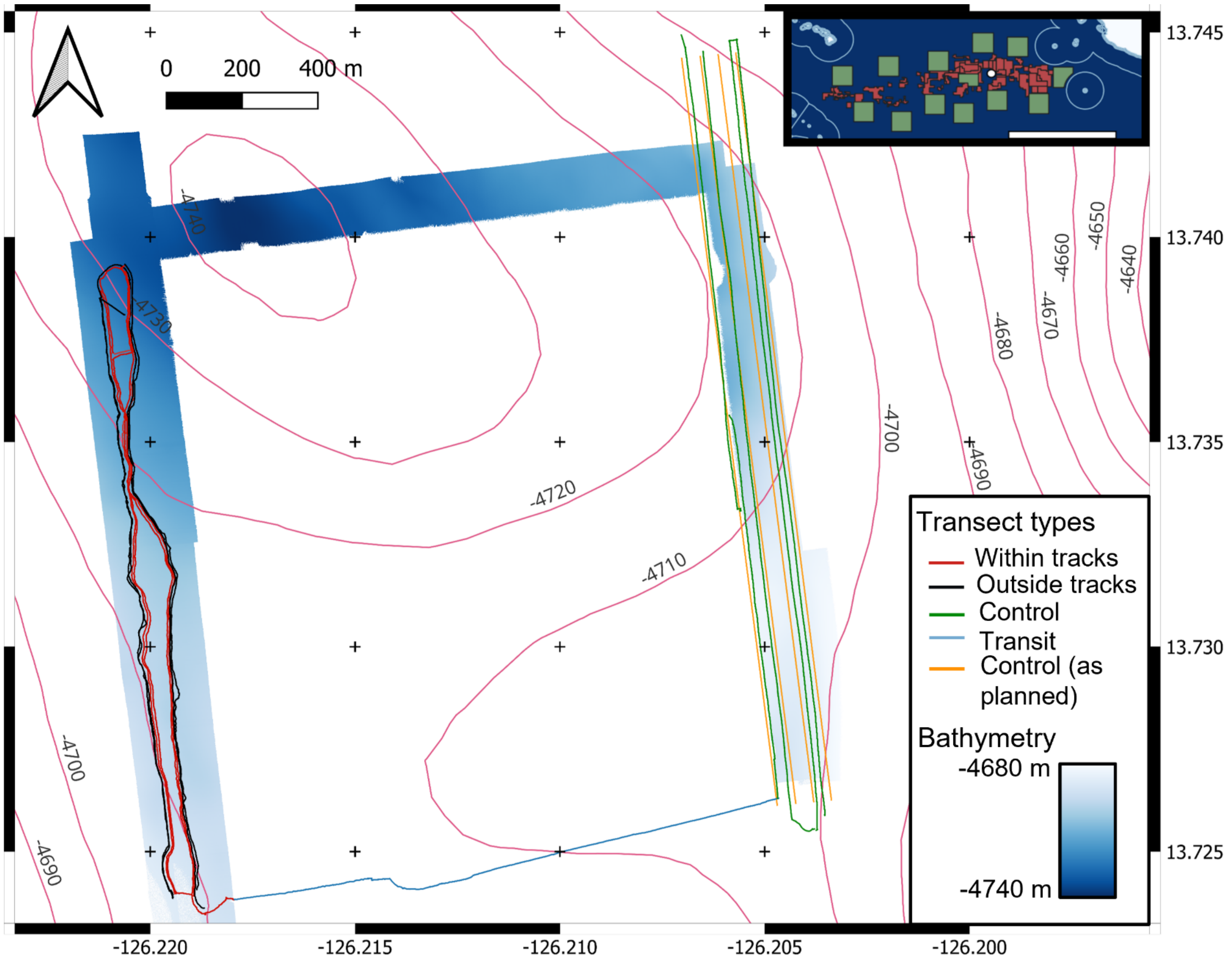


Figure 14.6 – Corrected DVL navigation of the ROV Isis during the 12 transects carried out during dives D403, D404, D405 and 409. Transects represented with lines, were performed for assessment of 'physical disturbance' inside the tracks (red), for assessment of 'plume disturbance' in proximity of the tracks (black) and for control (green). Transit (blue) and waypoints for the control (orange) are also delimited. The blue line shows the transit from the OMCO mining tracks to the control area during D403. Bathymetry was acquired during D402 and D410. The upper right inset positions the OMCO tracks (white circle) over a map of the Clarion-Clipperton Zone delimited with APEIs (green), contractor areas (red) and Exclusive Economic Zone (EEZ). Coordinate reference system: WGS84.

Table 14.8 – Imaging dives performed with Isis at JC241. Those dives were performed in combination with ROCSI sampling.

		On bottom				Off bottom			
Station	Dive	Date, time (UTC)	Lat.	Long.	Depth	Date, time (UTC)	Lat.	Long.	Depth
JC241-059	D403	27-02-2023, 17:05	13˚44.316'N	-126˚13.379'W	4702	28-02-2023, 21:42	13˚44.146'N	-126˚12.352'W	4690
JC241-060	D404	01-03-2023, 09:37	13˚44.002'N	-126˚12.335'W	4690	01-03-2023, 13:26	13˚44.705'N	-126˚12.426'W	4680
JC214-062	D405	02-03-2023, 03:21	13˚44.676'N	-126˚12.381'W	4674	02-03-2023, 20:37	13˚43.472'N	-126˚12.234'W	4671
JC241-082	D409	08-03-2023, 21:52	13˚44.285'N	-126˚13.240'W	4713	09-03-2023, 23:00	13˚43.437'N	-126˚13.127'W	4689

Table 14.9 – Details on survey transects of imaging dives of JC241 (Distance covered, direction of transect (S = southward, N = northward), time of DVL reset (NA = no data), Time and position of start and end of transect, dive and sequential numbers (1 being the first transect carried out, 12 being the last). Note that DVL positions were recorded only for D409, while all other coordinates were logged from the USBL navigation.

		Dist. [km]	Dir.	DVL reset	Start				End				Dive	Seq. #
					Date, time	Coordinates		Depth	Time	Coordinates		Depth		
Physical disturbance inside tracks														
	A1	1.72	S	00:07	28-02-23, 00:08	13˚44.2849'	-126˚13.2439'	4714	03:56	13˚43.3657'	-126˚13.1493'	4706	D403	1
	A2	1.88	N	04:11	28-02-23, 04:14	13˚43.4306'	-126˚13.1413'	4693	09:32	13˚44.4446'	-126˚13.1299'	4729	D403	2
	A3	1.96	S	09:49	28-02-23, 09:49	13˚44.343'	-126˚13.2360'	4719	16:06	13˚43.4121'	-126˚13.0996'	4694	D403	3
	A4	1.80	N	NA	09-03-23, 12:17	13˚43.4417'	-126˚13.1621'	4693	17:44	13˚44.3523'	-126˚13.2341'	4733	D409	11
Plume disturbance in proximity of the tracks, 5 or 10 m away														
5 m	B1	1.76	S	22:13	08-03-23, 22:50	13˚44.3059'	-126˚13.2737'	4713	02:06	13˚43.4349'	-126˚13.1668'	4706	D409	8
	B2	1.85	N	07:17, 07:46	09-03-23, 07:21	13˚44.3144'	-126˚13.2747'	4713	11:33	13˚43.4176'	-126˚13.1209'	4694	D409	10
10 m	B3	1.76	N	02:30	09-03-23, 02:40	13˚44.4477'	-126˚13.1756'	4705	07:15	13˚44.3093'	-126˚13.2725	4713	D409	9
	B4	1.76	S	18:00	09-03-23, 18:01	13˚44.3603'	-126˚13.2341'	4720	23:00	13˚43.4620'	-126˚13.1251'	4689	D409	12
Control area														
	C1.1	1.05	N	18:52	28-02-23, 18:53	13˚43.5810'	-126˚12.2801'	4691	21:46	13˚44.1235'	-126˚12.3413'	4703	D403	4
	C1.2	3.38	N	09:49	01-03-23, 10:08	13˚44.0043'	-126˚12.3463'	4696	13:15	13˚44.6830'	-126˚12.4196'	4682	D404	4
	C2	2.09	S	04:00	02-03-23, 04:03	13˚44.6697'	-126˚12.3893'	4681	09:38	13˚43.5447'	-126˚12.2633'	4690	D405	5
	C3	2.09	N	09:46	02-03-23, 09:55	13˚43.5717'	-126˚12.2185'	4690	15:49	13˚44.6875'	-126˚12.3495'	4679	D405	6
	C4	2.11	S	15:49	02-03-23, 16:26	13˚44.6875	-126˚12.3439'	4679	20:23	13˚43.5521'	-126˚12.2091'	4687	D405	7

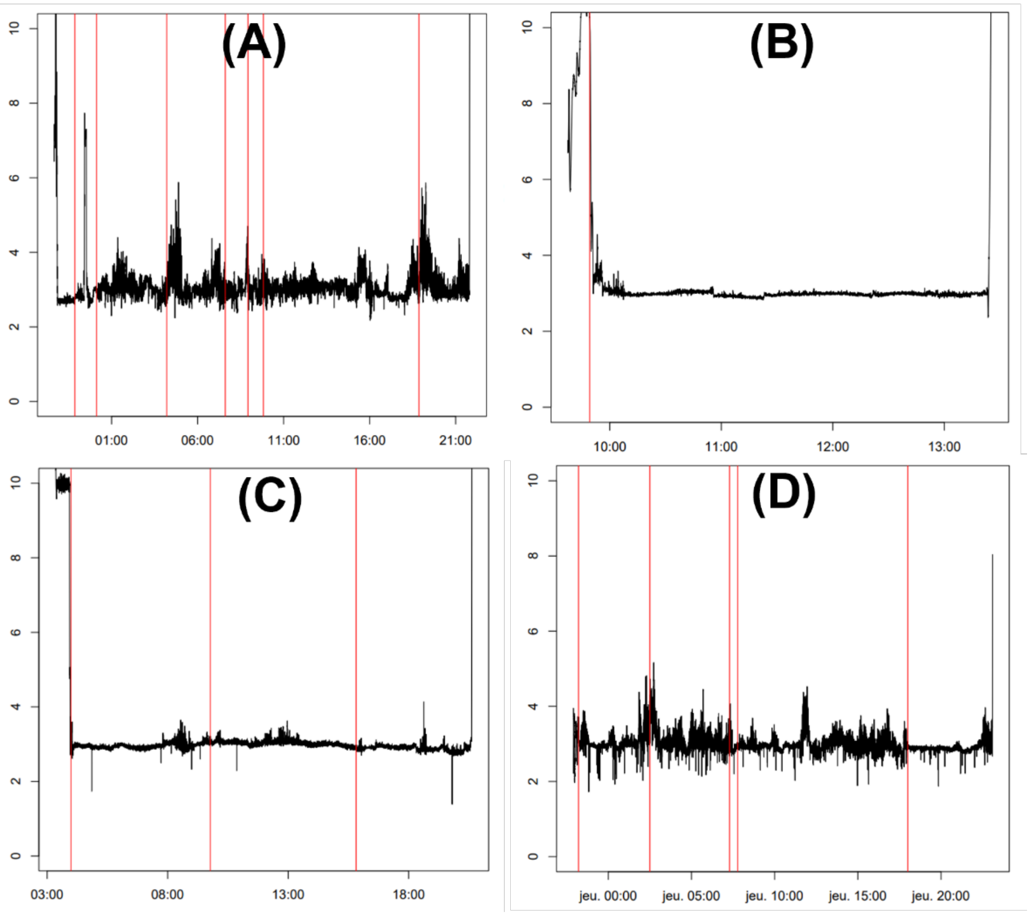


Figure 14.7 – Altitude [m] of the ROV altimeter over time, for dives (A) D403, (B) D404, (C) D405, (D) D409. Red vertical lines mark a Doppler reset.

Imaging dataset

Tables 14.10 & 14.11 provide a detailed lists of data acquired.

Table 14.10 – Details of the picture set acquired by the cameras AESA (8-bit .raw files, 2448x2048 pixels, 4,896 KB/image) and SCORPIO (.JPG files, 4672x2628 pixels, ~ 5,000 to 6,000 KB/image) at the OMCO mining tracks.

Dive	Mission duration	Count AESA pictures	Size [GB]	Count SCORPIO pictures	Size [GB]
D403	21:44:04	26,083	120	8,623	39.3
D404	04:55:39	5,844	27.2	1,233	5.75
D405	17:12:16	20,339	95.4	7,375	31.1
D409	24:00:45	30,232	141	9,374	43.7
Total	67:52:44	82,498	256.7	26,605	119.85

Table 14.11 – Details of video sequences acquired by the SCORPIO camera mounted on the ROV Isis during dives D403, 404, 405 and 409.

Dive	Video names	Start (UTC)	Size on disk [GB]
D403	JC241_SCO_DIVE403_12	27/02/2023 17:24	109
	JC241_SCO_DIVE403_13	19:25	117
	JC241_SCO_DIVE403_14	21:58	99
	JC241_SCO_DIVE403_15	23:43	96
	JC241_SCO_DIVE403_16	28/02/2023 01:33	106
	JC241_SCO_DIVE403_17	03:33	108
	JC241_SCO_DIVE403_18	05:34	105
	JC241_SCO_DIVE403_19	07:31	111
	JC241_SCO_DIVE403_20	09:35	104
	JC241_SCO_DIVE403_21	11:32	111
	JC241_SCO_DIVE403_22	13:36	109
	JC241_SCO_DIVE403_23	15:39	109
	JC241_SCO_DIVE403_24	17:40	111
	JC241_SCO_DIVE403_25	19:48	109
	JC241_SCO_DIVE403_26	21:50	42
		End at 23:43	Total = 1,400
D404	JC241_SCO_DIVE404_27	01/03/2023 04:54	121
	JC241_SCO_DIVE404_28	07:23	115
	JC241_SCO_DIVE404_29	09:08	111
	JC241_SCO_DIVE404_30	11:08	132
		End at 13:50	Total = 456
D405	JC241_SCO_DIVE405_31	01/03/2023 23:16	109
	JC241_SCO_DIVE405_32	02/03/2023 01:14	111
	JC241_SCO_DIVE405_33	03:12	103
	JC241_SCO_DIVE405_34	05:13	109
	JC241_SCO_DIVE405_35	07:14	111
	JC241_SCO_DIVE405_36	09:18	110
	JC241_SCO_DIVE405_37	11:20	109
	JC241_SCO_DIVE405_38	13:20	127
	JC241_SCO_DIVE405_39	15:44	110
	JC241_SCO_DIVE405_40	17:48	109

	JC241_SCO_DIVE405_41	19:49	118
	JC241_SCO_DIVE405_42	22:01	107
		End at 00:02	Total = 1,240
D409	JC241SCO_DIVE409_64	08/03/2023 21:54	108
	JC241_SCO_DIVE409_65	23:55	111
	JC241_SCO_DIVE409_66	09/03/2023 01:58	109
	JC241_SCO_DIVE409_67	04:01	110
	JC241_SCO_DIVE409_68	06:03	110
	JC241_SCO_DIVE409_69	08:06	112
	JC241_SCO_DIVE409_70	10:10	105
	JC241_SCO_DIVE409_71	12:07	118
	JC241_SCO_DIVE409_72	14:20	113
	JC241_SCO_DIVE409_73	16:26	110
	JC241_SCO_DIVE409_74	18:29	107
	JC241_SCO_DIVE409_75	20:27	106
	JC241_SCO_DIVE409_76	22:25	38
		End at 23:07	Total = 1,260

Table 14.12 – Details of video sequences acquired by the HDSCI camera mounted on the ROV Isis during dives D403, 404, 405 and 409.

Dive	Video names	Start (UTC)	Size on disk [GB]
D403	JC241_HDSCI_DIVE403_14	27/02/2023 17:24	107
	JC241_HDSCI_DIVE403_15	19:25	129
	JC241_HDSCI_DIVE403_16	21:58	96
	JC241_HDSCI_DIVE403_17	23:43	99
	JC241_HDSCI_DIVE403_18	28/02/2023 01:33	109
	JC241_HDSCI_DIVE403_19	03:33	110
	JC241_HDSCI_DIVE403_20	05:34	107
	JC241_HDSCI_DIVE403_21	07:31	113
	JC241_HDSCI_DIVE403_22	09:35	106
	JC241_HDSCI_DIVE403_23	11:32	113
	JC241_HDSCI_DIVE403_24	13:36	110
	JC241_HDSCI_DIVE403_25	15:39	108
	JC241_HDSCI_DIVE403_26	17:40	113
	JC241_HDSCI_DIVE403_27	19:48	110
	JC241_HDSCI_DIVE403_28	21:50	101
	JC241_HDSCI_DIVE403_29	23:43	99
		End at 01:34	Total = 1,390
D404	JC241_HDSCI_DIVE404_30	01/03/2023 04:54	114
	JC241_HDSCI_DIVE404_31	07:23	109
	JC241_HDSCI_DIVE404_32	09:08	110
	JC241_HDSCI_DIVE404_33	11:08	135
		End at 13:50	Total = 446
D405	JC241_HDSCI_DIVE405_34	01/03/2023 23:16	100
	JC241_HDSCI_DIVE405_35	02/03/2023 01:14	111
	JC241_HDSCI_DIVE405_36	03:12	108
	JC241_HDSCI_DIVE405_37	05:13	110
	JC241_HDSCI_DIVE405_38	07:14	109
	JC241_HDSCI_DIVE405_39	09:18	108
	JC241_HDSCI_DIVE405_40	11:20	110
	JC241_HDSCI_DIVE405_41	13:20	130

	JC241_HDSCI_DIVE405_42	15:44	112
	JC241_HDSCI_DIVE405_43	17:48	109
	JC241_HDSCI_DIVE405_44	19:49	114
	JC241_HDSCI_DIVE405_45	22:01	108
		End at 00:02	Total = 1,230
D409	JC241_HDSCI_DIVE409_66	08/03/2023 21:54	100
	JC241_HDSCI_DIVE409_67	23:55	102
	JC241_HDSCI_DIVE409_68	09/03/2023 01:58	101
	JC241_HDSCI_DIVE409_69	04:01	101
	JC241_HDSCI_DIVE409_70	06:03	102
	JC241_HDSCI_DIVE409_71	08:06	105
	JC241_HDSCI_DIVE409_72	10:10	97
	JC241_HDSCI_DIVE409_73	12:07	110
	JC241_HDSCI_DIVE409_74	14:20	106
	JC241_HDSCI_DIVE409_75	16:26	102
	JC241_HDSCI_DIVE409_76	18:29	96
	JC241_HDSCI_DIVE409_77	20:27	94
	JC241_HDSCI_DIVE409_78	22:25	35
		End at 23:07	Total = 1,250

Post processing

The Doppler Velocity Logger (DVL) navigation was corrected by removing the offset between the ultra-short baseline (USBL) and the original DVL navigations, based on a rolling average of 30 minutes (Van Audenhaege, Hendry, Broad and Huvenne [2021], Frontiers in Marine Science, 10.3389/fmars.2021.669372). No change was made to the pictures (Figures 14.8 & 14.9).

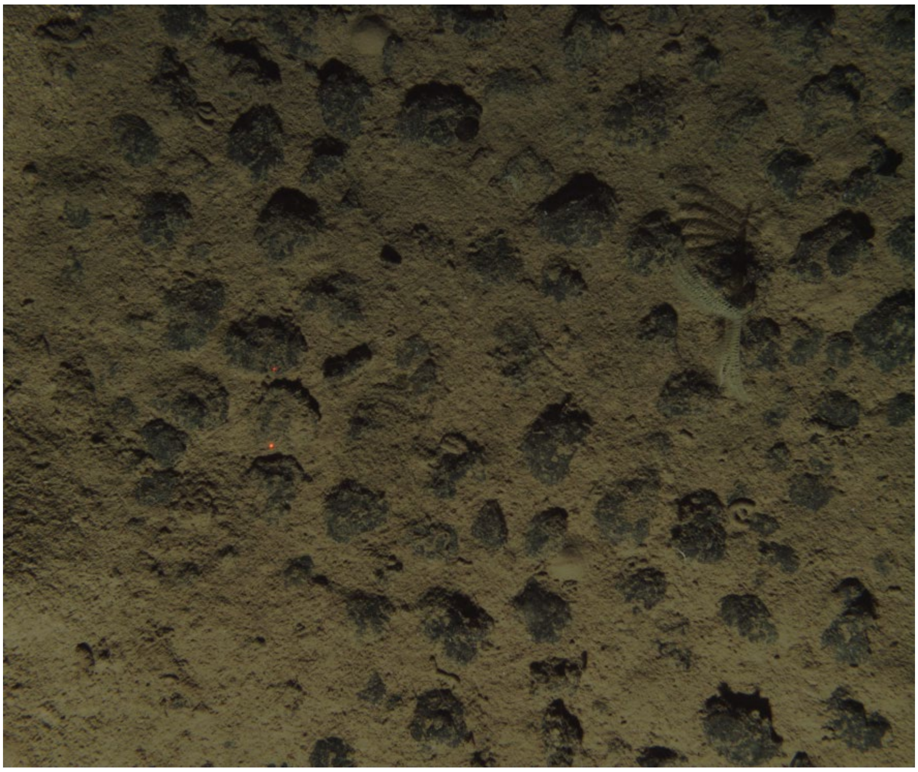


Figure 14.8 – Example of a picture collected with the AESA camera (Dive 409, 11370385_13322841594828.raw). See the lasers for a 10-cm scale.

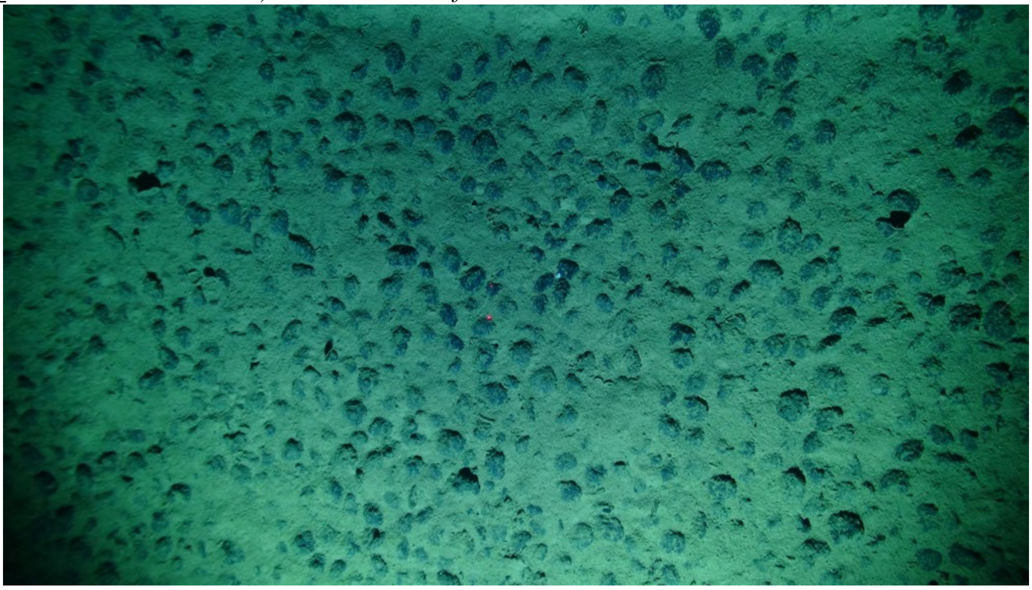


Figure 14.9 – Example of a picture collected with the SCORPIO camera (Dive 409, DSC09772.JPG). See the lasers for a 10-cm scale.

Figure 14.9B: Example picture of the same sponge collected with the Scorpio (left) and AESA (right) camera. Note both images are comprised on several images mosaicked together to ensure the whole sponge is in shot.

Lessons learnt

Initially, the use of the AUV *Autosub6000* was planned to perform imaging dives. The ROV *Isis* acquired images at a much slower pace (~0.1 - 0.15 m/s) than the AUV (typically 1.2 m/s). For the first time, those dives demonstrated the successful mounting of the AUV AESA camera on a ROV. Furthermore, the SCORPIO and the AESA were successfully mounted side by side in order to acquire complementary datasets.

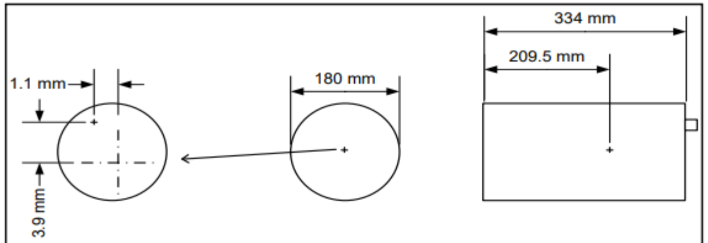
Supplementary Figure S14.1 – Isis instrument offsets considering a downward-looking configuration of the AESA and SCORPIO cameras used during D403, D404, D405 and D409.

Isis Instrument offsets

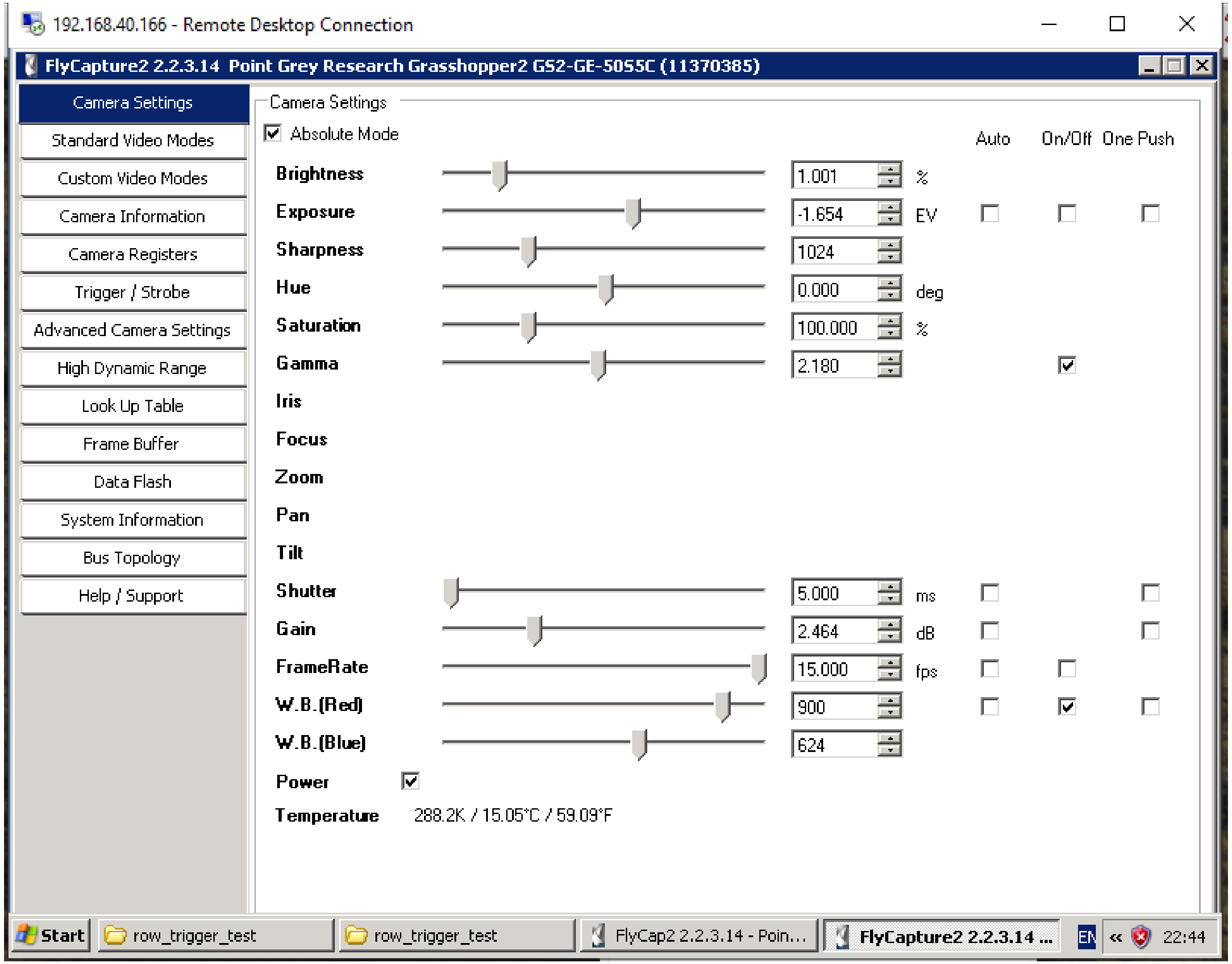
CFP (Common Refrence Point) relative offsets.
The CRP is located in the centre of the main cross beam fwd of the vehicle.
Dimensions are in metres.

	X Stbd = +	Y Fwd = +	Z Up = +
Compatt 5	-0.99	-0.35	1.55
6G WMT	0.97	-0.35	1.50
OCTANS	0.00	-0.88	-0.51
DVL Doppler	0.71	-2.48	-0.25
Para Scientific pressure senser	-0.45	-1.72	-0.04
Reson Sonar Reference Point	-0.47	-1.78	-0.82
Reson Receiver Reference Point	-0.47	-1.57	-0.82
Altimeter	-0.67	-2.54	-0.20
CTD	-0.85	-1.89	1.35
Tritech Super Sea King Sonar	-0.45	0.23	1.53
AUV Stills Camera (Vertically Mounted)	-0.24	0.46	-0.71
Scorpio Stills (Vertically Mounted)	-0.60	0.41	-0.71

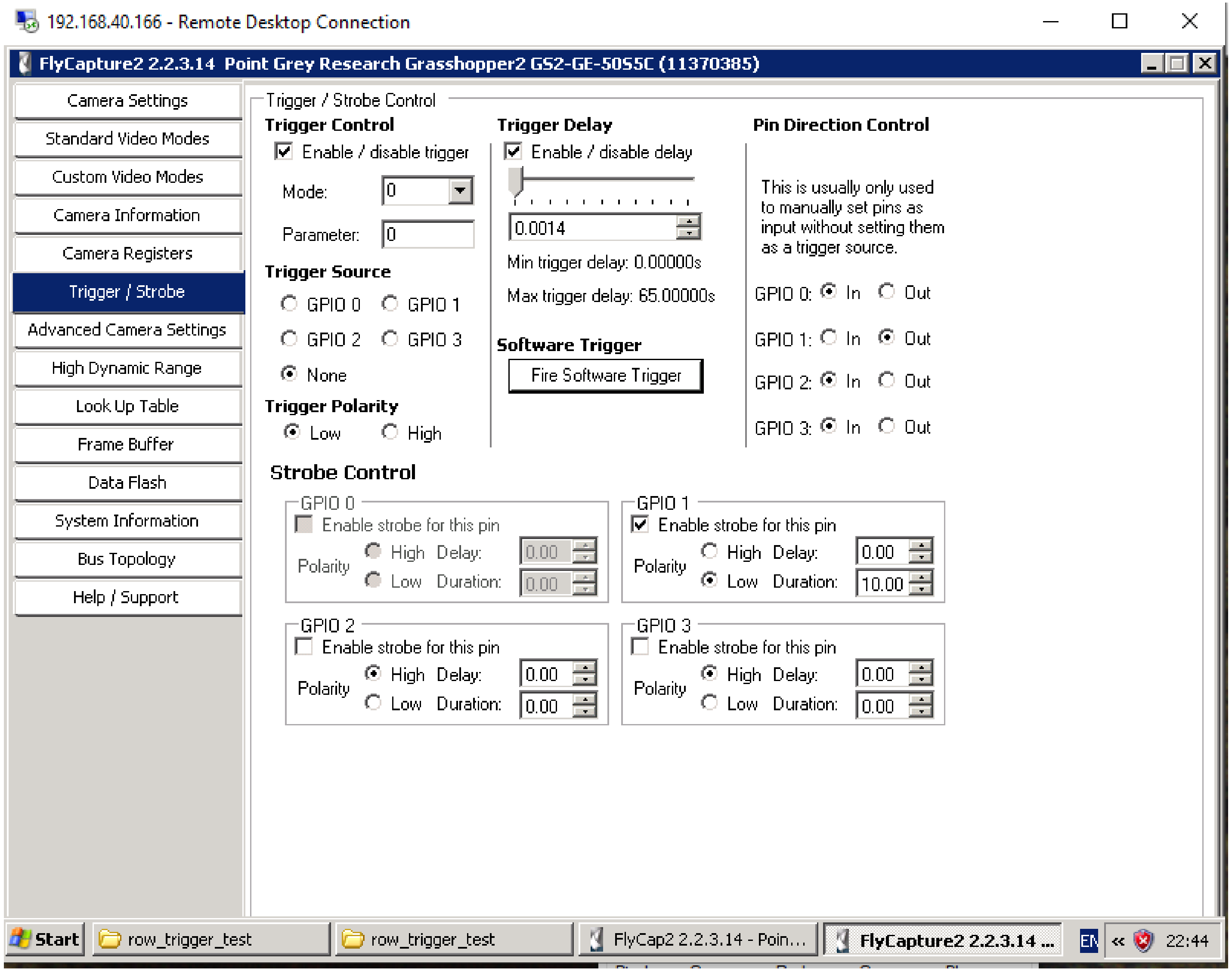
Note
1 Sonardyne use + = Down for their refrencing.
2 The position given from Sonardyne has the offset already computed within its calculation for position so for such post processing as SWATH, this offset should be entered as 0,0,0 (the CRP) of the vehicle.
3 CARAIBES uses + = down for their referencing.



OCTANS lever arm reference point



Supplementary Figure S14.2 – Screenshot of the AESA camera settings



Supplementary Figure S14.3 – Screenshot of the trigger setting

Imaging survey of the megafaunal benthic communities in the UK-1 contractor area

Material and set-up

The same camera setup for dives D403, D404, D405 and D409 was used (i.e. cameras SCORPIO and AESA vertically mounted, see section "Assessment of disturbance of benthic activities on benthic communities").

Survey design

The dive D413 was intended to characterise benthic megafaunal communities of the UK-1 contract area and to investigate scales of community variability. Furthermore, as that area has been targeted for a future mining test in the UK-1 and the Singaporean contract area, this study could become a pre-disturbance investigation.

Five consecutive transects of 2 km each were randomly allocated over space in ArcGIS. Furthermore, the last transect was replicated with two parallel transects located 50 m from the latter, to investigate community variability over smaller spatial scales.

Isis was flown at an altitude of 3 m and a speed ranging from 0.10 to 0.15 m/s.

Dive narrative

Isis arrived at the bottom at 08:21 (17-03-2023). After the characterisation of the fauna colonisation observed on nodules disposed on a settlement frame deployed in 2020 by A. Glover, the first transect started South. A few interesting observations were made during this such as abundant fragments of salp over the seabed. A close-up of a salp was carried out with the SCORPIO camera on the 18-03-2023 (13:11). No major issue had to be reported. Only half of the last replicate of the fifth transect (T5.3; Figure 14.10, Table 14.13) was carried out due to time constraints. *Isis* left the bottom at 17:09 (18-03-2023).

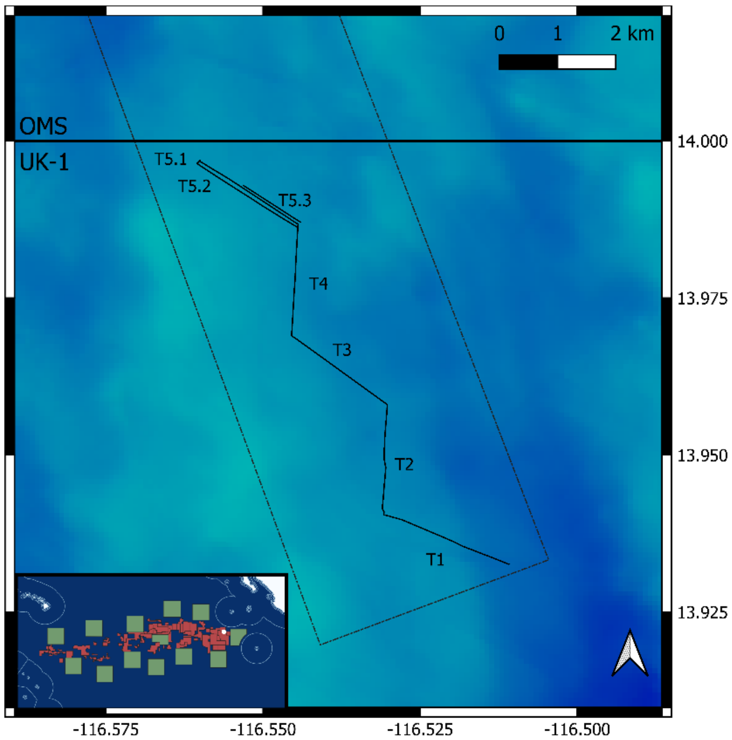


Figure 14.10 – Map of the imaging transects performed during the dive D413. The AOI-2 is delimited by a dashed rectangle, located on the contractor areas UK-1 and OMS (Singapore). The inset on the bottom left shows the location (white circle) within the CCZ (contractor areas in red, APEIs in green). Navigation results of the merging of the DVL and USBL navigations based on a rolling average of 30 minutes (Van Audenhaege et al. 2021, Front. in Mar. Sc.).

The ROV was maintained at steadier altitudes than previous dives (Figure 14.11).

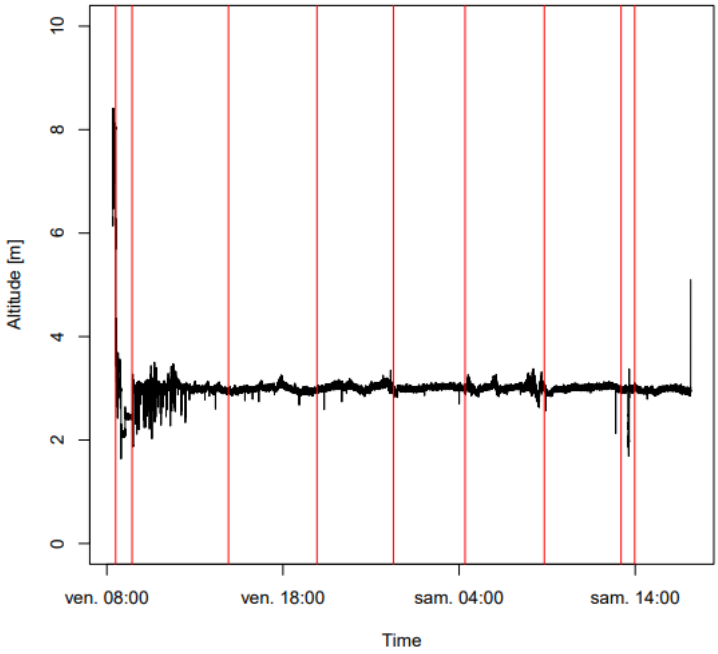


Figure 14.11 – Altitude [m] of the ROV over time for dive D413. Red lines mark Doppler resets.

Table 14.13 – Details on survey transects of imaging dives of JC241 (Distance covered, ROV heading, time of DVL reset, Time and position of start and end of transect. Note that positions were retrieved from the DVL sensor.

Transect number	Dist. [km]	Head. [˚]	DVL reset	Start				End			
				Date, time	Coordinates		Depth	Time	Coordinates		Depth
T1	2.310	68	09:26	17-03-23, 09:32	13˚55.9590’	-116˚30.6627’	4138	14:54	13˚56.4294’	-116˚31.8470’	4091
T2	1.965	0	14:54	17-03-23, 15:00	13˚56.4294’	-116˚31.8470’	4091	19:43	13˚57.4832’	-116˚31.8152’	4099
T3	2.035	55	19:56	17-03-23, 19:43	13˚57.4832’	-116˚31.8152’	4099	00:14	13˚58.1348’	-116˚32.7259’	4084
T4	1.970	0	00:16	18-03-23, 00:16	13˚58.1386’	-116˚32.7810’	4084	04:19	13˚59.2025’	-116˚32.6664’	4085
T5.1	2.025	58	04:20	18-03-23, 04:22	13˚59.2025’	-116˚32.6664’	4085	08:31	13˚59.7932’	-116˚33.5967’	4089
T5.2	2.065	125	08:50	18-03-23, 09:10	13˚59.7822’	-116˚33.6290’	4083	13:11	13˚59.1923’	-116˚32.6769’	4085
T5.3	1.175	58	13:58	18-03-23, 13:58	13˚59.2200’	-116˚32.6478’	4086	17:09	13˚59.5771’	-116˚33.1938’	4080

Imaging data

Table 14.14 – Details of the picture set acquired by the cameras AESA (8-bit .raw files, 2448x2048 pixels, 4,896 KB/image) and SCORPIO (.JPG files, 4672x2628 pixels, ~ 5,000 to 6,000 KB/image) at UK-1.

Dive	Mission duration	Count AESA pictures	Size [GB]	Count SCORPIO pictures	Size [GB]
D413	39:01:00	39,575	184	11,862	54

Table 14.15 – Details of video sequences acquired by the SCORPIO camera mounted on the ROV Isis during dives D413.

Dive	Name of videos	Start (UTC)	Size on disk [GB]
D399	JC241_SCO_DIVE413_90	16/03/2023 22:39	57
	JC241_SCO_DIVE413_91	17/03/2023 04:40	106
	JC241_SCO_DIVE413_92	06:45	107
	JC241_SCO_DIVE413_93	08:47	103
	JC241_SCO_DIVE413_94	10:50	105
	JC241_SCO_DIVE413_95	12:53	103
	JC241_SCO_DIVE413_96	14:55	103
	JC241_SCO_DIVE413_97	16:57	103
	JC241_SCO_DIVE413_98	18:32	80
	JC241_SCO_DIVE413_99	20:34	103
	JC241_SCO_DIVE413_100	22:33	101
	JC241_SCO_DIVE413_101	18/03/23 00:33	101
	JC241_SCO_DIVE413_102	02:36	103
	JC241_SCO_DIVE413_103	04:43	107
	JC241_SCO_DIVE413_104	06:45	103
	JC241_SCO_DIVE413_105	08:55	110
	JC241_SCO_DIVE413_106	11:22	124
			Total = 1719

Time-lapse imaging survey of benthic megafauna

Material and set-up

Intra-annual ecological dynamics was assessed with the deployment of Bathysnap lander systems in the UK-1 contractor area.

Bathysnap is composed of a pressure case containing the computer (Jetson nano 2GB) and the battery. The computer operates a flash with an adjustable pitch and a FLIR Blackfly S BFS-PGE-200S6C camera with a V1624-MPZ lens (Table 14.17, Supplementary Figure S14.4).

Table 14.16 Optical details of the FLIR Blackfly S BFS-PGE-200S6C camera.

Model	BFS-PGE-200S6C
Sensor	Sony ICX183, CMOS, 1"
Resolution	5472x3648
Output	
Photo	20MP, RAW 16-bit (38,988 KB) .DNG images (1107x738 px, 9,242 KB) plus .XMP file (6 KB each)

Table 14.17 – Optical specificities of the V1624-MPZ lens.

Model	V1624-MPZ
Focus range	0.1m - Infinity
Aperture	F2.4-16.0
Lens Focal Length	
Photo	16 mm
Angle of view in water	1"
Diagonal	53.2˚
Horizontal	43.8˚
Vertical	33.6˚

The computer is positioned on the 'back' of the frame while cameras and flashlight are positioned on the 'front'. The acoustic release and weight (approx. 35 kg) are positioned in the centre of the frame. The frame was attached to a mooring with 35m of line to a series of 5 glass float buoys, plus 15m of line to a Billings float (with flag) and an additional 15m of recovery line (Figure 14.12).

The pitch of the camera was set to 30˚ (Figure 14.13).

Deployments and data collected

Time-series image acquisition was tested by deploying one Bathysnap on 12/02/2023 (06:35). Image acquisition was set to a frequency of 15 minutes (Gain = 0).

The lander was successfully recovered on 15/03/2023 (13:32; Table 14.16). Forty-six .RAW files were retrieved (38,988 KB per image). 143 .DNG images (1107x738 pixels, 9,242 KB per image, total = 3.96 GB) accompanied by a .XMP file (6 KB each) were readable in IrfanView. From the 143 .DNG images, 83 were collected under water every 15 minutes from 12/02/2023 06:51:58 to 13/02/2023 03:21:56 when it unexpectedly stopped. As no apparent physical damage was noticed, test of the battery at low temperature (i.e., battery in freezer) indicated that the power supply system was not effective at the low temperatures of the seabed (~ 2˚C; i.e. problem of electric signal transmission), which possibly explains the camera stopped recording prematurely. The low light intensity of the images showed the inadequacy of the null gain as well as the orientation of the flashlight (Figure 14.14).

As a result, we recalibrated the flash angles to 0˚ (i.e., horizontal). The camera angle was not changed (30˚, Figure 14.4). The gain was set to 17.0086db. Battery type was not changed (one battery that was deployed had already been depleted to approx. 1/3-1/2 owing to the computer running for a couple of days on deck), but the acquisition frequency was switched to 24h instead of the 8h initially planned. Both landers were deployed on the 18/03/2023 and left on the seabed for a year of image acquisition (Table 14.18).

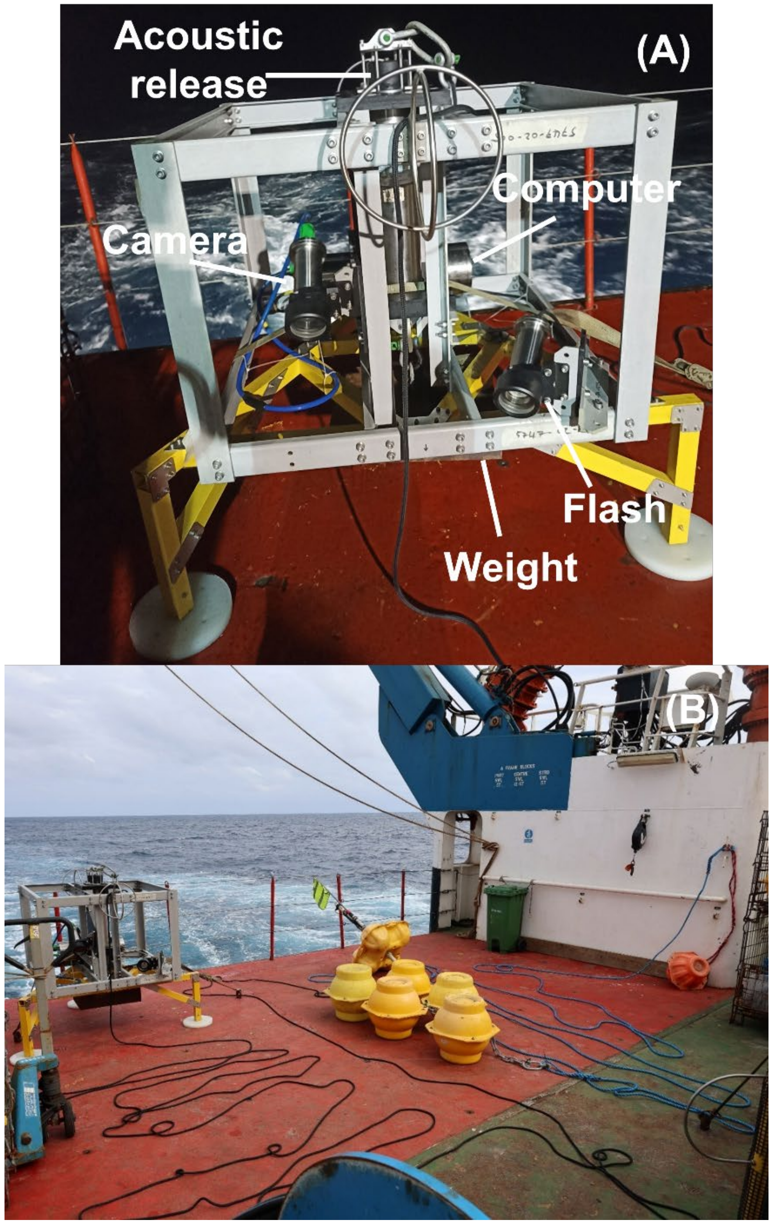


Figure 14.12 – (A) Close-up picture of the Bathysnap deployed on 12 February 2023, (B) Picture of the Bathysnap mooring ready to be deployed on 12 February 2023.

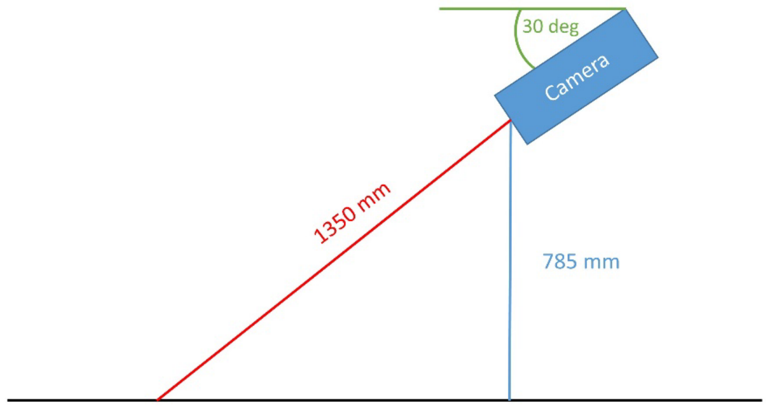


Figure 14.13 – Offset and angle of the Bathysnap camera.

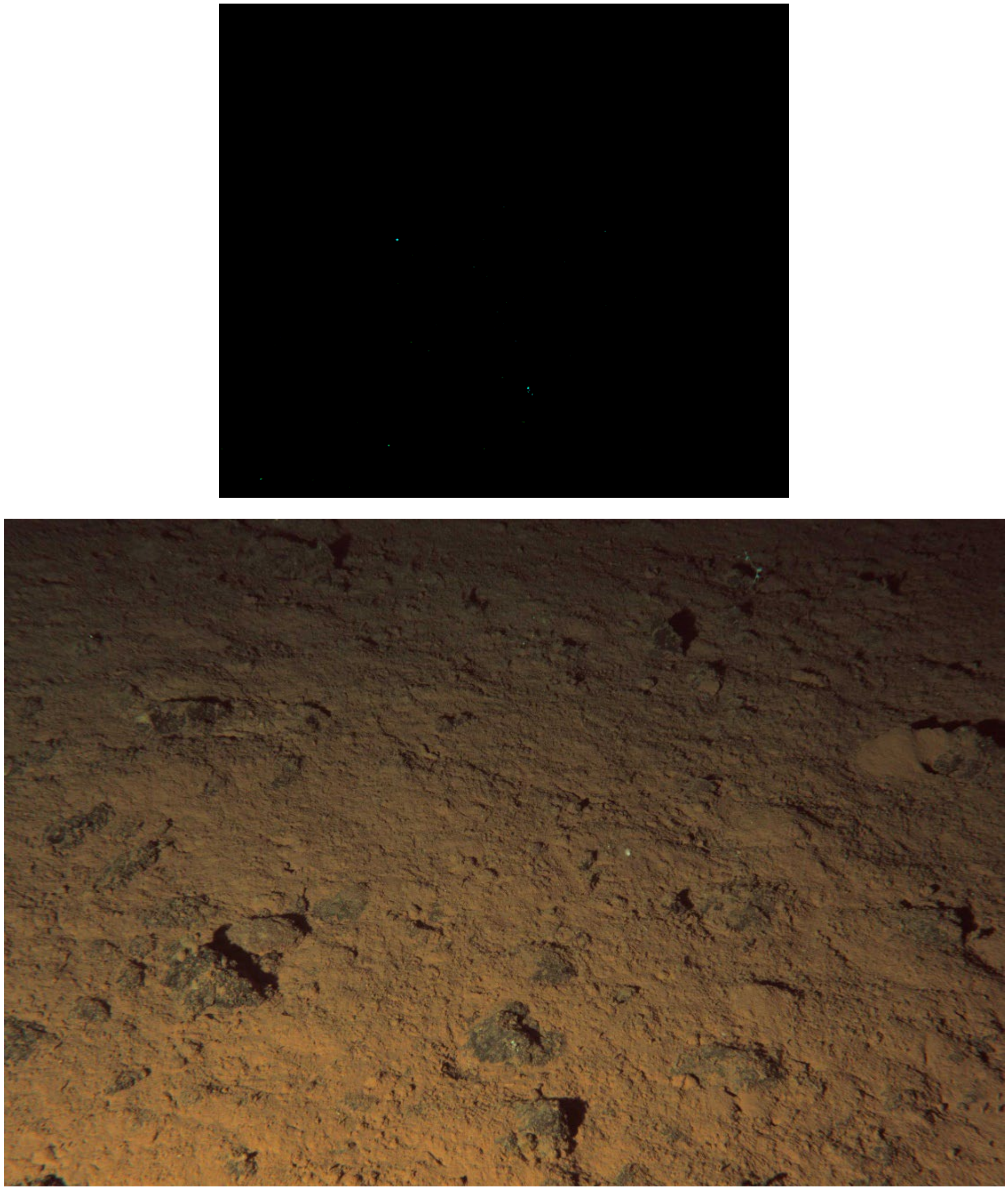


Figure 14.14 – A) Screenshot of a .DNG image taken at the seabed. Note the presence of blue pixels which slightly change of colour intensity over time. B) example of a colour corrected .jpg of the seafloor. Colour correction was done using Darktable.

Table 14.18 – Details of Bathysnap deployment/recovery.

Station		Deployment			Recovery		
		Date, Time (UTC)	Position	Sounding	Date, Time (UTC)	Position	Sounding
Bathysnap 1 for test	JC241-007	12/02/2023, 06:35	13°54.6109'N 116°33.1222'W	4074 m	15/03/2023, 13:32	13°6110'N 116°33.1220'W	4084 m
Bathysnap 2	JC241-099	18/03/2023, 22:00	13°54.86'N 116°31.26'W	4100 m			
Bathysnap 3	JC241-100	18/03/2023, 22:36	13°53.27'N 116°30.59'W	4146 m			

Post Recovery Images workflow

Converting from RAW to DNG

Requires the AESA II QC python package.

Use the command "convert_bathysnap_images" in a terminal window to convert the raw images to adobe DNG images. The output images will then be editable in a standard image porcessing tool like darktable, lightroom, photoshop or gimp.

The package requires python 3.10 and the ability to build cpython bindings. e.g. python3.10-dev or vsstuidos cpp14 toolchain.

1.After connecting the image drive to the PC, run the Run the convert_bathysnap_images command on the folder

i.convert_bathysnap_images -h will return a help message

ii.There should be a bathysnap_config.yaml file with the arguments filled in already

2.The conversion process may take a few minutes depending on drive speed, output folder location and the number of images

Correcting DNGs light and colour

3. When the job is finished, open Darktable. In the top left corner of the Lighttable window, there is an Import tab. Click add to library and add the DNG ouput folder. Darktable will generate an XMP file for each image. The output folder will be added to under the collections tab. All images from the deployment will appear in the main window.

4. Double click on an image that is representative of the dataset. Darktable will open the image in the darkroom window.

5. The darkroom window shows history on the left and tools on the right. The history tracks the number of operations preformed on an image. Clicking on one of the records in the history will apply the operations up to that record in the history. This is a handy way to

6. Adjust image to bring out the best of the seafloor using the darkroom tools on the right side of the. **Recommended tools include**

1. raw black/ white point. Set raw blacks to zero, reduce white point to brighten the image

2. white balance. Increase temperature and tint to make the image more brown/red than blue/green.

These two settings will get you pretty far.

3. Exposure. Play with exposure but don't make the image too bright.

4. Color correction. Correct for the amount of blue and lack of red

5. Tone curve.

6. Contrast brightness saturation

7. Color rgb

Note the "history" on the left of the screen allows you to return to the original image. You to start over again. The changes to the image are non-destructive, so it's safe to edit until you're happy.

7. When your happy with the image, return to the lighttable view. Select your corrected image, on the right side under "History Stack" press selective copy.

8. In the selective copy window, selective all.

9. Using Ctrl -A select all the images, on the right under history stack, press paste.

10. If the paste is successful, you will see that all the images match the colour correction of your manually edited image.

11. From here you are done, you can export the images to tiff or jpegs, compress the history, etc.

1. Note that the DNGs files have not changed, only the XMP file has changed.

computar

V1624-MPZ

f=16mm F2.4
for 1" type cameras, Fixed-focal
C-Mount

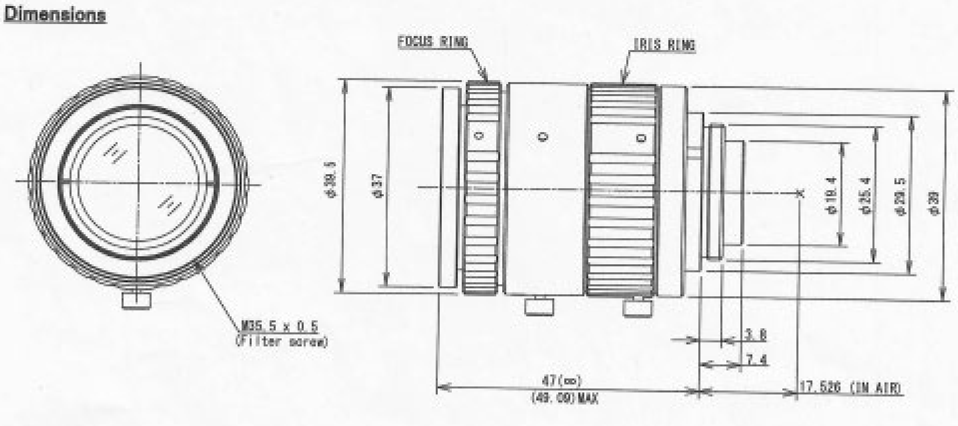
Model No.		V1624-MPZ
Focal Length		16mm
Max. Aperture Ratio		1:2.4
Max. Image Format		12.8mm x 9.6mm(φ16mm)
Operation Range	Iris	F2.4- F16.0
	Focus	0.1m - Inf.
Control	Iris	Manual
	Focus	Manual
Object Dimension at M.O.D.	1 Type	88.6mm x 66.3mm
	2/3 Type	60.7mm x 45.5mm
	1/1.8 Type	49.6mm x 37.2mm

Effective Lens Aperture	Front	φ20.9mm
	Rear	φ15.9mm
Distortion	1 Type	0.0%
	2/3 Type	-0.4%
	1/1.8 Type	-0.4%
Back Focal Length		10.7mm
Flange Back Length		17.526mm
Mount		C-Mount
Filter Size		M35.5 P=0.5mm
Dimensions		φ39.5mm x 47mm
Weight		108.1g

Angle of View	D	1 Type	53.2°	2/3 Type	38.1°	1/1.8 Type	31.6°
	H		43.8°		30.9°		25.5°
	V		33.6°		23.4°		19.2°
Operating Temperature	-10°C - +50°C						

M.O.D. : Minimum Object Distance

Dimensions



Specification is subject to change without any notice.

2020.05

Supplementary Figure S14.4 – Technical details of the V1264-MPZ lens used for the Bathysnap.

15. Biological sampling for megafauna

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15.1 Summary

Megafaunal sampling with the ROV Isis was carried out on JC241 to support two SMARTEX objectives and was highly successful. A total of 257 megafaunal specimens were collected during six sampling dives.

Samples will be returned to the NHM London for further analysis by the SMARTEX team.

15.2 Introduction and objectives

The megafauna sampling was designed to collect samples to deliver on the SMARTEX Proposal Objectives 5 (Biodiversity, community structure and trophic dynamics) and Objective 4 (Life history, Reproduction and Connectivity). Further details of these objectives are provided in the SMARTEX proposal, but in summary the main goals were to:

- Collect a wide range of megafauna, representative of the biodiversity, using the ROV Isis in three areas:
    - OMCO Track (a region disturbed by a trial mining machine in 1979)
    - OMCO Control (a region we designated ~2km to the E of the OMCO Track site as a control)
    - UK-1
- Target widespread and abundant species for connectivity studies
- Collect megafauna using high-quality preservational cold-chain methods (sensu Glover et al 2016) that can be used for taxonomic, population genomics, transcriptomic and life-history studies
- Provide *in situ* images and videos to inform taxonomic, behavioural, life history, and ecological studies

15.3 Methods and protocols

15.3.1 ROV configuration for megafaunal sampling

The Remotely Operated Vehicle 'ROV Isis' was utilised for megafauna collection. This ROV possess two manipulator arms (Titan and Predator), and one slurp gun for capturing and handling specimens and tools. For sampling, the ROV was configured to include storage for specimens, with four canisters available for the slurp gun, as well as three bioboxes, one on each of the port and starboard swing arms and a larger one on the front tray (Fig. 15.3.1.1). ROV Isis is also equipped with three high resolution cameras recording simultaneously: 1) Super Scorpio (hereafter referred to as Scorpio), 2) Konsberg Eye Ball Cam (hereafter referred to as Science Cam), and 3) Insite Mini Zeus Mk2 Cam (hereafter referred as Pilot Cam). Both the Scorpio and Pilot cameras have a set position, but the zoom can be controlled, and Pilot is mounted on Pan & Tilt. The Scorpio camera has a higher resolution, 12.3 MEGA-PIXEL for Ultra-High Definition, against 1080p (High definition) of the pilot camera and can be set to take stills at regular intervals. The Science camera is designed to be controlled by the scientists. It is located on top of the ROV Isis to provide a peripheral view ca. 180 degrees, providing unique visibility for finding specimens on the seafloor. It has a resolution of 1080 pixels and has both pan and tilt integrated. In order to obtain different angles from the *in situ* images, the Scorpio camera was positioned on the starboard side of the sliding tray, which provided a lateral view of the specimens instead of the typical oblique angle. This configuration was implemented for the last two collection dives (Dives 411 and 412), with Scorpio being placed higher up on an oblique angle on previous dives.

Figure 15.3.1.1. Left: ROV Isis configuration for sampling dives. Three bioboxes are mounted, a larger one on the front tray (grey biobox), and two smaller ones, one on each port and starboard swing arms. The slurp gun was also available on all sampling dives. Right: Canisters for collecting specimens with the slurp gun on the ROV Isis. The carousel is located at the back on the starboard side. Credit: G Bribiesca-Contreras.

The ROV Isis was maintained and deployed in good order by the ROV Isis team on board (Russell Locke, Will Handley, Martin Yeomans, Antonio Calado, Josue Rivera, Emre Mutlu and Steve McDonald). Most deployments and recoveries occurred between 10 am and 10 pm, all from the port A-frame of the RRS James Cook.

The NHM team oversaw megafaunal sampling, including the logging and recording of the dive, starting from the moment that it was off the deck until it was back on deck. All events logged required at least USBL coordinates (from ROV), depth, and GMT time; and video files were generated every two hours, with two copies made of each. All events were recorded in the dive log, the sample log and the media log for details on the dive, details of all samples collected, and details of all media collected including media back-up. Target megafaunal specimens were selected to include a wide range of megafaunal taxa representative of the abyssal biodiversity of the area. Additionally, multiple specimens of target species for population genomic studies (i.e., *Psychronaetes hanseni*, *Plesiodiadema globulosum*, Elpidiidae sp.) were collected. These species were selected based on abundance and previous knowledge of widespread distribution in the CCZ. Specimens were mostly collected with the manipulator and placed in the bioboxes or a wooden box on the front tray. Delicate specimens (e.g., fragile sea cucumbers that autolyse upon recovery), highly mobile, or specimens that were difficult to reach with the manipulator were sampled using the slurp gun.

General protocol was as follows, including a modified protocol for *in situ* imagery and video recording:

1. Before the dive starts, load and calibrate a new map of the dive site for use on the OFOP software.
2. Initiate a new OFOP protocol following manual provided by ROV Isis.
3. Log details of ROV in the water and start recording video for the Pilot camera only.
4. Change Pilot video tape and start recording the other two cameras (Scorpio and Science cam) when ROV is almost at the seabed (~100 m off the seabed).
5. Log ROV on bottom and make sure all camera videos are being recorded and set Scorpio stills to be taken every 30 seconds.
6. Use Science cam to locate target megafaunal specimens for collection.
7. Land ROV on seabed with specimen aligned with the Scorpio camera and make sure lasers are on.
8. Take some Scorpio stills.
9. Turn off the lasers and stop automatic stills on Scorpio. Depending on how mobile the specimen is, take at least 1 min of video or at least 10 mins if the animals is moving.
10. Turn off the main lights and turn on the lights on the starboard manipulator (Predator) to take Scorpio stills with lighting from different angles.
11. Turn the lasers back on, resume Scorpio stills, and proceed with collection using either slurp gun or manipulator. Log the event in the sample log, OFOP, and create a waypoint.
12. Log when ROV leaves the bottom and stop video recording in all cameras except for PILOT.
13. Ensure everything is logged until ROV is on deck.

15.3.2 Megafaunal samples processing (deck)

All dissection tools, trays and buckets were bleached in advance by immersing in a 1% bleach solution for at least 15 min, then rinsed three times with Milli-Q water. Areas for dissection were cleaned once per day with 1% bleach and between specimens with 80% non-denatured ethanol to avoid cross contamination. Disposable gloves were worn throughout the processing of megafaunal samples and cleaning of equipment and work areas. Between 100 and 200 m before the ROV Isis reached the surface, buckets and trays were taken out to deck and filled with Cold Filter Seawater (CFSW). Once the ROV was on deck and securely fastened, all specimens were recovered from the ROV. Any water in the bioboxes was syphoned off with hoses into a 300 µm sieve to improve visibility of megafauna. Specimens were recovered with gloves, and each placed in a clean bucket filled with CFSW (Fig. 15.3.2.1). When possible, sponges were placed in buckets previously cleaned with 1% bleach. Remaining sediment in bioboxes was recovered, sieved and kept at 4° C for posterior sorting in the wet lab. All samples were transferred to a temperature-controlled room at 4° C or to a fridge following the cold-chain process described in Glover et al. (2016).

Figure 15.3.2.1. Recovery of collected samples from ROV Isis. Left: water is syphoned out into a 300 um sieve to have a good view of the collected megafauna in the biobox while recovering small animals on the sieve. Right: Sea cucumber being carefully transferred from the starboard biobox to a bucket filled with cold-filtered seawater. Credit: D Jones.

15.3.3 Megafaunal samples imaging and processing (wet lab)

Megafaunal samples collected with the ROV and a few opportunistic samples recovered in the box core were processed in the same way. Specimens were individually photographed using a Canon EOS850d with Canon 430EXII speedlights (x2) before any subsampling or preservation was carried out. For some specimens, details of some taxonomically informative characters were also photographed under a Leica MZ9.5 stereo microscope with trinocular head and Leica phototube attached to a Best Scientific Canon EOS camera adaptor, and finally to a Canon EOS90d also equipped with 2x Canon 430EXII speedlights. Specimens were placed in different size aquaria or trays, depending on size, with a non-reflective black cloth as background and scale bar with a colour correction chart. For large or long animals (e.g., big sea cucumbers or sponges with long stalk) a big tray (L 56 x W 36 x H 14 cm) was used. For medium and small specimens, a small tray (L 43 x W 29 x H 12 cm) or a tank aquarium (L 30 x W 18 x H 17 cm) were used. These containers were filled with CFSW and ice packs were added to keep the water as cold as possible. CFSW was replaced in the aquaria after a couple specimens, and any specimens producing slime or autolysing were photographed last to avoid contamination of samples. Image numbers were recorded on a spreadsheet along with unique identifiers and any other relevant information.

All megafauna specimens were photographed, but samples were processed differently if they were considered voucher specimens (i.e., first individual collected of each species) or if they were targeted for population genomics. After photographs were taken, most voucher specimens were subsampled for different purposes. Ideally three different subsamples were taken (i.e., in 96% non-denatured ethanol (etoh96), RNAlater, and flash frozen in liquid Nitrogen) without damaging the voucher specimen too much and keeping the taxonomically informative characters as intact as possible. Different tissues were sampled depending on the groups; for instance, fragments of arms were taken from brittle-stars; body wall from anemones and sea cucumbers; tube feet from seastars, sea cucumbers, and sea urchins; and body tissue from sponges. All subsampling took place on a clean bench (previously cleaned with bleach or ethanol) in the wet lab, using decontaminated tools that were also cleaned between samples. Most specimens were examined for the presence of gonadal tissue, and if this was present, most subsamples were taken from it. Additionally, some gonadal tissue was preserved in 10% formalin, and sometimes in O.C.T. (Optimal Cutting Temperature) compound too, for histological studies on life-history traits. Voucher specimens were then fixed in 80% non-denatured ethanol and stored at 4° C. Subsamples in etoh96 were immediately transferred to -10° C, flash frozen were kept in liquid Nitrogen, and RNAlater were kept at 4° C for 12 hrs following manufacturer instructions.

Figure 15.3.3.1 Subsampling of voucher specimens collected during the ROV Isis dives, Left: Subsampling of Freyastera cf. benthophila. Right: subsampling of Elpidiidae sp. Image credit: G Bribiesca-Contreras, A Glover.

Each specimen and each subsample was assigned a unique identifier (NHM number), along with all other relevant information (e.g., deployment number, photo number, relevant notes). For subsamples, it was clearly indicated the type of tissue taken, the preservation method and the NHM number from the specimen it was taken from. As soon as tissue samples were processed, all flash frozen and etoh96 subsamples were stored at -80° C. Subsamples in RNAlater were kept at -80° C after 12 hours. For most voucher specimens, 80% ethanol was changed after 24–48 hours to ensure good preservation. For most samples, this ethanol was kept and assigned a unique identifier (NHM number) to be analysed as part of the DEEPEND Biodiscovery project.

15.3.4 Megafaunal samples for population genomics (dissections)

Three species were targeted for population genomic studies based on their relative abundance and their widespread distribution in the CCZ: 1) *Plesiodiadema globulosum*, 2) *Psychronaetes hanseni*, and 3) Elpidiidae sp. After all specimens were photographed and kept in the temperature-controlled room at 4° C, dissections were performed in the same room following the protocol described below.

Before dissection, all surfaces and instruments that would be in contact the specimens were disinfected with 1% bleach. Also, to avoid cross contamination, any surface that would have direct contact with the specimens was covered in foil, which was replaced for each specimen. Sea cucumbers (i.e., *Psychronaetes hanseni* and Elpiididae sp.) were displayed in the ventral position to start dissections with a central incision from mouth to anus, maintaining the digestive tube intact. After examining the anatomy and inspecting for internal parasites, removal of different tissues was carried out. Gonads and respiratory tree were subsampled and fixed in 10% formalin, O.C.T. compound, RNAlater, etoh96, and/or flash frozen. The digestive tract was subsampled from the mouth-anterior region, middle, hind, and cloaca, with subsamples of each region preserved RNAlater, etoh96, and flash frozen. The longitudinal muscles (four per specimen) were removed avoiding any body wall tissue and preserved in O.C.T compound, flash frozen, etoh96, and RNAlater. Finally, body wall tissue samples, without outer skin tissue, were taken (for Liverpool isotope, amino acid, and lipid analysis only). The rest of the animal was kept in non-denatured ethanol at 80% as a voucher.

Figure 15.3.4.1. Dissection of Psychronaetes hanseni*. Specimen is lying on dorsal surface, with a longitudinal incision running from the mouth to anus on the ventral surface. Credit: L King.*

The subsamples were fixed in different ways for different types of work. For instance, flash frozen and RNAlater tissues can be used for whole-genome and transcriptome sequencing. Ethanol subsamples for other DNA analyses, mainly barcoding. Formalin and O.C.T. will be used for histological analyses including life-history traits. Stable isotope, amino acid and lipid samples were frozen at - 80˚C and fatty acid desaturase gene samples were flash frozen, then stored at - 80˚C.

A different incision dissection technique was implemented with the sea urchin *Plesiodiadema globulosum*. The periproct was removed to access internal structures such as gonads while keeping the test intact. This also allowed to keep the gonads intact while avoiding contamination from digestive tissue and contents.

15.4 Results and samples distribution

A total of six megafaunal collection dives were carried out by ROV Isis. All were hugely successful, with 96 samples collected from the seabed (only three of those lost during ascent). Some of this samples contained more than one specimen and yielded an impressive collection of 257 specimens from 12 different phyla (Figs. 15.4.1–15.4.2), including four opportunistic megafauna samples from the box core and a metal pipe from the collector vehicle (Table 15.4.1). Additionally, nine pushcores were taken with the ROV Isis for the DEEPEND Biodiscovery Project.

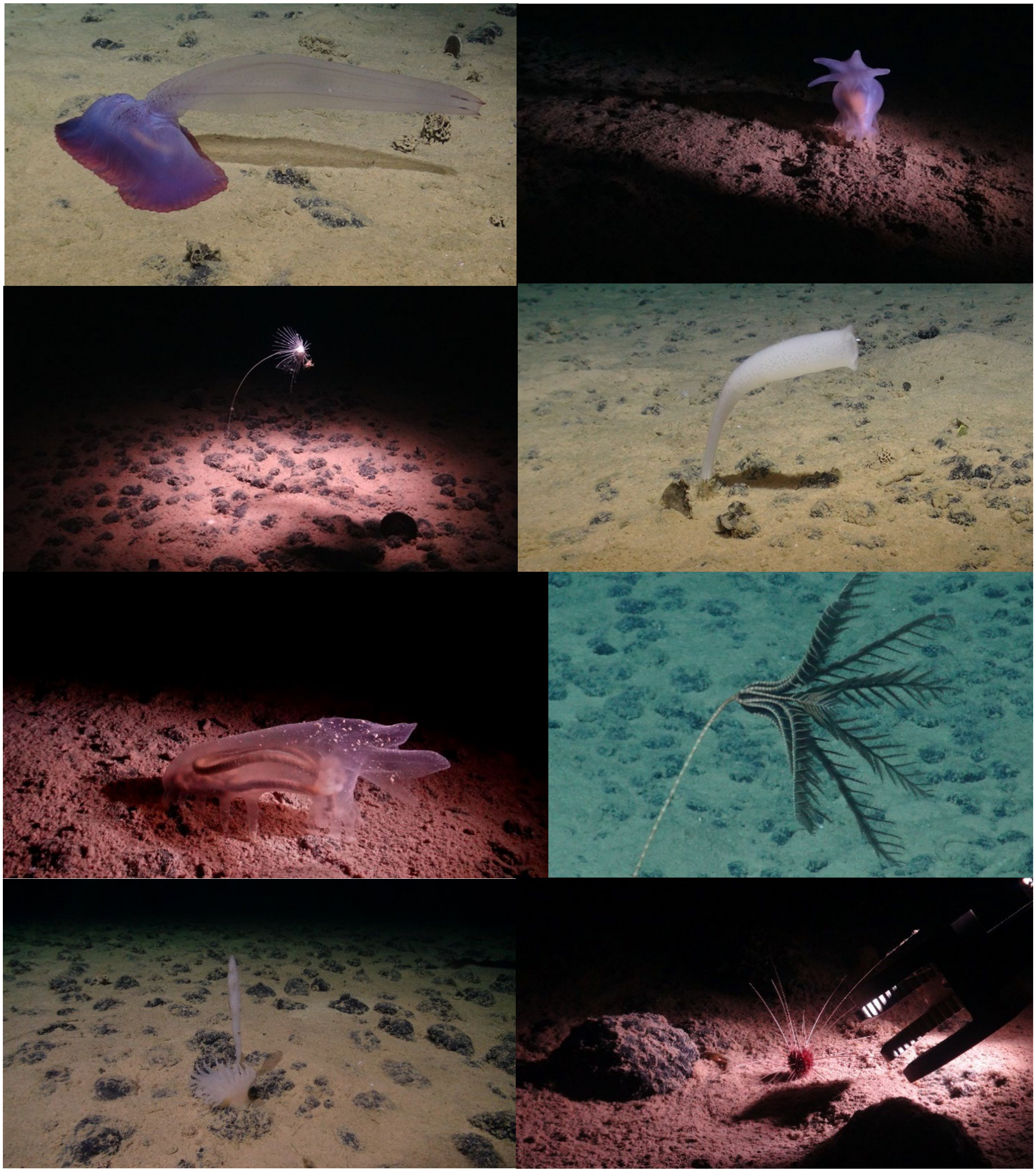

Figure 15.4.1. In situ images of benthic megafauna taken with the Scorpio camera placed on the starboard side on the front tray of the ROV Isis.

Figure 15.4.2. Diversity of benthic megafauna collected with ROV Isis during JC241, from images taken in the lab. Credit: NHM JC241 Team.

Table 15.4.1 Number of metazoan specimens collected during each of the ROV Isis collection dives, including a metal pipe from the collector vehicle and additional opportunistic megafaunal samples recovered from the box cores.

Phylum	Dive 399	Dive 406	Dive 407	Dive 408	Dive 411	Dive 412	Box core	Total indiv. per phylum
Cnidaria			77	1	1	11		90
Echinodermata	4	5	23	10	17	12	3	74
Arthropoda	10		19	1	4			34
Annelida	1		9	5	3	5		23
Porifera		1	2	2	8	6		19
Metazoa indet.			2	1	1	2		6
Bryozoa		1	1					2
Chordata			1			1		2
Mollusca	1					1		2
Cetacea						1		1
Chaetognatha	1							1
Nemertea						1		1
Hemichordata							1	1
Metal pipe*				1				1
Total indiv. per dive	**17**	**7**	**134**	**21**	**34**	**40**	**4**	**257**

15.4.1 Megafaunal 'omics' studies

The megafauna collection aimed to sample the benthic biodiversity of the OMCO and UK-1 sites. The two most abundant phyla in the samples were cnidarians and echinoderms. Many more echinoderms were targeted for collection, but several of the glass sponges collected had numerous anemones attached to the stalks, increasing the number of collected cnidarians.

In addition to the remarkable collection of specimens, there is an equally impressive number of subsamples taken that will allow us to conduct several other studies. A total of 474 subsamples were taken in five different preservation methods (Table 15.4.1.1) including snap frozen, RNAlater, etoh96, formalin, and O.C.T. Snap frozen and RNAlater preserved tissues yield higher quality DNA, thus permitting the sequencing of genomes and transcriptomes, respectively. DNA is also well preserved in etoh96, but will be most likely used for sequencing few specific genes (e.g. cytochrome oxidase I for barcoding). Both formalin and O.C.T. preserved tissues can be used for histological analyses, so we mainly targeted gonadal tissue that could provide insight into life-history traits.

Table 15.4.1.1. Total number of subsamples taken from all megafauna samples, including the opportunistic ones recovered from the box cores.

	Fixation method				
Phylum	**Snap frozen**	**RNAlater**	**etoh96**	**formalin**	**O.C.T.**
Arthropoda	2	2	2	1	
Chordata	1	1	1		
Cnidaria	11	11	11	3	
Echinodermata	103	99	117	22	42
Mollusca		1			
Porifera	12	15	12		5
Hemichordata			3		
Total	**129**	**129**	**146**	**26**	**47**

Over half of the subsamples taken (264 out of 474) were from specimens belonging to species targeted for population genomic studies. Subsamples from several different tissues were taken for carrying out a wide range of 'oomic' studies (e.g., microbiome, spatial trancriptomics). There were a considerable number of subsamples taken for the sea cucumbers *Psychronaetes hanseni* (Table 15.4.1.2) and Elpidiidae sp. (Table 15.4.1.3) compared to the sea urchin *Plesiodiadema globulosum* (Table 15.4.4), which had much smaller body sizes.

Table 15.4.1.2. Total tissue subsampled from Psychronaetes. hanseni organised by the different fixation methods implemented.

Subsample tissue	**Fixation method**					**Total**
	Snap	**RNAlater**	**etoh96**	**formalin**	**OCT**	
Muscle	19	14	12		10	**55**
body wall			3			**3**
gonads	5	7	6	6	12	**36**
mouth		6	8			**14**
middle intestine	8	4	7		8	**27**
cloaca	7	6	8			**21**
respiratory tree			3	1		**4**
unidentified tissue			1			**1**
Total	**39**	**37**	**48**	**7**	**30**	**161**

Table 15.4.1.3. Total tissue subsampled from Elpiididae sp organised by the different fixation methods implemented

Subsample tissue	Fixation method					**Total**
	Snap	**RNAlater**	**etoh96**	**formalin**	**OCT**	
Muscle	8	8	7		7	30
body wall						0
gonads	2	2	1	2	2	9
mouth		8	8			16
middle intestine	5	5	5			15
cloaca	7	7	7			21
Total	**22**	**30**	**28**	**2**	**9**	**91**

Table 15.4.1.4. Total tissue subsampled from Plesiodiadema globulosum organised by the different fixation methods implemented.

Subsample tissue	Fixation method		**Total**
	Snap	**etoh 96%**	
gonads	4	2	**6**
other tissues	4	2	**6**
Total	**8**	**4**	**12**

15.4.2 Food webs

Louisa Norman and Rachel Jeffreys, University of Liverpool.

Stable isotopes of ^{13}C and ^{15}N of faunal tissues will be used to quantify characteristics of food webs including, trophic position, food chain length, and trophic niche width (Jackson et al., 2011; Layman et al., 2012). Conservative ^{15}N-amino acids (e.g. phenylalanine) will be used to indirectly characterise the base of the food web (POM) & 'trophic' amino acids (e.g. glutamic acid) will be characterised in the tissues to determine trophic position & food chain length (Chikaraishi et al., 2009). Carbon isotopes in essential amino acids (^{13}C-EAA) and lipid distributions in faunal tissues will be used to fingerprint food sources in the deposit feeding holothurians (McMahon et al.,2018; Jeffreys et al.,2009; van Oevelen et al., 2018)

15.4.2.1 Megafaunal samples

Megafaunal sampling during ROV Isis dives were led by the team from NHM. Details of the collections and dissection of the faunal samples can be found earlier in this section. Samples for the food web studies were taken where sufficient fauna and tissue was available. In summary, longitudinal muscle, body wall, hind gut samples were taken from four *Psychronaetes hanseni*, three collected at the OMCO track site and one from the OMCO control site, and from three Elpiididae sp. collected from the OMCO track site. Stable isotopes of C and N, ^{15}N & ^{13}C amino acids and lipid biochemistry will be analysed from these tissues. Additionally, cloaca, gonad and respiratory tree samples were taken from these same individuals for fatty acid desaturase gene analysis which is part of a NEOF pilot funding project. At the OMCO track and control sites, five *Plesiodiadema globulosum*, two and three individuals, respectively, were collected. Due to the size of the individuals, only small amounts of gonad tissue were taken from each and so lipid biochemistry may not be possible for these. Tissue samples were placed in cryovials and either frozen at - 80˚C (isotope, amino acids, lipids) or snap frozen in liquid nitrogen and then stored at - 80˚ C (fatty acid desaturase genes). Details of samples collected including, station number, species, Liverpool ID, NHW voucher number, size, tissue collected and analysis can be found in table 15.4.2.1.1.

Table 15.4.2.1.1 Details of megafauna samples collected for stable isotope, amino acid and lipid analysis at OMCO including, station number, species, Liverpool ID, size, tissue collected and analysis to be conducted.

Station	Location	Species	Liverpool ID (NHM voucher No)	Size (Length or Height & Width, mm)	Samples taken	Analysis at Liverpool
JC241_069 ROV 406	OMCO-Track	*Psychronaetes hanseni*	P.hanseni_1	L = 322mm W = 106mm	4 x Muscle 4 x Body wall 2 x Hind gut	1. Stable isotopes of C &N; 2. d15N & d13C amino acids; 3. Lipid biochemistry
			P.hanseni_1	L = 322 mm W = 106mm	1 x Muscle, 1 x Body wall, 1 x Hind gut, 1 x Cloaca, 1 x Gonad/Resp.tree	Fatty acid desaturase genes (NEOF's pilot funding project).
		Psychronaetes hanseni	P.hanseni_2	L = 412 mm W = 141 mm	4 x Muscle 4 x Body wall 3 x Hind gut	1. Stable isotopes of C &N; 2. d15N & d13C amino acids; 3. Lipid biochemistry
			P.hanseni_2	L = 412 mm W = 141 mm	1 x Muscle, 1 x Body wall, 1 x Hind gut, 1 x Cloaca, 1 x Gonad/Resp.tree	Fatty acid desaturase genes (NEOF's pilot funding project).
		Psychronaetes hanseni	P.hanseni_3	L = 304 mm W = 224 mm*	4 x Muscle 4 x Body wall 3 x Hind gut	1. Stable isotopes of C &N; 2. d15N & d13C amino acids; 3. Lipid biochemistry
			P.hanseni_3	L = 304 mm W = 224 mm*	1 x Muscle, 1 x Body wall, 1 x Hind gut, 1 x Cloaca, 1 x Gonad/Resp.tree	Fatty acid desaturase genes (NEOF's pilot funding project).
JC241_070 ROV 408	OMCO-Track	*Plesiodiadema globulosum*	P.globulosum_1		1 x Gonad	1. Stable isotopes of C &N; 2. d15N & d13C amino acids (both analyses only if sufficient material available).
		Plesiodiadema globulosum	P.globulosum_2		1 x Gonad	1. Stable isotopes of C &N; 2. d15N & d13C amino acids (both analyses only if sufficient material available).
JC241_073	OMCO-Track	Elpiididae sp.	Sea pig_1	L = 83 mm W = 60 mm	2 x Muscle 4 x Body wall	1. Stable isotopes of C &N; 2. d15N & d13C amino acids; 3. Lipid biochemistry
			Sea pig_1	L = 83 mm W = 60 mm	1 x Muscle, 1 x Body wall, 1 x Hind gut, 1 x Cloaca, 1 x Gonad	Fatty acid desaturase genes (NEOF's pilot funding project).
		Elpiididae sp.	Sea pig_2	L = 95 mm W = 65 mm	3 x Muscle 4 x Body wall 4 x Hind gut	1. Stable isotopes of C &N; 2. d15N & d13C amino acids; 3. Lipid biochemistry
			Sea pig_2	L = 95 mm W = 65 mm	1 x Muscle, 1 x Body wall, 1 x Hind gut, 1 x Cloaca,	Fatty acid desaturase genes (NEOF's pilot funding project).
		Elpiididae sp.	Sea pig_3	L = 76 mm W = 58 mm	1 x Muscle 4 x Body wall	1. Stable isotopes of C &N; 2. d15N & d13C amino acids; 3. Lipid biochemistry
			Sea pig_3	L = 76 mm W = 58 mm	1 x Muscle, 1 x Body wall, 1 x Hind gut, 1 x Cloaca N.B. Parasitic gastropod present where gonads would be.	Fatty acid desaturase genes (NEOF's pilot funding project).
	OMCO-Control	*Psychronaetes hanseni*	P.hanseni_4	L = 378 mm W = 101 mm	4 x Muscle 4 x Body wall 4 x Hind gut	1. Stable isotopes of C &N; 2. d15N & d13C amino acids; 3. Lipid biochemistry
			P.hanseni_4	L = 378 mm W = 101 mm	1 x Muscle, 1 x Body wall, 1 x Hind gut, 1 x Cloaca, 1 x Gonad/Resp.tree	Fatty acid desaturase genes (NEOF's pilot funding project).
		Plesiodiadema globulosum	P.globulosum_3	L = 21 mm W = 19.3 mm	1 x Gonad	1. Stable isotopes of C &N; 2. d15N & d13C amino acids (both analyses only if sufficient material available).
		Plesiodiadema globulosum	P.globulosum_4	L = 19.4 mm W = 18.3 mm	1 x Gonad	1. Stable isotopes of C &N; 2. d15N & d13C amino acids (both analyses only if sufficient material available).
		Plesiodiadema globulosum	P.globulosum_5	L = 17.2 mm W = 16.1 mm	1 x Gonad	1. Stable isotopes of C &N; 2. d15N & d13C amino acids (both analyses only if sufficient material available).

15.4.2.2 Fish trap samples

Details of the fish trap deployments can be found in section 16 (Scavengers, Sweetman and Hartl)

Nine muscle samples, two cusk eel and seven rattail, from four fish trap deployments were provided by A. Sweetman and M. Hartl. In addition, triplicate samples of the amphipod *Paralicella tenuipes* were obtained from three of the deployments. Fish muscle samples were bagged, labelled with the station number, AKS number, species and tissue type. Amphipods were placed in cryovials, labelled with station number, species and number of individuals. All samples were placed at - 80˚ C for storage. All analyses will be undertaken at the home laboratory at the University of Liverpool. Details of samples collected including station number, species, Liverpool ID, AKS number, tissue collected and analysis to be conducted can be found in table 15.4.2.2.1.

Table 15.4.2.2.1. Details of samples collected for stable isotope, amino acid and lipid analysis at OMCO including, station number, species, Liverpool ID, AKS number, tissue collected and analysis to be conducted.

Station & AKS no.	Latitude N	Longitude W	Deployment location & water depth	Collected	Samples taken	Analysis at Liverpool
JC241_015 AKS339	13˚44.1618	- 126˚12.0320	OMCO 4668 m	Fish 2_Cusk Eel	1 x muscle (~25 x 25 mm)	Bulk stable isotopes of C and N, d15N & d13C amino acids. Lipid biochemistry if material available
				Amphipod *Paralicella. tenuipes*	3 x 8 large individuals (>5 mm) 3 x 16 small individuals (<5 mm)	Bulk stable isotopes of C and N, d15N & d13C amino acids Lipid biochemistry if material available
JC241_027 AKS340	13˚44.1510	- 126˚12.9010	OMCO 4722m	Fish 3_Rattail Fish 4_Rattail Fish 5_Rattail Fish 6_Rattail	1 x muscle (~25 x 25 mm) 1 x muscle (~25 x 25 mm) 1 x muscle (~25 x 25 mm) 1 x muscle (~25 x 25 mm)	Bulk stable isotopes of C and N, d15N & d13C amino acids Lipid biochemistry if material available
JC241_056 AKS341	13˚43.0199	- 126˚11.0262	OMCO - Track 4580 m	Fish 7_Cusk Eel	1 x muscle (~25 x 25 mm)	Bulk stable isotopes of C and N, d15N & d13C amino acids Lipid biochemistry if material available
				Fish 8_Rattail	1 x muscle (~25 x 25 mm)	Bulk stable isotopes of C and N, d15N & d13C amino acids Lipid biochemistry if material available
				Amphipod *Paralicella. tenuipes*	2 x 11 individuals 1 x 10 individuals Mixed sizes	Bulk stable isotopes of C and N, d15N & d13C amino acids Lipid biochemistry if material available
JC241_064 AKS342	13˚44.8420	- 126˚13.2110	OMCO – Depression 4732m	Fish 9_ Rattail	1 x muscle (~25 x 25 mm)	Bulk stable isotopes of C and N, d15N & d13C amino acids Lipid biochemistry if material available
				Amphipod *Paralicella. tenuipes*	3 x 11 individuals, mixed sizes	Bulk stable isotopes of C and N, d15N & d13C amino acids Lipid biochemistry if material available

15.4.3 Life history

To deliver on one of SMARTEX objectives related to life-history studies, all specimens collected (including macrofauna from the box cores) were analysed for the presence of eggs or any gonadal tissue (Fig. 15.4.3.1). When present, these tissues were removed and fixed in formalin. A total of 30 subsamples were taken for life history analyses (Table 15.4.3.1). Echinoderms comprised 80% of life history samples, but this group was also the most sampled. Holothurian, asteroid, and cnidarian gonadal tissue was obtained by carefully dissecting the specimens to preserve morphological characters.

Figure 15.4.3.1. Example of gonads recovered from the sea cucumber Psychropotes verrucicaudatus*.*

Table 15.4.3.1. Count of JC241 life history subsamples fixed in formalin per phyla.

Phylum	No. subsamples
Arthropoda	2
Cnidaria	3
Echinodermata	24
Metazoa	1
Total	**30**

Table 15.4.3.2. Detail of specimens subsampled during JC241 for life history analyses.

Deployment Number	Site	Sampler	Sample Number	NHM_#	Phylum	ID
JC241_013	OMCO Track	ROV	Dive399	10396	Arthropoda	Munidopsis
JC241_013	OMCO Track	ROV	Dive399	10399	Echinodermata	Freyella
JC241_018	OMCO Control	BC		10465	Echinodermata	cf. Ophiohelus
JC241_51	PRA_04	BC		10907	Arthropoda	Isopoda
JC241_67	OMCO Track	BC		11033	Echinodermata	Plesiodiadema globulosum
JC241_69	OMCO Track	ROV	DIVE406	11100	Echinodermata	Psychronaetes hanseni
JC241_69	OMCO Track	ROV	DIVE406	11117	Echinodermata	Psychronaetes hanseni
JC241_69	OMCO Track	ROV	DIVE406	11135	Echinodermata	Psychronaetes hanseni
JC241_73	OMCO Track	ROV	DIVE407	11228	Echinodermata	Dytaster sp.
JC241_73	OMCO Track	ROV	DIVE407	11261	Echinodermata	Elpidiidae
JC241_73	OMCO Track	ROV	DIVE407	11274	Echinodermata	Elpidiidae
JC241_73	OMCO Track	ROV	DIVE407	11386	Echinodermata	Brisingida
JC241_73	OMCO Track	ROV	DIVE407	11399	Cnidaria	Anemone
JC241_73	OMCO Track	ROV	DIVE407	11408	Cnidaria	Anemone
JC241_76	OMCO Track	ROV	DIVE408	11427	Cnidaria	Anemone
JC241_76	OMCO Track	ROV	DIVE408	11436	Echinodermata	Psychropotes verrucicaudatus
JC241_76	OMCO Track	ROV	DIVE408	11441	Echinodermata	Benthodytes marianensis?
JC241_76	OMCO Track	ROV	DIVE408	11467	Echinodermata	Psychronaetes hanseni
JC241_76	OMCO Track	ROV	DIVE408	11469	Echinodermata	Psychronaetes hanseni
JC241_76	OMCO Track	ROV	DIVE408	11496	Echinodermata	Psychronaetes hanseni
JC241_76	OMCO Track	ROV	DIVE408	11522	Echinodermata	Dytaster sp.
JC241_81	OMCO Control 2	BC		11598	Metazoa	Nemertea?
JC241_89	OMCO Control	ROV	DIVE411	11828	Echinodermata	Amperima
JC241_89	OMCO Control	ROV	DIVE411	11855	Echinodermata	Psychronaetes hanseni
JC241_89	OMCO Control	ROV	DIVE411	11893	Echinodermata	Elpidiidae
JC241_095	UK-1	ROV	DIVE412	11955	Echinodermata	Mesothuriidae
JC241_095	UK-1	ROV	DIVE412	12000	Echinodermata	cf. Benthodytes
JC241_095	UK-1	ROV	DIVE412	12016	Echinodermata	Synallactidae
JC241_095	UK-1	ROV	DIVE412	12022	Echinodermata	Molpadiodemas
JC241_095	UK-1	ROV	DIVE412	12033	Echinodermata	cf. Ophiuroglypha

15.4.4 Colonisation

Recolonisation after a disturbance event is of great interest, and relevant if deep-sea mining activities take place. During the cruise, there was the opportunity to collect and visit sites with human-made structures deployed within known time ranges. The recovery and imagery of these can provide insight into colonisation rates in the CCZ.

During ROV ISIS Dive 403 (27 Feb 23:28 UTC) a mooring was discovered to the NW of the northern part of the track site (location 13°44.3435’N 126°13.2910’W). This was around 10 m long with an intact glass sphere and an instrument below (Figure 15.4.4.3). It was ballasted with an iron weight. We did not get very close because of potential danger to the ROV, but we could see evidence of colonizing anemones in the pilot video.

During the ROV Isis Dive 408, a piece of metal pipe possibly from the frame of the OMCO collector vehicle was found (13°44.331’N 126°13.237’W) and recovered (Fig. 15.4.4.1). This piece has been on the seabed for about 40 years, and had at least two serpulid worms, a scyphozoan polyp, and another polychaete tube attached (Fig. 15.4.4.2).

Figure 15.4.4.1. Piece of metal pipe possibly from the frame of the OMCO collector vehicle. Credit: NHM JC241 Team

During Dive 411 another mooring was found (12 Mar 2023 15:50) in the control area at 13°43.925’N 126°12.276’W. This had a ring of 4 intact glass spheres on a rope to a heavy instrument (Figure 15.4.4.4), which was attached with a rope and what looked like an electrical cable to a large box on the seafloor (possibly a battery). The box may have “78” written on it, potentially indicating its year of manufacture.

Figure 15.4.4.2. Detail of serpulid worms attached to the piece of metal pipe possibly from the frame of the OMCO collector vehicle. Credit: NHM JC241 Team

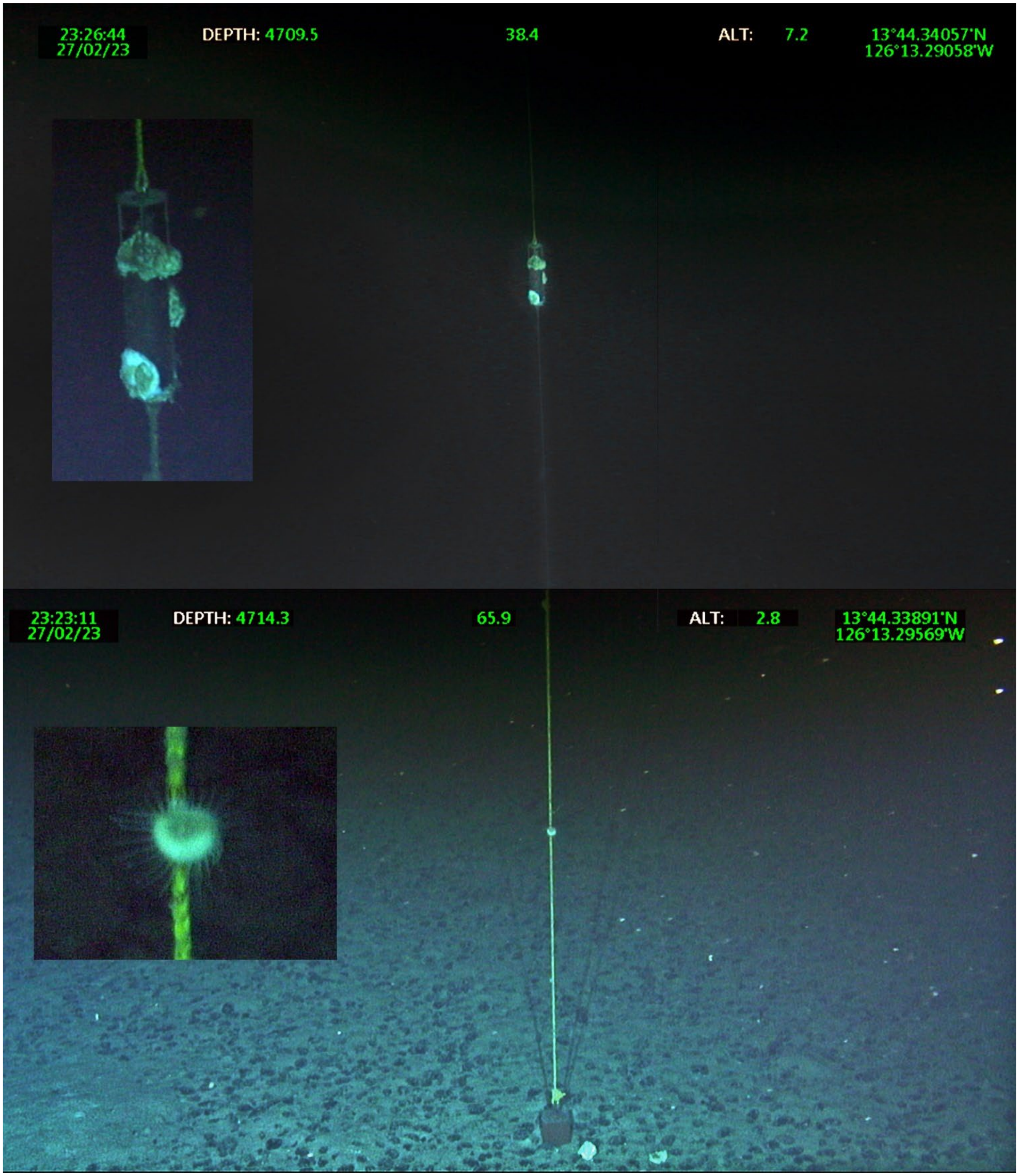

Figure 15.4.4.3 Details of mooring found near north western part of track. Mooring consists of metal weight, polypro line, an unidentified metal instrument and glass sphere buoyancy. Note the anemone growing on the rope. This mooring was expected to have been deployed in 1979.

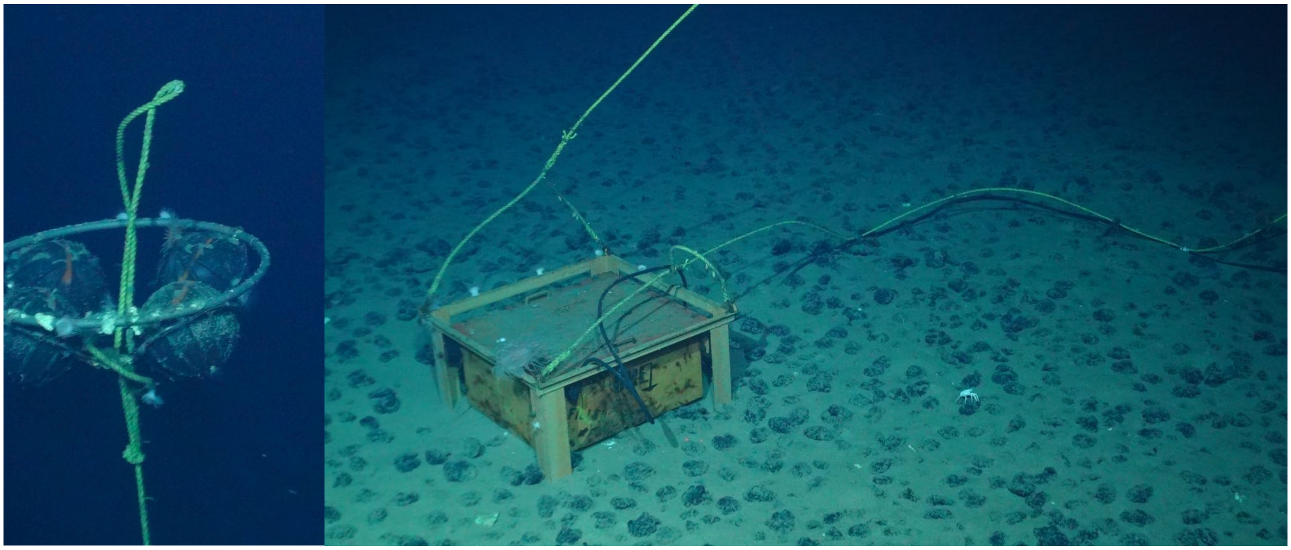

Figure 15.4.4.4 Details of mooring found at control site on 12 March 2023 Dive 411.

In 2013 (Smith et al, 2013), a lander was left on the seabed and this was explored during Dive 412 in the UK-1 area (Fig. 15.4.4.5). Only a small piece of frame was collected, which had no fauna attached after 10 years. The lander was explored in detail during the Dive, using the high-resolution camera Scorpio and no sessile fauna were observed attached to it. The only invertebrates identified were a squat lobster, two brittle stars, and a crinoid.

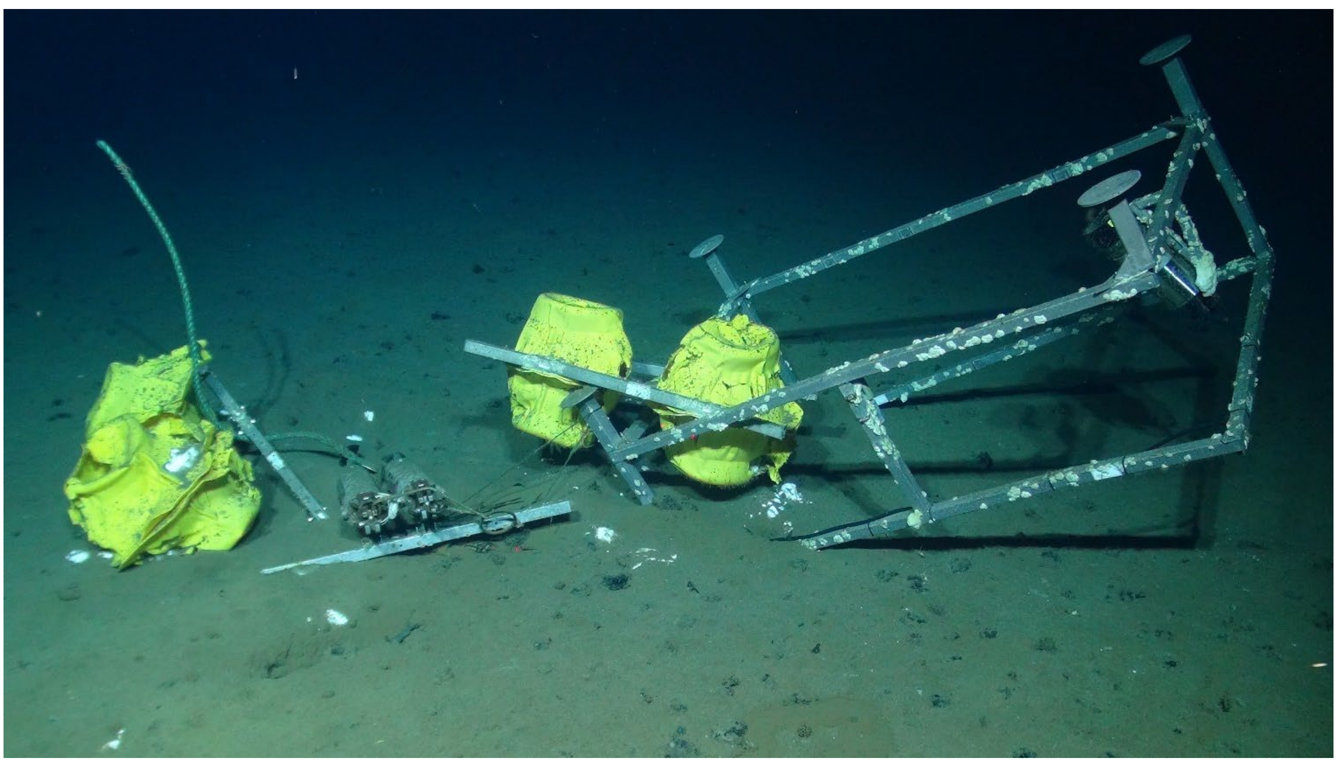

Figure 15.4.4.5. Lander deployed in 2013 in the UK-1 area.

In UK-1, the ROV Isis also dived a site where three colonisation experiments were deployed in 2020 (Fig. 15.4.4.6). These consisted of a 50 x 50 cm PVC frame with ten small pieces of basalt each. The purpose of these were to provide an insight into colonisation rates in the area. While these structures were not recovered, the ROV Isis hovered above each, providing high-resolution imagery of each of the rocks. These images, along with the metal pipe, will be further analysed at the Natural History Museum, London.

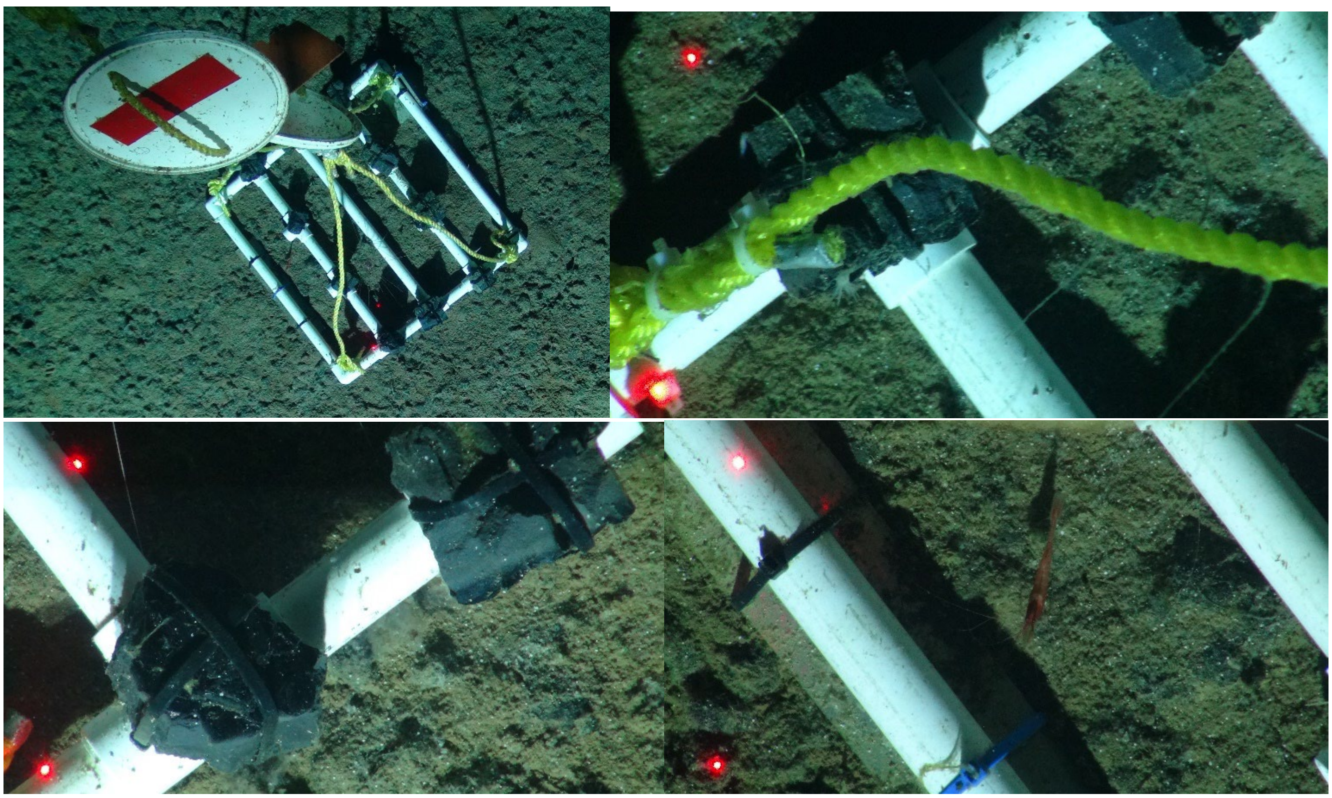

Figure 15.4.4.6. Colonisation experiment on seabed in UK-1 deployed during the RC01 cruise in 2020. Top left: structure with 10 small pieces of basalt. Top right: Detail of a basalt with a cnidarian attached. Bottom left: detail of two basalt fragments. Bottom right: close-up of shrimp near the structure.

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16. Scavenger Collections and Ecotoxicology

16.1 Fish collections and ecotoxicology

Mark G J Hartl – Heriot-Watt University

One aspect of the environmental impact of deep-sea mining is the effect of the resulting sediment plume on benthic and demersal organisms in terms of their toxicological response. Baited fish traps (Figure 16.1 & 16.3) were deployed on the seafloor to catch benthic and demersal scavenging fish species likely to encounter a mining plume and take various tissue samples for ecotoxicological biomarker assessment. The rational on this cruise was to establish baseline values for the species recovered in the trap to be used as a reference for estimating the ecotoxicological impact of future mining operations or other related disturbances.

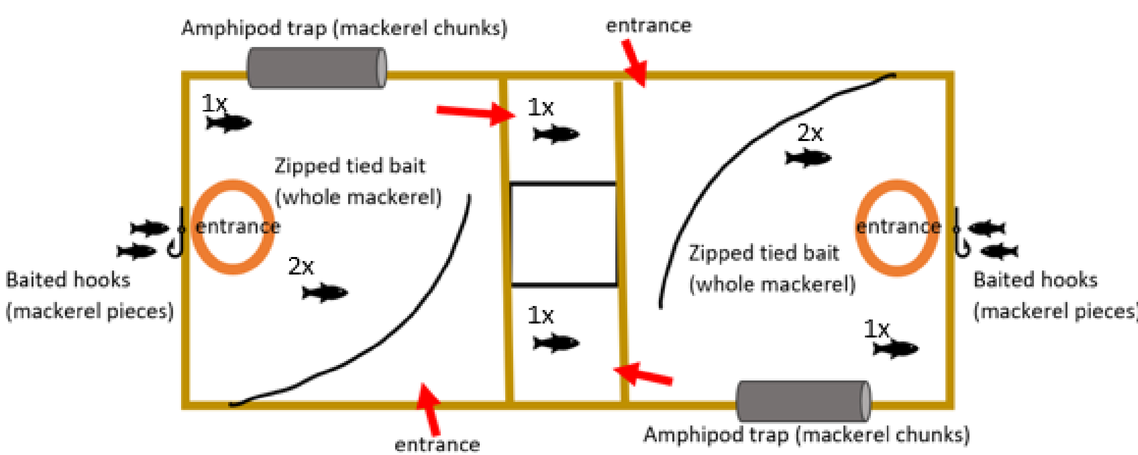

Figure 16.1 Bird's eye view of the fish trap deployed on JC241.

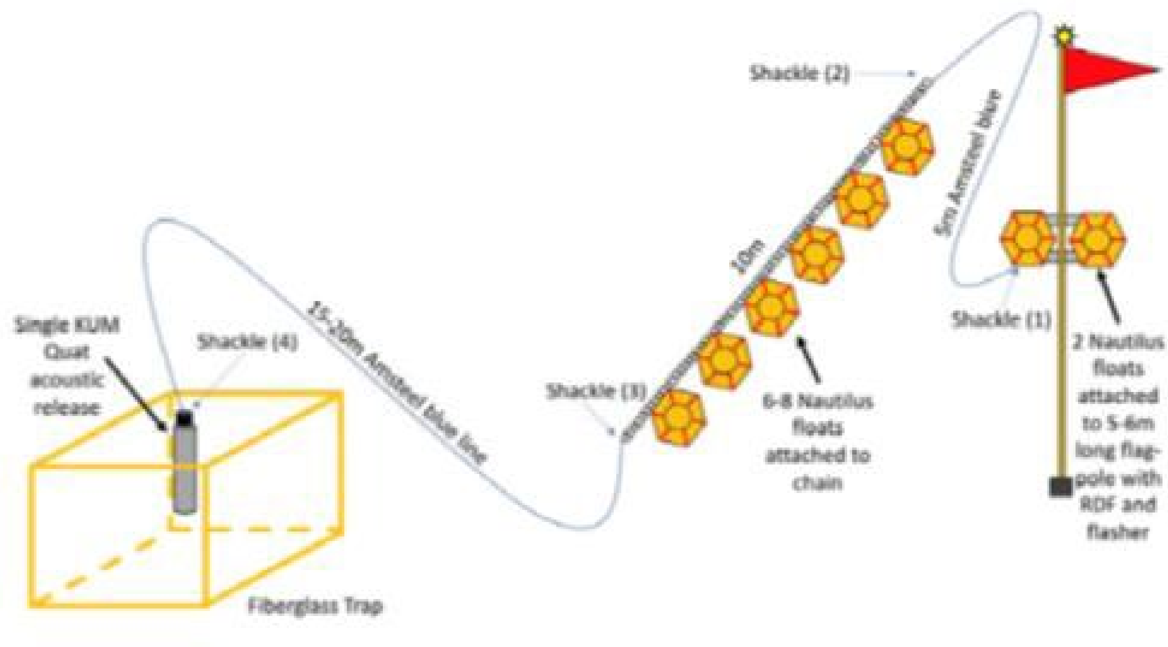

Figure 16.2 Diagrammatic depiction of the fish trap and its typical mooring configuration. Only three Nautilus floats were used on JC241 deployments.

The traps were deployed on 4 occasions for between 36 and 48 hours at depths ranging from 4,580m to 4,732m (Table 16.1; Figure 16.3).

Table 16.1 List of deployments during JC241.

StationID	Reference	Region	Deployment date/time (Ship_time)	Deployment date/time (UTC)	Recovery date/time (UTC)	Position Lat/Long		Depth (m)
JC241-15	AKS339	OMCO	16.02.23_13:30	16.2.23_21:30	18.02.23_01:04	13º44.1618N	126º12.031W	4,668
JC241-27	AKS340	OMCO	19.02.23_09:38	19.2.23_17:58	21.02.23_17:48	13º44.151N	126º12.901W	4,722
JC241_56	AKS341	OMCO	27.02.23_23:09	27.2.23_07:09	29.02.23_03:10	13º43.019N	126º11.026W	4,580
JC241_64	AKS342	OMCO	02.03.23_21:55	03.03.23_05:55	05.03.23_04:14	13º74736N	126º22018W	4,732

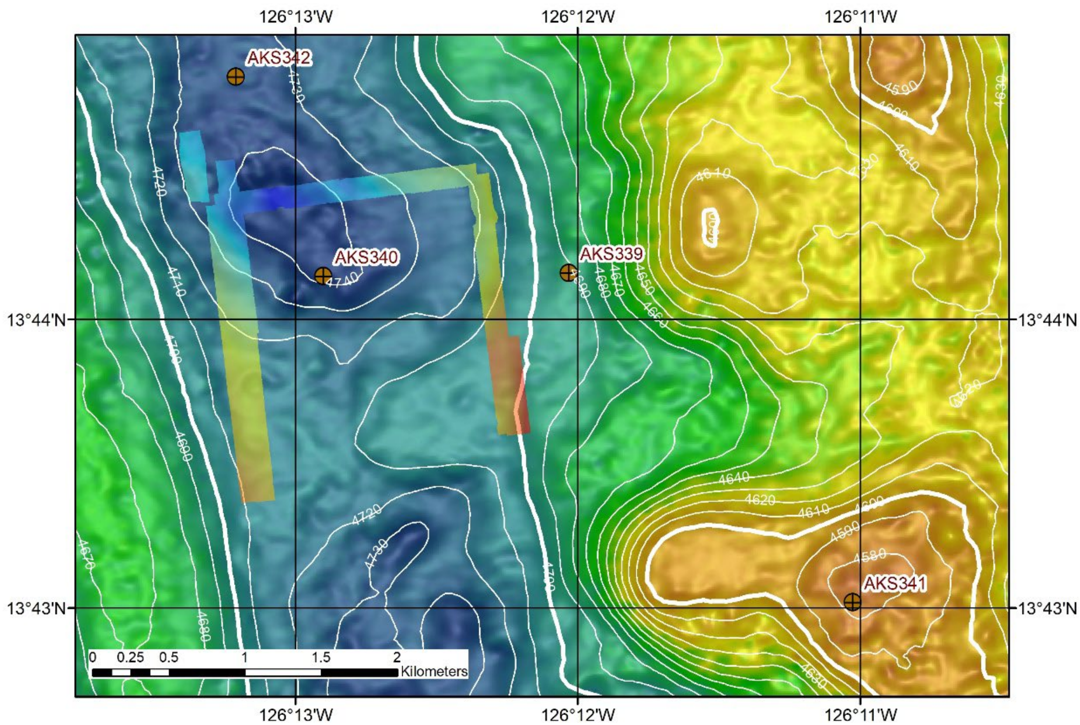

Figure 16.3: OMCO site. Positions of trap deployments.

Nine specimens, 7x *Coryphenoides sp* and 2x *Barathrites sp*, were caught. Fish length was measured, and photos taken for later identification (Figures 16.4-16.12). Specimens were immediately transferred to the CT room and processed.

Figure 16.4 JC241_15; AKS339-1. *Coryphenoides sp*

Figure 16.5 JC241_15; AKS339-2. *Barathrites sp*

Figure 16.6 JC241_27; AKS339-3. *Coryphenoides sp*

Figure 16.7 JC241_27; AKS339-4. *Coryphenoides sp*

Figure 16.8 JC241_27; AKS340-5. *Coryphenoides sp*

Figure 16.9 JC241_27; AKS340-6. *Coryphenoides sp*

Figure 16.10 JC241_56; AKS341-7. *Barathrites sp*

Figure 16.11 JC241_56; AKS341-8. *Coryphenoides sp*

Figure 16.12 JC241_64; AKS342-9. *Coryphenoides sp*

Blood samples were taken and processed for Comet assay on board and also frozen (-80ºC) in 10%DMSO/HBSS as a cryoprotector for analysis back in Edinburgh. The purpose of this was to determine whether the considerable effort of on board electrophoresis would quench the background in DNA strand breaks brought about by the freezing process applied on previous cruises. Gill samples were taken and also immediately processed for Comet assay assessment.

Cell suspensions were prepared as described by Kilemade et al (2004). Briefly, all handling during tissue dissection were performed in a CT room at 4ºC. Dissected gill tissue was transferred to a petri dish, gently minced in 2.5ml HBSS using fresh disposable scalpels, transferred to 15 ml Falcon tubes, and incubated (10 mins at room temp) in 5 ml trypsin (final concentration 0.05%) on a gyro rocker (very slowly). The enzyme digestion was interrupted/reduced by the addition of a further 5ml HBSS, after which the cell suspension was passed through a 40µm cell strainer

Figure 16.13 Lab setup in the chemistry lab of RRS James Cook

was passed through a 40µm cell strainer, centrifuged at 800rpm for 10mins, the supernatant decanted and the resulting pellet resuspended in fresh HBSS.

The Comet assay was performed as described by Coghlan et al (2002). Briefly, two microscope slides per sample were prepared by applying 100 µl 1% NGA to the slide and smearing it out allowing the smear to dry for at least 12 hours resulting in a thin "frosted" layer on the slides for the subsequent gel sandwich to adhere to. Cells were immobilised in the gel sandwich consisting of three separate layers of gel applied to each slide. The three layers were as follows: layer 1; 100 µl 1% NGA, layer 2; 70 µl 1% LMP + 30 µl sample cell suspension, layer 3; 100 µl 1% LMP (Figure 13).

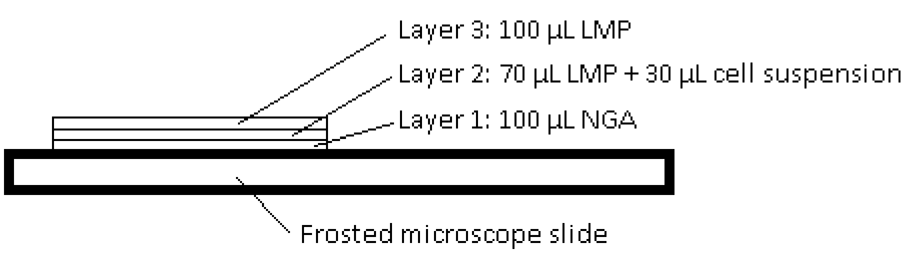

Figure 16.14: Microscope slide with gel sandwich.

After the third layer had set for 15 minutes in at 4°C, the coverslips were removed and the slides placed in a black, light proof lysis tank, containing lysis solution, and stored at 4°C for at least 90 minutes but up to 48 hours. Following the lysis step, all work was performed under non-fluorescent light in the CT room until after the electrophoresis step. The slides were placed in a horizontal electrophoresis tank in a random pattern (all in the same orientation) and covered with electrophoresis solution to allow for alkaline unwinding of the DNA. A custom-built gimbal table (Figure 14) was used to keep the electrophoresis tank stable in a horizontal position so that the electrophoresis buffer in the tank was level at all times and the current flow through the buffer maintained at 300mA, despite the movement of the ship.

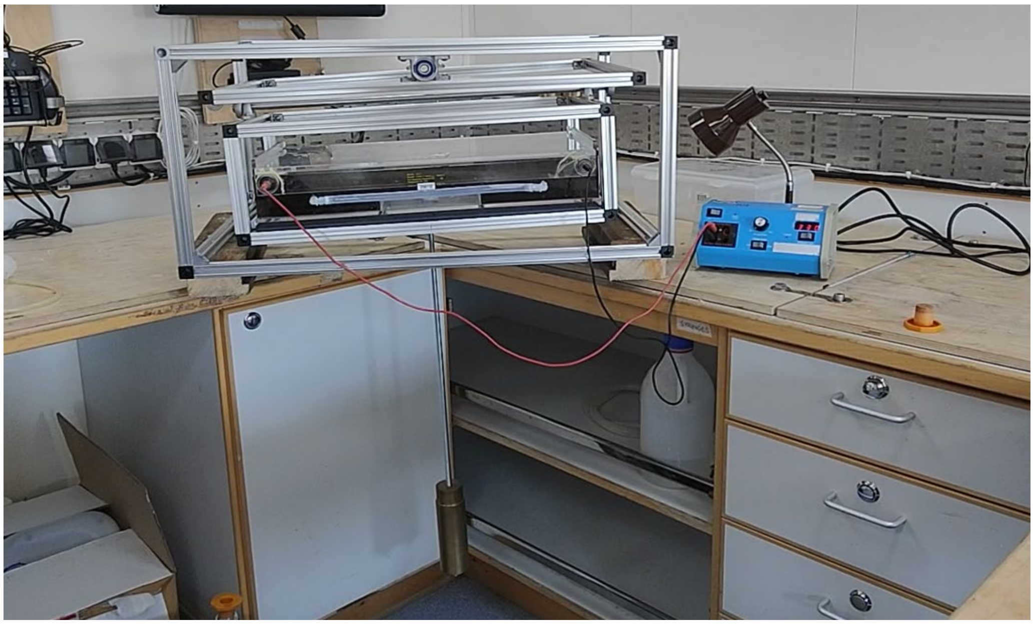

Figure 16.15 Electrophoresis tank mounted on a gimbal table which was screwed to the sacrificial benchtop in the CT room of RRS James Cook.

After 30 minutes the power was turned on and additional electrophoresis solution was added to the tank to afford a current of 300 mA and the slides were left for 25 minutes before turning the power off. The slides were removed from the electrophoresis tank and each gel neutralised by adding three times five drops of neutralisation solution for five minutes, pouring off the excess solution between each neutralisation. The slides were rinsed gently using distilled water and after the excess water was poured off, each gel was stained using five drops of Gelred per slide. After five minutes each slide was rinsed gently using distilled water, air-dried, following the procedure of Woods et al (1999), and stored in a slide box until further image analysis assessment in Edinburgh.

Additionally, further gill tissue samples were shock frozen in liquid nitrogen and preserved for oxidative stress assessment in Edinburgh. Further opportunistic tissue samples taken were: liver samples preserved in 4% formalin for histological assessment; muscle samples were frozen for stable isotope assessment; the stomach was frozen for later microplastic content assessment.

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16.2 Biological samples from the amphipod traps

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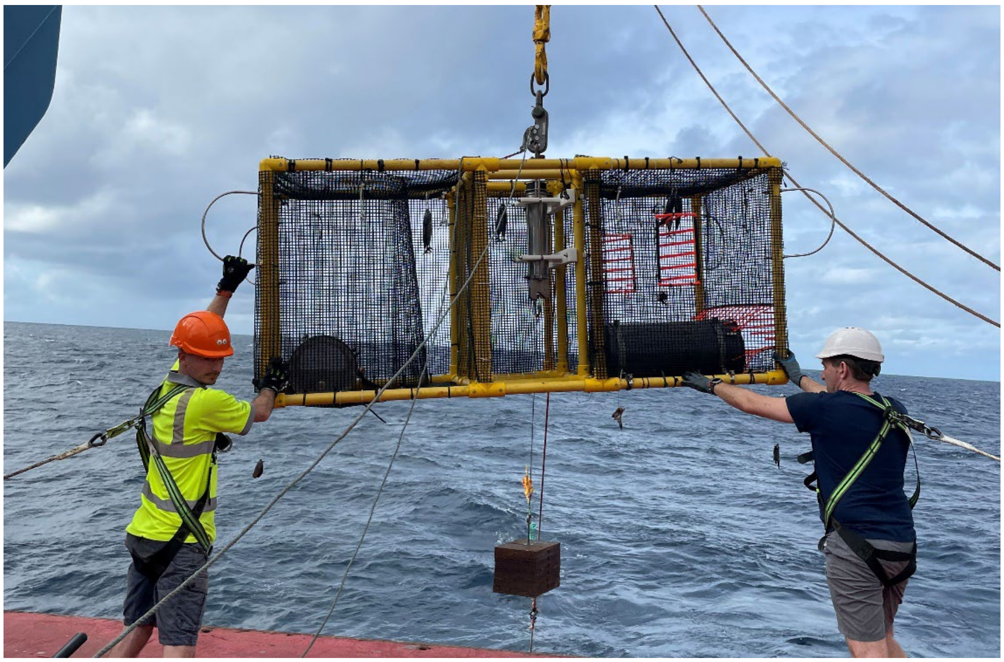

Figure 16.16 Deployment of the fish trap with amphipod traps (grey cylinders) inside.

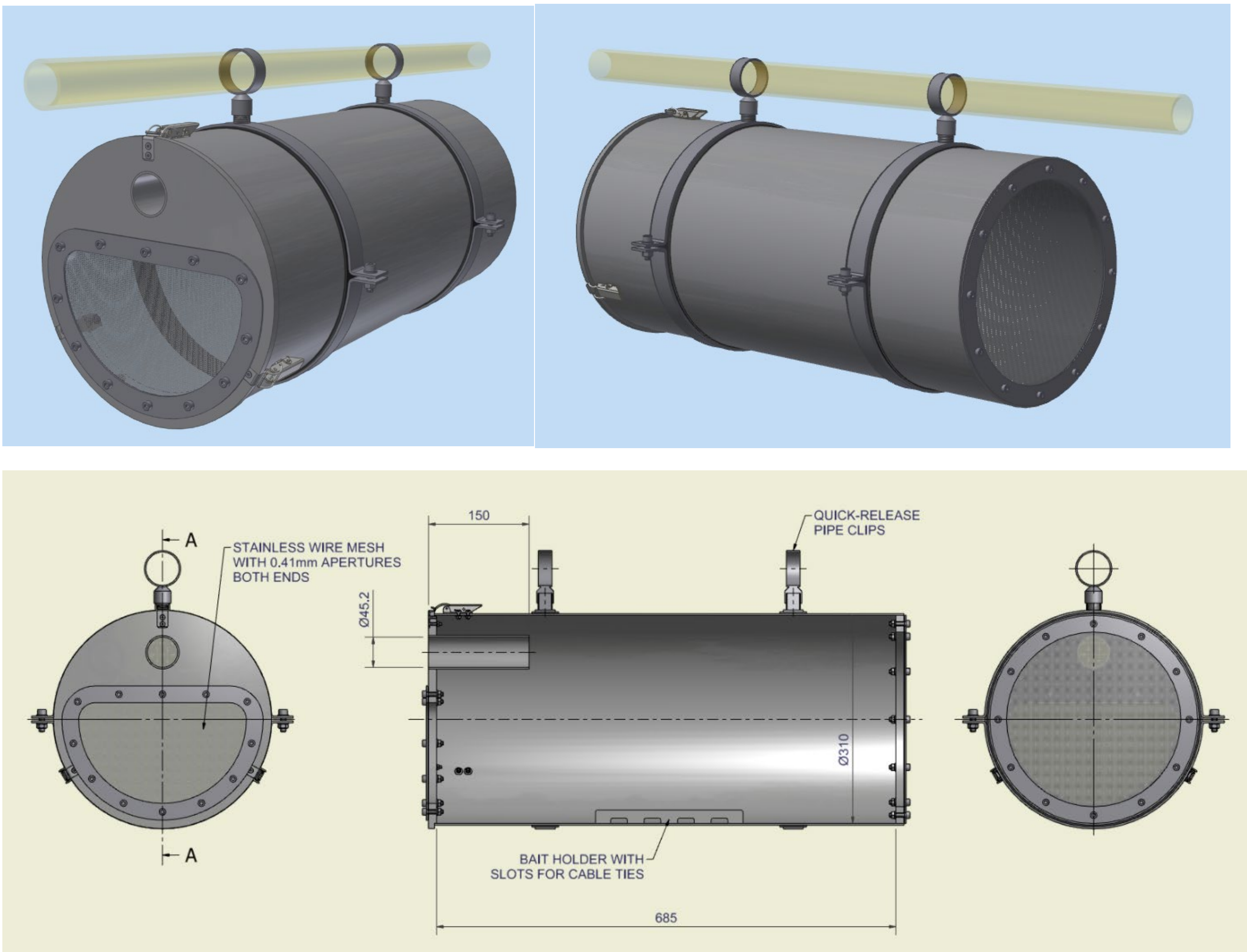

Figure 16.17. Design of amphipod trap. Internal volume = 51.7 litres, construction is rigid PVC and stainless steel. Weight in air ~8kg.

The amphipod traps were deployed in the fish trap lander (Figure 16.17) on the same deployments (Table 16.1). There were two traps (see Figure 16.18 for design) each baited with 2-4 mackerel. There were also baits within the fish trap and outside the amphipod trap, which was unfortunate as they appeared to be more highly scavenged by amphipods than the baits within the traps.

On recovery the specimens from each trap were placed in cold filtered seawater, before being live-sorted under stereomicroscopes by the NHM team. In addition to the traps, amphipod specimens attached to the bait within the fish trap were also collected. Representative individuals from each species were photographed, and specimens were bulk-fixed in 80% ethanol and labelled according to preliminary species ID. Specimens will be returned to the NHM and used for future taxonomic studies. Approximately 30 individuals of *Paralicella tenuipes* from each trap at each deployment were given to the University of Liverpool team and frozen at -80˚ C.

Table 16.2 Summary of amphipod specimens taken by NHM team. AT = Amphipod Trap.

	JC241_015		JC241_027			JC241_058		JC241_064		Total
Species	AT1	AT2	AT1	AT2	Fish bait	AT1	AT2	AT1	AT2	
Abyssorchomene spp.	18		16	4	378	1	1	2	30	450
Eurythenes sp.			1		1		1		1	4
Paralicella cf. caperesca	50	1	0	14	1	6	12	36	33	153
Paralicella cf. tenuipes	150	6	586	114	25	17	3	31	90	1022
Tectovalopsis sp.					1					1
Total	218	7	603	132	406	24	17	69	154	1630

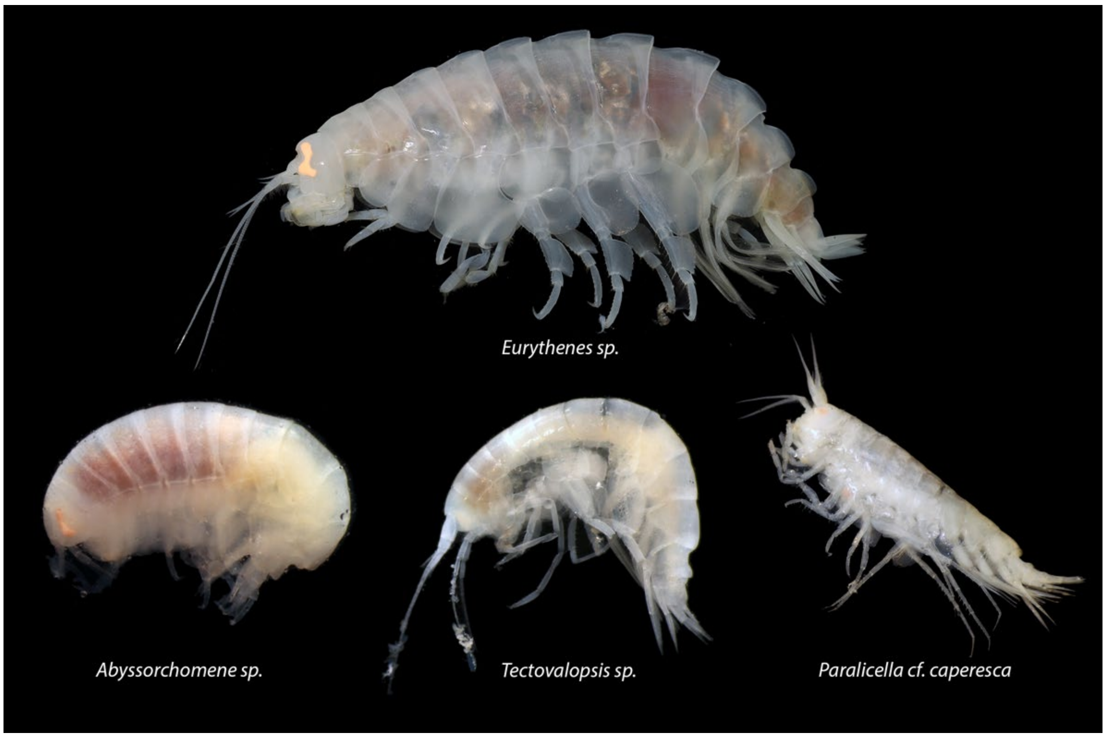

Figure 16.18 Amphipod species recovered from the amphipod trap on JC241 SMARTEX

17. Biological sampling for macrofauna

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17.1 Summary

Spade box core sampling was carried out on JC241 to support two SMARTEX objectives and was highly successful. 43 box core deployments were made, returning 40 samples of which 32 are fully-quantitative. Replicated sampling was achieved at several stations in the CCZ and samples being returned to the NHM London for further analysis by the SMARTEX team.

17.2 Introduction and objectives

The macrofaunal sampling was designed to collect samples to deliver on the SMARTEX Proposal Objectives 5 (Biodiversity, community structure and trophic dynamics) and Objective 4 (Life history, Reproductiong and Connectivity). Further details of these objectives are provided in the SMARTEX proposal, but in summary the main goals were to:

- Collect quantitative, replicated 50x50cm USNEL spade box core samples for macrofauna retained on a 300micron sieve using a randomised stratified design in three areas:
    - OMCO Track (a region disturbed by a trial mining machine in 1979)
    - OMCO Control (a region we designated ~2km to the E of the OMCO Track site as a control)
    - PRA (A preservational area surveyed by NOAA in 1989 using replicated box core samples of the same design we used on SMARTEX)
- Collect macrofauna using high-quality preservational cold-chain methods (sensu Glover et al 2016) that can be used for taxonomic, population genetic and life-history studies
- Provide images of an intact seafloor surface to enable observation and photography of nodule abundance
- Provide quantitative samples of polymetallic nodules to the BGS geological team (see section 11)

17.3 Methods and protocols

17.3.1 Box core deployment and recovery

The NMF marine equipment pool box core, based on the USNEL spade box core design of Hessler & Jumars (1974) was used for all sampling (Figure 17.1). The NMF box core is an all-stainless steel design that mirrors the design of the commercially-available Ocean Instruments BX-650 USNEL spade box core, with the exception that the lower frame does not have an opening to remove the box from the core (the frame is instead lifted off the box after recovery by the winch wire, which in practice worked perfectly well). The box core was maintained and deployed in good order by the NOC techincal team (Jeremy Evans, Howard King and Richard Phipps) and well supported by the ships crew in operation of the winch and safe deployment and recovery.

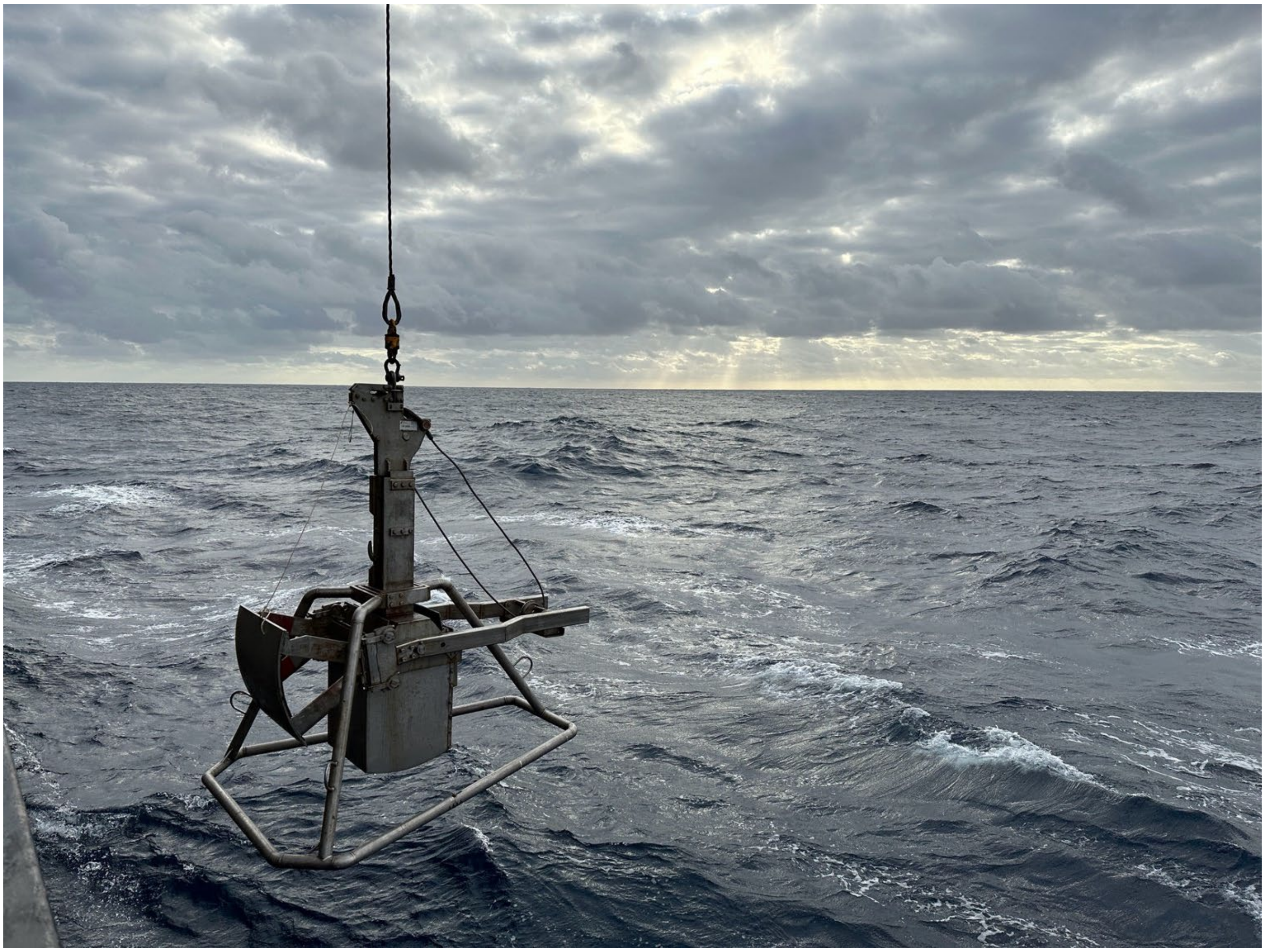

Figure 17.1 The NMF spade box core (USNEL spade box core design) being deployed on SMARTEX JC241 at the OMCO Track site. Photo: AG Glover.

In all cases the box core was deployed from the starboard gantry of the James Cook using the 3x25 core wire. The deployment procedure was as follows:

1. The box core was checked and armed by the NOC technical team and prepared for deployment
2. The ship was placed on station using DP with the positions provided to the bridge centered on the starboard gantry (at the OMCO control site, the positions were only required to be within 100m accuracy)
3. The box core was deployed over the side, safety pins removed and wire-out meter zeroed at the water surface
4. The box core was lowered to 50m and the USBL beacon attached to the wire (only at OMCO Track sites)
5. Lowering then proceeded at 45m/min to within 100m of the seafloor
6. All stop at 100m off bottom to let the box core settle for 5mins
7. Lowering then proceeded at 20m/min (adjusted after some initial failures at 10m/min) until touchdown was recorded on the tensiometer (Figure 17.2)
8. Additional 10m of wire paid out after touchdown
9. All stop at max wire out, with position of ship and USBL recorded
10. Haul at 10m/min until max tension recorded and box core off bottom (max Te, max wire out and positions all logged in the Station Log)
11. Haul at 60m/min to surface

12. Box core landed on deck with the box/spade allowed to rest on the deck surface to prevent loss of sample, while dolly cart is wheeled under the box and jacked up.
13. The retaining nuts and cams that hold the box and spade in are released
14. The box is lifted with the winch wire about 1m off the deck and the box, lower part of the spade arm are wheeled out to be secured for sampling in a shaded area by the biology team
15. The box core safety pins are placed in the box core and the corer lowered to the deck and secured.

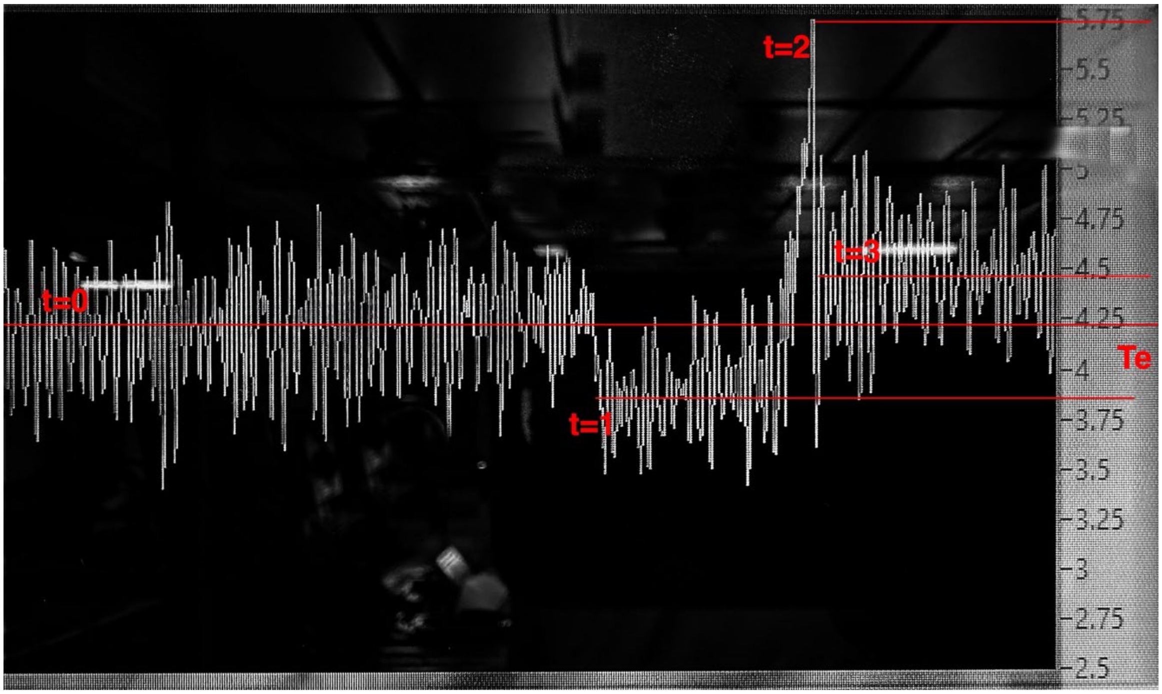

Figure 17.2 Graphical read out from the James Cook winch tensiometer during box core deployment on the seafloor. t=0 the box core is being lowered at 10m/min; t=1 the box core has touched down and ~0.5 ton of weight has come off the wire; t=2 after hauling at 10m/min the box core spade has closed and tension has peaked at 5.75 ton (at ~4800m wire out); t=3 the box core is released from the seabed and tension settles at approximately 0.25 ton larger than t=0 indicating a successful sample has been taken. Y-axis Te is Tension in metric tons. Image: AG Glover.

Figure 17.3 The box core is secured on deck, the box and spade arm cams released and the box wheeled out using a dolly cart while the corer head is raised 1m by the winch wire. Photo: AG Glover.

17.3.2 Cold Filtered Sea Water (CFSW) system

With surface water temperatures in the CCZ being approximately 27C and bottom water at 1.8C it is essential to have a seawater chilling system to chill large volumes of seawater that can be used to sieve the large volume of sediment recovered (Glover et al 2016). If CFSW is not used, the specimens are highly degraded and DNA not obtainable. On JC241, 2x 1000l tanks (FDL Packaging IBC 1000L natural metal pallet) were installed in the 4C reefer van on the Mezzanine deck above the deck lab, these were plumbed in with a steady supply of seawater that was passed through a 10 µm water filtration system (Deltaqua BB20FF1RV with 2045PP10 sediment cartridge) and into the tanks by means of a ball-cock. Outflow was at the base of the tanks into a hose that led out of the van where there was an isolator valve, and then onwards across the top of the ROV LARS to the sieving table on the aft deck, starboad side (Figure 17.4). The rate of outflow onto the sieve table was relatively low compared to the volume of seawater in the tanks, thus the water temperature could be maintained, it was typically in the range of 8-9C depending on usage, but in some cases the temperature went up to about 12C during heavy use (owing to the inflow of warm surface water into the tanks by the ball cock). In general the system worked very well but could be improved by having the inflow into the innermost tank and the outflow from the outermost tank, thus increasing the residence time of water in the cold van during heavy use. In addition, the extremely warm conditions and sun at times was likely to cause the reefer van to struggle to maintain the set temperature of 4C.

Figure 17.4. Left- 2 x Cold Filtered Seawater (CFSW) 1000L tanks setup in the 4C reefer van, Right - CFSW supply with 2 hoses to the Sieve table on the aft deck, starboard side. Photo: AG Glover.

17.3.3 Sieve table

An outdoor sieve table placed next to where the box core is processed, with sunshade and large-volume drainage over the side is essential to enable box core processing as the volumes are too large to be taken to labs inside the vessel. The NOC sieve table was used which is a heavy-duty stainless construction with a 1cm mesh size and connected to a drain that leads over the side. This worked perfectly although an additional normal table was required for storing jars, wash bottles, notebooks, cameras and general equipment (Figure 17.5).

Figure 17.5. Complete sieving station with sieve trays, buckets, wash table and extra storage table. Note the sun-shade essential for work in the tropics, and the large volume drainage hose running across the starboard deck. The box core was stored on the right next to the white buckets during sieving operations. Photo: AG Glover.

17.3.3 Box core processing (deck)

Responsible on watch for box core processing were Adrian Glover (midnight - noon) and Guadalupe Bribiesca Contreras (noon-midnight). They were supported by the entire team and additional sieving helpers from the other SMARTEX work teams. The support was hugely appreciated. The box core processing protocol took place in two main stages - (1) the deck processing of the core which included nodule washing, slicing and sieving and (2) the laboratory processing which included the cold-chain live sorting process for the nodules and sub-sample of the sediment. In general procedures followed Glover et al (2016) with the addition of steps for quantitative analysis of the fauna.

General box core deck processing protocol (Figure 17.6/7)

1. PREPARATION when BC 500m from surface.
    a. Live Sort / Nodule Wash Bucket (x2), 0-2cm Bucket, 2-5cm Bucket, 5-10cm Bucket (x2) half-filled with Cold Filtered Seawater (CFSW) by sieve station
    b. Nodule/megafauna Tupperware tubs x4 with CFSW (with ice baths)
    c. BC topshot camera with label and marker pen
    d. Jars for samples (250ml jars for 0-2cm live sort, 0-2 cm, 2-5cm, 5-10cm quantitative
    e. Chilled 99% ethanol on ice for initial sample fixation (this is later transferred to 80% ethanol for longer-term storage)
    f. Misc sieving equipment: sieve trays, hoses, turkey basters, forceps, wash bottles, slicing trowels, temperature log
2. Complete STATION LOG in Main Lab (Time in water, Time on bottom, Max wire out, Depth, Position on bottom, Max pull out tension, Time on deck, Core quality (after examining -e.g good core / top water lost / slumped / over penetrated etc)

3. GLOVES ON.
4. MOVE box core to shade and secure
5. Measure TEMP of Sed-Water interface > add to STATION LOG
6. DRAIN topwater to 1-2cm above sediment using hose on 300µm sieve > wash into LIVE SORT/nodule wash bucket
7. DRAIN remaining topwater with hose/or turkey basters into LIVE SORT /nodule wash bucket
8. TOPSHOT photograph. COLOUR BAR and JC241_ station label. Use BoxCore Canon 600D. Spare battery in deck lab.
9. MEGAFAUNA / Xenophyophores. Macrophotos of megafauna. Remove any LARGE motile megafauna / best xenos > Tupperware ice baths. (QUICK)
10. MICROBIOLOGY / eDNA. Nodules without fauna & sediment sample. NOC LEAD.
11. NODULE PICKING & WASH. > wash into LIVE SORT / NODULE WASH bucket. Nodules with fauna > Tupperware ice trays and lab. Nodules without fauna > Geo nodule buckets 0-2 / 2-5 / 5-10 / >10cm (label with JC241_XX)
12. LIVE-SORT Sub-sample 0-2cm. Cut 15x15cm 0-2 into LIVE SORT / NODULE WASH bucket with trowels > Sieve station
13. QUANTITATIVE sample 0-2cm. Cut remaining 0-2cm layer in 0-2cm bucket > Sieve station
14. QUANTITATIVE sample 2-5cm. Cut 2-5cm layer in 2-5cm bucket. > Sieve station
15. QUANTITATIVE sample 5-10cm. Cut 5-10cm layer in 5-10cm bucket. > Sieve station
16. [Optional: if time allows shovel remaining mud onto sieve station 1cm mesh and wash through with DECK HOSE SEAWATER not CFSW for deep burrowing megafauna]
17. SIEVING. Sieve LIVE SORT / 0-2cm / 2-5cm / 5-10cm in trays with 300micron sieve underwater in CFSW. If doing back-to-back coring, do not use CFSW for 5-10cm layer, use deck hose.
18. BURIED NODULES. Pass to GEO buckets for 0-2, 2-5, 5-10 >10cm layers. > BGS
19. CLEAN UP. Clean up box and cart with deck hose near side of ship. Power wash all sieves and tools upside down on table to remove debris.

The end result of the protocol outlined above was in general the following samples ready for storage or further analysis at sea:

- Free-living megafauna where present ready for lab photography, ID and fixation (Figure 17.6/7)
- Nodules with fauna on for immediate photography, ID and fixation in the lab
- 0-2cm 15x15cm 300 micron sieved sub-sample ready for cold-chain live-sorting process in the lab
- 0-2cm, 2-5cm and 5-10cm quantitative sample residues from sieving on 300micron sieves - these are kept cold on 96% ethanol until the live-sort process is finished
- Nodules without fauna to be transferred to geology team (the nodules with fauna are then returned to this sample)
- Depending on other team needs the following additional samples were taken from the box core without impacting the quantitative methodology:
    - 3x microbiology push core samples (3cm wide) from the centre of the core (Susan Evans / NOC group)
    - 3x nodules for microbiology (Susan Evans / NOC Group)
    - nodules for oxygen production experiments (Sweetman group / SAMS)

Figure 17.6 Deck processing of box cores on JC241 (1 of 2) Left to right from top row: 1) preparation of jars, ethanol, ice, camera equipment, 2) box core with clear topwater being moved to shade, 3,4) draining of topwater, 5) megafauna (Plesiodiadema sp.) sitting on surface of box core after draining, 5) washing nodules into live-sort bucket. Photos by AG Glover.

Figure 17.7 Deck processing of box cores on JC241 (2 of 2) Left to right from top row; 1) slicing the core, 2) sieving the sliced layers of mud in CFSW, 5) detail of sieving process showing submerged sieve to minimise impact on samples, 6) a Porcellanaster sp asteroid recovered from the surface of the box core. Photos: AG Glover.

17.3.4 Box core processing (lab)

The end result of the deck processing of box cores was a range of samples that were taken into the lab for further processing (Figure 17.8):

1. Free-living megafauna where present ready for lab photography, ID and fixation (Figure 17.6/7)
2. Nodules with fauna on for immediate photography, ID and fixation in the lab
3. Live Sort 0-2cm 15x15cm 300 micron sieved sub-sample ready for cold-chain live-sorting process in the lab
4. 0-2cm, 2-5cm and 5-10cm quantitative sample residues from sieving on 300micron sieves - these are kept cold on 96% ethanol until the live-sort process is finished, then transferred to 80% ethanol

Lab protocols for the free-living megafauna and nodule fauna followed the cold-chain approach of Glover et al (2016). In summary:

- Large (>2cm) megafauna were photographed with a Canon EOS850d with Canon 430EXII speedlights (x2) while submerged in CFSW in water baths that are submerged themselves in larger water baths filled with ice or iced water, a black non-reflective cloth was placed in the larger water bath to create a black background for the images

- Nodules were treated in a similar way with an image of the nodule taken showing the specimen and then subsequently the specimen was removed by scalpel and photographed a second time under a Leica MZ9.5 stereo microscope with trinocular head and Leica phototube attached to a Best Scientific Canon EOS camera adaptor, and finally to a Canon EOS90d also equipped with 2x Canon 430EXII speedlights.
- Individual specimens were placed into small 2ml - 20ml vials pre-filled with chilled 80% ethanol in DI water, the specimen number was obtained from a pre-labelled set of cryo-stickers each with an individual number and barcode, the photograph numbers from any of the cameras were added to the database.

Lab protocols for the live-sort fraction of the quantitative sediment sample followed Glover et al (2016):

- The residue retained on a 300micron sieve was live-sorted in petri dishes kept in ice, specimens were picked out and placed in separate dishes by phylum
- Each specimen was photographed and given an individual barcoded cryo-label, in some cases more than one specimen were placed in the vials if of the same species
- All residues and any unsorted material was returned to the 0-2cm quantitative sample to ensure no loss of data or specimens

Quantitative residues (the residues retained on a 300micron sieve from the slicing of the entire box core surface) comprised 3 samples: 0-2cm, 2-5cm and 5-10cm. These jars (already filled with 96% ethanol from the deck operation) had the liquid decanted onto a sieve and then washed back into the jar with 80% ethanol in DI water, the vial was then topped up with 80% ethanol and stored in the -20 freezer.

Finally, nodules being examined for fauna were returned to buckets with the other nodules from the core and passed to the geology team.

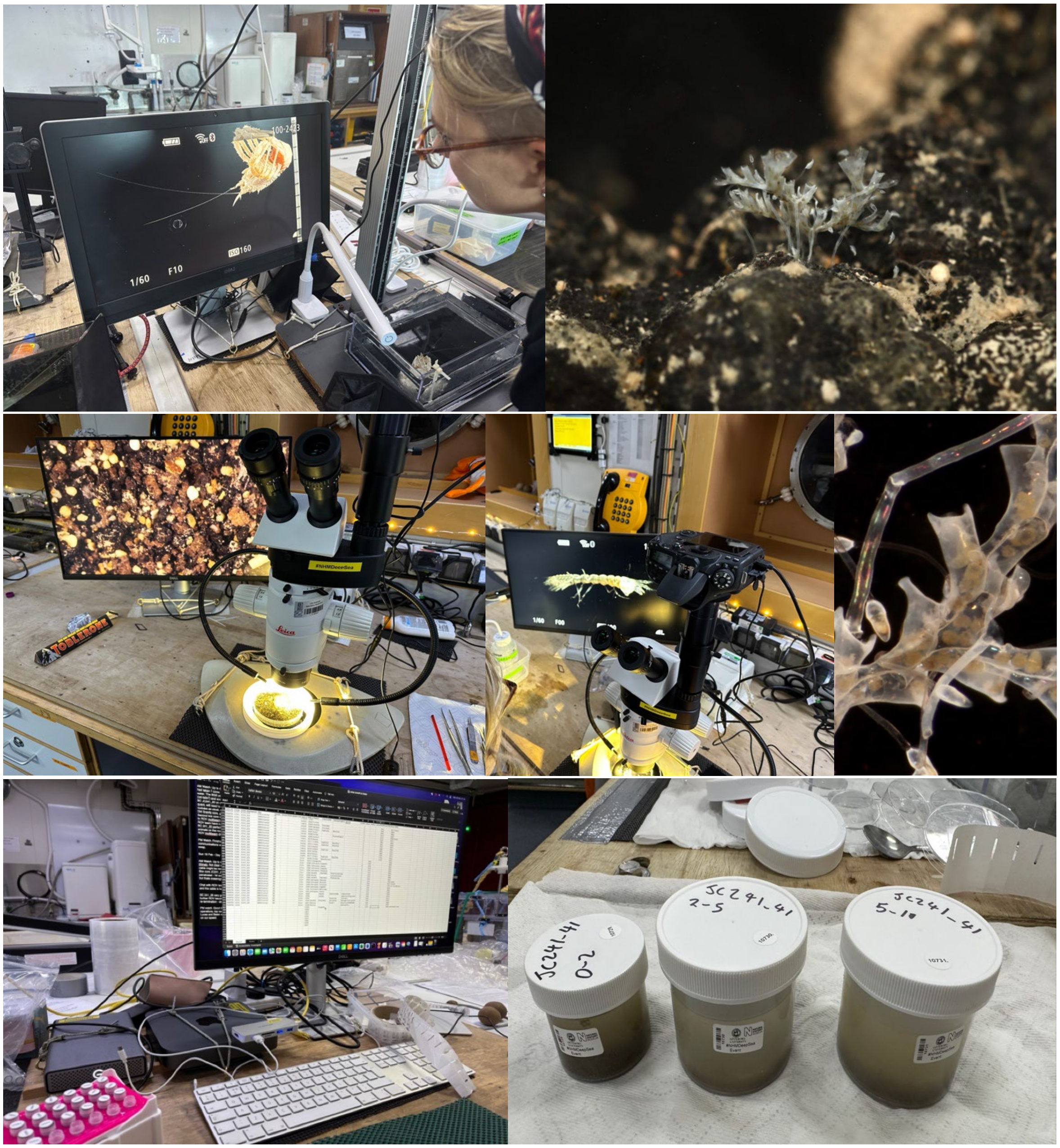

Figure 17.8 Laboratory sorting of box core samples on JC241 including left to right from top left 1) examining specimens and nodules in a CFSW water bath at low temperature for imagery, 2) detail of nodule bryozoan, 3) sorting macrofaunal samples from the live-sort fraction under ice, 4) imaging a tanaid macrofaunal sample, 5) detail of bryozoan, 4) specimen database and 2ml microtubes (pre chilled in ice pack), 5) final quantitative samples for the entire box core sample preserved in 80% ethanol and frozen. Photos: AG Glover.

17.4 Results and sample distribution

17.4.1. Box core deployments

A total of 43 box core deployments were made on JC241, of which 3 failed owing to the device not triggering. 8 samples lost the topwater or were compromised to the extent that they could only be used for qualitative live-sort sampling, but they still returned useful samples. 32 deployments returned high-quality quantitative samples (Table 17.1).

In general the box core operations went very well and an excellent set of samples obtained. The failure of the triggering mechanism in 3 deployments was something of a mystery but based on past experience the box core does sometimes take a few deployments before it works, addition of grease to the slider bar may have helped. The loss of the topwater in 8 samples took sometime to troubleshoot but was eventually diagnosed as an issue with the metal lip of the spade arm catching on the bottom of the box before it sealed on the rubber seal, creating a tiny mm-sized gap and bending the box. It was discovered that one box/spade combination had this problem but the other did not. Once this issue was resolved, success rate of deployments was 100%.

Table 17.1 Box core deployments on JC241 SMARTEX.

Station	Site	Date	Deployment Start (UTC)	Latitude (decimal)	Longitude (decimal)	Depth (m)	Sample	Notes
JC241_012	OMCO Control	15/02/2023	15:40:00	13.7368	-126.2033	4685	No sample	didn't trigger
JC241_014	OMCO Control	16/02/2023	17:19:00	13.7368	-126.2033	4688	No sample	didn't trigger
JC241_016	OMCO Control	16/02/2023	22:05:00	13.7369	-126.2035	4689	Quantitative sample	good sample
JC241_018	OMCO Control	17/02/2023	22:41:00	13.7325	-126.2042	4693	Quantitative sample	good sample
JC241_020	OMCO Control	18/02/2023	07:13:00	13.7384	-126.2057	4664	Live-sort sample only	Partial sample, non-quant
JC241_022	OMCO Control	18/02/2023	14:59:00	13.7375	-126.2048	4696	Quantitative sample	good sample, best so far
JC241_025	OMCO Control	19/02/2023	09:24:00	13.7352	-126.2054	4702	No sample	failed, didnt trigger
JC241_026	OMCO Control	19/02/2023	13:15:00	13.7375	-126.2057	4710	Quantitative sample	good sample
JC241_029	OMCO Control	20/02/2023	00:41:00	13.7309	-126.2042	4694	Quantitative sample	good sample
JC241_033	OMCO Plume	21/02/2023	10:24:00	13.7350	-126.2206	4728	Quantitative sample	good sample
JC241_036	PRA Station 5	22/02/2023	12:10:00	12.9468	-128.3440	4728	Quantitative sample	good sample
JC241_037	PRA Station 17	22/02/2023	16:35:00	12.9719	-128.3661	4875	Live-sort sample only	lost topwater
JC241_038	PRA Station 17	22/02/2023	20:20:00	12.9718	-128.3660	4848	Quantitative sample	good sample
JC241_039	PRA Station 20	23/02/2023	00:14:00	12.9739	-128.3500	4869	Quantitative sample	good sample, had enteropneust
JC241_040	PRA Station 14	23/02/2023	04:30:00	12.9686	-128.3272	4799	Live-sort sample only	lost topwater
JC241_041	PRA Station 14	23/02/2023	08:00:00	12.9685	-128.3272	4810	Quantitative sample	good sample, no surface nodules
JC241_042	PRA Station 21	23/02/2023	12:05:00	12.9774	-128.3165	4840	Quantitative sample	good sample, no surface nodules
JC241_043	PRA Station 18	24/02/2023	06:57:00	12.9772	-128.2973	4835	Live-sort sample only	topwater washed out, likely as not penetrated v far and v soupy
JC241_044	PRA Station 18	24/02/2023	10:25:00	12.9772	-128.2973	4835	Live-sort sample only	topwater washed out, likely as not penetrated v far and v soupy
JC241_045	PRA Station 22	24/02/2023	14:20:00	12.9987	-128.2958	4810	Quantitative sample	good sample, with seastar
JC241_046	PRA Station 13	25/02/2023	01:13:00	12.9179	-128.2826	4777	Live-sort sample only	non-quant, slowly washed out
JC241_047	PRA Station 13	25/02/2023	05:23:00	12.9178	-128.2827	4769	Live-sort sample only	non-quant, lost top water
JC241_048	PRA Station 16	25/02/2023	09:34:00	12.9305	-128.3150	4844	Live-sort sample only	non-quant, lost top water, some big nodules
JC241_049	PRA Station 16	25/02/2023	13:55:00	12.9306	-128.3150	4906	Quantitative sample	Good sample! Big nodules
JC241_050	PRA Station 15	25/02/2023	18:14:00	12.9250	-128.3205	4743	Quantitative sample	Good sample! Big nodules
JC241_051	PRA Station 4	25/02/2023	22:54:00	12.9247	-128.3267	4710	Quantitative sample	Good sample, big nodules
JC241_052	PRA Station 8	26/02/2023	03:17:00	12.9095	-128.3447	4788	Quantitative sample	Good sample. Just 1 tiny nodules
JC241_053	PRA Station 6	26/02/2023	07:38:00	12.9212	-128.3588	4829	Quantitative sample	Good sample, 3 small buried nods
JC241_054	PRA Station 2	26/02/2023	11:55:00	12.9343	-128.3657	4805	Quantitative sample	Good sample
JC241_061	OMCO Track	01/03/2023	18:38:00	13.7362	-126.2038	4712	Quantitative sample	Good sample, many nodules, possibly in track
JC241_065	OMCO Track	03/03/2023	06:22:00	13.7352	-126.2205	4732	Quantitative sample	Ok sample, definitely hit track. Lots of water came out

								bit all looked clear. Very shallow.
JC241_067	OMCO Plume	03/03/2023	14:11:00	13.7335	-126.2201	4724	Quantitative sample	Good sample, lots of big nodules + big urchin, probably didn't hit track
JC241_070	OMCO Track	05/03/2023	04:45:00	13.7357	-126.2207	4705	Quantitative sample	Good sample, whole layer of big buried nodules, probably hit the track
JC241_072	OMCO Track	05/03/2023	13:53:00	13.7346	-126.2205	4705	Quantitative sample	Good sample, definitely hit the track, massive slope and strange sediment. Buried nodules 30cm below surface
JC241_077	OMCO Plume	07/03/2023	22:02:00	13.7347	-126.2205	4716	Quantitative sample	Good sample, lots of nodules, probably not track
JC241_078	OMCO Plume	08/03/2023	02:42:00	13.7338	-126.2203	4735	Quantitative sample	Good sample, lots of nodules, probably not track
JC241_079	OMCO Plume	08/03/2023	07:07:00	13.7357	-126.2206	4707	Quantitative sample	Good sample, lots of nodules, probably not track
JC241_081	OMCO Control 2	08/03/2023	11:47:00	13.6353	-126.1997	4702	Quantitative sample	good sample, lots of tiny strange nodules
JC241_083	OMCO Plume	10/03/2023	03:34:00	13.7357	-126.2207	4700	Quantitative sample	Good sample, didn't hit track
JC241_084	OMCO Plume	10/03/2023	08:11:00	13.7360	-126.2206	4700	Quantitative sample	Good sample, didn't hit track
JC241_085	OMCO Plume	10/03/2023	12:43:00	13.7346	-126.2205	4703	Quantitative sample	Good sample, didn't hit track
JC241_087	OMCO Plume	11/03/2023	18:59:00	13.7338	-126.2204	4700	Quantitative sample	Good sample, didn't hit track
JC241_088	OMCO Control	11/03/2023	23:23:00	13.7358	-126.2025	4682	Quantitative sample	Good sample, control

Sampling followed a stratified randomised design, with replicate samples taken in each of three areas: OMCO Control (n=6), OMCO Track (n= 13) and PRA (n=12). An additional control site was chosen OMCO Control 2 but in the end only a single sample was taken there. The OMCO Track sites were split into two treatments- samples confirmed to have landed in the track based on visual observation (n=4) and those that landed close to the track in the area impacted by the plume (OMCO Plume, n=9) (Table 17.2).

Table 17.2 Summary of total number of quantitative box cores taken at each site on JC241.

Site	# Quantitative box cores
OMCO Control	6
OMCO Control 2	1
OMCO Plume	9
OMCO Track	4
PRA	12
Grand Total	32

17.4.2 Sample analysis and distribution

The live-sort cold-chain process allowed for some preliminary data on abundance and species composition, although these are not quantitative data until all samples are analysed (Table 17.3). 783 individual samples (mostly individual specimens) were picked out during the live-sort process, these are all photographed and given unique individual IDs to enable future quantitative and taxonomic work (Figures 17.10, 17.11, 17.12).

In general, the fauna is typical of the CCZ with Annelida (polychaete worms) being the most abundant group, followed by Arthropoda (mainly tanaids, isopods and amphipods) (Table 17.3, Figure 17.9).

Table 17.3 Total number of individuals sampled in the JC241 macrofaunal box cores broken down by phylum, including non-quantitative box cores but excluding macrofaunal material collected by other devices (amphipod traps / ROV). These data exclude the samples taken in the quantitative analysis, which will contain a much larger number of individuals once sorting is complete at the end of the project.

Phylum	# individuals
Annelida	215
Arthropoda	177
Brachiopoda	22
Bryozoa	61
Chaetognatha	19
Chordata	25
Cnidaria	92
Echinodermata	30
Entoprocta	2
Hemichordata	5
Metazoa	30
Mollusca	56
Nematoda	8
Nemertea	3
Porifera	37
Priapulida	1
Total	783

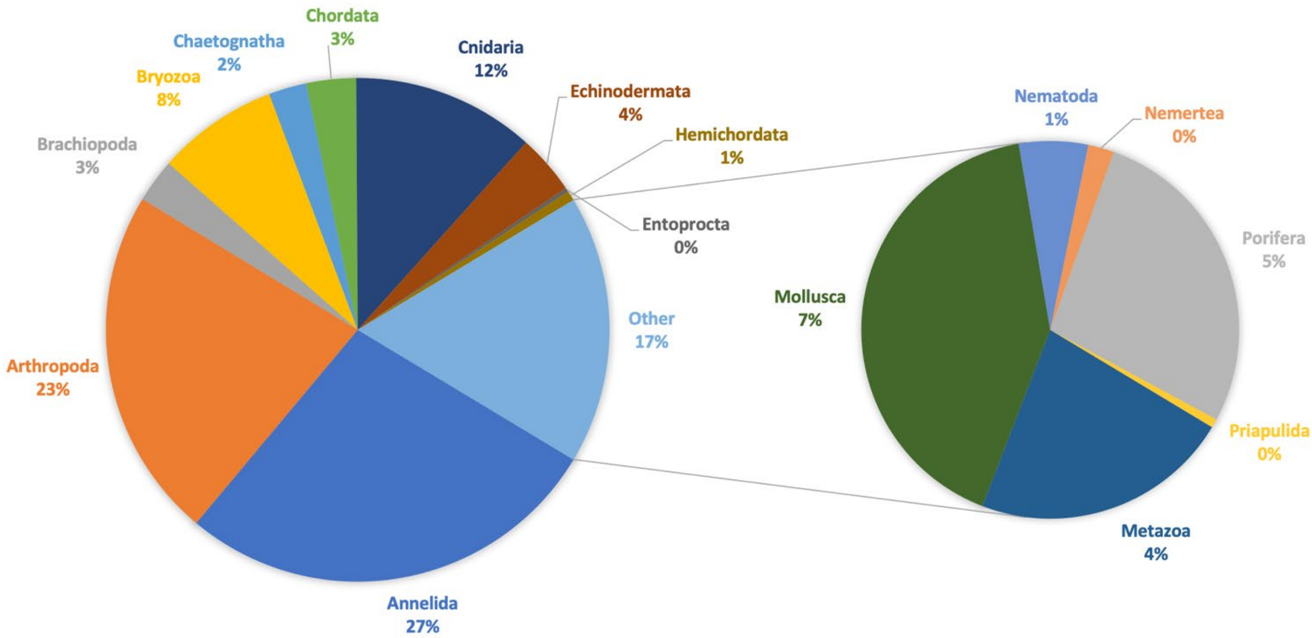

Figure 17.9 Breakdown by phylum of the live-sort fraction of the box core samples on JC241 including nodule and sediment-dwelling fauna.

Figure 17.10 Annelida (polychaetes) collected from the JC241 cruise box cores. Photo: NHM JC241 Team

Figure 17.11 Arthropoda (crustaceans) collected from the JC241 cruise box cores. Photo: NHM JC241 Team

Figure 17.12 Molluscs collected from the JC241 cruise box cores. Photo: NHM JC241 Team

The box core samples from JC241 are being returned to NHM London for further sorting and analysis by the SMARTEX team, with the exception of the geological specimens of nodules, which have gone to BGS.

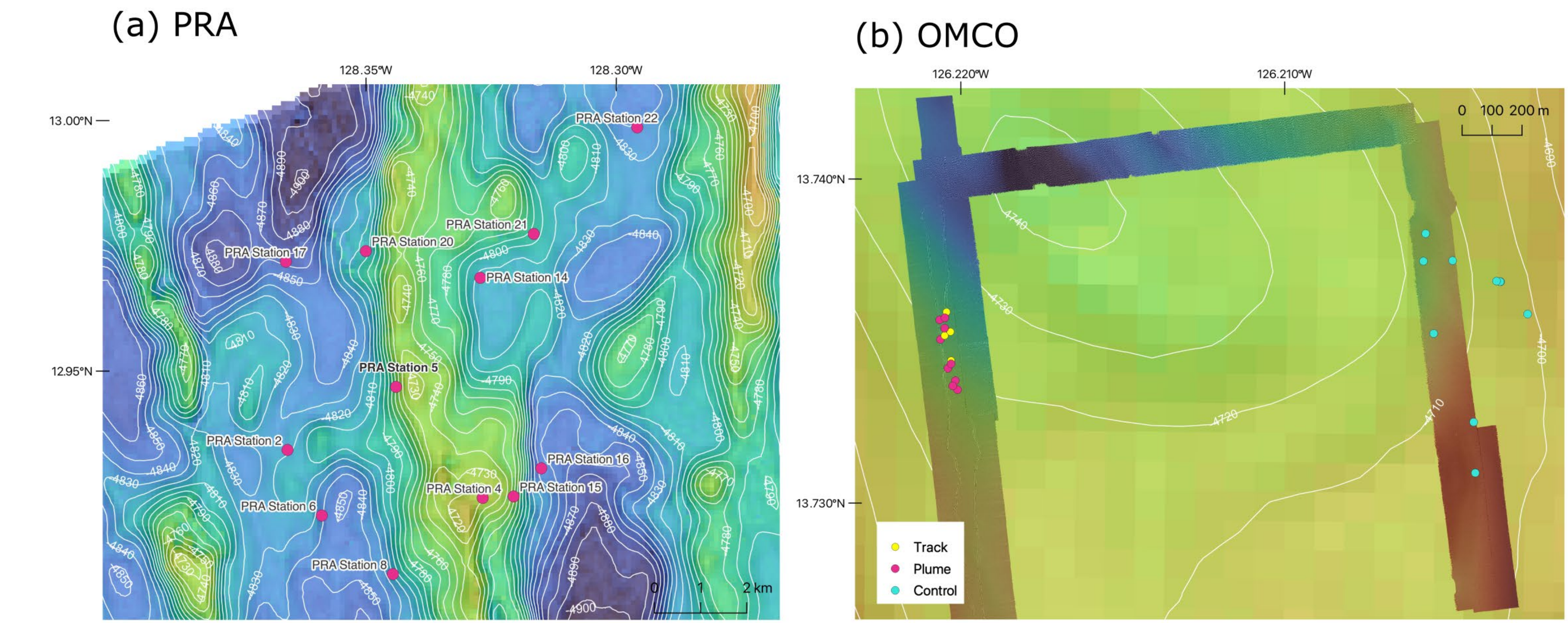

Figure 17.13: Map of Boxcore locations sampled during SMARTEX

Sampling for DEEPEND Defra GCBC biodiscovery project

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Summary

Samples were taken to support a new project funded by Defra to search for potentially new antibiotics and natural product chemistry from deep-sea samples. The project is being coordinated by NHM London. A total of 11 pushcores and 55 ethanol samples were taken for the project using the ROV and Megacore.

Introduction and objectives

To-date, DEEPEND has built an international multidisciplinary capacity-building network with Pacific Small Island Developing States (PSIDS), discovered novel chemistry and bioactives from deep sea invertebrates and microbes with potent antimicrobial bioactivity against some of the most clinically relevant pathogens and provided direct policy impact within international biodiversity agreements. The project has been the first to directly address a key societal need: an understanding of the value of biodiversity in unexplored regions to enable critical policy decisions on climate change and biodiversity loss. Leveraging current UKRI deep-ocean exploration programs in the Pacific (SMARTEX) and Atlantic (PAP time series) provides a unique opportunity to develop DEEPEND into a long-term project.

On SMARTEX the objectives are to provide added value to the sampling by

- sampling microbial community using genomic and culturing approach to detect bioactivity against ESKAPE pathogens (U Strathclyde)
- additional data for the OMCO track/off track comparision
- sampling ethanol extracts from the megafaunal samples for bioactive secondary metabolites and novel chemistry (e.g anti-cancer properties) (U Aberdeen)

Methods

Core samples

Core samples were obtained in replicated sets of cores on the OMCO track, off track and control (Table). Immediately on recovery, the entire core including the tube was frozen at minu80. After 24hours in the freezer, the outside of the core tube was warmed up under hot water briefly to allow the mud core to be released into a plastic heat sealed bag, which was double-bagged, sealed and re-frozen at minus80.

Table. Samples obtained for the University of Strathclyde for DEEPEND microbes

JC241_#	Site	Sampler	Sample Number	Sub-sample number	NHM_#	Phylum	fixation
JC241_013	OMCO Track	ROV	Dive399	PUSHCORE Y1	10411	DEEPEND Biodiscovery core	minus80
JC241_013	OMCO Track	ROV	Dive399	PUSHCORE W1	10447	DEEPEND Biodiscovery core	minus80
JC241_024	OMCO Control	MC	MC4		10607	DEEPEND Biodiscovery core	minus80
JC241_024	OMCO Control	MC	MC1		10608	DEEPEND Biodiscovery core	minus80
JC241_013	OMCO Track	ROV	Dive399	PUSHCORE P17	10609	DEEPEND Biodiscovery core	minus80
JC241_71	OMCO Control	MC			11210	DEEPEND Biodiscovery core	minus80
JC241_73	OMCO Track	ROV	DIVE407	PUSHCORE G1	11416	DEEPEND Biodiscovery core	minus80
JC241_73	OMCO Track	ROV	DIVE407	PUSHCORE W2	11417	DEEPEND Biodiscovery core	minus80
JC241_73	OMCO Track	ROV	DIVE407	PUSHCORE W3	11418	DEEPEND Biodiscovery core	minus80
JC241_73	OMCO Track	ROV	DIVE407	PUC W1	11947	DEEPEND Biodiscovery core	minus80
JC241_89	OMCO Control	ROV	DIVE411	PUC R1	11948	DEEPEND Biodiscovery core	minus80

Ethanol samples

After photography and sub-sampling (see section 16) selected megafauna were fixed in 96% ethanol, which was decanted after 24-48hours for DEEPEND sampling. These were assigned additional numbers linked to the original voucher specimen, which had new ethanol (80%) added. The ethanol samples were maintained intact. In some cases a second ethanol change occured in which case another sample was taken for DEEPEND.

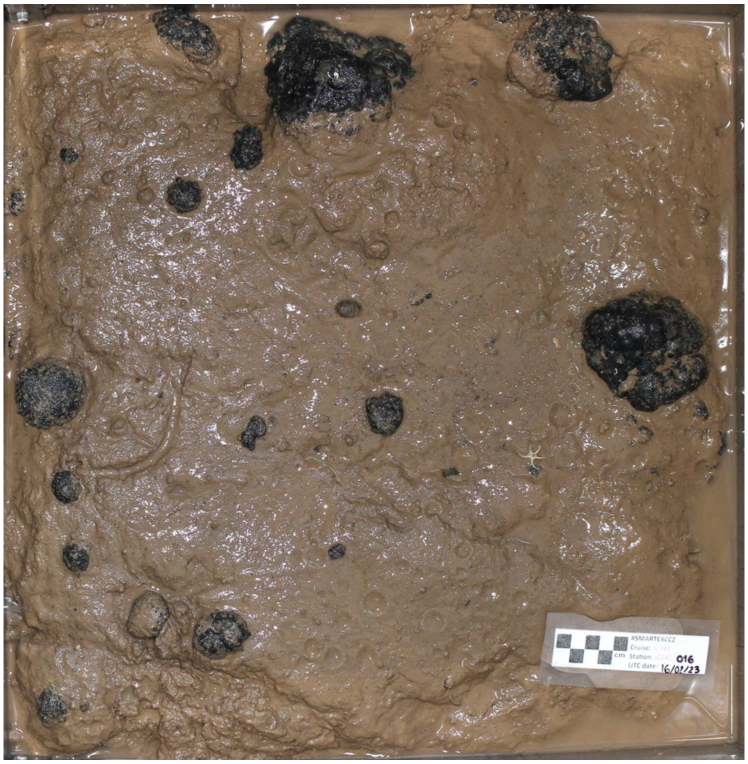
Table. Ethanol samples taken from the megafaunal preservation on JC241 SMARTEX for University of Aberdeen

JC241_#	Site	Sampler	Sample Number	Sub-sample number	Voucher specimen	NHM_#	Sample	vialsize	fixation	Notes
JC241_015	OMCO Control	FishTrap				10525	DEEPEND Biodiscovery ethanol	15ml	etoh	first ethanol from rattail tissue 10524; AKS339
JC241_015	OMCO Control	FishTrap				10528	DEEPEND Biodiscovery ethanol	15ml	etoh	first ethanol from cusk eel tissue 10526; AKS339
JC241_013	OMCO Track	ROV	Dive399	SUCTION2	10394	10529	DEEPEND Biodiscovery ethanol	50ml	etoh	First ethanol from munidopsis 10394
JC241_013	OMCO Track	ROV	Dive399	BIOBOX Port	10400	10530	DEEPEND Biodiscovery ethanol	500ml	etoh	First ethanol from Plesiodiadema 10400
JC241_013	OMCO Track	ROV	Dive399	BIOBOX Front	10407	10531	DEEPEND Biodiscovery ethanol	250ml	etoh	First ethanol from Mesoturiida 10407
JC241_013	OMCO Track	ROV	Dive399	BIOBOX Front	10402	10532	DEEPEND Biodiscovery ethanol	1.5L	etoh	First ethanol from Psychronaetes hanseni 10402
JC241_013	OMCO Track	ROV	Dive399	BIOBOX Stbrd	10397	10533	DEEPEND Biodiscovery ethanol	500ml	etoh	First ethanol from Freyella 10397
JC241_45	PRA_22	BC			10776	10951	DEEPEND Biodiscovery ethanol	60ml	etoh	ethanol change from Porcellanaster 10776
JC241_013	OMCO Track	ROV	Dive399	SUCTION2	10394	11599	DEEPEND Biodiscovery ethanol		etoh	Munidopsis second ethanol
JC241_013	OMCO Track	ROV	Dive399	BIOBOX Front	10402	11600	DEEPEND Biodiscovery ethanol		etoh	Hanseni second ethanol
JC241_69	OMCO Track	ROV	DIVE406	wooden box	11091	11601	DEEPEND Biodiscovery ethanol		etoh	Hanseni first ethanol
JC241_73	OMCO Track	ROV	DIVE407		11382	11602	DEEPEND Biodiscovery ethanol	1180mL	etoh	Brisingida first ethanol
JC241_73	OMCO Track	ROV	DIVE407		11362	11603	DEEPEND Biodiscovery ethanol	5.6L	etoh	Tunicata first ethanol
JC241_69	OMCO Track	ROV	DIVE406	STBD biobox	11085	11604	DEEPEND Biodiscovery ethanol		etoh	Hymenaster cf. echinulatus first ethanol
JC241_73	OMCO Track	ROV	DIVE407		11395	11605	DEEPEND Biodiscovery ethanol		etoh	Barrel anemone first ethanol
JC241_73	OMCO Track	ROV	DIVE407		11404	11606	DEEPEND Biodiscovery ethanol		etoh	Large nodule anemone first ethanol
JC241_73	OMCO Track	ROV	DIVE407	yellow or black slurp	11195	11607	DEEPEND Biodiscovery ethanol		etoh	Elpidiidae first ethanol
JC241_73	OMCO Track	ROV	DIVE407	yellow or black slurp	11195	11608	DEEPEND Biodiscovery ethanol		etoh	Elpidiidae first ethanol
JC241_73	OMCO Track	ROV	DIVE407	yellow or black slurp	11196	11609	DEEPEND Biodiscovery ethanol		etoh	Elpidiidae first ethanol
JC241_73	OMCO Track	ROV	DIVE407	yellow or black slurp	11196	11610	DEEPEND Biodiscovery ethanol		etoh	Elpidiidae first ethanol
JC241_73	OMCO Track	ROV	DIVE407		11200	11611	DEEPEND Biodiscovery ethanol		etoh	Plesiodiadema globulosum first ethanol
JC241_73	OMCO Track	ROV	DIVE407		11229	11612	DEEPEND Biodiscovery ethanol		etoh	Trianguloscalpellum gigas first ethanol
JC241_73	OMCO Track	ROV	DIVE407	yellow or black slurp	11193	11613	DEEPEND Biodiscovery ethanol	1180mL	etoh	Elpidiidae first ethanol
JC241_73	OMCO Track	ROV	DIVE407		11387	11614	DEEPEND Biodiscovery ethanol		etoh	Barrel anemone first ethanol
JC241_73	OMCO Track	ROV	DIVE407	yellow or black slurp	11197	11615	DEEPEND Biodiscovery ethanol	50mL	etoh	Elpidiidae first ethanol 1/6
JC241_73	OMCO Track	ROV	DIVE407	yellow or black slurp	11197	11616	DEEPEND Biodiscovery ethanol	50mL	etoh	Elpidiidae first ethanol 2/6

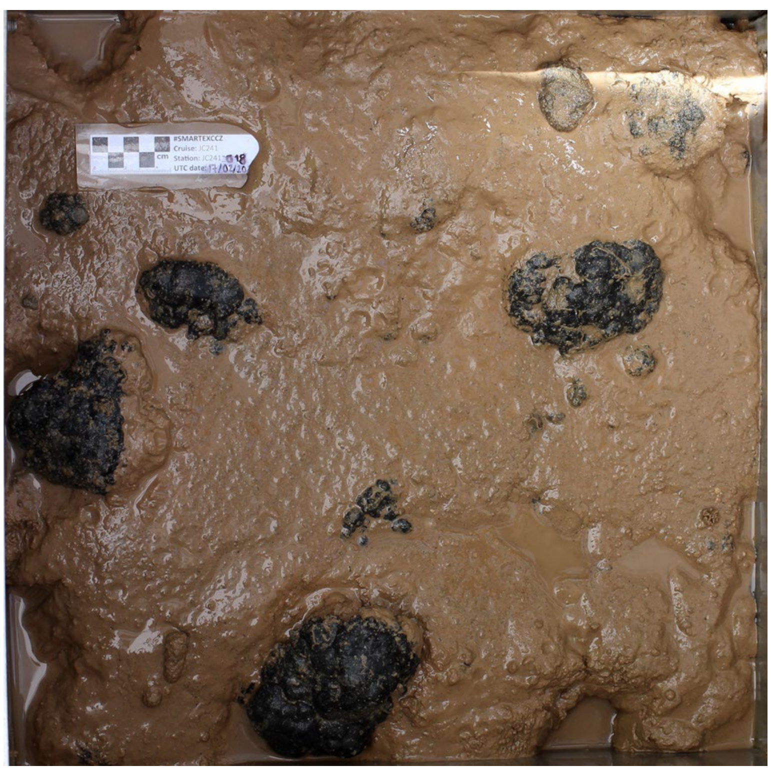
JC241_73	OMCO Track	ROV	DIVE407	yellow or black slurp	11197	11617	DEEPEND Biodiscovery ethanol	50mL	etoh	Elpidiidae first ethanol 3/6
JC241_73	OMCO Track	ROV	DIVE407	yellow or black slurp	11197	11618	DEEPEND Biodiscovery ethanol	50mL	etoh	Elpidiidae first ethanol 4/6
JC241_73	OMCO Track	ROV	DIVE407	yellow or black slurp	11197	11619	DEEPEND Biodiscovery ethanol	50mL	etoh	Elpidiidae first ethanol 5/6
JC241_73	OMCO Track	ROV	DIVE407	yellow or black slurp	11197	11620	DEEPEND Biodiscovery ethanol	50mL	etoh	Elpidiidae first ethanol 6/6
JC241_73	OMCO Track	ROV	DIVE407		11208	11621	DEEPEND Biodiscovery ethanol		etoh	Hymenaster sp. first ethanol
JC241_73	OMCO Track	ROV	DIVE407	yellow or black slurp	11190	11622	DEEPEND Biodiscovery ethanol		etoh	Elpidiidae first ethanol
JC241_73	OMCO Track	ROV	DIVE407	yellow or black slurp	11198	11623	DEEPEND Biodiscovery ethanol		etoh	Elpidiidae first ethanol
JC241_73	OMCO Track	ROV	DIVE407	yellow or black slurp	11192	11624	DEEPEND Biodiscovery ethanol	1180mL	etoh	Elpidiidae first ethanol
JC241_73	OMCO Track	ROV	DIVE407	yellow or black slurp	11194	11625	DEEPEND Biodiscovery ethanol	50mL	etoh	Elpidiidae first ethanol 1/6
JC241_73	OMCO Track	ROV	DIVE407	yellow or black slurp	11194	11626	DEEPEND Biodiscovery ethanol	50mL	etoh	Elpidiidae first ethanol 2/6
JC241_73	OMCO Track	ROV	DIVE407	yellow or black slurp	11194	11627	DEEPEND Biodiscovery ethanol	50mL	etoh	Elpidiidae first ethanol 3/6
JC241_73	OMCO Track	ROV	DIVE407	yellow or black slurp	11194	11628	DEEPEND Biodiscovery ethanol	50mL	etoh	Elpidiidae first ethanol 4/6
JC241_73	OMCO Track	ROV	DIVE407	yellow or black slurp	11194	11629	DEEPEND Biodiscovery ethanol	50mL	etoh	Elpidiidae first ethanol 5/6
JC241_73	OMCO Track	ROV	DIVE407		11358	11630	DEEPEND Biodiscovery ethanol	1.5L	etoh	ethanol from black coral
JC241_73	OMCO Track	ROV	DIVE407		11209	11631	DEEPEND Biodiscovery ethanol	1180mL	etoh	ethanol from slimy sea cucumber - orange color!
JC241_73	OMCO Track	ROV	DIVE407		11371	11632	DEEPEND Biodiscovery ethanol	5.6L	etoh	first ethanol change from Psychropotes cf. longicauda
JC241_73	OMCO Track	ROV	DIVE407		11207	11633	DEEPEND Biodiscovery ethanol		etoh	Hyalonema first ethanol
JC241_69	OMCO Track	ROV	DIVE406	STBD biobox	11079	11634	DEEPEND Biodiscovery ethanol	5.6L	etoh	First ethanol change of Deima sp.
JC241_73	OMCO Track	ROV	DIVE407		11221	11635	DEEPEND Biodiscovery ethanol	5.6L	etoh	Dytaster sp. First ethanol
JC241_76	OMCO Track	ROV	DIVE408		11431	11636	DEEPEND Biodiscovery ethanol	1180mL	etoh	Psychropotes verrucicaudatus first ethanol
JC241_76	OMCO Track	ROV	DIVE408	STBD biobox	11518	11638	DEEPEND Biodiscovery ethanol	1180mL	etoh	Dytaster sp. First ethanol
JC241_76	OMCO Track	ROV	DIVE408		11437	11639	DEEPEND Biodiscovery ethanol	5.6L	etoh	First ethanol from cf. Benthodytes marianensis
JC241_73	OMCO Track	ROV	DIVE407		11250	11640	DEEPEND Biodiscovery ethanol	1180mL	etoh	First ethanol change from cf. Abyssopathes lyra
JC241_76	OMCO Track	ROV	DIVE408		11505	11641	DEEPEND Biodiscovery ethanol	50mL	etoh	First ethanol change from mystery cuke
JC241_89	OMCO Control	ROV	DIVE411	Starboard biobox	11924	11949	DEEPEND Biodiscovery ethanol	1180mL	etoh	ethanol Holasus cf. taraxarum 11924
JC241_76	OMCO Track	ROV	DIVE408		11509	11950	DEEPEND Biodiscovery ethanol	500mL	etoh	
JC241_095	UK-1	ROV	DIVE412	Starboard biobox	11966	12049	DEEPEND Biodiscovery ethanol	5.6l	etoh	from Benthodytes no. 11966 - looks like Ribena!
JC241_095	UK-1	ROV	DIVE412	Yellow canister	12012	12051	DEEPEND Biodiscovery ethanol	1000ml	etoh	from Synallactidae 12012 - looks like Iron Brew
JC241_095	UK-1	ROV	DIVE412	Grey biobox	12018	12052	DEEPEND Biodiscovery ethanol	500mL	etoh	from the Xe- cumber 12018

JC241 Box Core top shots

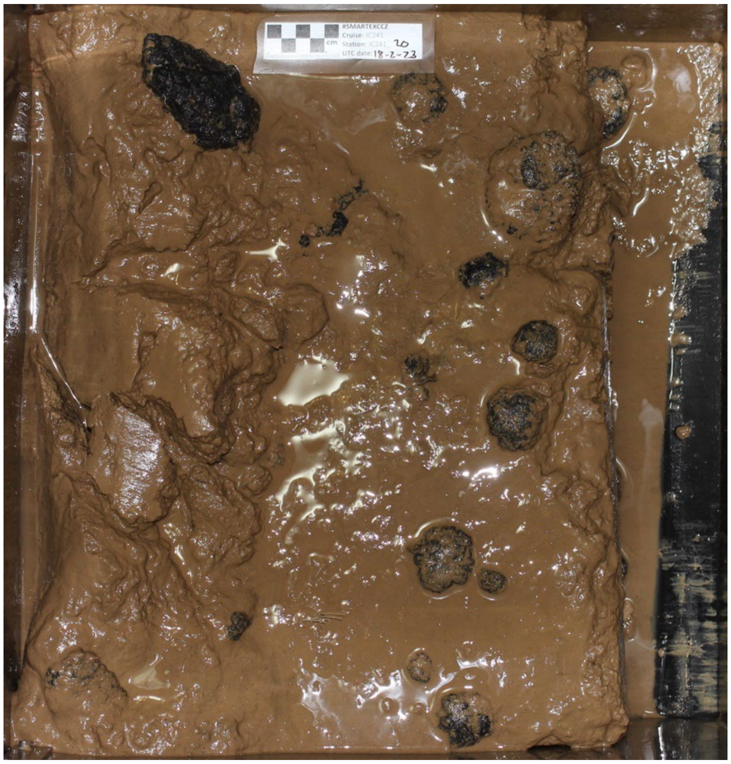
Compilation of box core top shots including box core number (BC_##), station number (JC241_###), sample site, date, and position of deployment. Note: failed and non-quantitative box cores are not included.



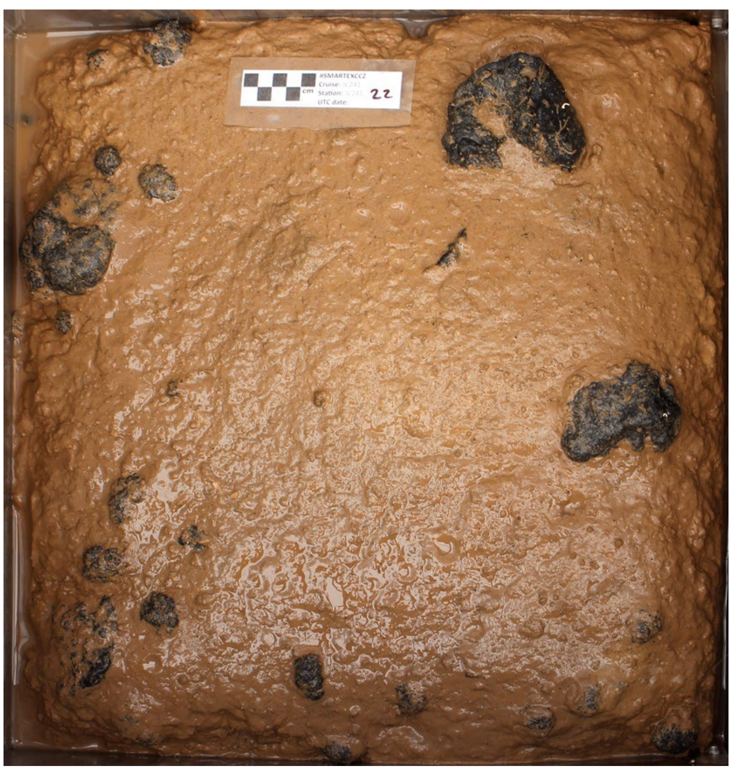
BC_03, JC241_016, OMCO Control, 16/02/2023, 13.7369 N -126.2035 W



BC_04, JC241_018, OMCO Control, 17/02/2023, 13.7325 N -126.2042 W

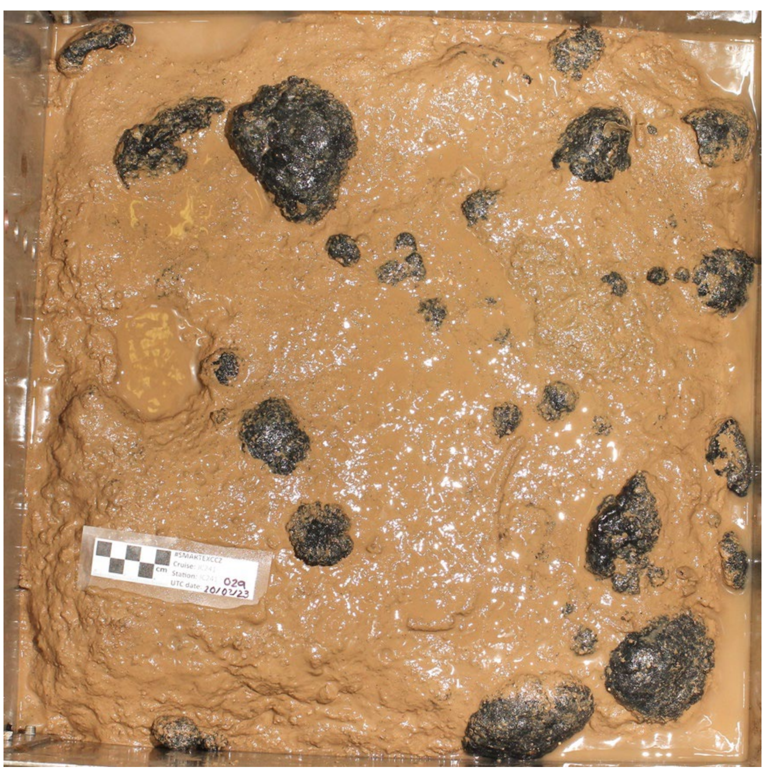


BC_05, JC241_020, OMCO Control, 18/02/2023, 13.7384 N -126.2057 W

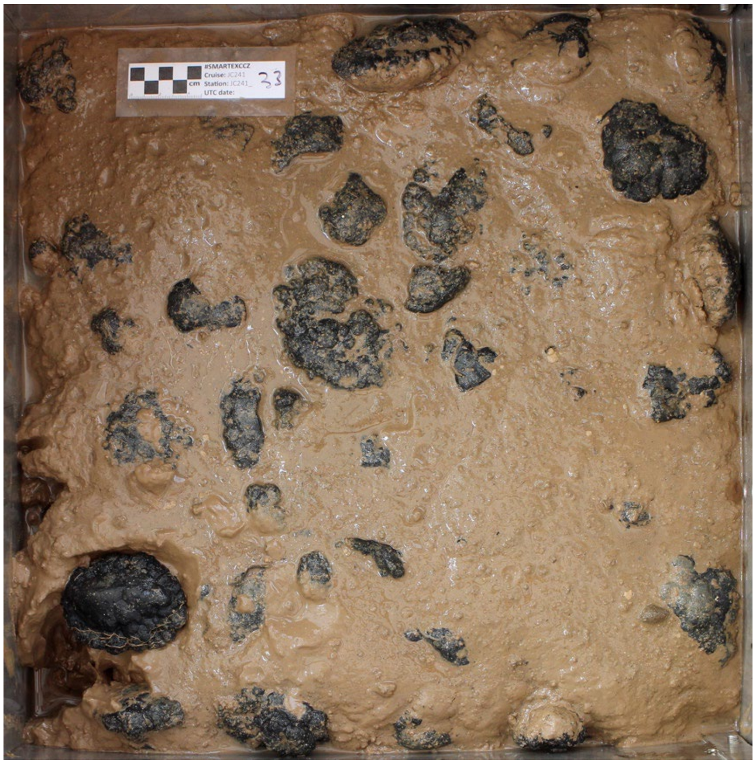


BC_06, JC241_022, OMCO Control, 18/02/2023, 13.7375 N -126.2048 W

BC_08, JC241_026, OMCO Control, 19/02/2023, 13.7375 N -126.2057 W



BC_09, JC241_029, OMCO Control, 20/02/2023, 13.7309 N, -126.2042 W



BC_10, JC241_033, OMCO Plume, 21/02/2023, 13.7350 N, -126.2206 W

BC_11, JC241_036, PRA Station 5, 22/02/2023, 12.9468 N, -128.3440 W

BC_13, JC241_038, PRA Station 17, 22/02/2023, 12.9719 N, -128.3661 W

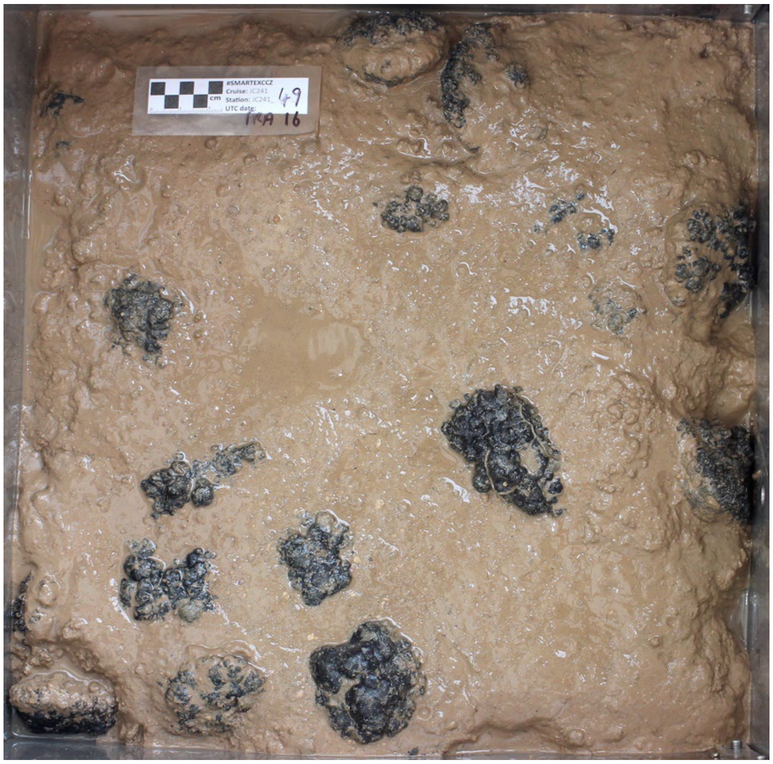
BC_14, JC241_039, PRA Station 20, 23/02/2023, 12.9739 N, -128.3500 W

BC_16, JC241_041, PRA Station 14, 23/02/2023, 12.9685 N, -128.3272 W

BC_17, JC241_042, PRA Station 21, 23/02/2023, 12.9774 N, -128.3165 W

BC_20, JC241_045, PRA Station 22, 24/02/2023, 12.9987 N, -128.2958 W

BC_21, JC241_046, PRA Station 13, 25/02/2023, 12.9179 N, -128.2826 W

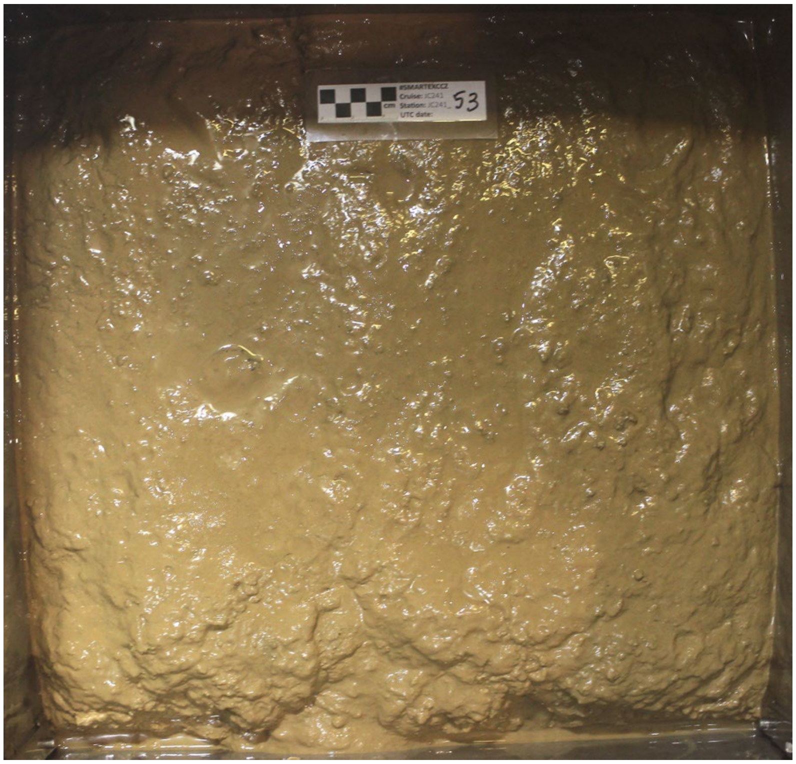


BC_24, JC241_049, PRA Station 16, 25/02/2023, 12.9306 N, -128.3150 W

BC_25, JC241_050, PRA Station 15, 25/02/2023, 12.9250 N, -128.3205 W

BC_26, JC241_051, PRA Station 4, 25/02/2023, 12.9250 N, -128.3205 W

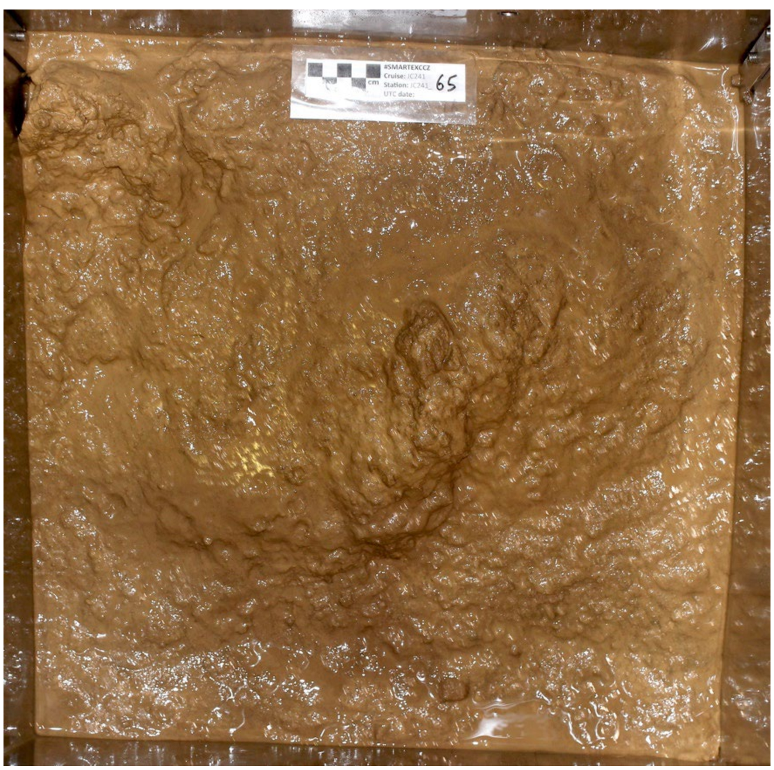
BC_27, JC241_052, PRA Station 8, 26/02/2023, 12.9095 N, -128.3447 W



BC_28, JC241_053, PRA Station 6, 26/02/2023, 12.9212 N, -128.3588 W

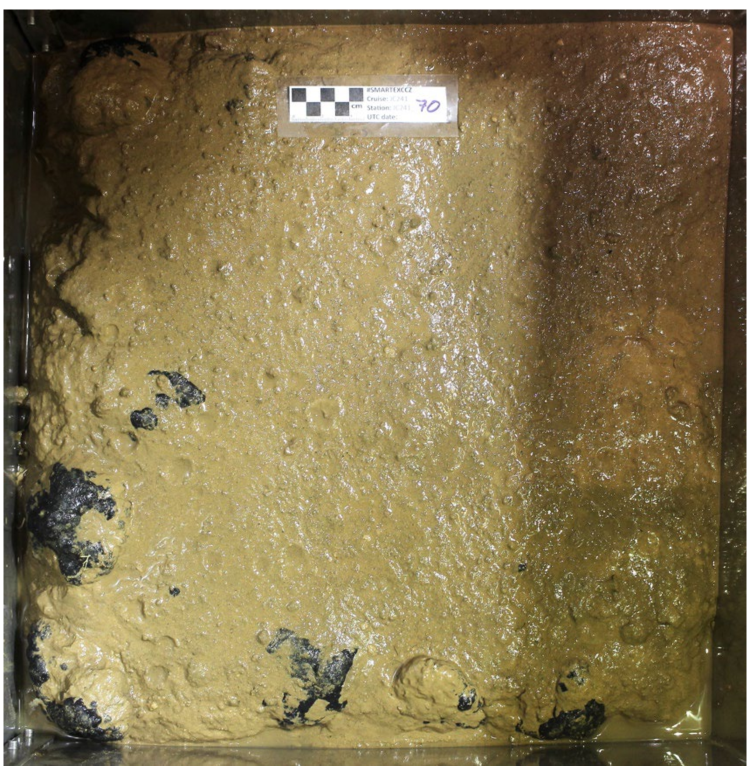
BC_29, JC241_054, PRA Station 2, 26/02/2023, 12.9343 N, -128.3657 W

BC_30, JC241_061, OMCO Track, 01/03/2023, 13.7362 N, -126.2038 W



BC_31, JC241_065, OMCO Track, 03/03/2023, 13.7352 N, -126.2205 W

BC_32, JC241_067, OMCO Plume, 03/03/2023, 13.7335 N, -126.2201 W



BC_33, JC241_070, OMCO Track, 05/03/2023, 13.7357 N, -126.2207 W

BC_34, JC241_072, OMCO Track, 05/03/2023, 13.7346 N, -126.2205 W

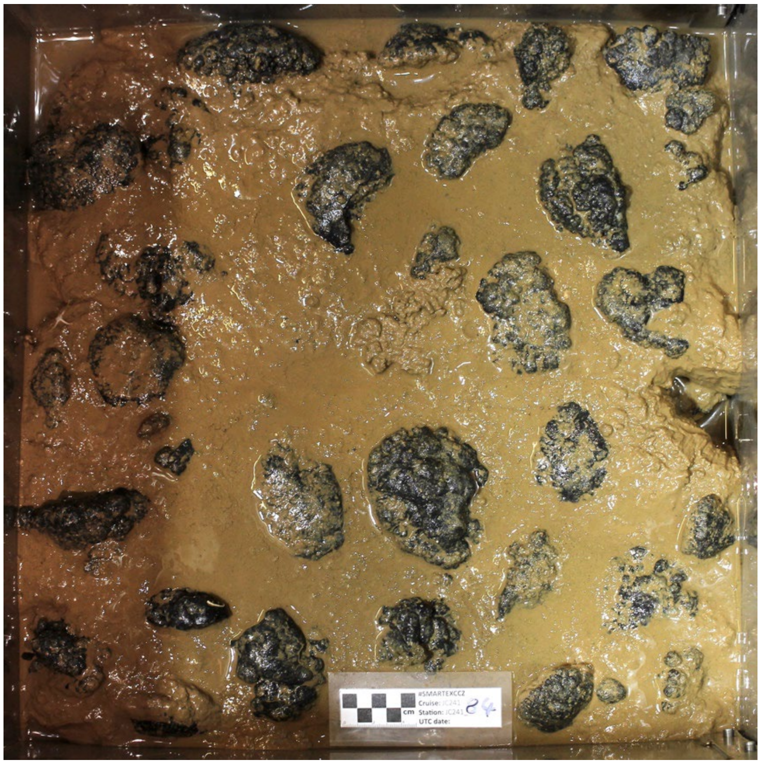
BC_35, JC241_077, OMCO Plume, 07/03/2023, 13.7347 N, -126.2205 W

BC_36, JC241_078, OMCO Plume, 08/03/2023, 13.7338 N, -126.2203 W

BC_37, JC241_079, OMCO Plume, 08/03/2023, 13.7357 N, -126.2206 W

BC_38, JC241_081, OMCO Control 2, 08/03/2023, 13.6353 N, -126.1997 W

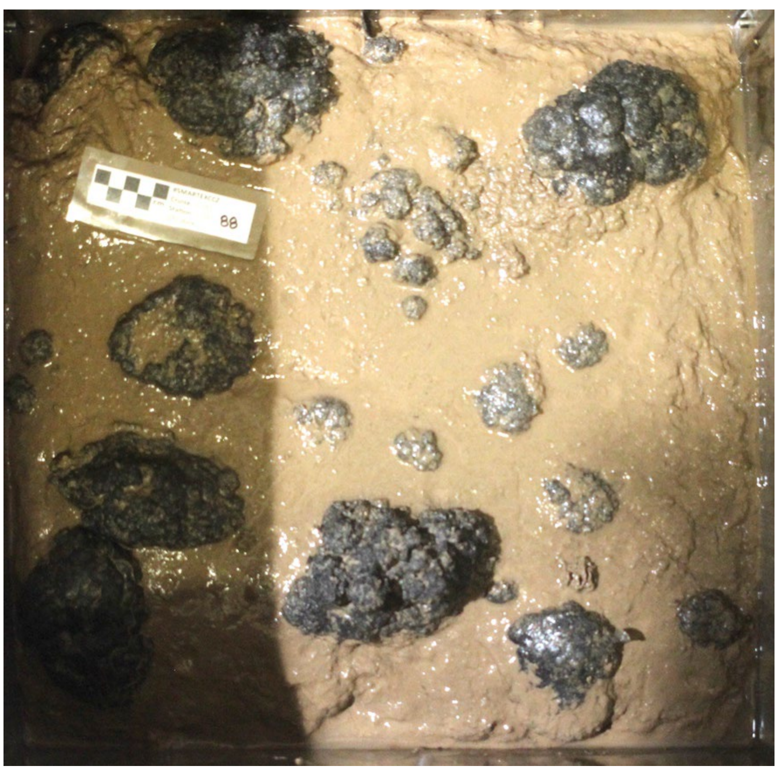
BC_39, JC241_083, OMCO Plume, 10/03/2023, 13.7357 N, -126.2207 W



BC_40, JC241_084, OMCO Plume, 10/03/2023, 13.7360 N, -126.2206 W

BC_41, JC241_085, OMCO Plume, 10/03/2023, 13.7346 N, -126.2205 W

BC_42, JC241_087, OMCO Plume, 11/03/2023, 13.7338 N, -126.2204 W



BC_43, JC241_088, OMCO Control, 11/03/2023, 13.7358 N, -126.2025 W

18. Benthic foraminifera

Bryan J. O'Malley[1,2], Adrian G. Glover[3]

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3.) *Natural History Museum, London, England*

18.1 Summary

Benthic foraminifera were successfully sampled on JC241 using a variety of sampling equipment including ROV pushcores, multicores, boxcores, and ROV manipulators. 27 ROV pushcores, 9 multi-cores, and 16 xenophyophore samples were taken across OMCO and UK-1 regions adding up to 215 total samples. 6 push cores replicates were taken at three different treatments at the OMCO tracks (track, near track, control). 4 specimens of *Reticulammina* sp., the prominent sessile recolonizer of the tracks and 11 other xenophyophores were collected for biomass and DNA analyses.

Figure 18.1 Micrograph of benthic foraminiferal species found throughout the Clarion-Clipperton Zone (note: not to scale).

18.2 Introduction & Objectives

Benthic foraminifera are single-celled protistan amoebas that are characterized by their tests (either organic, calcareous, or agglutinate) and their use of pseudopodia, the cytoplasmic net-like extensions that allow them feed, move, and burrow. Benthic foraminifera are the most prominent eukaryotes in abyssal plains, making up over 50% of the eukaryotic biomass of macro- and meio-faunal size classes (Gooday et al., 1992; Hauquier et al,, 2019; Gooday et al., 2021). They are ideal bioindicators for ecological monitoring because of their high biodiversity, short turnover rate, low trophic status, varying degrees of environmental sensitivities, and high abundance in all marine environments. Due to their high abundances and densities in abyssal plains they provide a reliable database for statistical analysis when restricted to small sample volumes, which is conducive to an efficient sampling program (Goineau and Gooday, 2017; Gooday and Goineau, 2019). Benthic foraminifera represent an important connective link across trophic levels as most species are direct consumers of sedimentary organic material, phytodetritus, and bacteria (Culver and Lipps, 2003; Nomaki et al., 2008). Foraminifera are also capable of assimilating dissolved organic matter into biomass, which is especially important in deep sea abyssal zones where organic matter is limited (DeLaca et al., 1981). Over the past two decades, a renewed interest in deep-sea mining has spurred a rise in benthic ecological studies, including the first quantitative foraminiferal surveys of the Clarion-Clipperton zone, which this study seeks to continue (Nozawa et al., 2006; Goineau and Gooday, 2017, 2019; O'Malley et al., 2023).

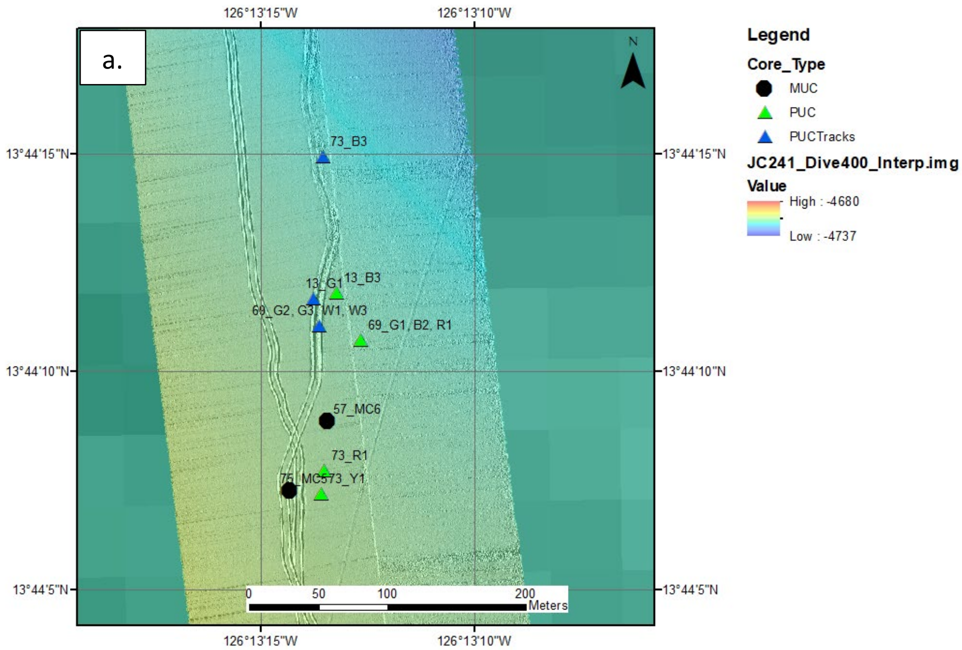
Objectives:
The SMARTEX research campaign to the Clarion-Clipperton Zone (CCZ) was conducted to evaluate the long-term ecological impacts and succession from a prototype polymetallic nodule collector vehicle (PCV) test conducted by OMCO in 1979. The objective of the benthic foraminifera workscope was to:

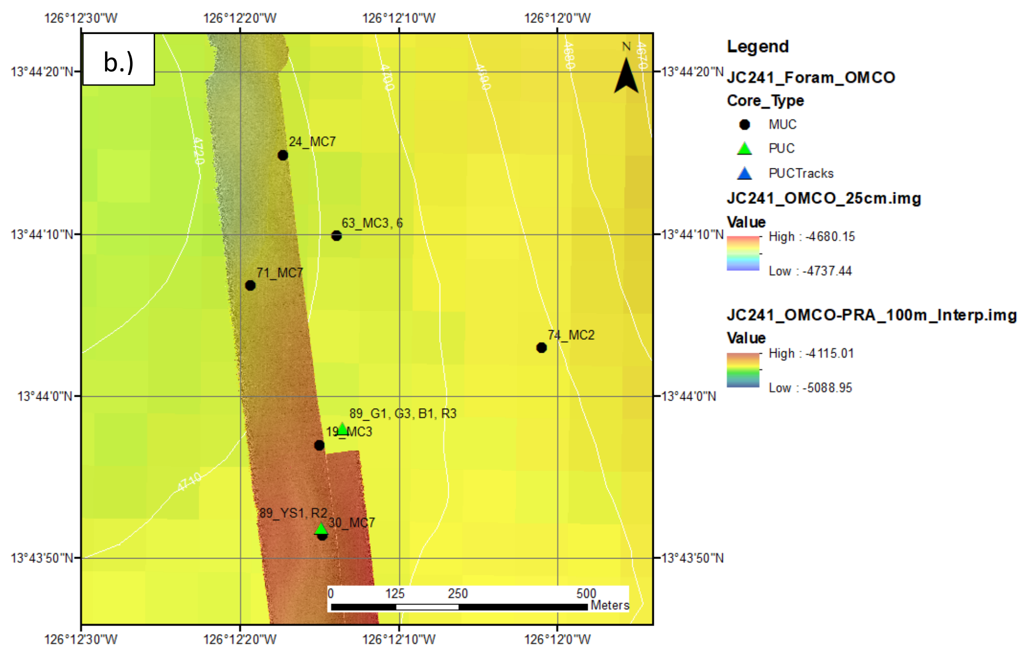
- Sample and characterize foraminiferal assemblages from areas directly disturbed by the PCV, areas affected by the sediment plume, and from control sites with no influence from the PCV test.
- Disentangle natural variability from the effects of sedimentation, sediment compaction, and nodule removal.
- Collect the megafaunal-sized xenophyophore species for DNA and Micro-CT scanning to further expand the understanding of the diversity and biomass of the most abundant megafaunal sized organisms of the CCZ.
- Continue the time series from UK-1 established by Gooday and Goineau (2019).
- An ongoing objective of this study is to determine relationships between environmental data, macrofaunal and megafaunal data, microbial communities, and benthic foraminifera through synthesis and statistical means.

18.3 Sampling Regime

Total Foraminifera Samples

- n (MUC cores): 9
- n (PUC cores): 27
- Sampling Increments for cores (PUC and MUC): 0-1 cm, 1-2 cm, 2-3 cm, 3-4 cm, 4-5 cm
- n samples (MUC cores): 45
- n samples (PUC cores): 135
- n Xenophyophore DNA samples: 16
- n Xenophyophore CT samples: 7


a.
126°13'15"W
126°13'10"W
13°44'15"N
13°44'10"N
13°44'5"N
73_B3
13_G1
13_B3
69_G2, G3, W1, W3
69_G1, B2, R1
57_MC6
73_R1
75_MC5
73_Y1
0
50
100
200
Meters
N
Legend
Core_Type
MUC
PUC
PUCTracks
JC241_Dive400_Interp.img
Value
High : -4680
Low : -4737


b.)
126°12'30"W
126°12'20"W
126°12'10"W
126°12'0"W
13°44'20"N
13°44'10"N
13°44'0"N
13°43'50"N
24_MC7
63_MC3, 6
71_MC7
74_MC2
89_G1, G3, B1, R3
19_MC3
89_YS1, R2
30_MC7
4720
4710
0
125
250
500
Meters
N
Legend
JC241_Foram_OMCO
Core_Type
MUC
PUC
PUCTracks
JC241_OMCO_25cm.img
Value
High : -4680.15
Low : -4737.44
JC241_OMCO-PRA_100m_Interp.img
Value
High : -4115.01
Low : -5088.95

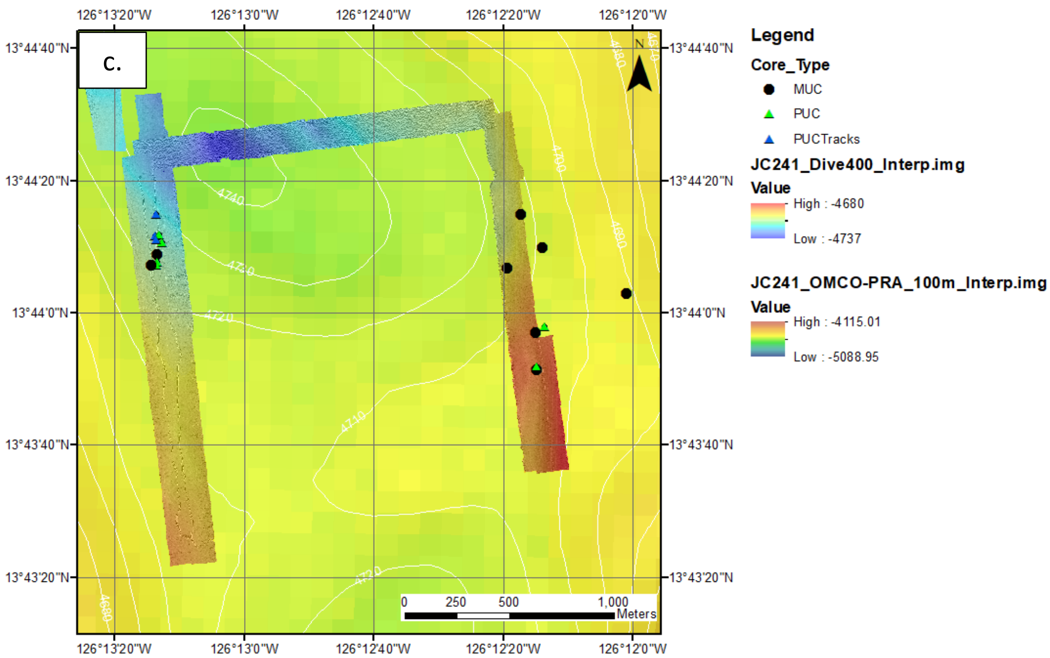
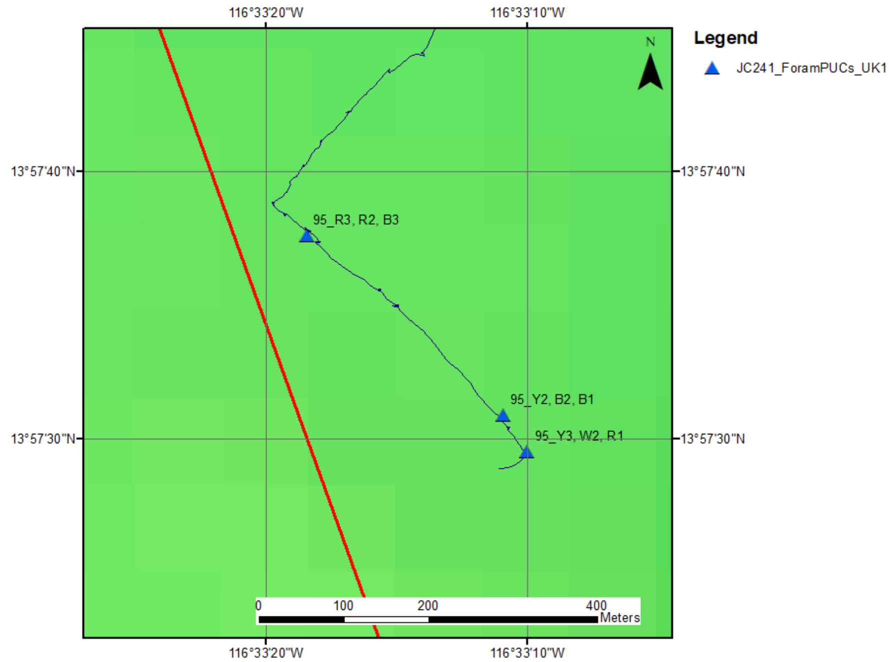

c.
126°13'20"W
126°13'0"W
126°12'40"W
126°12'20"W
126°12'0"W
13°44'40"N
13°44'20"N
13°44'0"N
13°43'40"N
13°43'20"N
Legend
Core_Type
MUC
PUC
PUCTracks
JC241_Dive400_Interp.img
Value
High : -4680
Low : -4737
JC241_OMCO-PRA_100m_Interp.img
Value
High : -4115.01
Low : -5088.95
0 250 500 1,000
Meters

Figure 18.2 Spatial sampling positions of ROV pushcores and multicores for foraminifera at the OMCO collector test area. (Black circle: multicore; Blue triangle: pushcore track; Green triangle: pushcore). a.) Close up map of foraminifera samples taken from the track area. Labels refer to deployment number followed by core number, (multiple may be taken at one site). b.) Close up map of foraminifera samples taken from the control site. Labels refer to deployment number followed by core number, (multiple may be taken at one site). c.) Zoomed out spatial sampling positions for foraminifera encompassing the entire OMCO region.

Figure 18.3 Spatial sampling positions of ROV pushcores at 3 sites (3 replicates at each site) in the "Area of Interest" in the UK-1 lease



area. Labels refer to deployment number followed by core number, (3 pushcores replicates taken at each site).

18.4 Methods

18.4.1 For each core:

Sample labelling protocol

Every sample layer has a unique code, linked to the multi-core number, and core number. An example of this logic is provided in Table 18.1. The increasing deployment ID and ensures no label is repeated twice in the sample log.

Table 18.1 Example of the layers added together to make the sampling code for labels, example shown on the right.

Campaign ID	Equipment	Deployment ID	Core ID	Core allocation	Avg sed depth cm	Sed. Depth cm	Label
JC241	MUC	001	CR08	Foraminifera	.5	0/1	JC241_MUC_001_CR08_forams 0-1
JC241	MUC	001	CR08	Foraminifera	1.5	1/2	JC241_MUC_001_CR08_forams 1-2
JC241	MUC	001	CR08	Foraminifera	2.5	2/3	JC241_MUC_001_CR08_forams 2-3

JC241	MUC	001	CR08	Foraminifera	3.5	3/4	JC241_MUC_001_CR08_forams 3-4
JC241	MUC	001	CR08	Foraminifera	4.5	4/5	JC241_MUC_001_CR08_forams 4-5
JC241	PUC	001	Green2	Foraminifera	0.5	0/1	JC241_PUC_001_G2_forams_0-1
JC241	PUC	001	Green2	Foraminifera	1.5	1/2	JC241_PUC_001_G2_forams_1-2
JC241	PUC	001	Green2	Foraminifera	2.5	2/3	JC241_PUC_001_G2_forams_2-3
JC241	PUC	001	Green2	Foraminifera	3.5	3/4	JC241_PUC_001_G2_forams_3-4
JC241	PUC	001	Green2	Foraminifera	4.5	4/5	JC241_PUC_001_G2_forams_4-5

1. Label bottles appropriately, ensuring unique identifier or code (including cruise, date, station, gear, event number, number/color of MUC/PUC core tube (#/12), number of the deployment, and slice depth in centimeters).
2. Remove bottom rubber stopper.
3. Keep top rubber stopper to secure vacuum seal.
4. Place tube on extruder.
5. Place tube on extruder plug (top rubber stopper stays on to avoid loss of mud from the bottom).
6. Slide core tube until 5-10 cm overlying water is accessible (avoid resuspension as much as possible. If resuspension occurs, collect all overlying water that may be cloudy).
7. Siphon off water onto 63-micron sieve.
8. Using clean forceps, pick off the nodules and rinse them with cold filtered seawater onto the 63-micron sieve. Store nodules in the designated nodule jars.

Figure 18.4 Steps 7 and 8 of the core extrusion protocol.

9. Collect sieve residue into the 0-1 cm 250 mL jar.
10. Place slicing ring onto core tube.
11. Push tube down so that the desired sediment slice sits within the slicing ring.
12. Using the slicing plate, slice the mud in between the core tube and the slicing ring. Using a smooth movement and with slight downward pressure, slide the plate off the tube, removing the slicing ring and mud with it. (If you pull the plate off in an upward movement, mud may stick to the bottom of the slicing plate and pull the mud (what remains in the core tube) out of the tube, resulting in inaccurate sediment horizons.)

Figure 18.5 Steps 10, 11, and 12 of the core extrusion protocol.

13. Rinse the core slicing ring into the funnel into the sample jar.
14. Using a putty knife, slice chunks of mud and place them in the wide mouth sample bottles. If the sediment is runny, then use the funnel to guide the sediment into the sample bottle. Wash top of extrusion plate into sample jar with cold filtered seawater. (Bottom side is part of the next increment. Slice the next increment with the dirty side up.)
15. Add cold filtered seawater to the sample bottle until the sediment volume is at least doubled.
16. Repeat step 7-15 until finished.

17. After the core is extruded the samples are then gently sieved with cold filtered seawater on a 63-um sieve without directly spraying them. This is best done with an up and down motion on a tray filled with cold filtered seawater. Sieve until the water underneath does not appear cloudy anymore.

Figure 18.6 Sieving the sediment with cold filtered seawater on a 63-um sieve.

18. Use a nozzled squirt bottle of 70% ethanol to concentrate the residue in one corner and tip it in a funnel to collect into the sample vial.

Notes on foram sampling:

- Sample Resolution: Nodules, 0-1, 1-2, 2-3, 3-4, 4-5 cm slices from each sediment core for foram analysis.

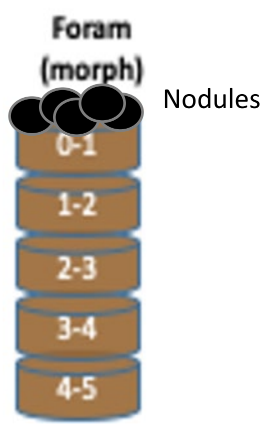


Figure 18.7 Sample resolution schematic for pushcores and multicores (cm).

18.4.2 At-Sea Photography

Polymetallic nodules and xenophyophores were photographed using a flat-lay (top-down) macrophotography set up with a Canon EOS camera and Kaiser RS2XA with a hand cranked height adjuster (Figure 18.8). Photos were taken at different focus depths and manually focus stacked in Helicon Pro to fully capture the depth of field of the xeno or nodule. After photographing, all nodules were preserved in 70% ethanol, while xenophyophores were subsampled for DNA and preserved in RNAlater at -80 degrees C and for CT scanning preserved in 4% buffered formalin.

Figure 18.8 Flat-lay macro-photography set up.

Foraminifera from qualitatative samples were also photographed at sea using an Olympus SZX12 photomicroscope equipped with an Amscope MU2003 digital camera with a 20 megapixel Sony CMOS sensor. Photos and videos were recorded with the open source ToupView software (Figure 18.9).

Figure 18.9 Photomicroscopy set up for qualitative live sorting and photography of specimens in cold filtered seawater.

18.5 Results & Initial Observations

Table 18.2 List of multicores and pushcores sampled for quantitative benthic foraminiferal analysis.

Core Type	Station	Label	Date	Latitude	Longitude	Depth (m)	Core number (CR-)	Core Diameter	Size Fraction (um)	Preservation	Purpose	Notes
PUC	013	JC241_013_PUC_6_FORAM_G1	2/16/23	13.736574	-126.220488	4712	G1	6 cm	63	ETOH (70%)	Foraminifera Morphology	Tracks
PUC	013	JC241_013_PUC_13_FORAM_B3	2/16/23	13.73661	-126.22034	4712	B3	6 cm	63	ETOH (70%)	Foraminifera Morphology	Near track
MUC	019	JC241_019_MUC_FORAM_Cr3	2/17/23	13.73250	-126.20417	4682	3	10 cm	63	ETOH (70%)	Foraminifera Morphology	Control Site
MUC	024	JC241_024_MUC_FORAM_Cr7	2/19/23	13.73747	-126.20480	4697	7	10 cm	63	ETOH (70%)	Foraminifera Morphology	Control Site
MUC	030	JC241_030_MUC_FORAM_Cr7	2/20/23	13.73095	-126.20412	4694	7	10 cm	63	ETOH (70%)	Foraminifera Morphology	Control Site
MUC	057	JC241_057_MUC_FORAM_Cr6	2/28/23	13.73580	-126.22040	4711	6	10 cm	63	ETOH (70%)	Foraminifera Morphology	Near track
MUC	063	JC241_063_MUC_FORAM_Cr3	3/3/23	13.73608	-126. 22053	4715	3	10 cm	63	ETOH (70%)	Foraminifera Morphology	Near Track
MUC	063	JC241_063_MUC_FORAM_Cr6	3/3/23	13.73608	-126.22053	4715	6	10 cm	63	ETOH (70%)	Foraminifera Morphology	Near Track
PUC	069	JC241_069_PUC_FORAM_G1	3/4/23	13.73631	-126.22018	4700	G1	6 cm	63	ETOH (70%)	Foraminifera Morphology	Near track
PUC	069	JC241_069_PUC_FORAM_B2	3/4/23	13.73631	-126.22018	4700	B2	6 cm	63	ETOH (70%)	Foraminifera Morphology	Near track
PUC	069	JC241_069_PUC_FORAM_R1	3/4/23	13.73631	-126.22018	4700	R1	6 cm	63	ETOH (70%)	Foraminifera Morphology	Near track
PUC	069	JC241_069_PUC_FORAM_G2	3/4/23	13.73640	-126.22045	4700	G2	6 cm	63	ETOH (70%)	Foraminifera Morphology	Tracks
PUC	069	JC241_069_PUC_FORAM_G3	3/4/23	13.73640	-126.22045	4700	G3	6 cm	63	ETOH (70%)	Foraminifera Morphology	Tracks
PUC	069	JC241_069_PUC_FORAM_W1	3/4/23	13.73640	-126.22045	4700	W1	6 cm	63	ETOH (70%)	Foraminifera Morphology	Tracks
PUC	069	JC241_069_PUC_FORAM_W3	3/4/23	13.73640	-126.22045	4700	W3	6 cm	63	ETOH (70%)	Foraminifera Morphology	Tracks
MUC	071	JC241_071_MUC_FORAM_Cr7	3/5/23	13.73523	-126.20537	4696	7	10 cm	63	ETOH (70%)	Foraminifera Morphology	Control Site
PUC	073	JC241_073_PUC_FORAM_B3	3/5/23	13.73748	-126.22043	4716	B3	6 cm	63	ETOH (70%)	Foraminifera Morphology	Tracks
PUC	073	JC241_073_PUC_FORAM_R1	3/5/23	13.73548	-126.22042	4716	R1	6 cm	63	ETOH (70%)	Foraminifera Morphology	Near track
PUC	073	JC241_073_PUC_FORAM_Y1	3/5/23	13.73533	-126.22044	4716	Y1	6 cm	63	ETOH (70%)	Foraminifera Morphology	Near track
MUC	074	JC241_074_MUC_FORAM_Cr2	3/5/23	13.73416	-126.20027	4670	2	10 cm	63	ETOH (70%)	Foraminifera Morphology	Control Site
MUC	075	JC241_075_MUC_FORAM_Cr5	3/5/23	13.73536	-126.22065	4730	5	10 cm	63	ETOH (70%)	Foraminifera Morphology	Near track
PUC	089	JC241_089_PUC_FORAM_YS1	3/12/23	13.73107	-126.20414	4700	YS1	6 cm	63	ETOH (70%)	Foraminifera Morphology	Control Site
PUC	089	JC241_089_PUC_FORAM_R2	3/12/23	13.73107	-126.20414	4700	R2	6 cm	63	ETOH (70%)	Foraminifera Morphology	Control Site

PUC	089	JC241_089_PUC_FORAM_G1	3/12/23	13.73277	-126.20377	4700	G1	6 cm	63	ETOH (70%)	Foraminifera Morphology	Control Site
PUC	089	JC241_089_PUC_FORAM_G3	3/12/23	13.73277	-126.20377	4700	G3	6 cm	63	ETOH (70%)	Foraminifera Morphology	Control Site
PUC	089	JC241_089_PUC_FORAM_B1	3/12/23	13.73277	-126.20377	4700	B1	6 cm	63	ETOH (70%)	Foraminifera Morphology	Control Site
PUC	089	JC241_089_PUC_FORAM_R3	3/12/23	13.73277	-126.20377	4700	R3	6 cm	63	ETOH (70%)	Foraminifera Morphology	Control Site
PUC	095	JC241_095_PUC_FORAM_Y2	3/15/23	13.958587	-116.553029	4086	Y2	6 cm	63	ETOH (70%)	Foraminifera Morphology	UK-1 site 1
PUC	095	JC241_095_PUC_FORAM_B2	3/15/23	13.958587	-116.553029	4086	B2	6 cm	63	ETOH (70%)	Foraminifera Morphology	UK-1 site 1
PUC	095	JC241_095_PUC_FORAM_B1	3/15/23	13.958587	-116.553029	4086	B1	6 cm	63	ETOH (70%)	Foraminifera Morphology	UK-1 site 1
PUC	095	JC241_095_PUC_FORAM_Y3	3/15/23	13.958204	-116.552772	4086	Y3	6 cm	63	ETOH (70%)	Foraminifera Morphology	UK-1 site 2
PUC	095	JC241_095_PUC_FORAM_W2	3/15/23	13.958204	-116.552772	4086	W2	6 cm	63	ETOH (70%)	Foraminifera Morphology	UK-1 site 2
PUC	095	JC241_095_PUC_FORAM_R1	3/15/23	13.958204	-116.552772	4086	R1	6 cm	63	ETOH (70%)	Foraminifera Morphology	UK-1 site 2
PUC	095	JC241_095_PUC_FORAM_R3	3/15/23	13.96045	-116.55512	4086	R3	6 cm	63	ETOH (70%)	Foraminifera Morphology	UK-1 site 3
PUC	095	JC241_095_PUC_FORAM_R2	3/15/23	13.96045	-116.55512	4086	R2	6 cm	63	ETOH (70%)	Foraminifera Morphology	UK-1 site 3
PUC	095	JC241_095_PUC_FORAM_B3	3/15/23	13.96045	-116.55512	4086	B3	6 cm	63	ETOH (70%)	Foraminifera Morphology	UK-1 site 3

Total Foraminifera Samples

- n (MUC cores): 9
- n (PUC cores): 27
- Sampling Increments for cores (PUC and MUC): 0-1 cm, 1-2 cm, 2-3 cm, 3-4 cm, 4-5 cm
- n samples (MUC cores): 45
- n samples (PUC cores): 135
- n Xenophyophore DNA samples: 16
- n Xenophyophore CT samples: 7

In summary, foraminiferal sampling was a successful endeavor with 18 pushcores taken at OMCO (6 in each treatment: track, near track, and control) as well as 7 supplementary multicores from the control site and 2 from the “near track” area. These multicores will provide more material and replicates to help understand the background natural variability of the area. This is important to help disentangle natural variability from any long-term effects of the PCV collector. 9 pushcores from 3 sites were also taken at the UK-1 lease area to temporally build on baseline studies from Gooday & Goineau (2017, 2019). This will help understand temporal or seasonal changes amongst deep sea foraminiferal communities.

A qualitative live sort of two rejected cores from the OMCO control area was carried out to get a broad initial insight into the types of foraminifera that inhabit the region. This live sort revealed a dominance of monothalamous (single-chambered) forms such as spheres, tubes, komokiaceans (Figure 18.10), and *Lagenammina*-like flasks. Common multi-chambered forms were agglutinated including *Pseudonodosinella nodulosa, Hormosinella distans, Reophax* sp. HOR_019, *Reophax dentaliniformis,* and *Hyperammina laevigata.* Calcareous forms such as *Epistominella exigua* and *Alabaminella weddellensis* were also present, however very rare throughout the sorted samples. The extreme depths of ~4,700 meters and the calcium carbonate compensation depth (CCD) seem to dictate the forms and species of foraminifera that live in the OMCO area. This would explain the dominance of organic and agglutinated forms as well as the lack of calcareous forms when compared to other CCZ areas like NORI-D and UK-1, which are both at around 4,100 meters water depth. This was especially visible in the ROV footage from the UK-1 area as globigerinid ooze speckled the sediment with white dots.

Xenophyophores were also opportunistically collected from ROV push cores, ROV manipulators, and boxcores (Figure 18.11). 16 xenophyophore specimens were collected and 23 samples were taken and preserved in RNALater at -80 degrees Celsius and for DNA sequencing (n=16) and in 4% buffered formalin for MicroCT scanning and structural analyses (n=7). 8 (RNALater: n=5; Formalin: n=3) of these samples came from a holothurian (*Pseudostichopus* sp.?) that had encrusted xenophyophore tests to its dorsal region (Figure 18.12). DNA sequencing and MicroCT scans will be run on these xenophyophores to determine if they were alive at the time of collection and living on the back of the holothurian. If they are, this could implicate the holothurian as a kind of “mobile reef”.

In the OMCO tracks and furrows, *Reticulammina* sp. (Figure 18.11.i) was the dominant large sessile organism to recolonize areas of nodule removal. This species is undescribed from the CCZ but the genus has been described from the abyssal Atlantic. DNA samples and MicroCT samples were taken from 4 specimens to help describe and clarify the taxonomy of this important recolonizer species.

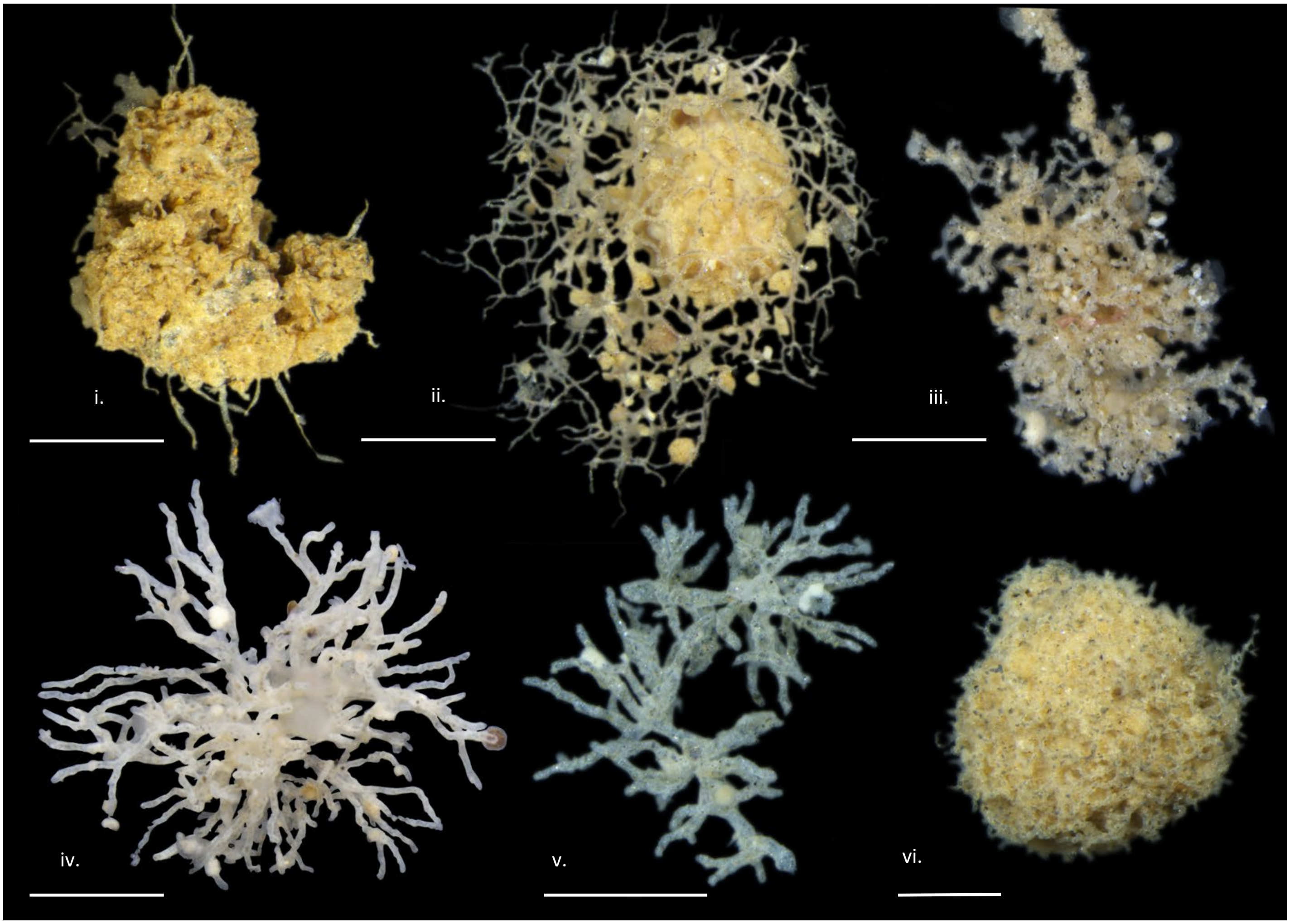


Figure 18.10 Micrograph of komokiaceans photographed from a qualitative live-sort from OMCO control sites. i.) Edgertonia argillispherula; ii.) Edgertonia sp.; iii.) Ipoa sp.; iv.) Septuma ocotillo; v.) Ipoa sp.; vi.) Reticulum sp. (Scale bar=500 um).

Figure 18.11 Macrophotography plate of xenophyophores sampled on JC241. Xenos were preserved for DNA and Micro-CT analysis. i.) Reticulammina sp.; ii.) Branching sp. indet.; iii.) Mud xeno indet.; iv.) Stannophyllum sp.; v.) Moannammina sp.; vi.) Psammina limbata; vii.) Spiculammina sp. (Scale bar= 1 cm).

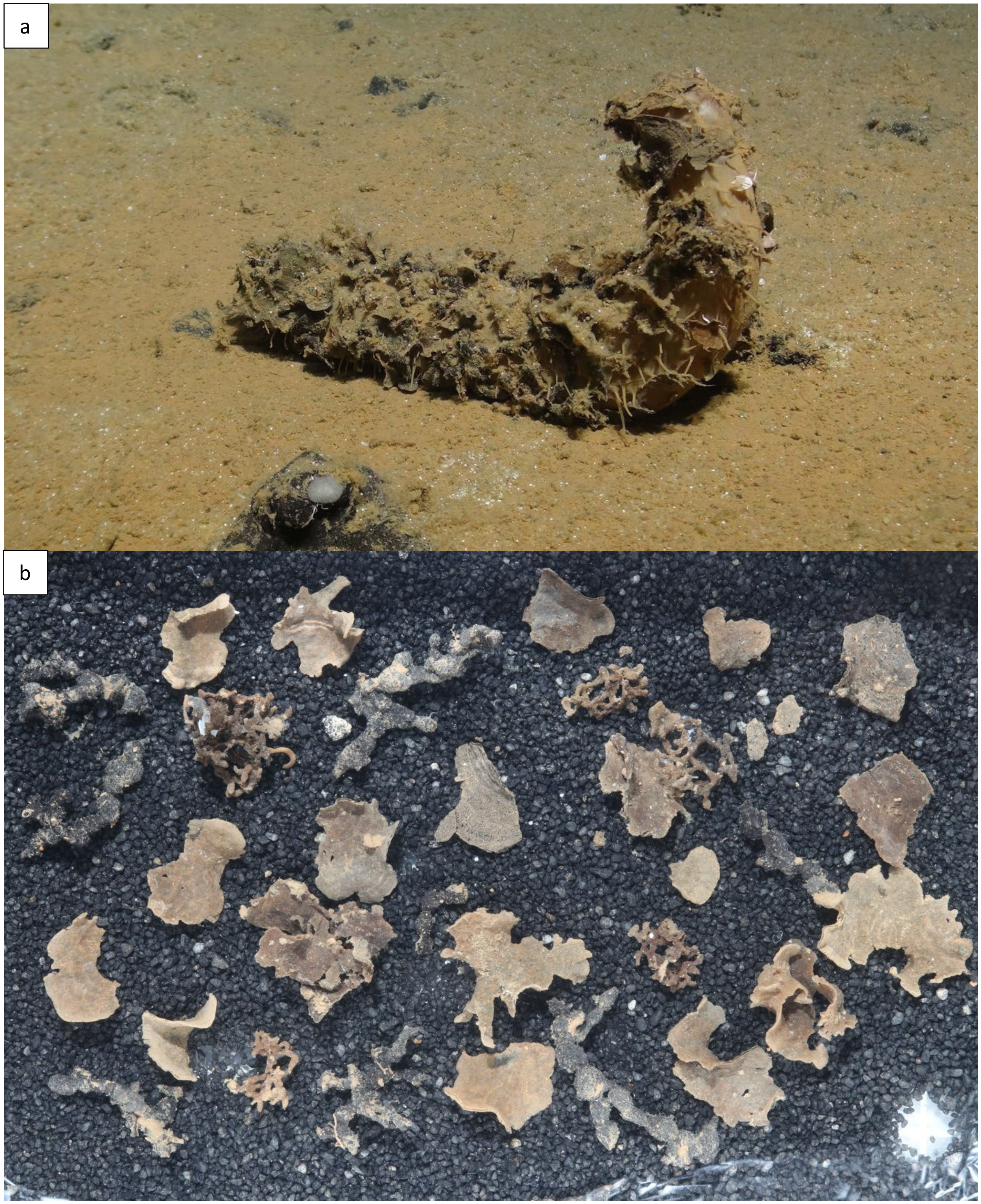


Figure 18.12 a.) Scorpio still of the xenophyophore-encrusted holothurian (Pseudostichopus sp. ?). b.) Xenophyophore morphotypes found stuck to the holothurians dorsal side.

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19. Microbial diversity and eDNA

Susan Evans, NOC

19.1 Background and aims

To meet objective 5.3 (Biodiversity, community structure and trophic dynamics) of the SMARTEX project, water, sediment and nodule samples were collected using multiple methods with the overall aim of determining the impact of mining on deep-sea microbial ecosystem function and to assess of spatial changes in microbial and macro/megafauna communities using environmental DNA (eDNA) metabarcoding. A diverse microbial community is typically found in the upper 20 cm of abyssal sediment in the CCZ (Hollingsworth et al., 2021), with spatial differences in functional potential previously identified between sites in CCZ. Samples collected during JC241, will be used to establish baseline conditions and assess spatial and temporal changes in microbial community composition and function in abyssal habitats across different regions, substrate types and across gradients in mining impact.

To understand the natural state of marine ecosystems, there is a need to characterise biological baselines in remote environments that are often challenging to sample. The use of emerging technologies to facilitate genetic observations has great potential to improve baseline data, especially in environments like the deep-sea and to monitor the impact of anthropogenic pressures such as deep-sea mining. eDNA analysis has the potential to characterise biological communities with high sensitivity and species-level accuracy without disturbing organisms in the environment, by sequencing DNA signatures from sloughed cells, scales, slime, faeces or other material left behind (Wood et al., 2020).

Initially the aim was to demonstrate simultaneous biological sampling using the high-resolution autonomous eDNA sampler, the Robotic Cartridge Sampling Instrument (RoCSI) developed at National Oceanography Centre, UK in the nose of the Autosub5, together with image and multibeam survey's at different altitudes from the seabed. However, owing to technical difficulties with Autosub5 prior to JC241, the RoCSI was integrated onto the ROV ISIS instead.

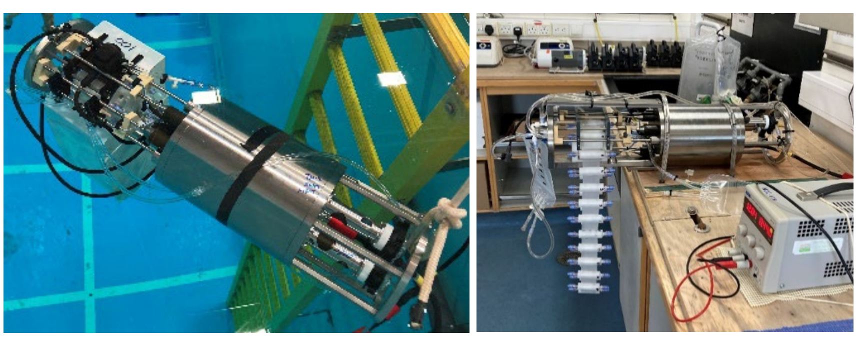


Figure 19.1: RoCSI in the NOC test tank and benchtop setup (photo credit: Susan Evans, NOC)

19.2 RoCSI

The RoCSI is designed to filter and preserve predefined volumes of water in-situ, collecting genetic material such as eDNA on a 0.22 µm filter (Figure 19.1). During JC241, a RoCSI rated to 5000 m was used to collect and preserve environmental DNA (eDNA) from the deep ocean. In addition, water samples were collected using the CTD-Rosette and ROV and then filtered using a peristaltic pump in the lab. These samples will be used to validate the eDNA samples collected autonomously but also to assess the biodiversity in OMCO track site and control site. As well demonstrating the ability of RoCSI to work autonomously at depth during the cruise, water and sediment samples were also collected for eDNA analysis using traditional CTD-rosette deployments and during ROV dives to validate and compare to the autonomously collected samples. Throughout the cruise, opportunistic eDNA samples were successfully collected from the CTD-rosette casts, ROV dives and also from the mega-core, gravity core, box core and ROV push cores.

RoCSI on ROV ISIS

The RoCSI was integrated onto the rear of ROV ISIS at the start of JC241 (Figure 19.2). The sample inlet consisted of a 2.2 m tubing at the front right of the nose (Figure 19.2A), away from the thrusters and in a forward position. The inlet was flushed using a syringe in between ROV dives with a 5% bleach solution followed by Milli-Q water and both ends capped to avoid contamination. Samples collected were either 3L or 4L depending on the ROV dive duration. During the ROV missions, RoCSI received 12V from the ROV and was programmed in real time directly from the ROV control van using the RoCSI GUI.

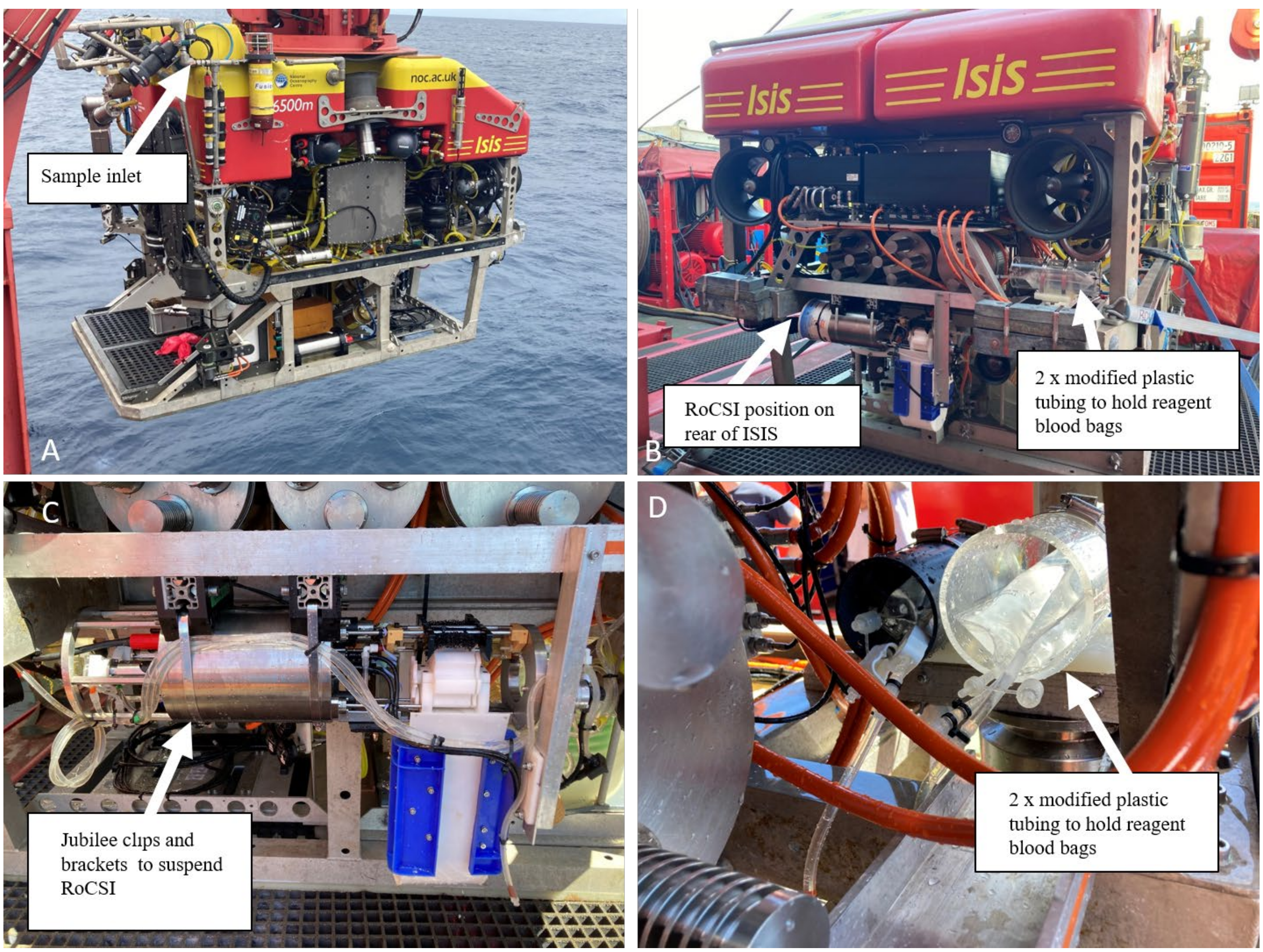


Figure 19.2 RoCSI on ROV ISIS with A) sample inlet on the ROV ISIS away from thrusters and at the front of the vehicle, B) RoCSI location on the rear of the ROV, C) metal framework and brackets suspend RoCSI with a reinforced sample bucket to protect the sampling cartridges, D) modified plastic tubing to hold reagent blood bags.

Sterile 0.22 µm Sterivex™ filter units were assembled into pre-labelled cartridge units by hand as close as possible prior to the deployment of the ROV. These were loaded into a 24 cartridge sampling belt which was loaded into RoCSI using the GUI to advance the magazine. The correct alignment of all the cartridge units was then checked at least twice. Fresh RNAlater preservative and cleaning solution (5% bleach) was prepared as close to deployment as possible to avoid extreme temperature changes on deck. The plumbing was checked for leaks. RoCSI was programmed directly using a GUI through RS232 serial coms via the ROV ISIS umbilical cord. After the ROV dive, the samples were removed from RoCSI as soon as possible, the cartridge units were disassembled, and the Sterivex units sealed. All samples were then immediately transferred to the -80°C freezer. In total, 17 samples were collected autonomously using the ROV ISIS from a total of 7 ROV dives (401, 403, 404, 405, 409, 410, 413) (Table 19.1) from the OMCO track and control site (Figure 19.3) and UK-1 (Figure 19.4). The dives were either multibeam dives at approximately 50 m altitude or imaging dive at 3 m altitude. Details about the multibeam and megafaunal imaging ROV dives can be found in sections 10 and 14 respectively.

Table 19.1. Summary of RoCSI samples collected on ROV ISIS during JC241

Station	Dive number	Number of RoCSI samples	Location	ROV dive type
23	401	1	OMCO	Multibeam
59	403	19	OMCO track	Photography on tracks and 1 km control transect
60	404	3	OMCO control	Photography at control site (1 km control transect)
62	405	17	OMCO control	Photography (3 x 2 km image transects at track sites)
82	409	24	OMCO tracks	Photography
86	410	19	OMCO tracks	Multibeam
98	413	24	UK-1-AOI2	3 m altitude photography (5 x 2 km transects)
		107		

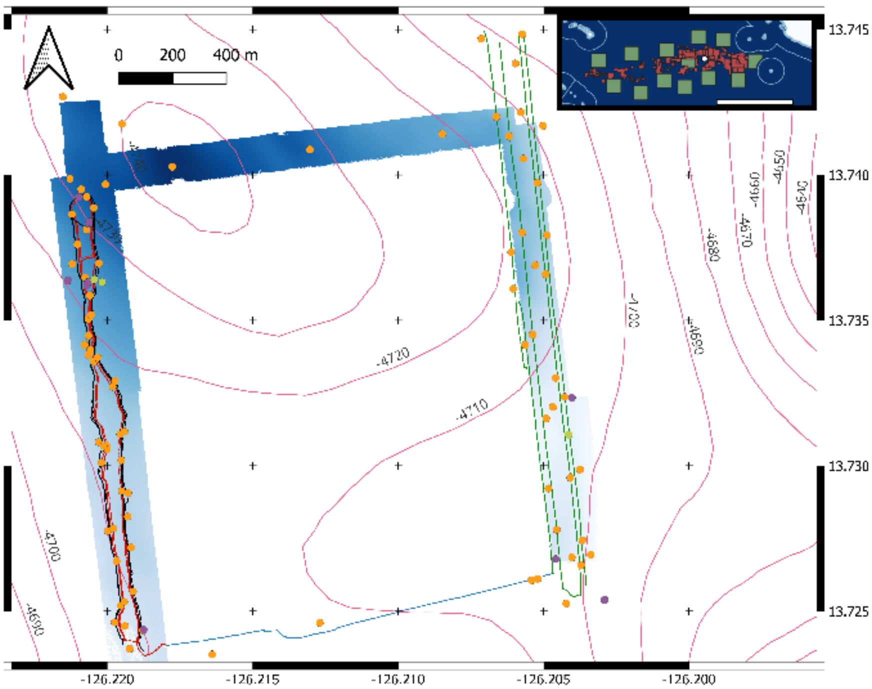


Table 19.3. Map of RoCSI samples (each orange dot is represents an individual RoCSI sample with sampling intervals set to either 60 or 70 minute depending on the length of the transect) obtained using ROV ISIS during imaging survey at the OMCO site throughout JC241.

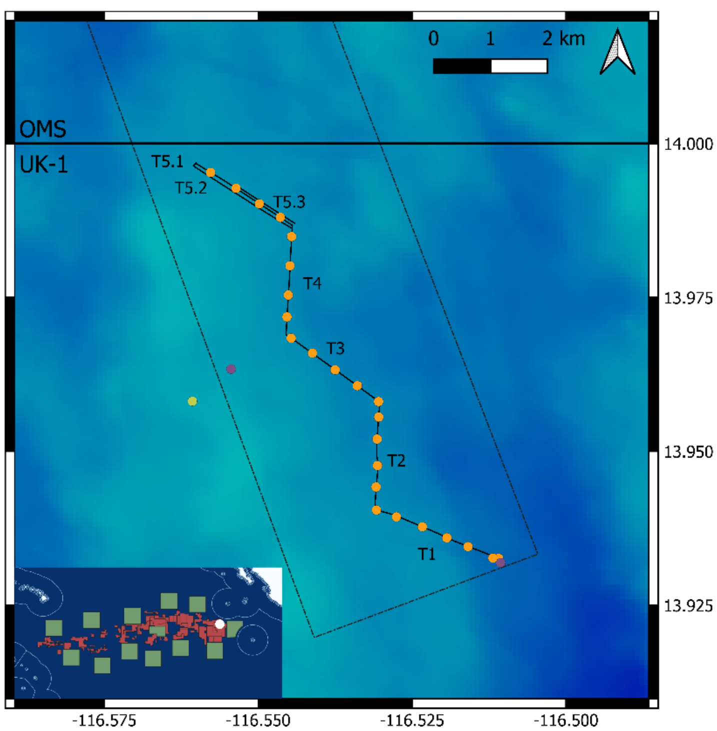


Figure 19.4. Location of RoCSI samples along ROV image transect (yellow), ROV push core (green) and ROV niskin (purple) at UK-1

19.3 Water Sampling

Water samples were collected from niskin bottles on both the CTD-rosette and on the ROV both within and outside plume impact area at OMCO to evaluate how plumes impact water column community composition and function as measured by eDNA and microbial functional genomic profiles.

CTD-rosette

Water was collected from the 10L OTE sampling bottles mounted on the CTD-Rosette from a total of 3 casts at 3 of the sites (OMCO track, OMCO control and UK-1) with 3 x 10 L niskin bottles fired at 5 depths (bottom, bottom -10 m, lowest dissolved oxygen, chlorophyll-a maximum and 5 m). In total, 45 samples were collected for eDNA and microbial analysis (Table 19.1).

Table 19.1. Location and number of eDNA samples filtered from CTD-rosette casts during JC241

Station number	Location	Number of samples	Total volume of water filtered (L)
9	OMCO	15	48
31	OMCO control	15	48
96	UK-1	15	48
		45	**144**

Following the collection and retrieval of the CTD on deck, seawater was immediately filtered in triplicate through 0.2 µm Sterivex™ filters using a Masterflex peristaltic pump in a laboratory (Figure 19.5) which was kept free of sediment and fish biomass. 4 L of seawater was filtered per sample with the exception of the surface and chlorophyll-a maximum depths, where only 2 L was filtered due to high biomass. The eDNA on the filter was immediately preserved using RNAlater preservative and then stored at -80°C onboard.

Figure 19.5. Filtration setup in the lab using peristaltic pumps.

Water Sampling from the ROV niskins

Water was also collected using ROV ISIS and then filtered in the lab for subsequent eDNA analysis. A 10 L OTE sampling bottle from the National Marine Equipment Pool was mounted on the left side forward and the bottle was triggered using the robotic arm which pulled a rope above the sampling tray (Figure 19.6).

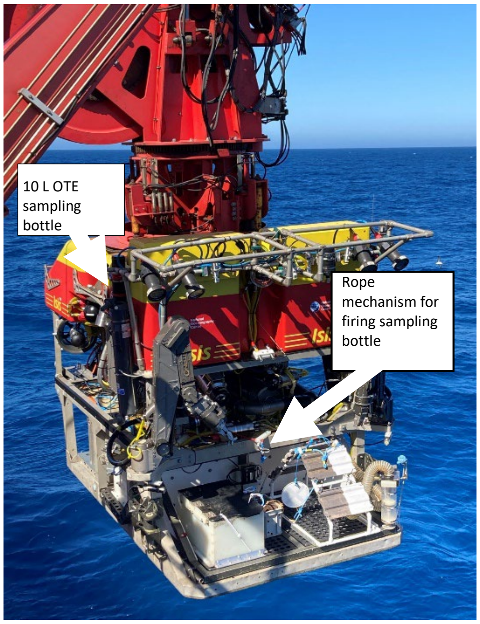


Figure 19.6 Location of 10L water sampling bottle on ROV ISIS and the triggering mechanism.

In addition, water was also collected using the 6 x 1.2 L niskin bottles positioned on the right side aft. The small niskin bottles were fired under the control of the ROV and were closed one at a time. It took between 3 and 4 minutes to fire all 6 bottles under the control of the ROV pilot. Care was taken to avoid disturbing the sediment during sampling so typically the niskin's were fired at the start of a sampling event. In total, 37 samples were filtered. Following the dive, seawater was collected from the bottles into 10L carboys using sterile tubing as soon as the ROV was secured on deck. The water was filtered in the same methodology as detailed above for the CTD samples.

Table 19.2. Location of ROV dives at OMCO and UK-1 where water was filtered from ROV niskin bottles for eDNA analysis

Station number	Location	ROV dive	Number of samples
13	OMCO track	399	6
60	OMCO control	404	4
62	OMCO control	405	4
69	CUBE deployment	406	7
82	OMCO track	409	4
86	OMCO tracks and control	410	4
89	OMCO track	411	4
95	UK-1	412	4
			37

19.4 Sediment sampling

Sediment samples for eDNA analysis and microbial ecology (abundance, biomass, community composition and functional diversity of microbes) were collected from 3 sites in total (OMCO, Preservation Reference Area (PRA) and UK-1) using multiple coring methods. At OMCO, sediment samples were taken with boxcore, gravity core, megacore and ROV pushcores (Figure 19.7) from both the track site and the control site. At UK-1, an ROV push core, megacore and a gravity core were obtained which will provide baseline information. Specific details about the sediment sampling methods can be found in section 11 of this report.

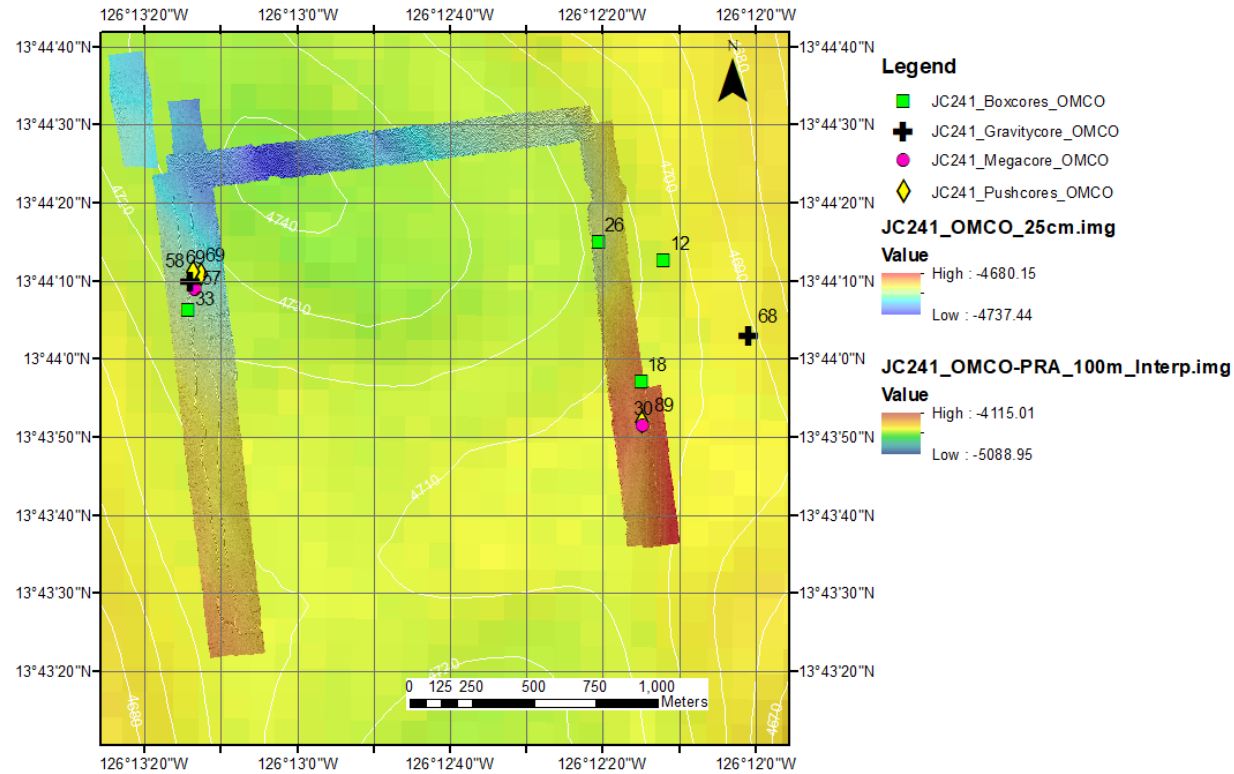


Figure 19.7, Location of sediment sampling at the OMCO track and control areas

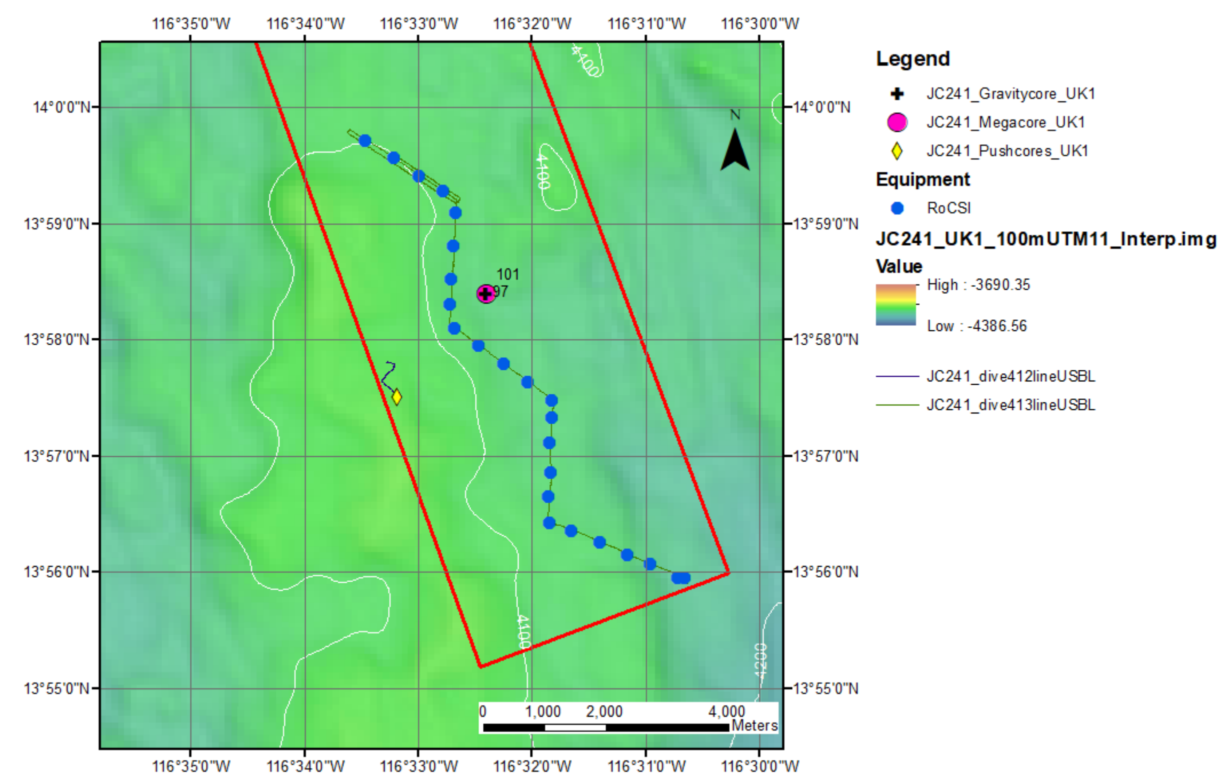


Figure 19.8, Location of gravity core, ROV push core and megacore sampled for eDNA/ microbial work in relation to the ROV image survey transect line at UK-1 where RoCSI samples were also taken.

Gravity core

Following retrieval of the core on deck, samples for molecular analysis were collected from every 1 m section from 3 gravity cores (Table 19.3). Immediately after each 1 m section of core was cut, a ~ 8 g sub-sample was taken from the bottom and top of each 1 m section using a 10 ml syringe with the end cut off and the sediment sample dispensed into a sterile sampling bag, then frozen at -80°C. The saw used to cut the core liner was cleaned in between each section using 95% ethanol and gloves were changed in between.

Table 19.3. Summary of molecular samples collected from the gravity core at 3 locations

Station	Location	Number of samples	Length of core (cm)
58	OMCO track	10	486
68	OMCO control	8	335
97	UK-1	8	328

Megacore

Sediment for microbial and eDNA were collected from 1 megacore at each of the OMCO track and control sites and 1 from UK-1 (Table 19.4). The core was sectioned in a 4°C cold room within 1 hour of being back on deck. Approximately 10 ml of the sediment water interface was sampled using a sterilised tubing and syringe. Each core was then sectioned into 2 cm intervals, down to 20 cm and the sectioning equipment was rinsed, then dipped in 5% bleach followed by a rinse in Milli-Q in between each section. A 2 g subsample of each section was taking using a sterile plastic spoon and transferred into a 1.5 ml eppendorf tube which was flash frozen in liquid nitrogen then stored at -80°C for subsequent metatranscriptomic analysis. The remaining section was transferred into a sterile bag for metagenomics and then stored at -80°C.

Table 19.4. Summary of megacore samples taken during JC241 including nodule presence in each core

Station	*Location*	*Samples collected*	*Nodule*
30	*OMCO Control*	*21*	*0*
57	*OMCO Track*	*21*	*1*
101	*UK-1*	*21*	*1*

ROV push core

Sediment for microbial and eDNA were collected from 1 push core at each of the OMCO track and control sites and 1 from UK-1 (Table 19.5). Prior to sectioning, all equipment (core extruder, rings) were cleaned with 5% bleach and rinsed thoroughly with Milli-Q water. The core was sectioned in a 4°C cold room within 1 hour of being back on deck. Each core was sectioned into 2 cm intervals, down to 20 cm and the sectioning equipment was rinsed, then dipped in 5% bleach followed by a rinse in Milli-Q in between each section. A 2 g subsample of each section was taking using a sterile plastic spoon and transferred into a 1.5 ml eppendorf tube which was flash frozen in liquid nitrogen then stored at -80°C for subsequent metatranscriptomic analysis. The remaining section was transferred into a sterile bag for metagenomics and then stored at -80°C.

Table 19.5. Summary of ROV push core samples taken during JC241

Station	ROV dive	Location	Number of sediment samples
69	406	OMCO track	21
69	406	OMCO control	21
89	411	OMCO plume	21
95	412	UK-1	21

Box core

Sediment for eDNA and microbial ecology was collected from selected boxcores performed at the OMCO track and control sites and the PRA site first sampled in 1989 (Table 19.6). In total, eDNA samples were collected from the successful boxcores performed at 13 of the original PRA sites (Figure 19.9). These samples will be compared to the original Wilson dataset but also act as a control reference site for eDNA metabarcoding from the OMCO site.

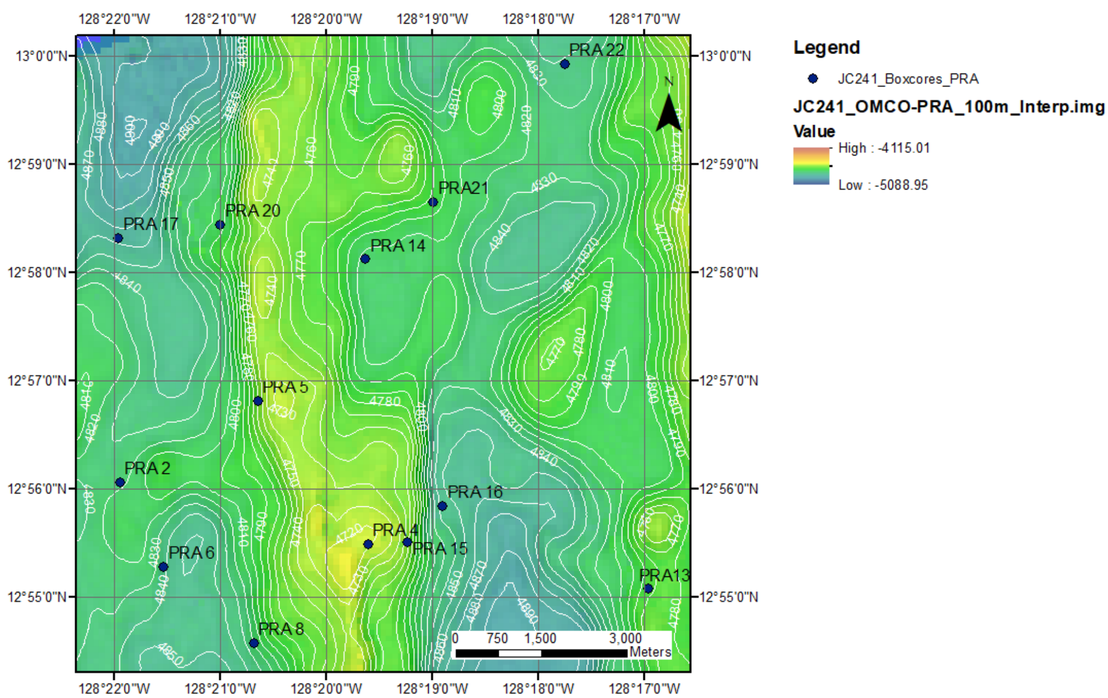


Figure 19.9, location of PRA sites from 1989 where boxcore samples for eDNA analysis were taken.

Table 19.6. Location of boxcores sub-sampled for molecular analysis during JC241, including whether nodules were collected or not.

Station	Location	Nodules collected
12	OMCO control	3
18	OMCO control	3
26	OMCO control	3
33	OMCO tracks	0
36	PRA 5	3
38	PRA 17	0
39	PRA 20	0
41	PRA 14	0
42	PRA21	0
45	PRA 22	0
46	PRA 13	0
49	PRA 16	1
50	PRA 15	3
51	PRA 4	0
52	PRA 8	0
53	PRA 6	0
54	PRA 2	0

Following the draining of the overlying water from the boxcore, 3 nodules (if present) were removed using sterile tweezers and placed in separate sterile bags. These nodules were photographed, measured and then rinsed using Milli-Q water. The wash was collected in 50 ml falcon tube and then frozen at -80°C. 3 x 30 cm sediment cores were taken from the centre of the box core using sterilised piping (2 cm diameter) which were then extruded into separate bags using a homemade metal extruder. All equipment used was cleaned thoroughly between each core extrusion.

19.4 Processing methodology

Once all the filter units and sediment samples are transported to the UK, eDNA/DNA/RNA in the samples will be extracted in a dedicated clean lab at NOC. DNA metabarcoding (multiple markers) will be performed on the samples for eDNA analysis to provide an overview of biodiversity and targeted single species detection will be carried out using quantitative PCR (qPCR) with species-specific primers. The specific qPCR assays conducted will be largely informed using information from the ROV video transects and based on species of interest and importance. For the metabarcoding approach, eDNA will be extracted from all samples and gene fragments will be amplified and sequenced (paired end) using an Illumina MiSeq system. DNA markers from four gene regions (cytochrome c oxidase I, 18S rRNA, 12S rRNA, and 16S rRNA) will be used to assess biodiversity in these samples. The raw sequence reads will be demultiplexed, quality filtered and then clustered into operational taxonomic units (OTUs). The OTUs will be denoised and taxonomically assigned to the best possible taxonomic resolution using several sequence databases. The results from both multiple marker and single marker analysis will be used to assess biodiversity at the sample areas (OMCO on and off track, PRA-1 and UK-1). In addition to metabarcoding of eDNA, abyssal microbial communities will be characterized at each site from the sediment and water samples collected.

Between sampling areas, the number of OTUs detected and number of unique OTUs for each metabarcoding marker will be compared to give an indication of deep-sea community composition at each sampling area. To validate the eDNA data collected autonomously, this will be compared to eDNA data collected using the CTD-rosette casts and ROV dives. Results from these samples will be discussed in the context of biodiversity assessment and also compared to eDNA samples collected by traditional rosette sampling.

DNA/RNA will also be extracted from specific samples and 16S (bacteria and archaea) and 18S (eukaryotes) rRNA gene amplicon screening, microbial metagenomics and metatranscriptomics will be performed using established protocols. Metagenomic data will be used for taxonomic identification and to identify potential metabolic pathways. Metagenomic assembly will be used to determine which 16S markers are associated with metabolic functions and as the basis for metatranscriptomic mapping. Metatranscriptomics will be used to identify which genes are actively expressed allowing the pathways involved in microbial ecosystem function to be determined.

Bioinformatics and data analysis will be conducted using QIIME 2 and genomic sequencing and bioinformatics will follow established methodologies (e.g Ottesen et al., 2011). However, bioinformatic pipelines will be optimised to ensure the most appropriate methods are applied to the datasets generated.

19.5 References

Hollingsworth, A., Jones, D.O.B., Young, R.C. (2021) Spatial Variability of Abyssal Nitrifying Microbes in the North-Eastern Clarion-Clipperton Zone. Frontiers in Marine Science (8)

Ottensen, E., Marin, R., Preston, C., Young, R.C., Ryan, J.P., Scholin, C.A., DeLong, E.A. (2011) Metatranscriptomic analysis of autonomously collected and preserved bacterioplankton, ISME J 5, 1881-1895.

Wood, S., Biessey, L., Latchford, J., Zaiko, A., von Ammon, U., Audrezet., F., Cristescu, M., Pochon, X (2020). Release and degradation of environmental DNA and RNA in a marine system. Science of the Total Environmental (704)

20. Outreach

Drone

Daniel Jones

A DJI Mini 2 drone was used to obtain photographs and video of the *James Cook*. This is a small (249 gram) drone equipped with a gimballed 4k video camera that can also take still photographs.

Figure 20.1: Drone in flight

Most of the time during the cruise the wind was too strong to fly this drone safely >20 knots. Ideal flying conditions are <15 knots. Ideal flying position is lifeboat deck forward of the bridge. It is possible to fly from the rear deck in light winds. The drone is easy to control and highly recommended. The battery lasts around 20 minutes and having three batteries charged for each flight was optimal (the drone can be landed and the battery changed relatively quickly). Holing the drone above the metal deck helps with the initiation (to prevent compass calibration issues). We did not fly the drone when the ship was moving. Drone operations were only carried out when and in locations where it was safe to operate.

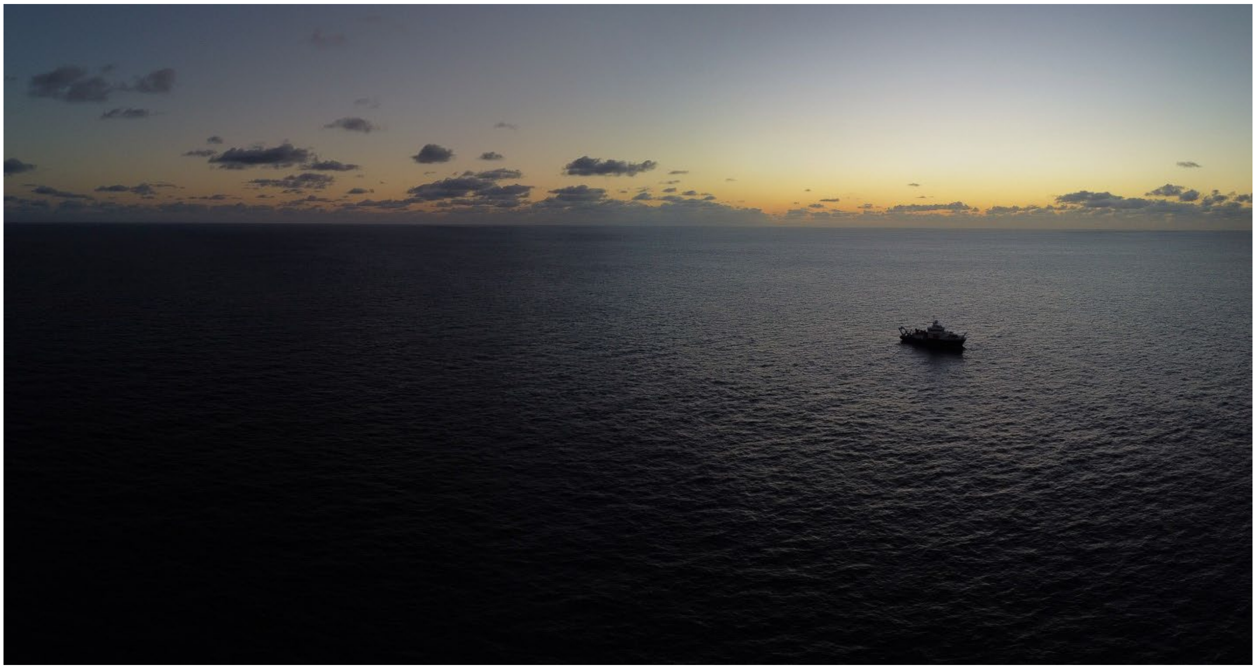
Image resolution is relatively poor at night. Photographs obtained using JPEG and RAW and video at 4K resolution. Pro mode was needed to control aperture, shutter speed and ISO for most low light photographs and video but automatic settings mostly worked well in the day except in high sun. It would be worth considering a polarizing filter for the camera for sunny operations.

Table 20.1 Drone deployments. Note times in local (GMT -8) not UTC. Files include still images and video.

Date	Start Time	End Time	Number of files	Comments
7 March 2023	08:00	08:23	28	Day. Images of ship
8 March 2023	06:00	07:13	186	Dark to day. Sunrise. Images of ship
9 March 2023	18:40	19:07	33	Night. Images of ROV recovery
12 March 2023	14:46	14:56	57	Day. ROV recovery
15 March 2023	07:12	07:40	33	Day. Short Mooring Deployment
15 March 2023	11:25	11:42	49	Day. Long Mooring Deployment
15 March 2023	14:25	14:28	17	Day. Short Mooring Deployment
17 March 2023	17:57	18:06	36	Sunset. Images of ship
18 March 2023	10:51	10:59	53	Day. Images of turtle


JAMES COOK


MLRM6
JAMES COOK
LONDON



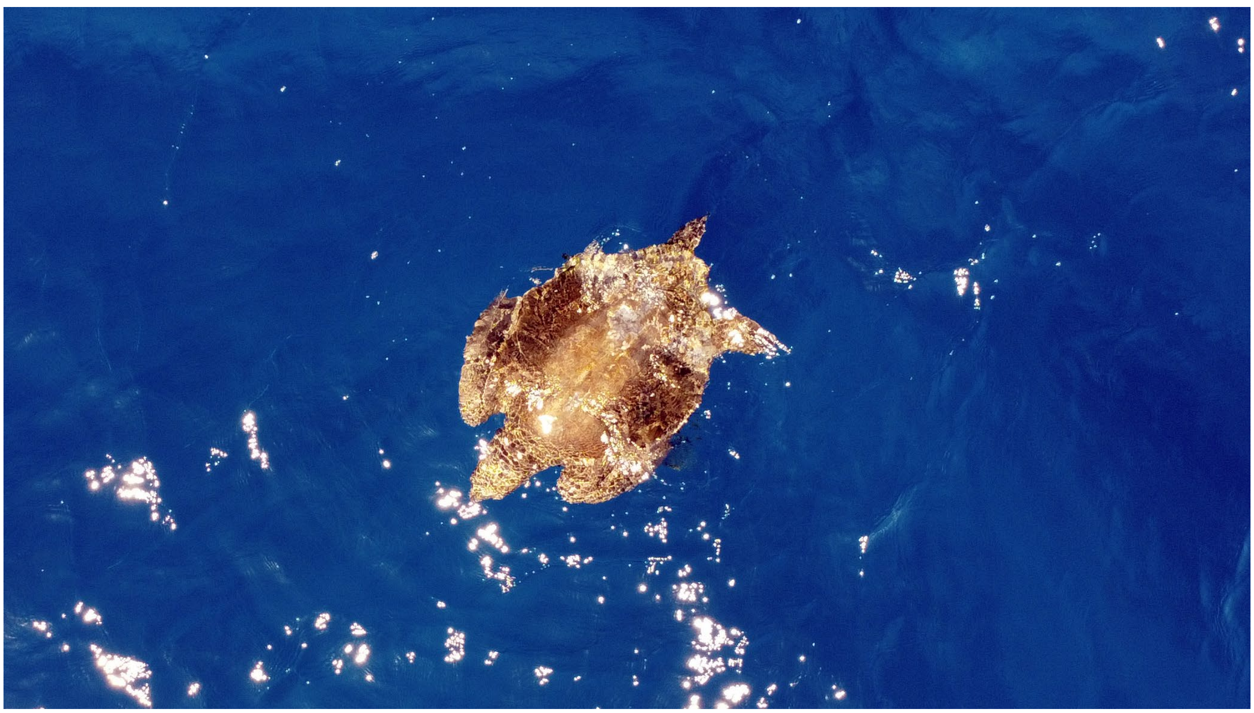


Figure 20.2 The best drone shots

Film making

Eleanor Mortimer

I joined the SMARTEX expedition as an observer/filmmaker at very short notice. Together with Jacob Thomas & Muriel Rabone, I had been making a documentary about deep sea taxonomy (with a focus on the team at NHM working on CCZ abyssal plains) for a year and grown very keen to capture the 'discovery' part of this work, which happens at sea. The idea of traveling somewhere so remote and unknown (and exploring a place so deep) is incredibly exciting to most people, and they want to know what this experience is like. For this reason I resolved to capture the human side of being at sea, intertwined with the details of the scientific processes on board. As a filmmaker I am interested in the human relationship to the natural world, and our endeavours to make sense of the unknown.

My process is observational, which means that generally I try not to influence or direct what's happening, in an attempt to capture an unfolding experience. Arriving on board having never been on a ship before, I felt like there was a tremendous amount to get my head around. My initial activities involved learning the layout and rhythms of the ship, getting to know the crew, technicians and scientists and making sure everyone understood why I was there filming, and felt comfortable with what I was doing.

As I got the hang of the rhythms of the ship and the science processes, I began to make lists of the types of scenes I needed and with who, as well as follow-up moments from scenes I had already filmed. I was really grateful for the fact that everybody made me feel welcome to film, at any given hour of the day and night, and trusted me to be there.

As a solo director / camera / sound person I faced a lot of challenges. Every day many things would be happening at once, often changing at short notice, making it hard to 'switch off' from filming. I would have to think carefully about which scenes I needed to get and how, as well as carrying all my equipment up and down flights of stairs and through very heavy doors. Getting good audio was extremely difficult due to the quantity of machines and air conditioning units on board a ship (microphones are not good at filtering this kind of sound). In the ROV van, I devised a system to have 5 microphones going at once, capturing people talking in different parts of the room (to be synced in post production).

Over the 8 weeks I was able to capture on camera some beautiful, dramatic, curious, and funny moments of humans encountering the unknown; the excitement in the ROV van when a new animal appears, the anticipation as a box core lands on deck and everyone peers over it and sees a giant worm or starfish sitting amongst the nodules, and the quieter moments of contemplation

I also captured the collaborative nature of what was happening on board – the bridge communicating with the deck team and ROV team, and the biologists comparing thoughts on the animals. Towards the end of the cruise I am picking up brief in-situ interviews with various people (scientists, techs & crew) about the nature of life on the ship and our relationship with the deep sea. (Further in-depth voiceover interviews will be carried out on land, in a sound controlled environment). I am incredibly grateful to have had this experience, and I look forward to editing together a film which draws audiences into the experience of those on board, inciting curiosity and wonder about this part of the world. I have filmed around 90 hours of footage, and will be using the deep sea ROV footage as well as some epic gopro and drone footage filmed by Adrian Glover, Daniel Jones & Lotty Astbury, as well as microscope footage filmed by Bryan O'Malley and the NHM team.


DEEP-SEA
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WoRMS

Figure 20.3: Example shots from the filming

GoPro filming

Daniel Jones and Adrian Glover

We filmed some material with several GoPro Cameras (models 10 and 11). The most successful was the deployment of the GoPro camera on a carbon fibre extendable pole. The pole was 2m long when folded and consisted of 8 sections all of a similar length. The GoPro was attached with a movable mount to the end. Note that the GoPro cameras are rated to 10 m depth but they stop filming automatically at about 3 m depth. A separate diving housing needs to be purchased for use deeper, which would certainly be a worthwhile addition for a future cruise.

Figure 20.4: Example 4K video grabs taken with GoPro camera on pole.

21. Acknowledgements

This work was funded by the UK Natural Environment Research Council through the Seabed Mining And Resilience To EXperimental impact (SMARTEX) project (Grant Reference NE/T003537/1)

22. Appendix 1: Station List

Station	Event	Gear	Site	Date	UTCTime	Depth	Latitude	Longitude
JC241_001	Underway ADCP	Multibeam	Edge of Costa Rica EEZ	06/02/2023	17:00:00	3638	10.073100	-92.087017
JC241_002	Multibeam	Underway ADCP	Edge of Costa Rica EEZ	06/02/2023	17:00:00	3638	10.073100	-92.087017
JC241_003	CTD01	CTD	Eddy Edge	09/02/2023	09:17:00	3210	11.817767	-104.710083
JC241_004	CTD02	CTD	Eddy Centre	09/02/2023	16:54:00	3400	11.821330	-105.638287
JC241_005	Underway ADCP	Underway ADCP	Edge of Clipperton EEZ	11/02/2023	02:28:00	4080	12.677283	-111.691800
JC241_006	Multibeam	Multibeam	Edge of Clipperton EEZ	11/02/2023	02:28:00	4080	12.677283	-111.691800
JC241_007	Bathysnap1	Bathysnap	UK-1	12/02/2023	06:35:00	4074	13.910182	-116.552037
JC241_008	Multibeam	Multibeam	UK-1	12/02/2023	07:05:00	4075	13.908583	-116.553083
JC241_009	CTD03	CTD	OMCO track	14/02/2023	20:38:00	4717	13.731247	-126.222662
JC241_010	CASIUS Beacon Test	Mooring	OMCO	15/02/2023	00:52:00	4697	13.715000	-126.236000
JC241_011	Gravity core 1	Gravity Corer	OMCO control	15/02/2023	12:19:00	4685	13.736783	-126.202867
JC241_012	Box core 1	Boxcore	OMCO control	15/02/2023	15:40:00	4685	13.736833	-126.203333
JC241_013	ROV dive 399	ISIS ROV	OMCO	15/02/2023	20:25:00	4712	13.731246	-126.211895
JC241_014	Box core 2	Boxcore	OMCO control	16/02/2023	17:19:00	4688	13.736833	-126.203350
JC241_015	Fish trap	Fish trap	OMCO fish trap 1	16/02/2023	21:36:00	4668	13.736030	-126.200533
JC241_016	Box core 3	Boxcore	OMCO control	16/02/2023	22:05:00	4689	13.736851	-126.203461
JC241_017	ROV dive 400	ISIS ROV	OMCO	17/02/2023	02:37:00	4715	13.740000	-126.220000
JC241_018	Box core 4	Boxcore	OMCO control	17/02/2023	22:41:00	4693	13.732500	-126.204167
JC241_019	Megacore 1	Megacore	OMCO control	18/02/2023	02:19:00	4682	13.732500	-126.204167
JC241_020	Box core 5	Boxcore	OMCO control	18/02/2023	07:13:00	4664	13.738317	-126.205650
JC241_021	Gravity core 2	Gravity Corer	OMCO control	18/02/2023	11:27:00	4705	13.738350	-126.205667
JC241_022	Box core 6	Boxcore	OMCO control	18/02/2023	14:59:00	4696	13.737483	-126.204817
JC241_023	ROV dive 401	ISIS ROV	OMCO	18/02/2023	19:24:00	4720	13.742117	-126.203983
JC241_024	Megacore 2	Megacore	OMCO control	19/02/2023	04:42:00	4697	13.737467	-126.204800
JC241_025	Box core 7	Boxcore	OMCO control	19/02/2023	09:24:00	4702	13.735233	-126.205400
JC241_026	Box core 8	Boxcore	OMCO control	19/02/2023	13:15:00	4710	13.737467	-126.205717
JC241_027	Fish trap	Fish trap	OMCO	19/02/2023	17:38:00	4722	13.735850	-126.215017
JC241_028	Gravity core 3	Gravity Corer	OMCO control	19/02/2023	21:09:00	4600	13.730333	-126.204500
JC241_029	Boxcore 9	Boxcore	OMCO control	20/02/2023	00:41:00	4694	13.730933	-126.204117

Station	Event	Gear	Site	Date	UTCTime	Depth	Latitude	Longitude
JC241_030	Megacore 3	Megacore	OMCO control	20/02/2023	15:01:00	4694	13.730950	-126.204117
JC241_031	CTD04	CTD	OMCO control	20/02/2023	19:31:00	4699	13.737500	-126.205000
JC241_032	ROV Dive 402	ISIS ROV	OMCO track	20/02/2023	23:18:00	4722	13.737100	-126.221083
JC241_033	Boxcore 10	Boxcore	OMCO track	21/02/2023	10:24:00	4728	13.735050	-126.220630
JC241_034	Gravity core 4	Gravity Corer	OMCO track	21/02/2023	18:49:00	4707	13.735000	-126.220633
JC241_035	Multibeam	Multibeam	Multibeam OMCO to PRA	21/02/2023	21:54:00	4176	13.736500	-126.219333
JC241_036	Boxcore 11	Boxcore	PRA Station 5	22/02/2023	12:10:00	4728	12.946833	-128.344000
JC241_037	Boxcore 12	Boxcore	PRA Station 17	22/02/2023	16:35:00	4875	12.971883	-128.366067
JC241_038	Boxcore 13	Boxcore	PRA Station 17	22/02/2023	20:20:00	4848	12.971833	-128.366000
JC241_039	Boxcore 14	Boxcore	PRA Station 20	23/02/2023	00:14:00	4869	12.973950	-128.350033
JC241_040	Boxcore 15	Boxcore	PRA Station 14	23/02/2023	04:30:00	4799	12.968583	-128.327150
JC241_041	Boxcore 16	Boxcore	PRA Station 14	23/02/2023	08:00:00	4810	12.968617	-128.327167
JC241_042	Boxcore 17	Boxcore	PRA Station 21	23/02/2023	12:05:00	4840	12.977383	-128.316483
JC241_043	Boxcore 18	Boxcore	PRA Station 18	24/02/2023	06:57:00	4835	12.977167	-128.297333
JC241_044	Boxcore 19	Boxcore	PRA Station 18	24/02/2023	10:25:00	4835	12.977167	-128.297333
JC241_045	Boxcore 20	Boxcore	PRA Station 22	24/02/2023	14:20:00	4810	12.998650	-128.295817
JC241_046	Boxcore 21	Boxcore	PRA Station 13	25/02/2023	01:13:00	4777	12.917833	-128.282667
JC241_047	Boxcore 22	Boxcore	PRA Station 13	25/02/2023	05:23:00	4769	12.917833	-128.282667
JC241_048	Boxcore 23	Boxcore	PRA Station 16	25/02/2023	09:34:00	4844	12.930500	-128.315000
JC241_049	Boxcore 24	Boxcore	PRA Station 16	25/02/2023	13:55:00	4906	12.930583	-128.314967
JC241_050	Boxcore 25	Boxcore	PRA Station 15	25/02/2023	18:14:00	4743	12.924967	-128.320500
JC241_051	Boxcore 26	Boxcore	PRA Station 4	25/02/2023	22:54:00	4710	12.924670	-128.326690
JC241_052	Boxcore 27	Boxcore	PRA Station 8	26/02/2023	03:17:00	4788	12.909462	-128.344633
JC241_053	Boxcore 28	Boxcore	PRA Station 6	26/02/2023	07:38:00	4829	12.921171	-128.358825
JC241_054	Boxcore 29	Boxcore	PRA Station 2	26/02/2023	11:55:00	4805	12.934250	-128.365683
JC241_055	Multibeam	Multibeam	PRA to OMCO	26/02/2023	15:50:00	4803	12.916667	-128.351667
JC241_056	Fish trap	Fish trap	OMCO	27/02/2023	07:02:00	4580	13.716998	-126.183770
JC241_057	Megacore 4	Megacore	OMCO track	27/02/2023	07:48:00	4711	13.735800	-126.220400
JC241_058	Gravity Core 5	Gravity Corer	OMCO track	27/02/2023	13:02:00	4739	13.736083	-126.220550
JC241_059	ROV Dive 403	ISIS ROV	OMCO track	27/02/2023	17:05:00	4702	13.738595	-126.222982
JC241_060	ROV Dive 404	ISIS ROV	OMCO control	01/03/2023	05:01:00	4698	13.733372	-126.205585
JC241_061	Boxcore 30	Boxcore	OMCO track	01/03/2023	18:38:00	4712	13.735900	-126.220440
JC241_062	ROV Dive 405	ISIS ROV	OMCO control	01/03/2023	23:15:00	4679	13.744608	-126.206350
JC241_063	Megacore 5	Megacore	OMCO track	03/03/2023	01:11:00	4715	13.736083	-126.220533

Station	Event	Gear	Site	Date	UTCTime	Depth	Latitude	Longitude
JC241_064	Fish trap	Fish / Amphipod trap lander	OMCO	03/03/2023	05:55:00	4732	13.747367	-126.220183
JC241_065	Boxcore 31	Boxcore	OMCO track	03/03/2023	06:22:00	4732	13.735170	-126.220500
JC241_066	Gravity core 6	Gravity Corer	OMCO control	03/03/2023	10:43:00	4701	13.735233	-126.205400
JC241_067	Boxcore 32	Boxcore	OMCO track	03/03/2023	14:11:00	4724	13.733500	-126.220100
JC241_068	Gravity core 7	Gravity Corer	OMCO control	04/03/2023	03:12:00	4666	13.734150	-126.200250
JC241_069	ROV Dive 406	ISIS ROV	OMCO track	04/03/2023	08:24:00	4700	13.736167	-126.220833
JC241_070	Boxcore 32	Boxcore	OMCO track	05/03/2023	04:45:00	4705	13.735288	-126.220319
JC241_071	Megacore 6	Megacore	OMCO control	05/03/2023	09:16:00	4696	13.735233	-126.205367
JC241_072	Boxcore 33	Boxcore	OMCO track	05/03/2023	13:53:00	4705	13.734400	-126.220300
JC241_073	ROV Dive 407	ISIS ROV	OMCO track	05/03/2023	20:28:00	4716	13.736400	-126.220400
JC241_074	Megacore 7	Megacore	OMCO control	06/03/2023	19:55:00	4670	13.734162	-126.200273
JC241_075	Megacore 8	Megacore	OMCO tracks	07/03/2023	00:24:00	4730	13.735359	-126.220645
JC241_076	ROV Dive 408	ISIS ROV	OMCO tracks	07/03/2023	05:15:00	4711	13.736496	-126.219880
JC241_077	Boxcore 33	Boxcore	OMCO tracks	07/03/2023	22:02:00	4716	13.734158	-126.220399
JC241_078	Boxcore 34	Boxcore	OMCO tracks	08/03/2023	02:42:00	4735	13.733780	-126.220170
JC241_079	Boxcore 35	Boxcore	OMCO tracks	08/03/2023	07:07:00	4707	13.735400	-126.220500
JC241_080	Multibeam	Multibeam	Transit to new control site	08/03/2023	10:39:00		13.735600	-126.220600
JC241_081	Boxcore 36	Boxcore	OMCO control 2	08/03/2023	11:47:00	4702	13.635347	-126.199675
JC241_082	ROV Dive 409	ISIS ROV	OMCO tracks	08/03/2023	17:53:00	4713	13.738090	-126.220673
JC241_083	Boxcore 37	Boxcore	OMCO tracks	10/03/2023	03:34:00	4700	13.735656	-126.220654
JC241_084	Boxcore 38	Boxcore	OMCO tracks	10/03/2023	08:11:00	4700	13.735720	-126.220491
JC241_085	Boxcore 39	Boxcore	OMCO tracks	10/03/2023	12:43:00	4703	13.734300	-126.220300
JC241_086	Rov Dive 410	ISIS ROV	OMCO tracks	10/03/2023	17:02:00		13.733033	-126.219650
JC241_087	Boxcore 40	Boxcore	OMCO tracks	11/03/2023	18:59:00	4700	13.733617	-126.220250
JC241_088	Boxcore 41	Boxcore	OMCO tracks	11/03/2023	23:23:00	4682	13.735833	-126.202500
JC241_089	ROV Dive 411	ISIS ROV	OMCO tracks	12/03/2023	04:05:00	4700	13.726690	-126.204440
JC241_090	CTD05	CTD	OMCO tracks	12/03/2023	23:13:00	4690	13.730071	-126.204475
JC241_091	Multibeam	Multibeam	Multibeam OMCO to UK1	13/03/2023	02:32:00	4693	13.727917	-126.203100
JC241_092	Short Mooring 2	Mooring	UK-1	15/03/2023	15:51:00	4187	13.941983	-116.505650
Station	Event	Gear	Site	Date	UTCTime	Depth	Latitude	Longitude
JC241_093	Long Mooring 1	Mooring	UK-1	15/03/2023	19:30:00	4222	13.890083	-116.489733
JC241_094	Short Mooring 3	Mooring	UK-1	15/03/2023	21:39:00	4051	13.874217	-116.549200

JC241_095	ROV Dive 412	ISIS ROV	UK-1	15/03/2023	23:52:00	4086	13.957700	-116.554317
JC241_096	CTD06	CTD	UK-1	16/03/2023	20:25:00	4223	13.900632	-116.493961
JC241_097	Gravity Core 8	Gravity Corer	UK-1	17/03/2023	00:16:00	4118	13.973305	-116.540057
JC241_098	ROV Dive 413	ISIS ROV	UK-1	17/03/2023	04:26:00	4153	13.932372	-116.510993
JC241_099	Bathysnap2	Bathysnap	UK-1	18/03/2023	21:50:00	4103	13.914333	-116.521000
JC241_100	Bathysnap3	Bathysnap	UK-1	18/03/2023	22:33:00	4147	13.887500	-116.510167
JC241_101	Megacore 9	Megacore	UK-1	18/03/2023	23:25:00	4118	13.973317	-116.540050
JC241_102	Multibeam	Multibeam	Multibeam UK1 to Costa Rica	19/03/2023	04:00:00	4100	13.938233	-116.476350

23. Appendix 2: ROV sample Log

Dive	Date	Time	Equipment	Equip_No	Name	Lat	Lon	Comments	Recipient
Dive399	16/02/2023	05:55	PUC	PUC1	JC241_013_SE1	13.7366355	-126.2203400	Between tracks, soft seds	BGS
Dive399	16/02/2023	05:59	PUC	PUC2	JC241_013_SE2	13.7366355	-126.2203400	Between tracks, soft seds	BGS
Dive399	16/02/2023	06:03	PUC	PUC3	JC241_013_SE3	13.7366017	-126.2203967	Between tracks, soft seds	Louisa
Dive399	16/02/2023	06:09	PUC	PUC4	JC241_013_G3	13.7366055	-126.2204833	Between tracks, soft seds	Louisa
Dive399	16/02/2023	06:11	PUC	PUC5	JC241_013_G2	13.7365940	-126.2205057	Between tracks, soft seds	Louisa
Dive399	16/02/2023	06:14	PUC	PUC6	JC241_013_G1	13.7365743	-126.2204880	Between tracks, soft seds	Bryan
Dive399	16/02/2023	06:18	PUC	PUC7	JC241_013_Y1	13.7365743	-126.2204880	Between tracks, soft seds	BIODISCOVERY
Dive399	16/02/2023	06:34	PUC	PUC8	JC241_013_R1	13.7366128	-126.2203367	Edge of tracks,soft sediment between nodules (type II)	BGS
Dive399	16/02/2023	06:39	PUC	PUC9	JC241_013_Y2	13.7366128	-126.2203367	Edge of tracks,soft sediment between nodules (type II)	BGS
Dive399	16/02/2023	06:44	PUC	PUC10	JC241_013_Y3	13.7366128	-126.2203367	Edge of tracks,soft sediment between nodules (type II)	BGS
Dive399	16/02/2023	06:51	PUC	PUC11	JC241_013_B1	13.7366128	-126.2203367	Edge of tracks,soft sediment between nodules (type II)	Bryan
Dive399	16/02/2023	06:56	PUC	PUC12	JC241_013_B2	13.7366128	-126.2203367	Edge of tracks,soft sediment between nodules (type II)	Bryan
Dive399	16/02/2023	07:01	PUC	PUC13	JC241_013_B3	13.7366128	-126.2203367	Edge of tracks,soft sediment between nodules (type II)	Failed
Dive399	16/02/2023	07:06	PUC	PUC14	JC241_013_W1	13.7366128	-126.2203367	Edge of tracks,soft sediment between nodules (type II)	BIODISCOVERY
Dive399	16/02/2023	07:30	BIOB	BIOB1	JC241_013_Biob1	13.7366128	-126.2203367	Holothurian spiky red. Still I location 2, polynoid bonus on holoth	NHM
Dive399	16/02/2023	07:42	BIOB	BIOB2	JC241_013_Biob2	13.7366128	-126.2203367	Holothurian with sediment brown	NHM
Dive399	16/02/2023	08:11	PUC	PUC15	JC241_013_R2	13.7363422	-126.2205808	Between tracks, soft seds	BGS
Dive399	16/02/2023	08:19	PUC	PUC16	JC241_013_W2	13.7363422	-126.2205808	Between tracks, soft seds	BGS
Dive399	16/02/2023	08:30	PUC	PUC17	JC241_013_R3	13.7362083	-126.2203183	To side of tracks, pore water, possibly too deep	BGS
Dive399	16/02/2023	08:35	PUC	PUC18	JC241_013_W3	13.7362317	-126.2204217	To side of tracks	BGS
Dive399	16/02/2023	09:03	SUC	SUC	JC241_013_Suc2	13.7368400	-126.2203667	Squat lobster munnidopsis	NHM

Dive	Date	Time	Equipment	Equip_No	Name	Lat	Lon	Comments	Recipient
Dive399	16/02/2023	09:31	BIOB	BIOBSTB	JC241_013_BiobStb	13.7375833	-126.2203800	Brisingid starfish	NHM
Dive399	16/02/2023	09:44	BIOB	BIOBPORT	JC241_013_BiobPort	13.7376275	-126.2204123	Urchin + mud	NHM
Dive399	16/02/2023	10:01	NIS	Bottle1	JC241_013_NIS_bottle1	13.7383217	-126.2206397	5 not closed propery	Susan
Dive399	16/02/2023	10:09	NIS	10L_Bottle	JC241_013_NIS_10L	13.7383853	-126.2206217	-	Susan
DIVE400	17/02/2023	10:54	Multibeam	Reson7125		13.7408797	-126.2224735		Tim and Cat
DIVE401	18/02/2023	21:27	RoCSI	RoCSI	JC241_060_RoCSI	13.7426900	-126.2215308		Susan
DIVE403	28/02/2023	00:09	RoCSI	RoCSI	JC241_059_RoCSI	13.7381167	-126.2207117		Susan
DIVE403	28/02/2023	01:20	RoCSI	RoCSI	JC241_059_RoCSI	13.7350500	-126.2206500		Susan
DIVE403	28/02/2023	02:30	RoCSI	RoCSI	JC241_059_RoCSI	13.7306950	-126.2200325		Susan
DIVE403	28/02/2023	03:41	RoCSI	RoCSI	JC241_059_RoCSI	13.7251990	-126.2195292		Susan
DIVE403	28/02/2023	04:50	RoCSI	RoCSI	JC241_059_RoCSI	13.7256833	-126.2191167		Susan
DIVE403	28/02/2023	06:18	RoCSI	RoCSI	JC241_059_RoCSI	13.7301915	-126.2195167		Susan
DIVE403	28/02/2023	07:30	RoCSI	RoCSI	JC241_059_RoCSI	13.7338150	-126.2206500		Susan
DIVE403	28/02/2023	08:40	RoCSI	RoCSI	JC241_059_RoCSI	13.7395083	-126.2209000		Susan
DIVE403	28/02/2023	09:50	RoCSI	RoCSI	JC241_059_RoCSI	13.7396800	-126.2200617		Susan
DIVE403	28/02/2023	11:00	RoCSI	RoCSI	JC241_059_RoCSI	13.7358500	-126.2206000		Susan
DIVE403	28/02/2023	12:10	RoCSI	RoCSI	JC241_059_RoCSI	13.7327183	-126.2198167		Susan
DIVE403	28/02/2023	13:20	RoCSI	RoCSI	JC241_059_RoCSI	13.7291333	-126.2195050		Susan
DIVE403	28/02/2023	16:12	RoCSI	RoCSI	JC241_059_RoCSI	13.7235183	-126.2163950		Susan
DIVE403	28/02/2023	17:22	RoCSI	RoCSI	JC241_059_RoCSI	13.7246088	-126.2126948	Approx coords	Susan
DIVE403	28/02/2023	18:32	RoCSI	RoCSI	JC241_059_RoCSI	13.7261167	-126.2052000		Susan
DIVE403	28/02/2023	18:55	RoCSI	RoCSI	JC241_059_RoCSI	13.7252677	-126.2042217		Susan
DIVE403	28/02/2023	19:55	RoCSI	RoCSI	JC241_059_RoCSI	13.7292217	-126.2048333		Susan
DIVE403	28/02/2023	20:55	RoCSI	RoCSI	JC241_059_RoCSI	13.7260667	-126.2054167	RoCSI 18 stopped	Susan
DIVE403	28/02/2023	21:55	RoCSI	RoCSI	JC241_059_RoCSI	13.7360833	-126.2060500		Susan
Dive404	01/03/2023	10:06	RoCSI	RoCSI	JC241_060_RoCSI	13.7341570	-126.2056417	-	Susan
Dive404	01/03/2023	11:16	RoCSI	RoCSI	JC241_060_RoCSI	13.7373405	-126.2061133	-	Susan
Dive404	01/03/2023	12:27	RoCSI	RoCSI	JC241_060_RoCSI	13.7419962	-126.2066237	-	Susan
Dive404	01/03/2023	13:16	RoCSI	RoCSI	JC241_060_RoCSI	13.7446787	-126.2071370	13:37 stopped 4542 13º44.73081 N 126º45787 W	Susan
DIVE405	02/03/2023	04:03	RoCSI	RoCSI	JC241_062_RoCSI	13.7438292	-126.2059678		Susan

Dive	Date	Time	Equipment	Equip_No	Name	Lat	Lon	Comments	Recipient
DIVE405	02/03/2023	05:03	RoCSI	RoCSI	JC241_062_RoCSI	13.7413348	-126.2061897		Susan
DIVE405	02/03/2023	06:03	RoCSI	RoCSI	JC241_062_RoCSI	13.7380180	-126.2057320		Susan
DIVE405	02/03/2023	07:03	RoCSI	RoCSI	JC241_062_RoCSI	13.7345153	-126.2053768		Susan
DIVE405	02/03/2023	08:03	RoCSI	RoCSI	JC241_062_RoCSI	13.7316290	-126.2049100	Forgot to log coords	Susan
DIVE405	02/03/2023	09:03	RoCSI	RoCSI	JC241_062_RoCSI	13.7278043	-126.2045532	Fish churned in thruster - may affect DNA	Susan
DIVE405	02/03/2023	10:03	RoCSI	RoCSI	JC241_062_RoCSI	13.7265862	-126.2037065		Susan
DIVE405	02/03/2023	11:03	RoCSI	RoCSI	JC241_062_RoCSI	13.7295895	-126.2040802		Susan
DIVE405	02/03/2023	12:03	RoCSI	RoCSI	JC241_062_RoCSI	13.7330215	-126.2045882		Susan
DIVE405	02/03/2023	13:03	RoCSI	RoCSI	JC241_062_RoCSI	13.7365863	-126.2049285		Susan
DIVE405	02/03/2023	14:03	RoCSI	RoCSI	JC241_062_RoCSI	13.7397310	-126.2052107		Susan
DIVE405	02/03/2023	15:03	RoCSI	RoCSI	JC241_062_RoCSI	13.7421520	-126.2057740	Forgot to log coords	Susan
DIVE405	02/03/2023	16:09	RoCSI	RoCSI	JC241_062_RoCSI	13.7448363	-126.2057318		Susan
DIVE405	02/03/2023	17:04	RoCSI	RoCSI	JC241_062_RoCSI	13.7416880	-126.2050188		Susan
DIVE405	02/03/2023	18:04	RoCSI	RoCSI	JC241_062_RoCSI	13.7379340	-126.2048827		Susan
DIVE405	02/03/2023	19:04	RoCSI	RoCSI	JC241_062_RoCSI	13.7323680	-126.2042755		Susan
DIVE405	02/03/2023	20:04	RoCSI	RoCSI	JC241_062_RoCSI	13.7274403	-126.2036628		Susan
DIVE405	02/03/2023	20:27	NIS	10L_Bottle	JC241_062_NIS_10L	13.7253903	-126.2029037		Susan
DIVE406	04/03/2023	12:28	NIS	small	small	13.7361967	-126.2206962	No sediment disturbance	Susan
DIVE406	04/03/2023	12:33	NIS	10L_Bottle	JC241_069_NIS_10L	13.7363117	-126.2206685	No sediment disturbance	Susan
DIVE406	04/03/2023	12:34	NIS	Bottle4	JC241_069_NIS_bottle4	13.7363117	-126.2206685		Susan
DIVE406	04/03/2023	12:35	NIS	Bottle5	JC241_069_NIS_bottle5	13.7363117	-126.2206685		Susan
DIVE406	04/03/2023	12:36	NIS	Bottle6	JC241_069_NIS_bottle6	13.7363117	-126.2206685		Susan
DIVE406	04/03/2023	12:37	NIS	Bottle1	JC241_069_NIS_bottle1	13.7363117	-126.2206685		Susan
DIVE406	04/03/2023	12:38	NIS	Bottle2	JC241_069_NIS_bottle2	13.7363117	-126.2206685		Susan
DIVE406	04/03/2023	12:39	NIS	Bottle3	JC241_069_NIS_bottle3	13.7363117	-126.2206685		Susan
DIVE406	04/03/2023	14:47	PUC	Blue1		13.7363117	-126.2203135		Bryan
DIVE406	04/03/2023	14:50	PUC	Blue2		13.7363117	-126.2201802	Good	Bryan
DIVE406	04/03/2023	14:53	PUC	Blue3		13.7363117	-126.2201802	Pushed too high?	Susan
DIVE406	04/03/2023	14:56	PUC	Green1		13.7363117	-126.2201802	good	Bryan
DIVE406	04/03/2023	14:59	PUC	Red1		13.7363117	-126.2201802	drilled	Bryan
DIVE406	04/03/2023	15:02	PUC	Red2		13.7363117	-126.2201802	drilled	BGS

Dive	Date	Time	Equipment	Equip_No	Name	Lat	Lon	Comments	Recipient
DIVE406	04/03/2023	15:41	PUC	White1		13.7364000	-126.2204500	with xeno	Bryan
DIVE406	04/03/2023	15:53	PUC	Red3		13.7364000	-126.2204500	drilled	BGS
DIVE406	04/03/2023	16:01	PUC	White2		13.7364000	-126.2204500	good	Susan
DIVE406	04/03/2023	16:08	PUC	White3		13.7364000	-126.2204500	good	Bryan
DIVE406	04/03/2023	16:12	PUC	Green2		13.7364000	-126.2204500	good	Bryan
DIVE406	04/03/2023	16:14	PUC	Green3		13.7364000	-126.2204500	good	Bryan
DIVE406	04/03/2023	16:28	BIOB	BIOBSTB		13.7362000	-126.2204500	Psychronaetes hanseni	NHM
DIVE406	04/03/2023	17:20	BIOB	BIOBSTB		13.7359450	-126.2204533	Hymenaster cf	NHM
DIVE406	04/03/2023	18:30	BIOB	BIOBSTB		13.7357817	-126.2205383	Deimatidae	NHM
DIVE406	04/03/2023	20:29	BIOB	Basket		13.7339733	-126.2204167	Psychronaetes hanseni no. 2	NHM
DIVE406	04/03/2023	21:44	BIOB	Basket		13.7328167	-126.2198333	Hexantinellidae -> sponge + anemone	NHM
DIVE406	04/03/2023	22:10	BIOB	Basket		13.7324733	-126.2196387	Psychronaetes hanseni no. 3	NHM
DIVE407	06/03/2023	00:52	NIS	Bottle1		13.7363833	-126.2213500		Susan
DIVE407	06/03/2023	00:52	NIS	Bottle2		13.7363833	-126.2213500		Susan
DIVE407	06/03/2023	00:52	NIS	Bottle3		13.7363833	-126.2213500		Susan
DIVE407	06/03/2023	00:52	NIS	Bottle4		13.7363833	-126.2213500		Susan
DIVE407	06/03/2023	00:52	NIS	Bottle5		13.7363833	-126.2213500		Susan
DIVE407	06/03/2023	00:52	NIS	Bottle6		13.7363833	-126.2213500		Susan
DIVE407	06/03/2023	00:56	NIS	10L_Bottle	JC241_073_NIS_10L	13.7363667	-126.2213600		Susan
DIVE407	06/03/2023	01:44	PUC	Red3		13.7374833	-126.2204250		Louisa
DIVE407	06/03/2023	01:52	PUC	Red2		13.7374833	-126.2204250		Louisa
DIVE407	06/03/2023	01:55	PUC	Blue1		13.7374833	-126.2204250		Louisa
DIVE407	06/03/2023	02:00	PUC	Green1		13.7374833	-126.2204250		BIODISCOVERY
DIVE407	06/03/2023	02:02	PUC	Blue3		13.7374833	-126.2204250		Bryan
DIVE407	06/03/2023	02:31	PUC	White1		13.7367167	-126.2204667		BIODISCOVERY
DIVE407	06/03/2023	02:34	PUC	Green2		13.7367167	-126.2204667		Bryan
DIVE407	06/03/2023	02:41	PUC	Green3		13.7367167	-126.2204667		Louisa
DIVE407	06/03/2023	02:45	PUC	Stripey2		13.7367167	-126.2204667		Louisa
DIVE407	06/03/2023	02:48	PUC	Stripey3		13.7367167	-126.2204667		Louisa
DIVE407	06/03/2023	02:55	SUC	BIOBSTB		13.7367167	-126.2204667	Urchin	NHM

Dive	Date	Time	Equipment	Equip_No	Name	Lat	Lon	Comments	Recipient
DIVE407	06/03/2023	03:56	BIOB	BIOBSTB		13.7378400	-126.2205433	Urchin	NHM
DIVE407	06/03/2023	04:26	BIOB	BIOBSTB		13.7378400	-126.2205433	Anemone	NHM
DIVE407	06/03/2023	04:54	SUC	CanisterRed2		13.7377583	-126.2207383	Slimy sea cucumber	NHM
DIVE407	06/03/2023	05:11	BIOB	GreyBIOB		13.7376917	-126.2209167	Barnacle stalk	NHM
DIVE407	06/03/2023	05:19	BIOB	GreyBIOB		13.7377333	-126.2209200	Anemone	NHM
DIVE407	06/03/2023	05:59	SUC	CanisterYellow3		13.7372335	-126.2210073	Sea pig	NHM
DIVE407	06/03/2023	06:05	SUC	CanisterYellow3		13.7373292	-126.2209507	Sea pig	NHM
DIVE407	06/03/2023	06:06	SUC	CanisterYellow3		13.7373292	-126.2209507	Anemone	NHM
DIVE407	06/03/2023	06:11	SUC	CanisterYellow3		13.7373292	-126.2209507	Rolling anemone and sea pig	NHM
DIVE407	06/03/2023	06:21	SUC	CanisterBlack		13.7373292	-126.2209507	Sea pig	NHM
DIVE407	06/03/2023	06:28	SUC	CanisterBlack		13.7373292	-126.2209507	3 sea pigs and anemone	NHM
DIVE407	06/03/2023	06:56	SUC	CanisterBlack		13.7369817	-126.2208867	3 more sea pigs	NHM
DIVE407	06/03/2023	07:24	BIOB	GreyBIOB		13.7365410	-126.2208512	Brisingida	NHM
DIVE407	06/03/2023	08:10	BIOB	GreyBIOB		13.7351850	-126.2206085	Asteropechnidae	NHM
DIVE407	06/03/2023	08:21	BIOB	GreyBIOB		13.7351933	-126.2206667	Xeno	NHM
DIVE407	06/03/2023	08:34	BIOB	GreyBIOB		13.7350417	-126.2207850	Purple psychropotes	NHM
DIVE407	06/03/2023	09:03	PUC	Yellow1		13.7353283	-126.2204383		Bryan
DIVE407	06/03/2023	09:11	PUC	Yellow2		13.7353217	-126.2204083		Louisa
DIVE407	06/03/2023	09:13	PUC	Yellow3		13.7353217	-126.2204083		Louisa
DIVE407	06/03/2023	09:19	PUC	YellowStriped		13.7353217	-126.2204083		Louisa
DIVE407	06/03/2023	09:26	PUC	White2		13.7353217	-126.2204083		BIODISCOVERY
DIVE407	06/03/2023	09:39	PUC	DoubleYellow3		13.7354767	-126.2204217		Louisa
DIVE407	06/03/2023	09:45	PUC	DoubleYellow2		13.7354767	-126.2204217		Louisa
DIVE407	06/03/2023	09:50	PUC	DoubleYellow1		13.7354767	-126.2204217		Louisa
DIVE407	06/03/2023	09:59	PUC	White3		13.7354767	-126.2204217		BIODISCOVERY
DIVE407	06/03/2023	10:08	PUC	Red1		13.7354767	-126.2204217		Bryan
DIVE407	06/03/2023	10:18	BIOB	BIOBPORT		13.7354767	-126.2204217	Leafy tunicate	NHM
DIVE407	06/03/2023	10:38	BIOB	BIOBPORT		13.7357650	-126.2203700	Sponge/Anemone/Brittle star	NHM
DIVE407	06/03/2023	10:46	PUC	Red3		13.7357650	-126.2203700	Red 3 with double yellow	Louisa
DIVE407	06/03/2023	10:54	PUC	Red2		13.7357650	-126.2203700	Red 2 with double yellow	Louisa
DIVE407	06/03/2023	11:00	PUC	Red1		13.7357650	-126.2203700	Red 1 with double yellow	Louisa

Dive	Date	Time	Equipment	Equip_No	Name	Lat	Lon	Comments	Recipient
DIVE407	06/03/2023	11:14	BIOB	BIOBPORT		13.7357650	-126.2203700	Black coral	NHM
DIVE407	06/03/2023	11:41	BIOB	BIOBPORT		13.7356900	-126.2206867	Black coral no. 2	NHM
DIVE407	06/03/2023	12:14	BIOB	BIOBPORT		13.7349500	-126.2207833	Starfish	NHM
DIVE407	06/03/2023	12:31	BIOB	BIOBPORT		13.7349168	-126.2207362	Bryozoan	NHM
DIVE407	06/03/2023	13:31	BIOB	Drawer		13.7337000	-126.2204000	Psychronaetes hanseni	NHM
DIVE407	06/03/2023	13:47	BIOB	BIOBPORT		13.7336167	-126.2202667	Big starfish	NHM
DIVE407	06/03/2023	14:19	BIOB	Drawer		13.7313167	-126.2198833	Psychronaetes hanseni	NHM
DIVE407	06/03/2023	14:38	PUC	Blue2		13.7327167	-126.2196833		BGS
DIVE407	06/03/2023	15:09	BIOB	BIOBPORT		13.7324833	-126.2196000	Starfish	NHM
DIVE408	07/03/2023	09:30	Cube	Cube4		13.7364483	-126.2204817		AKS
DIVE408	07/03/2023	09:42	PUC	Green1		13.7364483	-126.2204817		AKS
DIVE408	07/03/2023	09:48	PUC	Green3		13.7364483	-126.2204817		AKS
DIVE408	07/03/2023	09:53	PUC	White1		13.7364483	-126.2204817		AKS
DIVE408	07/03/2023	10:00	Cube	Cube1		13.7363030	-126.2204817		AKS
DIVE408	07/03/2023	10:18	PUC	Green2		13.7363030	-126.2204420		AKS
DIVE408	07/03/2023	10:25	PUC	Red1		13.7363030	-126.2204420		AKS
DIVE408	07/03/2023	10:28	PUC	Red2		13.7363030	-126.2204420		AKS
DIVE408	07/03/2023	10:37	Cube	Cube3		13.7363350	-126.2202867		AKS
DIVE408	07/03/2023	10:48	PUC	White2		13.7363350	-126.2202867		AKS
DIVE408	07/03/2023	10:52	PUC	Red3		13.7363350	-126.2202867		AKS
DIVE408	07/03/2023	10:56	PUC	Yellow1		13.7363350	-126.2202867		AKS
DIVE408	07/03/2023	11:01	Cube	Cube2		13.7364500	-126.2202533		AKS
DIVE408	07/03/2023	11:12	Cube	Cube2		13.7364483	-126.2202500		AKS
DIVE408	07/03/2023	11:16	PUC	White3		13.7364483	-126.2202500		AKS
DIVE408	07/03/2023	11:18	PUC	Yellow2		13.7364483	-126.2202500		AKS
DIVE408	07/03/2023	11:24	PUC	Yellow3		13.7364483	-126.2202500		AKS
DIVE408	07/03/2023	12:42	BIOB	BIOBPORT		13.7373833	-126.2205167	Urchin	NHM
DIVE408	07/03/2023	13:13	BIOB	BIOBSTB		13.7374117	-126.2205250	Urchin	NHM
DIVE408	07/03/2023	13:29	BIOB	BIOBSTB		13.7375167	-126.2204917	Stalked sponge + anemone	NHM
DIVE408	07/03/2023	13:34	BIOB	BIOBPORT		13.7375167	-126.2204917	Psychronaetes hanseni	NHM
DIVE408	07/03/2023	14:00	BIOB	BIOBPORT		13.7385500	-126.2206167	Purple cucumber	NHM

Dive	Date	Time	Equipment	Equip_No	Name	Lat	Lon	Comments	Recipient
DIVE408	07/03/2023	14:27	BIOB	BIOBSTB		13.7386617	-126.2205883	Dytaster	NHM
DIVE408	07/03/2023	14:35	ROVDrawer	Drawer		13.7388467	-126.2206183	Yellow pipe from collector	NHM
DIVE408	07/03/2023	14:57	BIOB	BIOBSTB		13.7392567	-126.2204950	Purple cucumber	NHM
DIVE408	07/03/2023	15:40	BIOB	BIOBSTB		13.7397683	-126.2207767	Psychronaetes hanseni no. 2	NHM
DIVE408	07/03/2023	15:57	BIOB	BIOBSTB		13.7395417	-126.2208450	Ophiuroid	NHM
DIVE408	07/03/2023	16:10	BIOB	BIOBSTB		13.7395617	-126.2208650	Psychronaetes hanseni no. 3	NHM
DIVE408	07/03/2023	16:58	BIOB	BIOBSTB		13.7395067	-126.2210283	Amperima	NHM
DIVE408	07/03/2023	17:25	BIOB	BIOBPORT		13.7393883	-126.2209500	Spiny urchin	NHM
DIVE408	07/03/2023	17:33	NIS	Niskin		13.7392333	-126.2209833	Niskin water	Susan
DIVE409	08/03/2023	22:48	RoCSI	RoCSI	JC241_082_RoCSI	13.7386537	-126.2212122		Susan
DIVE409	08/03/2023	23:48	RoCSI	RoCSI	JC241_082_RoCSI	13.7344700	-126.2206433		Susan
DIVE409	09/03/2023	00:48	RoCSI	RoCSI	JC241_082_RoCSI	13.7301070	-126.2201900		Susan
DIVE409	09/03/2023	01:48	RoCSI	RoCSI	JC241_082_RoCSI	13.7417500	-126.2195000	At bottom of line 1	Susan
DIVE409	09/03/2023	02:48	RoCSI	RoCSI	JC241_082_RoCSI	13.7246217	-126.2197450	Start of line 2	Susan
DIVE409	09/03/2023	03:48	RoCSI	RoCSI	JC241_082_RoCSI	13.7277610	-126.2199900		Susan
DIVE409	09/03/2023	04:48	RoCSI	RoCSI	JC241_082_RoCSI	13.7308167	-126.2203017		Susan
DIVE409	09/03/2023	05:53	RoCSI	RoCSI	JC241_082_RoCSI	13.7341583	-126.2207767		Susan
DIVE409	09/03/2023	06:48	RoCSI	RoCSI	JC241_082_RoCSI	13.7369367	-126.2212083		Susan
DIVE409	09/03/2023	07:48	RoCSI	RoCSI	JC241_082_RoCSI	13.7388667	-126.2204700		Susan
DIVE409	09/03/2023	08:48	RoCSI	RoCSI	JC241_082_RoCSI	13.7351850	-126.2205567		Susan
DIVE409	09/03/2023	09:48	RoCSI	RoCSI	JC241_082_RoCSI	13.7310817	-126.2195583		Susan
DIVE409	09/03/2023	10:48	RoCSI	RoCSI	JC241_082_RoCSI	13.7271950	-126.2191820		Susan
DIVE409	09/03/2023	11:48	RoCSI	RoCSI	JC241_082_RoCSI	13.7237117	-126.2192317		Susan
DIVE409	09/03/2023	12:48	RoCSI	RoCSI	JC241_082_RoCSI	13.7253267	-126.2194217		Susan
DIVE409	09/03/2023	13:48	RoCSI	RoCSI	JC241_082_RoCSI	13.7278500	-126.2197917		Susan
DIVE409	09/03/2023	14:48	RoCSI	RoCSI	JC241_082_RoCSI	13.7305783	-126.2200050		Susan
DIVE409	09/03/2023	15:59	RoCSI	RoCSI	JC241_082_RoCSI	13.7335883	-126.2204687		Susan
DIVE409	09/03/2023	16:48	RoCSI	RoCSI	JC241_082_RoCSI	13.7364783	-126.2207650		Susan
DIVE409	09/03/2023	17:48	RoCSI	RoCSI	JC241_082_RoCSI	13.7392550	-126.2207133		Susan
DIVE409	09/03/2023	18:48	RoCSI	RoCSI	JC241_082_RoCSI	13.7369600	-126.2202850		Susan

Dive	Date	Time	Equipment	Equip_No	Name	Lat	Lon	Comments	Recipient
DIVE409	09/03/2023	19:48	RoCSI	RoCSI	JC241_082_RoCSI	13.7337233	-126.2203417		Susan
DIVE409	09/03/2023	20:48	RoCSI	RoCSI	JC241_082_RoCSI	13.7311783	-126.2194167		Susan
DIVE409	09/03/2023	21:48	RoCSI	RoCSI	JC241_082_RoCSI	13.7282550	-126.2192983		Susan
DIVE409	08/03/2023	23:02	NIS	10L_Bottle	JC241_082_NIS_10L	13.7243667	-126.2187517	Fired just before seabed	Susan
DIVE410	10/03/2023	21:10	RoCSI	RoCSI	JC241_086_RoCSI	13.7329167	-126.2197383		Susan
DIVE410	10/03/2023	22:10	RoCSI	RoCSI	JC241_086_RoCSI	13.7290617	-126.2192883		Susan
DIVE410	10/03/2023	23:10	RoCSI	RoCSI	JC241_086_RoCSI	13.7243483	-126.2187767		Susan
DIVE410	11/03/2023	00:10	RoCSI	RoCSI	JC241_086_RoCSI	13.7245000	-126.2194000		Susan
DIVE410	11/03/2023	01:10	RoCSI	RoCSI	JC241_086_RoCSI	13.7267233	-126.2196783		Susan
DIVE410	11/03/2023	02:10	RoCSI	RoCSI	JC241_086_RoCSI	13.7306783	-126.2201300		Susan
DIVE410	11/03/2023	03:10	RoCSI	RoCSI	JC241_086_RoCSI	13.7339820	-126.2205800		Susan
DIVE410	11/03/2023	04:10	RoCSI	RoCSI	JC241_086_RoCSI	13.7376150	-126.2210317		Susan
DIVE410	11/03/2023	05:10	RoCSI	RoCSI	JC241_086_RoCSI	13.7398650	-126.2212850		Susan
DIVE410	11/03/2023	06:10	RoCSI	RoCSI	JC241_086_RoCSI	13.7402800	-126.2177650		Susan
DIVE410	11/03/2023	07:10	RoCSI	RoCSI	JC241_086_RoCSI	13.7408710	-126.2130395		Susan
DIVE410	11/03/2023	08:10	RoCSI	RoCSI	JC241_086_RoCSI	13.7414067	-126.2084850		Susan
DIVE410	11/03/2023	09:10	RoCSI	RoCSI	JC241_086_RoCSI	13.7405567	-126.2056983		Susan
DIVE410	11/03/2023	10:10	RoCSI	RoCSI	JC241_086_RoCSI	13.7368950	-126.2052850		Susan
DIVE410	11/03/2023	11:10	RoCSI	RoCSI	JC241_086_RoCSI	13.7320267	-126.2046783		Susan
DIVE410	11/03/2023	12:10	RoCSI	RoCSI	JC241_086_RoCSI	13.7268500	-126.2040250		Susan
DIVE410	11/03/2023	13:10	RoCSI	RoCSI	JC241_086_RoCSI	13.7269450	-126.2033800		Susan
DIVE410	11/03/2023	14:10	RoCSI	RoCSI	JC241_086_RoCSI	13.7298717	-126.2037483		Susan
DIVE410	11/03/2023	15:10	RoCSI	RoCSI	JC241_086_RoCSI	13.7323383	-126.2040283		Susan
DIVE410	11/03/2023	15:01	NIS	10L_Bottle	JC241_086_NIS_10L	13.7323383	-126.2040283		Susan
DIVE411	12/03/2023	08:13	NIS	10L_Bottle	JC241_089_NIS_10L	13.7267933	-126.2045800		Susan
DIVE411	12/03/2023	09:30	BIOB	GreyBIOB		13.7267000	-126.2044083	Big Crinoid	NHM
DIVE411	12/03/2023	08:35	BIOB	BIOBSTB		13.7267817	-126.2046017	Tulip sponge	NHM
DIVE411	12/03/2023	10:36	SUC	CanisterYellow3		13.7273850	-126.2042467	Elpidiidae and brittle star	NHM
DIVE411	12/03/2023	11:01	BIOB	BIOBSTB		13.7274788	-126.2042500	cf. Holoscus	NHM
DIVE411	12/03/2023	11:21	BIOB	BIOBSTB		13.7278533	-126.2042650	Pterasteridae	NHM
DIVE411	12/03/2023	11:43	SUC	CanisterYellow3		13.7282317	-126.2044150	Pinkish elpidiidae	NHM

Dive	Date	Time	Equipment	Equip_No	Name	Lat	Lon	Comments	Recipient
DIVE411	12/03/2023	12:15	BIOB	BIOBSTB		13.7282507	-126.2042872	Axoniderma	NHM
DIVE411	12/03/2023	12:40	BIOB	BIOBSTB		13.7285233	-126.2046017	Urchin	NHM
DIVE411	12/03/2023	12:43	BIOB	BIOBSTB		13.7285233	-126.2046017	Slimy foram or metazoa?	NHM
DIVE411	12/03/2023	12:48	BIOB	BIOBSTB		13.7285233	-126.2046017	Amperima	NHM
DIVE411	12/03/2023	13:18	BIOB	BIOBSTB		13.7292117	-126.2043400	Bucket sponge	NHM
DIVE411	12/03/2023	13:58	BIOB	BIOBPORT		13.7301800	-126.2044083	Psychropotes - good footage	NHM
DIVE411	12/03/2023	14:17	PUC	XLPUC		13.7303633	-126.2044650	Big XL pushcore	BGS
DIVE411	12/03/2023	14:49	PUC	Red1		13.7310683	-126.2041417	On top of ophiuroid	BIODISCOVERY
DIVE411	12/03/2023	14:55	PUC	Red2		13.7310683	-126.2041417	forams	Bryan
DIVE411	12/03/2023	14:58	PUC	Red3		13.7310683	-126.2041417	forams	Bryan
DIVE411	12/03/2023	15:05	PUC	Stripey1		13.7310683	-126.2041417	Xeno	Bryan
DIVE411	12/03/2023	15:13	PUC	Stripey2		13.7310683	-126.2041417		Susan
DIVE411	12/03/2023	15:35	BIOB	BIOBPORT		13.7314283	-126.2042533	Psychronaetes hanseni	NHM
DIVE411	12/03/2023	16:47	BIOB	BIOBSTB		13.7327683	-126.2037683	Brisingida	NHM
DIVE411	12/03/2023	16:51	PUC	Green1		13.7327683	-126.2037683	forams	Bryan
DIVE411	12/03/2023	16:55	PUC	Green2		13.7327683	-126.2037683	forams	Bryan
DIVE411	12/03/2023	16:59	PUC	Green3		13.7327683	-126.2037683	forams	Bryan
DIVE411	12/03/2023	17:03	PUC	Blue1		13.7327683	-126.2037683	forams	Bryan
DIVE411	12/03/2023	17:03	BIOB	GreyBIOB		13.7327683	-126.2037683	sponge	NHM
DIVE411	12/03/2023	17:21	BIOB	BIOBSTB		13.7328167	-126.2036367	Urchin	NHM
DIVE411	12/03/2023	17:40	BIOB	BIOBSTB		13.7326533	-126.2035183	Urchin	NHM
DIVE411	12/03/2023	18:06	BIOB	BIOBSTB		13.7324150	-126.2034817	Tall sponge	NHM
DIVE411	12/03/2023	18:10	BIOB	GreyBIOB		13.7324150	-126.2034817	Anemone	NHM
DIVE411	12/03/2023	19:04	BIOB	BIOBSTB		13.7310867	-126.2034683	Crinoid	NHM
DIVE412	16/03/2023	03:38	NIS	Niskin		13.9633815	-116.5543873		Susan
DIVE412	16/03/2023	04:26	BIOB	BIOBSTB		13.9628667	-116.5536267	Benthodytes	NHM
DIVE412	16/03/2023	04:46	BIOB	FrontBIOB		13.9593500	-116.5537333	Urchins	NHM
DIVE412	16/03/2023	04:57	BIOB	FrontBIOB		13.9626733	-116.5536833	Hairy sea cucumber	NHM
DIVE412	16/03/2023	04:59	BIOB	FrontBIOB		13.9626733	-116.5536833	Sponge	NHM
DIVE412	16/03/2023	05:26	BIOB	WoodenBox		13.9623667	-116.5538333	Metal bar	NHM
DIVE412	16/03/2023	05:56	BIOB	FrontBIOB		13.9621717	-116.5542167	Sponge	NHM

Dive	Date	Time	Equipment	Equip_No	Name	Lat	Lon	Comments	Recipient
DIVE412	16/03/2023	06:27	BIOB	BIOBSTB		13.9616683	-116.5546667	Mesothuriidae	NHM
DIVE412	16/03/2023	06:56	BIOB	FrontBIOB		13.9614075	-116.5549333	Octocoral	NHM
DIVE412	16/03/2023	06:59	BIOB	GreyBIOB		13.9614075	-116.5549333	Coral	NHM
DIVE412	16/03/2023	08:00	BIOB	BIOBSTB		13.9608167	-116.5555033	Round big sponge	NHM
DIVE412	16/03/2023	08:27	BIOB	GreyBIOB		13.9607933	-116.5554550	Small coral	NHM
DIVE412	16/03/2023	08:57	BIOB	GreyBIOB		13.9606900	-116.5553983	Pink brisingid	NHM
DIVE412	16/03/2023	09:27	PUC	Red3		13.9604500	-116.5551183	forams	Bryan
DIVE412	16/03/2023	09:32	PUC	Red2		13.9604500	-116.5551183	forams	Bryan
DIVE412	16/03/2023	09:38	PUC	Blue3		13.9604500	-116.5551183	forams	Bryan
DIVE412	16/03/2023	10:27	BIOB	BIOBPORT		13.9603633	-116.5550083	Anemone	NHM
DIVE412	16/03/2023	10:16	BIOB	GreyBIOB		13.9603633	-116.5550083	Coral	NHM
DIVE412	16/03/2023	11:31	BIOB	GreyBIOB		13.9599083	-116.5543567	Ophiuroid	NHM
DIVE412	16/03/2023	12:26	BIOB	BIOBSTB		13.9597150	-116.5541767	Psychropotes	NHM
DIVE412	16/03/2023	13:12	PUC	Blue2		13.9585870	-116.5530290	forams	Bryan
DIVE412	16/03/2023	13:18	PUC	Blue1		13.9585870	-116.5530290	LOUISA	Bryan
DIVE412	16/03/2023	13:39	PUC	Yellow2		13.9585870	-116.5530290	forams	Bryan
DIVE412	16/03/2023	13:45	PUC	Yellow1		13.9585870	-116.5530290		BIODISCOVERY
DIVE412	16/03/2023	13:49	PUC	White3		13.9585875	-116.5530707		Susan
DIVE412	16/03/2023	13:49	BIOB	GreyBIOB		13.9585870	-116.5530290		NHM
DIVE412	16/03/2023	14:32	SUC	Slurp2		13.9583803	-116.5529368		NHM
DIVE412	16/03/2023	15:35	SUC	Slurp3		13.9582040	-116.5527720		NHM
DIVE412	16/03/2023	15:39	PUC	Yellow3		13.9582040	-116.5527720		Bryan
DIVE412	16/03/2023	15:42	PUC	White1		13.9582040	-116.5527720		Bryan
DIVE412	16/03/2023	15:45	PUC	White2		13.9582040	-116.5527720		Bryan
DIVE412	16/03/2023	15:45	PUC	Red1		13.9582040	-116.5527720		Bryan
DIVE413	17/03/2023	08:28	NIS	10L_Bottle	JC241_098_NIS_10L	13.9319223	-116.5106238	Water Sample	Susan
DIVE413	17/03/2023	08:58	RoCSI	RoCSI	JC241_098_RoCSI	13.9326333	-116.5109500		Susan
DIVE413	17/03/2023	09:58	RoCSI	RoCSI	JC241_098_RoCSI	13.9326800	-116.5118700		Susan
DIVE413	17/03/2023	10:58	RoCSI	RoCSI	JC241_098_RoCSI	13.9345367	-116.5159133		Susan
DIVE413	17/03/2023	11:58	RoCSI	RoCSI	JC241_098_RoCSI	13.9359683	-116.5193583		Susan
DIVE413	17/03/2023	12:58	RoCSI	RoCSI	JC241_098_RoCSI	13.9377567	-116.5233317		Susan

Dive	Date	Time	Equipment	Equip_No	Name	Lat	Lon	Comments	Recipient
DIVE413	17/03/2023	13:58	RoCSI	RoCSI	JC241_098_RoCSI	13.9393750	-116.5275433		Susan
DIVE413	17/03/2023	14:58	RoCSI	RoCSI	JC241_098_RoCSI	13.9404900	-116.5307833		Susan
DIVE413	17/03/2023	15:58	RoCSI	RoCSI	JC241_098_RoCSI	13.9442300	-116.5308500		Susan
DIVE413	17/03/2023	16:58	RoCSI	RoCSI	JC241_098_RoCSI	13.9477100	-116.5306183		Susan
DIVE413	17/03/2023	17:58	RoCSI	RoCSI	JC241_098_RoCSI	13.9520050	-116.5306833		Susan
DIVE413	17/03/2023	18:58	RoCSI	RoCSI	JC241_098_RoCSI	13.9555817	-116.5304183		Susan
DIVE413	17/03/2023	19:58	RoCSI	RoCSI	JC241_098_RoCSI	13.9580967	-116.5304533		Susan
DIVE413	17/03/2023	20:58	RoCSI	RoCSI	JC241_098_RoCSI	13.9606730	-116.5338920		Susan
DIVE413	17/03/2023	21:58	RoCSI	RoCSI	JC241_098_RoCSI	13.9632457	-116.5375045		Susan
DIVE413	17/03/2023	22:58	RoCSI	RoCSI	JC241_098_RoCSI	13.9659683	-116.5411900		Susan
DIVE413	17/03/2023	23:58	RoCSI	RoCSI	JC241_098_RoCSI	13.9684000	-116.5446333		Susan
DIVE413	17/03/2023	00:58	RoCSI	RoCSI	JC241_098_RoCSI	13.9718615	-116.5453258		Susan
DIVE413	17/03/2023	01:58	RoCSI	RoCSI	JC241_098_RoCSI	13.9753933	-116.5451100		Susan
DIVE413	17/03/2023	02:58	RoCSI	RoCSI	JC241_098_RoCSI	13.9801548	-116.5448435		Susan
DIVE413	17/03/2023	03:58	RoCSI	RoCSI	JC241_098_RoCSI	13.9849005	-116.5445727		Susan
DIVE413	17/03/2023	04:58	RoCSI	RoCSI	JC241_098_RoCSI	13.9880237	-116.5463933		Susan
DIVE413	17/03/2023	05:59	RoCSI	RoCSI	JC241_098_RoCSI	13.9902243	-116.5498140		Susan
DIVE413	17/03/2023	06:59	RoCSI	RoCSI	JC241_098_RoCSI	13.9927498	-116.5535937		Susan
DIVE413	17/03/2023	07:58	RoCSI	RoCSI	JC241_098_RoCSI	13.9953015	-116.5577215		Susan