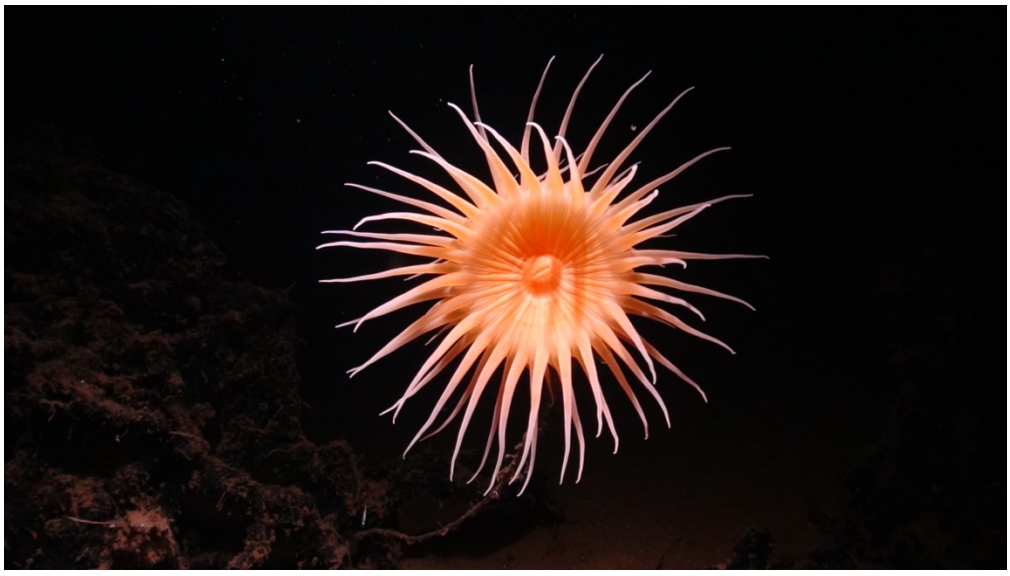
Cruise Report

RRS James Cook Cruise JC257



Uncorrected image from the ROV Isis SCORPIO camera of a sea anemone at 4100m depth, dive JC257_117 on 11 March 2024

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The scientific and technical team pose on the bow of the RRS James Cook on 19 March 2024 during the cruise JC257

Executive Summary

Cruise JC257 of the RRS James Cook was a 44-day oceanographic cruise out of Caldera, Costa Rica working in the international waters of the Clarion-Clipperton Zone and the second cruise of the NERC Highlight Topic project 'Seabed Mining and Resilience to Experimental Impact' (SMARTEX), a 4-year project that started in 2021 to study fundamental scientific questions to inform the debate on seabed mining for polymetallic nodules. The main objectives of the cruise were measurements of physical oceanography (mesoscale eddies and their impacts at the seafloor), geophysical survey (mapping the seabed at various resolutions), biology (the spatial scaling of biodiversity and natural geochemical drivers of biodiversity across a 100km transect), as well as studies of recolonisation rates, ecosystem function and ecotoxicology baselines. JC257 made use of a wide variety of equipment including the Remotely Operated Vehicle (ROV) Isis, the Autonomous Underwater Vehicle (AUV) Autosub, a megacorer, USNEL-type box corer, CTD, gravity core, glider, fish trap and in-situ pulse-chase experimental cubes. The main work area was the 'UK-1' region of the Clarion Clipperton Zone at 13°30 N, 116°20 W and a typical depth of 4100m.

The cruise departed Costa Rica on 7 February 2024 and returned on 21 March 2024. The ship's company consisted of 17 scientists, 15 technical support personnel and 21 ship's crew. The captain was John Leask, the chief scientist Adrian Glover, the co-chief scientist Daniel Jones and the head of the NMF technical team Dave Turner. A total of 122 over-the-side equipment deployments were made as well as continuous underway operation of the ship acoustic instruments (multibeam and ADCP systems). The largest proportion of the ship-time was dedicated to ROV operations, followed by box cores, then AUV, then megacorer followed by the remaining gears. A large volume of samples and data were obtained and all primary objectives of the cruise met, as well as opportunistic recovery of settlement experiments deployed on two previous cruises. All the moorings deployed on JC241 were recovered leaving nothing on the seafloor and good data were obtained from all the mooring instruments.

16 deployments of the ROV Isis were made on the cruise, all were successful. The ROV was deployed in various configurations including the ability to take in-situ close up imagery of seabed fauna using the highest resolution camera mounted low and at the front of the tool sled; this was very successful. Other configurations included deployment and recovery of the cube experiments and recovery of settlement panels deployed on previous cruises in 2013 and 2020. 16 deployments of the AUV Autosub5 were also made, some of these dives were tests and 9 dives returned useable data. 39 deployments were made of the box core, of which 34 returned samples and 32 were high-quality fully quantitative samples. The megacore was deployed 16 times, all of which returned good samples with typically 5-7 of the core tubes returning good quality samples for a variety of studies. 19 CTD casts were made on the cruise; several issues were encountered with the cable in the earlier part of the cruise requiring retermination and troubleshooting. These problems were eventually solved. The CTD included water sampling and a variety of sensors which are detailed in section 2, as well as ADCP data. The Gravity Core was deployed on 1 successful deployment at the end of the cruise. The NMF Deep Glider was deployed during the transit from Caldera to the UK-1 site but did not surface as planned and was eventually lost. The Fish Trap supplied by SAMS was deployed 4 times but was lost on the final deployment. 3 long-term oceanographic moorings deployed on JC241 were recovered with good data (physical sensors and sediment traps), as well as the Bathysnap time lapse cameras, which were also redeployed on JC257 for the duration of the cruise and recovered successfully at the end of the cruise with good data. The vessel-mounted underway instrumentation ADCP and EM122 multibeam were used throughout the cruise with good data obtained.

The cruise program was focussed on (1) the collection of geophysical data during transits to/from the UK-1 area, (2) the collection of physical oceanographic data during the transits with a focus on a region influenced by a mesoscale eddy and (3) the study of scale dependence of geophysical and biological properties of several sites distributed on a 100km transect starting in the northern area of UK-1 ('0km' site) on a vector of approximately 168° with sampling at 0km, 1km, 16km, 30km and 100km sites. The 30km site was only sampled by the AUV, the remaining sites were sampled with all equipment.

Geophysical (mapping) from the shipboard multibeam and AUV multibeam and side scan sonar was led by the NOC team. The shipboard data filled in a number of gaps in data in the UK-1 area as well as during the transits. The AUV was used to map in high-resolution the 0, 1, 16, 30 and 100km sites. Physical oceanographic work was led by SAMS and a large volume of data obtained from the CTD, ADCP and long-term moorings. Preliminary results suggested a strong influence of surface eddies associated to Central American mountain gap winds on abyssal hydrography, as well as temporal changes during the year. Megafaunal survey using imagery was led by the NOC team. Imagery data was obtained from the 0, 1, 16, 30 and 100km sites, a total of 68,985 seabed images (of which 52,951 are on photo transects). The NOC team also led the work on the Bathysnap data, with 4 sets of data obtained (2 long-term 1 year deployments [deployed on JC241] and 2 short term 1.5 month deployments during JC257). The images were of good quality and showed interesting temporal trends. Megafaunal sampling was led by the NHM team using the ROV Isis, high quality in-situ imagery was obtained of samples prior to collection by the ROV. Samples were then imaged and sub-sampled at sea in the labs. A total of 159 megafaunal

individuals were collected dominated by sponges and echinoderms. From the 159 samples, a total of 516 sub-samples were taken for morphological, genomic and stable-isotope work (in collaboration with the University of Liverpool team). Quantitative macrofaunal sampling (using box cores) was led by the NHM team with a large amount of assistance from the NOC team and all on board. Standard procedures were followed making all data collected compatible with a wide-range of macrofaunal datasets from the eastern CCZ region. Between 6 and 10 replicate samples were taken at each site (10 replicates at each of the 0km and 1km sites) with a total of 32 quantitative replicate samples. 1426 live-sorted individual specimens were taken from the box cores on board, with the remainder of the material in the quantitative fractions to be sorted in the NHM on return. The NHM and NOC also led quantitative samples for foraminifera and meiofauna although no specific team was on board to deal with the samples. We obtained 20 core samples from 12 deployments with 3 replicates at each site on the 100km transect. An additional 13 pushcores for foraminifera/meiofauna were also obtained from the ROV, these were focussed around the colonisation panels. Microbial sampling on JC257 was led by the NOC team using an in-situ eDNA sampler (RoCSI), water sampling from the CTD and ROV, and sediment sampling with ROV push cores, megacores and box cores. Although the RoCSI sampler was largely not successful owing to a leak, a wide variety of microbial samples were taken with the other gears. Ecosystem function studies were led by SAMS, with a total of 14 successful benthic cube experiments undertaken to study pulse-chase data using labelled algae, with 3 ROV push-cores taken within each benthic cube imprint. Ecotoxicological baseline studies were conducted by Heriot-Watt University, who sampled six abyssal fish specimens for Comet assay work to establish methods. The University of Liverpool team led work on the stable isotopes as well as sedimentary geochemical variables including amino acids, lipids and total carbon/nitrogen. Most of the work used the megacore and a total of 46 cores were studied from 16 deployments. An additional 61 tissue samples from 42 megafauna specimens were collected for stable isotope studies. Several other sets of data were collected on JC257 including geological collections of nodules from all box cores, pushcore samples for the Defra DEEPEND biodiscovery project, ethanol samples from the ethanol changes of the megafauna (also for Defra DEEPEND), the sediment trap samples for the NOC team, the hard substrate colonisation experiment deployed on RC01 cruise in 2020, the whale bone/ wood colonisation experiment deployed on the AB01 cruise in 2013, marine mammal observations during multibeam operations, surface water ecological observations (18 species) and drone observations. Two trainees from the Cook Islands and the University of Southampton were also on board JC257 and their training and observations are documented in this report.

During JC257 we maintained a public communication channel using the blog at www.smartexccz.org where 13 blog posts were published and a twitter/X feed which created 2.8m impressions globally using the hashtag #smartexccz. The cruise was mentioned during a BBC news piece on deep-sea mining including an interview with the PSO live from the ship and imagery from the cruise was released to the media with mentions in a wide variety of news outlets.

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Sailing from Puntarenas, Costa Rica at dawn, 7 February 2024. Photo by Daniel Jones.

1. Cruise Overview

1.1. Background and Objectives

The JC257 Deep-Sea Biodiversity cruise assessed fundamental scientific questions with regards the biodiversity, ecology, ecosystem function, ecotoxicology, geophysics, geochemistry and oceanography of the eastern Clarion-Clipperton Zone (CCZ), Pacific Ocean in a region being explored for seabed minerals (polymetallic nodules) and a potential target of future mineral extraction. The main focus of the work was in a 68,000 km^2 region of the eastern CCZ (termed hereafter UK-1) for which the United Kingdom is the Sponsoring State of a seabed mining exploration contract licensed by the International Seabed Authority (ISA) to the contractor UK Seabed Resources Ltd. The contractor was not involved in the study; the cruise forms part of the Seabed Mining and Resilience to Experimental Impact (SMARTEX) project, funded under the Natural Environment Research Council (NERC) Highlight Topic Strategic Programmes (grant number NE/S007210/1) to undertake baseline fundamental research in the area.

1.1.1. Main objectives

Physical oceanography

The main objective was to measure deep-water mesoscale eddies in the eastern Pacific that could be relevant to sediment resuspension at the seafloor, as well as CTD deployments and data from the long-term moorings (deployed on JC241) to support background baseline knowledge of the physical oceanography of the CCZ and eastern Pacific more generally.

Seafloor geophysical survey

The objectives of the geophysical survey were to (1) collect additional multibeam data during transits to further map the seafloor in regions not yet surveyed and (2) to collect high-resolution multibeam and sidescan data from the Autonomous Underwater Vehicle (AUV) Autosub to study local scale seafloor geophysics to guide biological sampling, the determination of the scaling study and discover scientifically novel seafloor features.

Biology, biodiversity, recolonisation, ecosystem function, ecotoxicology and geochemical studies

The main biological objective was to study the spatial scaling of biodiversity and natural geochemical drivers of biodiversity, as well as studies of food-webs, ecosystem function and ecotoxicological baselines over scales of approximately 1km, 10km and 100km in the UK-1 region. The main work area was in the northern section of UK-1 around 13° 55 N, 116° 32 W. This was chosen as it was a region sampled on two previous cruises in 2013 (RV Melville cruise AB02) and 2020 (RV Pacific Constructor cruise RC01) using some similar equipment including ROV, AUV and box core sampling. This created the opportunity to not only gain better spatial data but to also study temporal trends over decadal scales. Within the JC257 work area, four sites were chosen originally planned to cover scales of 0, 1, 10 and 100km. These were modified during the cruise to form 5 sites, one termed 0km (at the northernmost site, in a region likely to be a focus of future mining tests), 1km (termed as it was 1km on a transect from the 0km site), 16km (10km from 0km site), 30km and 100km. Site choice was informed by the geophysical survey and the sites were intended to be as similar as possible in terms of their habitats to maximise the study of spatial scaling. In the event of future mining tests being carried out in the area, these sites could also form impact and control sites to study the long-term impacts of seabed mining.

At each of the main sites (0km, 1km, 16km, 100km), we planned replicated sampling using a variety of equipment (summarised below). We planned the 30km site to be only studied using the Autosub. Additional sites studied included 3 recolonisation experiments put down in 2020 and 2013 as well as recovery of the moorings put down on JC241 close to the 1km site.

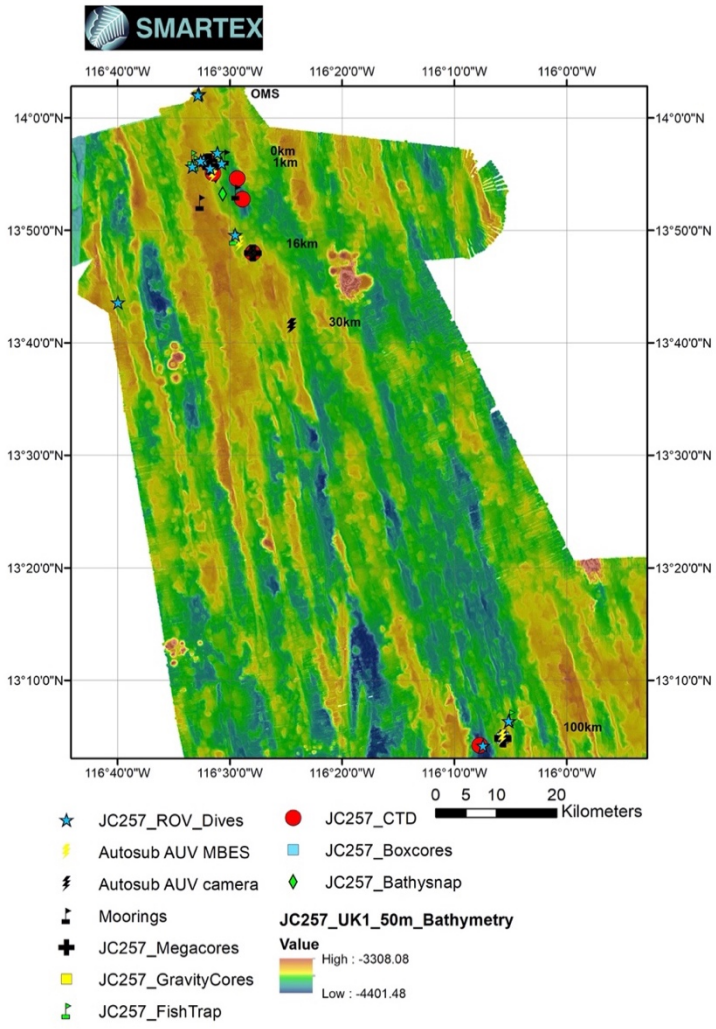


Figure 1.1.1 Principal work area of JC257 in the UK-1 region of the Clarion-Clipperton Zone. Map by Catherine Wardell (NOC).

1.1.2. Summary of equipment used and ship time allocations

Alongside the RRS James Cook underway instrumentation (principally the EM122 multibeam and ADCP current profilers), JC257 made use of a wide variety of National Marine Facility (NMF) sampling gears used over-the-side: the ROV Isis, AUV Autosub, Megacore, Box core, CTD, Gravity core, Glider as well as externally-provided equipment in the form of a free-vehicle Fish Trap (belonging to the Scottish Association of Marine Science (SAMS)) and pulse-chase cube experiments (also provided by SAMS).

A technical summary of all equipment used and deployments is provided in section 2 of this report. Figure 1.1.2 illustrates the allocation of ship-time across JC257 on the various equipment.

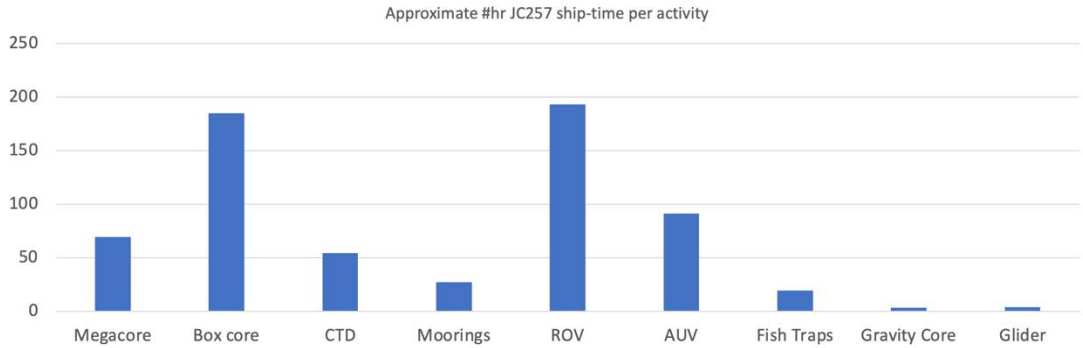


Figure 1.1.2 Approximate #hr of JC257 per over-the-side gear deployments over the whole cruise.

Bottlenose dolphins, Tursiops truncatus*, on passage to the eastern Pacific abyss. Image by Daniel Jones.*

1.2. Personnel

1.2.1. JC257 Scientific and technical personnel

Scientific personnel

Name	Role	Institution
Adrian Glover	Chief Scientist	Natural History Museum
Daniel Jones	Co-Chief Scientist, AUV lead	National Oceanography Centre
Belen Arias	Bio sampling lead	Natural History Museum
Thomas Dahlgren	Bio sampling	NORCE Norway
Regan Drennan	Bio sampling	Natural History Museum
Georgina Glaser	Bio sampling	Natural History Museum
Muriel Rabone	Bio sampling	Natural History Museum
Lucy Harris	Trainee	University of Southampton
Tanga Morris	Trainee	SBMA Cook Islands
Louisa Norman	Biogeochemistry/food-webs	University of Liverpool
Loïc Van Audenhaege	AUV image surveys	National Oceanography Centre
Bethany Fleming	AUV image surveys	National Oceanography Centre
Catherine Wardell	Mapping	National Oceanography Centre
Susan Evans	ROCSI/microbes	National Oceanography Centre
Andrew Sweetman	Function/fish-traps	SAMS
Mark Hartl	Ecotoxicology	Herriot-Watt University
Dima Aleynik	Physical oceanography	SAMS

Technical personnel

Name	Role	Institution
Dave Turner	ROV lead / senior tech	National Oceanography Centre
Russ Locke	ROV	National Oceanography Centre
Emre Mutlu	ROV	National Oceanography Centre
Will Handley	ROV	National Oceanography Centre

Martin Yeomans	ROV	National Oceanography Centre
Steve McDonagh	ROV	National Oceanography Centre
Alex Downer	ROV	National Oceanography Centre
Matt Kingsland	AUV lead	National Oceanography Centre
Richard Austin-Berry	AUV	National Oceanography Centre
Eoin Ó Hóbáin	AUV	National Oceanography Centre
Shivan Ramdhanie	AUV	National Oceanography Centre
Howard King	Coring	National Oceanography Centre
Billy Platt	CTD/moorings/coring	National Oceanography Centre
Mark Maltby	Ship systems	National Oceanography Centre
Josue Viera Rivero	Ship systems	National Oceanography Centre

1.2.2. Ship's crew

Name	**Role**	**Institution**
John Leask	Captain	National Oceanography Centre
Iain Macleod	Chief Officer	National Oceanography Centre
Tom Williams	2nd Officer	National Oceanography Centre
Max Bishop	3rd Officer	National Oceanography Centre
Keith Sneddon	Chief Engineer	National Oceanography Centre
Michael Murren	2nd Engineer	National Oceanography Centre
Kai Foreman	3rd Engineer	National Oceanography Centre
George Palmer	3rd Engineer	National Oceanography Centre
Paula McDougall	Purser	National Oceanography Centre
Mark Squibb	CPOS	National Oceanography Centre
Steven Duncan	CPOD	National Oceanography Centre
Ryan Paris	POS	National Oceanography Centre
Craig Gilfillian	POD	National Oceanography Centre
Robert McKeown	SG1A	National Oceanography Centre
Marnie Ross	SG1A	National Oceanography Centre
Peter Smith	SG1A	National Oceanography Centre
Lewis Smyth	SG1A	National Oceanography Centre
Darren Caines	Head Chef	National Oceanography Centre
Chris Crombie	Chef	National Oceanography Centre
Carl Piper	Steward	National Oceanography Centre
Daniel Higham	Assistant Steward	National Oceanography Centre

1.3. Itinerary and Cruise Track

Port of mobilisation: Caldera Costa Rica

Departure date: 7 February

Return date: 21 March 2023

Total days at sea: 44

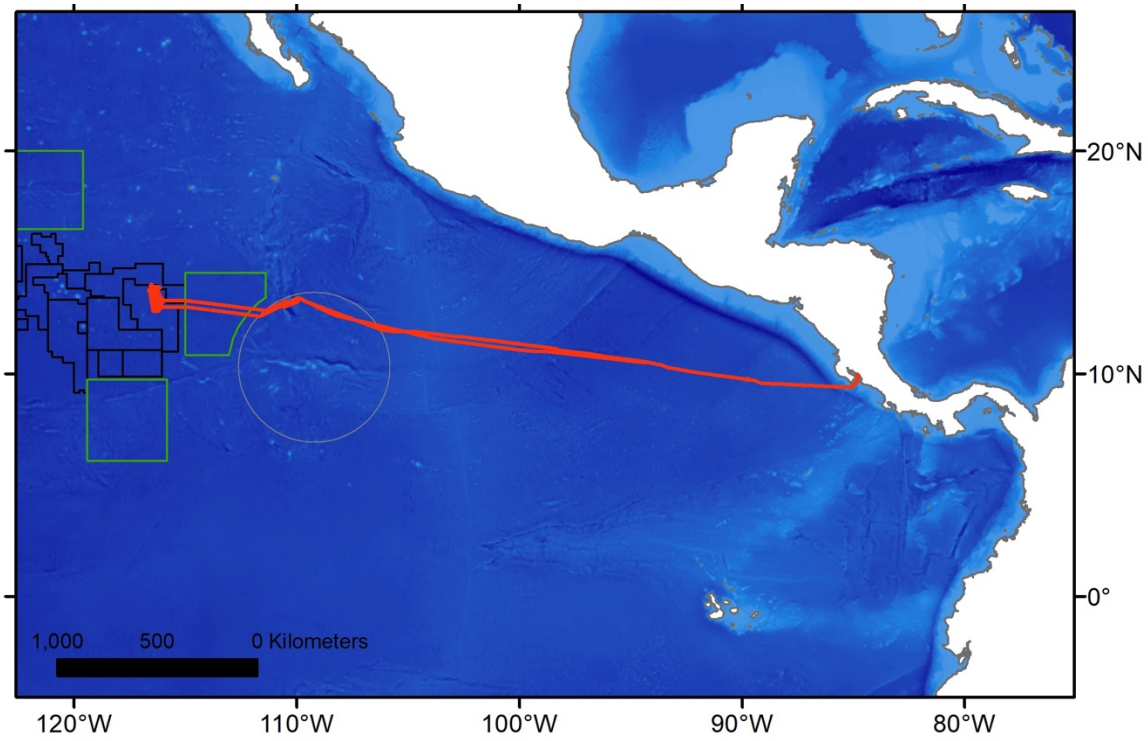


Figure 1.3.1 Cruise track of JC257 (created by Daniel Jones).

1.4. Cruise Narrative

By Adrian Glover, Chief Scientist, Natural History Museum. All date and times are local ship time.

Saturday 3 Feb 2024

The majority of the science party (13) arrived in San Jose, Costa Rica. The evening was spent in a hotel near the airport.

Sunday 4 Feb

After a drive from San Jose down to the coast organised by the agent (Armada de Navigacion) the science party moved on to the ship via small boat transfer from the port of Caldera in the Gulf of Nicoya. The weather was calm but there was some ocean swell coming in from a southerly direction. The science team were excited to board the Cook up a rope ladder.

Monday 5 Feb

The final scientists arrive from San Jose, again with a boat transfer and the team start to unpack and setup equipment. An electrical fault was discovered in the ROV tether that could not be repaired. The ROV team decided to remove the tether from the drum and replace with the Deep Tow cable. They started work on this tricky process and completed the removal of the old tether by the end of the day, storing it in 600m lengths in an open container. The scientists held a meeting and started to organise watches and the science plan, as well as go over all safety issues.

Tuesday 6 Feb

The Cook moved alongside at Puntarenas to take on water and some final stores. The water took longer to arrive than planned, and the flow rate into the ship was slower than expected, hence departure was delayed until 0500 7 Feb. The science team completed stowing and organising of all science equipment and started to plan protocols for the wide variety of equipment on board.

Wednesday 7 Feb - Day 1 at sea

The ship departed Puntarenas at 0500 in very calm clear weather. Dawn broke as we sailed down the Gulf of Nicoya and the views were enjoyed by all. During the day the ROV team wound the new Deep Tow cable back on to the ROV winch which was completed by mid afternoon. The scientists met and had an initial discussion on the science plan. After leaving the Gulf of Nicoya the ship headed to sea almost due W towards the shelf break and the edge of the Costa Rican EEZ. At 2100 local time the air temperature was 26°C and the wind 11 knots from the NE. The ships position at 2100 was 9 35.305N 89 11.053W. The water depth was 3240m.

Thursday 8 Feb - Day 2

We continued passage to the edge of the Costa Rican EEZ at 9° 35'N 89° 10.76'W and on arrival at ~0600 local conducted an MMO watch prior to switching on acoustics (multibeam and ADCP, plus standard underway instruments). The multibeam was switched on and the ship changed course slightly to the N to head to the ROV and Autosub test site. During the day the gap winds from the NE increased to about 20knots with a reasonably large swell. By the late evening these had dropped back down to 10knots. The Cook made good progress with SOG typically 11-12 knots.

Friday 9 Feb - Day 3

The James Cook continued a westerly heading of 277° towards the ROV and Autosub test site. The weather was fair and the winds light 12-13 knots. A strong westerly current and favourable winds gave the ship a SOG of approximately 12 knots, sometimes higher. We re-evaluated the position of the ROV test site and moved it further west to allow us to arrive in the hours of daylight. At the daily 1215 science meeting, the scientists presented individual science plans from each workgroup.

Saturday 10 Feb - Day 4

The ship went on DP at the ROV and Autosub test site at about 0600. The depth was 4040m at 11° 3.253 N 98° 14.687W. The weather was fair with light winds (<10knots) and remarkably little swell for the east Pacific. Autosub 5 (dive AS5MO83) was deployed in the calm weather and started a test dive. Systems checked out and the sub was commanded to start a full depth dive with a multibeam test pattern at 4000m. At the same time, the ROV cable wire stream was started. Autosub was on the surface at 1730, although recovery was slightly difficult as one of the attachment lines was lost at one point and had to be re-grappled. The sub was safely on deck at 1851. The data from the sub looked good and multibeam had been collected from the seafloor at 4000m. A third ROV wire stream was completed at 2200 which was also a success. The James Cook then started the long transit (almost 3 days) to the proposed 'Eddy site' N of Clipperton Island. It was a beautiful, moonless calm evening; Jupiter reflecting on the water as we steamed west.

Sunday 11 Feb - Day 5

The James Cook continued passage west in calm seas with winds 5-10 knots from the NE. The science party continued to setup some lab spaces and hold discussions on the science plan over the next days. A BBQ was held on the back deck at sunset.

Monday 12 Feb - Day 6

The Cook continued passage west for the most part on a heading of approximately 290°. During the day the currents that had been pushing us along at SOG of 12-13 knots disappeared and SOG reduced to about 10.5 knots. We continued to collect multibeam data on a track parallel to the one created on JC241, and in the afternoon passed into the EEZ of France, given its territory of Clipperton Island about 200nm to the S. With the Diplomatic Clearance for work in the French EEZ we were able to keep the multibeam and underway instruments on. The day was somewhat overcast and muggy, with the occasional light shower. At 2300 local the air temperature was still 27.5C and winds 10-11knots from the NNE with a gentle swell. Our position was 12° 29.13 N 107° 33.63 W.

Tuesday 13 Feb - Day 7

We continued passage west across the N end of the Clipperton Island EEZ and reached the Eddy site at approximately 1300. The weather was fairly calm, with NE trades about 10-15knots and a gentle swell. Comms were established with the Deep Glider on deck and the decision made to deploy it once the pilot team at NOC decided it was ready. The Glider was deployed (JC257_005) over the starboard side at 1324 local time. The depth was 4475m and the position 13° 23.989 N 110° 0.00 W. The glider was commanded from base to perform a test dive to 30m and disappeared from view (apparently diving normally) at 1404. We then proceed to do a CTD (JC257_006) which reached 2240m upon which the comms failed to the CTD. It was recovered and checked out OK on the surface. At this point we had not received any updated comms or position from the Glider (being monitored from NOC) and were instructed to perform a visual search at the last known position. We moved the ship to the position and began slowly tracking to the S following the direction of the surface current. The Glider was not located. It was not known whether it was on the surface or not as no information was received from it. At 1720 we went on DP and decided to try the CTD again. It reached 1400m and then lost comms. The CTD was recovered and the fault diagnosed in the cable and the decision made to re-terminate the cable before trying it again. We continued passage W on the multibeam track line.

Wednesday 14 Feb - Day 8

Overnight the technicians did a quick re-termination of the CTD while the James Cook continued on the multibeam track line west (next to the track line of JC241). Overnight the ship passed over the ‘Mathematician Ridge’ which is an extinct spreading centre that runs to the N of Clipperton Island. After passing over the ridge we recorded a depth of 5994m on the multibeam. This represents an unusually deep depression in a region of abyssal plain that is mostly up to 4000m deep. At about 0630 the ship was stopped at a point on the SW corner of the Eddy centre to conduct another CTD and test it. The CTD comms failed again at ~1400m. We recovered the CTD and decided to proceed to the main UK-1 sampling site. The weather got worse during the day and by evening the air temperature had dropped from 27C to 23C and the wind increased to 30 knots.

Thursday 15 Feb - Day 9

We continued passage towards UK1 taking a multibeam route in from the SE corner to complete a line up to the main work area the location of the samples taken in 2013 on the research cruise on RV Melville led by the University of Hawaii (AB01), and a possible location for future exploratory tests of deep-sea mining vehicles. On arrival we released Bathysnap time-lapse camera deployed in 2023 (JC241_100) which came up at about 45m/min and was recovered safely to the deck. We then recovered JC241_099 also safely to the deck. Excellent imagery was recovered from both cameras approx 340 images from JC241_100 and 240 images from JC241_099. The megacore was then deployed and returned an excellent sample (JC257_011). Finally, the box core JC257_012 was deployed at the end of the day. The wind eased throughout the day and at 2300 was down to 10-12 knots from the ENE.

Friday 16 Feb - Day 10

The James Cook continued work at the UK1 site on the coring location site JC257_1km (1km to the N of an area likely to be impacted by future test mining). The box core JC257_012 was brought on deck and was a good sample although was not processed for quantitative studies owing to disturbance on recovery. We proceeded to deploy the Autosub at about 0430 on a multibeam survey of the site which went well. The winds in the morning were light, around 10knots during deployment of the Autosub. A CTD was next attempted, but the deck unit reported an electrical fault at ~500m water depth and was recovered. This was the third CTD failure in a row, each time the depth at which the CTD failed was shallower than the previous. The ROV was deployed (Isis Dive431) at approximately 0800 configured to deploy some pulse-chase experimental cubes as well as collect megafauna. At noon the barometric pressure dropped and the winds started to increase, eventually reaching about 15-20knots

but did not impact work with the ROV. The ROV dive was a very successful with no issues and collected some excellent samples and video imagery. The ROV was recovered in 20knot winds and swell with no issues. We then proceeded to deploy the fish trap (JC257_016), followed by the megacore (JC257_017).

Saturday 17 Feb - Day 11

We continued work at site JC257_1km in the UK1 area recovering a good megacore sample (JC257_017) with 7 out of 8 good tubes. This was followed by a CTD which had been having issues. The CTD deck unit tripped out at about 500m but we continued to deploy it to full depth to collect an SVP profile and test a USBL marker for the ROV team. We then proceeded to recover the Autosub (JC257_013) after breakfast which was safely to the deck in mild conditions 10-15knot winds and a moderate swell. We then proceeded to deploy the ROV on Dive432 to image and collect megafauna which was in the water by 11am local time. ROV Dive 432 continued the remainder of the day, getting some excellent and imagery and samples. During the dive a fault developed with the port manipulator (Kraft) and the port vertical thruster making some tasks difficult but we continued the dive as we were still able to work.

Sunday 18 Feb - Day 12

We recovered the ROV Isis at approximately 0900 after a successful dive to image and collect megafauna. We then proceeded to deploy the Autosub (JC257_020) which went well followed by the Box core JC257_021. The box core returned a perfect sample with intact clear top water and a good surface. The afternoon watch processed it completing the sieving within 2hrs and the remainder of the imagery and faunal sorting some 2 hours later. During this period we recovered the Fish Trap which had been down for 2 days at the 1km site. The trap was difficult to recover owing to the lack of a floating line to grapple. The trap contained 2 fish and some amphipods in the amphipod trap, which had eaten about half a mackerel. We then proceeded to deploy the 2 Bathysnap time-lapse cameras JC257_022 and JC257_023 which went well, followed by another box core JC257_024 which arrived on deck in good condition at midnight. The weather was fine with NE trades at 10-20knots and a moderate swell.

Monday 19 Feb - Day 13

The James Cook continued work at the UK1 site. Box Core JC257_024 was processed while another Megacore JC257_025 was deployed, this was recovered and was a good sample. This was followed by another CTD attempt but comms was lost at 393m. This was followed by a failed Boxcore which appeared to not trip. We tested the Box core on the deck and repeated the deployment using a faster speed of 20m/min into the seafloor. The core was a success. This was followed by another good Megacore returning all tubes in perfect condition. Finally a box core JC257_030 was deployed again and recovered at the midnight watch change, the core sample was good with clear top water. During this long day of coring and wire time, repairs were completed to the ROV manipulator and thrusters and preparations for the ROV dive complete. Throughout the dark hours we have observed a large number of sharks surrounding the ship. The species are Silky sharks and Oceanic white tips. A very large Oceanic white tip shark was filmed with the GoPro camera on a stick, it attacked the stick a few times. At one point, two sharks were circling the box core as we deployed it. At 2300 the winds were moderate - 10-15 knots, and the air temperature 26C.

Tuesday 20 Feb - Day 14

The box core JC257_030 arrived on deck at the midnight watch change, this was followed by a good megacore sample then another boxcore. The coring went well until the ROV was deployed in the morning to recover the first set of pulse-chase experiments on the seafloor (4 cubes with labelled algae). The dive went well and 2 hours were spent on the seabed collecting megafauna opportunistically, the cubes were recovered and the the ROV ISIS was recovered at 1900. At this point the swell had increased considerably. Work continued - a megacore was deployed which was good and then the boxcore JC257_35 was deployed at the end of the day. At midnight the winds were about 18knots from the NE and a moderate swell.

Wednesday 21 Feb - Day 15

The James Cook continued work at the 1km and 0km sites in UK-1. Box core JC257_35 failed to trip at the seafloor and was followed by a CTD cast that again lost comms at 300m depth. The technicians swapped out the swivel and in a second attempt, it worked perfectly to full depth. This was followed by a good box core JC257_038. The box cores have mostly been coming up with clear and intact top water and temperatures at the surface-water interface of 14-16 °C. This indicates good sealing of the sample from water ingress through the doors, a common problem. We followed this deployment with deploying the Fish Trap, this time with a USBL beacon so it can be located by ROV if needed. This was followed by another good box core at the 1km site taking us to 7 out of 8 required at that site. We then proceeded to deploy Autosub. Deployment went find despite gusty winds up to 20knots and some moderate swell. However, shortly after Autosub was programmed to start its mission it aborted and returned to the surface. There were initially some issues communicating with it and it started to head towards the ship, however the AUV team were able to stop it and gain control of it. It was decided to leave it on the surface

and track it overnight while completing the ROV dive as the Captain decided it was too rough to recover at night. We proceeded to the ROV location with the Autosub following us, like a sort of faithful dog about 100m behind the ship. ROV Isis Dive 434 was deployed at about 2100 local time and reached the seafloor at 4100m at 2330. We commenced a planned 12hr dive to image and collect megafauna. At 2300 the wind was about 15knots from the NE and temperature 25°C.

Thursday 22 Feb - Day 16

The ROV Isis continued work at the seafloor on Dive 434 (JC257_042). Imaging and collecting went extremely well. Since JC241 we have been using the SCORPIO camera in a position mounted low and forward on the tool tray, allowing extreme close up imagery and video of invertebrates from a low perspective. This has resulted in spectacular imagery of even quite small invertebrates. Further refinement of the imaging is using the Schilling arm with a side light to provide side illumination of the living animal in situ. As far as we know, these are the first detailed images of abyssal fauna taken this way. Imaging and collecting continued until 0700 when the ROV was brought up and on deck safely at 1000. A highlight of the dive was a truly spectacular image of an anemone in situ on the seafloor. Unfortunately anemones are taxonomically extremely difficult to determine and very few people work on abyssal specimens. Once the ROV was secured we studied, imaged and sub-sampled the animals. One of the specimens was a sea-pen, *Umbellula*. Remembering that the one of the very first specimens recovered by HMS Challenger was also a sea-pen and observed to glow on disturbance, we took the specimen to the James Cook darkroom and sure enough it glowed quite brightly green when disturbed with a pair of forceps. The bioluminescent material seemed to shed slightly from the stem into the tray. All the scientists on board enjoyed seeing this remarkable, abyssal light. PSO thought about Prof Paul Tyler (who led many cruises on the JC and earlier NERC ships) and how much he would have enjoyed seeing *Umbellula* glowing from the abyss - one of his favourite deep-sea animals. Soon after the ROV was secured on the deck we safely recovered the Autosub which had spent a night following us around at the surface, piloted over WiFi from the AUV control van, or in fact, the AUV technicians iPhones. The fault in Autosub was diagnosed as a software issue, with the dive abort triggered by a low voltage reading in a battery and a solution developed. While the AUV team worked on this we switched to coring and obtained another excellent box core sample JC257_043. As with all the box cores taken so far, the nodule abundance is high - likely 15-20 kg/m2 - and almost entirely composed of relatively small (5-10cm) nodules that are broken and irregular. The fauna on the nodules is composed of mainly small sponges and bryozoans that are hard to see except with a trained eye. Fortunately we have a good team of experienced zoologists on board able to see and identify the fauna directly. The box core was followed by a good CTD to full depth with water samples taken for the microbiology and stable isotope groups. The earlier trouble with the CTD seems to have been fixed. The day ended with deploying another box core JC257_045. At 2300 the wind was 14 knots from the NE trades, temperature 25°C and the swell fairly low.

Friday 23 Feb - Day 17

Coring continued overnight. The boxcore JC257_045 was good and was followed by a good megacore returning 5 out of 8 tubes. The sediments in this area (the 0km site) appear to show some cracks and some bubbles moved through some of the cores, making it hard to get a perfect set of cores. We followed this by deploying the Autosub on a second attempt at the benthic photos dive. This was followed by another good box core, after which we went to deploy the ROV on Dive435 to survey the fish traps, add sediment to the traps and collect megafauna and imagery. The fish traps were located without trouble and we observed a large lizard fish *Bathysaurus ferox* sitting next to the trap, although seemingly not interested in the bait. Inside the trap were a couple of cusk eels and a swarm of amphipods could be seen around the bait. We moved to megafauna imagery and captured some excellent imagery and footage. It was interesting to observe the benthic (planulae) stage of the jellyfish *Nausithoe* attached to a *Sympagella* sponge. We see these cone shaped tubes on the nodules but have not yet seen one with an animal inside. The ROV was recovered safely at 2130 and then proceeded to deploy a megacore while we spent time photographing the megafauna. We got a second *Umbellula* sea pen, and again observed strong bioluminescence in the dark room - a sequences of green flashes as one rubbed the side of the tube. It was too dark to capture with any camera we had.

Saturday 24 Feb - Day 18

Work continued at the UK1 0Km site. This is the site of the original ABYSSLINE survey carried out in 2013 on which the PSO and several other scientists on board were present. This has brought back memories of working on the RV Melville, now retired, but at the time the oldest ship in the UNOLS fleet with many fixtures and fittings dating from the 1960s. The James Cook, although almost twenty years old herself still feels extremely modern. A megacore was recovered with good samples, which was followed by a good boxcore JC257_051. We moved the location of this 1km to the south (the 1km site) to avoid any ‘interaction’ with Autosub doing the photo survey transects. We then recovered the fish traps, undertook a short ROV dive to deploy the second set of pulse-chase cube experiments and then proceeded to recover Autosub. It proved a bit tricky to grapple as the sub was not aligned parallel to the ship, but she was finally onboard. There was a bump on the ship on recovery slightly damaging one propellor, which will be replaced. This was unlucky really, the sub just swung the wrong way as it

was being hauled up. The position of the LARS on the starboard side makes launch and recovery of Autosub quite challenging but the crew and technicians have been doing a great job with it. The bosun noted that they need some heavier ropes to do the grappling as the ones they have are too light (and get blown by the wind). The recovery was followed by an excellent box core. Following that, we deployed the CTD which unfortunately lost comms again at 3000m. Finally we deployed another box core (JC257_055) at midnight.

Sunday 25 Feb - Day 19

Some bad luck to start the day, the box core Jc257_055 drained top water on recovery and was only useful for qualitative studies. Autosub JC257_056 was deployed but suffered a battery issue at 1500m during the dive and aborted to the surface. We then recovered the Autosub and made a short transit N to the OMS site where a colonisation experiment had been placed on the seafloor during the RC01 cruise in 2020. We dived with the ROV on Dive437 JC257_057 and found the experiment within minutes of being on the seafloor. The experiment consisted of 10 nodule-sized basalt blocks collected on the East Pacific Rise so the experiment was designed to test the hypothesis that nodule dwelling fauna could live on non-nodule hard substrate habitats, potentially these could act as refugia from planned deep-sea mining. We surveyed and recovered the experiment noting a number of epifauna attached to the basalt blocks and the ropes and marker float. We then proceeded SW about 200m collecting and imaging megafauna and finished the dive at a small rocky outcrop about 20m in width and 2-3m in height. The outcrop of pillow basalt was an oasis of sponges and sessile fauna, including a large dead sponge stalk that was taller than the ROV. Several 1m long or more 'cauliflower' sponges were present on top of the rock. We spent 1hour collecting representative fauna and then returned to the surface with the colonisation experiment in a large biobox (made from a Zarges case) on the front of the ROV. The ROV was recovered at 2200 and work began on the samples. Interestingly, the colonisation experiment had started to be colonised with small serpulid tubeworms, some bryozoans and one specimen of a small white sponge that is very common in the eastern CCZ, *Plenaster craigi*. This is the first time we have obtained evidence of the growth rates of CCZ specimens on hard substrates. We followed the ROV recovery with deploying the box core, on the first of 3 back-to-back deployments.

Monday 26 Feb - Day 20

A night of box coring commenced to catch up with the core requirement at the 0km station. Two good samples JC257_058 and _059 were followed by a failed core that did not trip at the seabed. An additional fourth box core in a row was deployed on the PM shift which was successful, and we then proceeded to deploy the CTD after yet another re-termination. Thankfully it worked perfectly to full depth. The CTD was followed by deploying the ROV to recover the second set of nodule colonisation experiments deployed in 2020 on the RC01 cruise. We found the experiments within minutes of landing at the seabed, and imaged them before collecting them into a large biobox. As with the panel deployed at OMS site, brittle-stars were clinging to the ropes and we observed a number of chaetognaths, amphipods and isopods swimming and crawling over the surface of the rocks. It was remarkable to be able to observe behaviours at an abyssal site first hand. The ROV dive438 continued overnight to collect megafauna.

Tuesday 27 Feb - Day 21

The ROV dive 438 continued until the morning, and was recovered at 0930. Stunning imagery and in-situ observations of abyssal fauna were made including imagery and video close up of animals such as *Psychropotes* and a very large specimen of *Amperima*, which was sampled. A large crinoid was also imaged and collected. We then proceeded to deploy Autosub (JC257_064) on a multibeam survey of the 16km and 30km site. The CTD followed, which worked well to full depth and then we deployed the fish traps on their third outing, at the 16km site (JC257_066). We followed the fish trap with the first box core at the 16k site which worked well. The Autosub was in the vicinity of the box core site but using the ships USBL instrumentation we were able to track its position at the seafloor and time the box core when it was safely out of the way. At this exact moment in the cruise we were operating 9 seafloor instruments simultaneously in UK1: 3 long-term moorings, 2 time-lapse cameras, 1 in-situ pulse-chase experiment, 1 fish trap, Autosub and box core, plus underway instrumentation. We recovered the box core JC257_067 which was good, then proceeded to deploy the megacore JC257_068 which was also good. This concluded a successful day of sampling on the James Cook.

Wed 28 Feb - Day 22

Hump day on JC257 was observed with more coring and another ROV dive. Megacore JC257_068 was recovered from the 16km site and was a good sample. This was followed by another good boxcore, then a short multibeam survey to the ROV Dive439 location to recover the SAMS pulse-chase cube experiment at the 0km site which had been on the seabed for 4 days. The ROV team managed to get the ROV down at the seabed at 11AM, recover the cubes, take 12 push cores and get us back on deck at 1600, something of a record. We followed this with a transit of an hour back to the 16km to recover Autosub from its multibeam survey which went well. We then proceeded to collect another boxcore (JC257_072) and then deploy another megacore, taking us to the end of a successful day. The boxcores in the 16km region are quite patchy, some with large nodules and a soupy sediment

layer and others with very few nodules. Some good macrofauna were live sorted and imaged in the NHM Bio sorting team.

Thursday 29 Feb - Day 23

Sampling continued overnight at the 16km site, with a good megacore recovered (JC257_073) followed by a good boxcore JC257_074. With some spare time prior to the planned ROV dive, we decided to deploy the boxcore again. Unfortunately at 3773m wire out, very close to the seabed there was a catastrophic failure in one of the sheaves that runs the core wire through the gantry with the wire jumping about and making horrendous noises. We all stopped on the winch and the ships crew investigated the issue - unable to provide any work-around we brought the wire up through the broken sheave, extremely slowly at first and gradually increasing to about 10m/min. In the late afternoon we were able to finally get the boxcore and wire recovered to the deck. The sheave was not salvageable and the crew abandoned use of the core wire and switched coring operations to use of the trawl wire, which runs through a different sheave. At 1600 approximately we deployed the Autosub on a photos dive to the 16/30km sites, and while shadowing it ran some tests on the CTD system to help diagnose earlier issues with the CTD. With these tests concluded, we deployed the ROV on Dive440 to deploy the cube experiments for the 3rd time. The weather was marginal on launch, with winds of 20knots from the NE. The dive proceeded well but shortly before midnight, the Autosub aborted its photo dive and returned to the surface, likely encountering unexpected bathymetry. We continued the ROV dive while monitoring Austosub which we planned to recover in the morning. At 2300 the air temperature dropped noticeably to around 25C and the winds (20knots) and swell were quire noticeable. Although the weather has not stopped us doing anything, we have not had any really calm days at sea since arriving at the UK-1 site.

Friday 1 Mar - Day 24

ROV Dive440 (JC257_078) continued through the night. The fish traps were observed with a large number of fish (relative to normal abyssal background) including *Coryphaenoides* sp, *Bassozetus* sp cusk-eels and *Pachycara* sp. eelpouts in the trap or close to it. The ROV was able to close the fish trap mechanism. The dive then proceeded to image and collect megafauna on a transect headed away from the fish trap and was recovered to the deck at 0930 after another very good dive without any issues. We recovered the Autosub from dive JC257_076, which proved difficult again using the LARS on the starboard side. The main issue is that any rotation in the vehicle as it is brought out of the water causes either the propeller end or nose end to impact the ships hull, as there is limited separation in the LARS mechanism from the ship. Although this happened, the vehicle was recovered safely. We then proceeded to recover the fish trap (JC257_066) with the acoustic release which went well. Four fish were recovered (3 species) in the trap and a number of amphipods including the large *Eurythenes* sp. some of which were clinging to the bait packages. These were imaged and sampled by the NHM BIO team. We followed this by moving back to the 0km site to recover the first of the 3 long-term moorings (Short mooring 3 - JC257_079). This was recovered successfully in the afternoon despite 8 of the floats having imploded just above the SBE (CTD) package. The short mooring 3 consisted from the seabed upwards of twin acoustic releases, a Nortek ADCP, then 8 glass spheres (which imploded), followed by 80m of rope, then an SBE CTD, then another Nortek ADCP, then the sediment trap leading to a further 10 glass spheres and a surface float with recovery line. All instruments were still working on recovery and recorded data. The sediment trap bottles were secured and imaged, and showed signs of seasonality in sedimentation rates. After the mooring was recovered, we moved to deploy the remainder of the box cores at the 0km site, and JC257_080 was a good box core. We followed this with deploying the Autosub JC257_081 on a photos dive of the 0km site to cover the part of the survey that was missing from the last photo dive. Finally, we deployed another box core JC257_082 before the end of the day. The weather continued in much the same pattern as the last days - 15knot trade winds from the NE and air temperatures of around 26C.

Saturday 2 Mar - Day 25

The James Cook continued finishing up the coring activities at site 0km. The boxcore was recovered (JC257_082) which worked well, followed by a second box core of the night which was also good. We then proceeded to ping the second short mooring (Short Mooring 2) deployed on JC241. It responded initially and the release command was sent, but we were unable to range it or determine if it had released. We continued to range it for 2 hours, and sent the release command to the second release, but we were still unable to range it. After 3 hours we proceeded to deploy the box core (JC257_085). During deployment we checked the Iridium satellite link to see if the mooring had surfaced and at approximately 1130 it showed up as being on the surface a few miles from our position. We recovered the box core (a good sample) and proceeded to pick up the mooring which was on deck after lunch. The sediment trap looked to have worked well, and all instruments appeared to be still functional. We then proceeded to recover the Autosub from its photos dive at 0km which went well, then moved back to the 16km site to complete the coring at that site. The boxcore was deployed (JC257_086) but it was a fail - only a small sample of nodules and mud were returned. It appeared that the wire had caught on the spade arm on recovery as the wire had a bend in it just above the corer. We repeated the box core and got a good sample, then proceeded to deploy the megacore. Thus ended a fairly successful day. Winds through most of the afternoon were 15-20knots NE trade winds, air temperature 26°C, as has been the pattern throughout most of the cruise.

Sunday 3 Mar - Day 26

We recovered the Megacore (JC257_088), which was a good sample, then deployed the boxcore which also returned a good sample, completing 6 of 6 boxcores at the 16km site. At this point, in order to maximise the chances of obtaining good Autosub dives, we transited south to the 100km station to deploy the AUV on its multibeam survey (JC257_090), then headed back to the 16km site to recover the cube pulse-chase experiment as it needed to be on the seabed for 72hours. We just made it in time to be able to deploy the ROV at 2230 local time. The ROV dive proceeded well with 3 hours of collecting and imaging prior to recovering the cube experiments in the early hours of Monday. The weather was fine throughout the day 10-15 knot NE trade winds and clear weather.

Monday 4 Mar - Day 27

The ROV Dive441 JC242_091 continued overnight with some excellent samples obtained including 3 scleractinian (stony) corals which are very rarely encountered in the CCZ. The ROV was recovered safely and we proceeded to deploy the Megacore for the last sample at the 16km site which returned a good sample. We then proceeded back south to recover the Autosub from dive JC257_090 from the 100km site. A large swell from the N made the southerly transit to the 100km site quite enjoyable in the late afternoon, the ship surfing down the long Pacific rollers with the sun setting. Recovery of Autosub went well but some errors in the Autosub systems resulted in only partial coverage of the multibeam. We then moved to deploy the ROV on Dive442 to deploy the last set of cube (pulse-chase) experiments as well as recover megafauna. The ROV was configured in a hybrid mode with the SCORPIO high resolution camera mounted back on the front sled but with 2 boxes for the cubes rather than 4. Late in the evening, the science team were treated to some outstanding imagery of megafauna at the seafloor with incredible detail from the macro lens on the SCORPIO camera. The ROV dive continued through the night. The weather was reasonably calm, 10-15 knot trades and air temperature of 27C. The long swell from the N has made the ship roll and wallow occasionally but it has not stopped activities.

Tuesday 5 Mar - Day 28

The ROV Dive JC257_094 continued through the early hours, with more amazingly detailed shots of abyssal life using the sled-mounted SCORPIO camera. The site was noticeably rich in large sponges, and a stunning *Amperima* sp. holothurian was imaged and recovered in good condition. We attempted to do some 3D photogrammetry on the specimen prior to preservation. The ROV was recovered safely and the Fish Traps deployed at the 100km site for the last time. This was followed by Autosub deployment (JC257_096) for a photo survey dive at the 100km site despite not having the best multibeam data. With Autosub away on its mission, the afternoon shift did 2 boxcores back to back (JC257_097 and 099) which both worked well. The nodules were small but the density quite high - likely 20 kg/m2. Finally we deployed the Megacore for the first samples at the 100km site which worked well and was on deck shortly before midnight. As with previous evenings, the wind had strengthened again later at night to 15-20 knots from the NE. A long but quite large swell continues from the NW.

Wednesday 6 Mar - Day 29

We continued coring at the 100km site through the night, with a good box core (JC257_100), the 100th deployment of JC257 on deck around 0400, followed by a Megacore which was also good. Unfortunately Autosub dive JC257_096 had aborted, so we recovered this in the morning and then conducted a CTD JC257_102 while the AUV team turned Autosub around for a redeployment, after a fast battery charge and new dive plan. Autosub was then redeployed around 1500 local time, which we followed with a Megacore sample (JC257_101) while Autosub was being shadowed on its dive. Following the Megacore, we left Autosub on its survey and proceeded to deploy the ROV on Dive443 (JC257_105) to image and collect megafauna at the 100km site, the final planned dive at this site. The dive proceeded very well with some spectacular video and imagery of abyssal fauna, including a large *Amperima* sp. (nicknamed the Barbie-Pig) which appeared to be a different species to the one collected previously, and a very unusual sea cucumber with a long thin dorsal sail. The ROV dive continued through the night.

Thursday 7 Mar - Day 30

The ROV Dive443 continued until about 0500 local when it was recovered to the deck safely. An excellent haul of megafaunal animals was recovered with many species having outstanding in-situ imagery and video. We then recovered the Autosub from dive JC257_103 (its second attempt at the 100km site) before starting a transit north along a multibeam line to re-deploy Autosub in the evening following a battery recharge and position at the 30km site. At this point in the cruise we decided to maximise Autosub dives (in order to make up for some that had failed) which involved some movement back and forth between sites at the expense of ship time. Autosub was deployed at around 1930 local time on JC257_106. We then transited back to the 100km site with a plan to deploy the ROV on the last cube recovery dive JC257_107.

Friday 8 Mar - Day 31

Having moved back to the 100km site, we deployed the ROV on Dive444 JC257_107 which was a quick dive to recover the cube pulse-chase experiment for the last time. This was recovered and back on deck by 0900 and we then had to transit back to 16km to recover the Autousb from its dive at the 16/30km sites. The recovery went well but unfortunately the Autosub had not recorded a full set of photos, only the first 3 hours (mostly water column), so the dive had not worked. We then had to move back to the 100k to recover the fish trap which was released at 2230 local time and was due on deck around 0100. Meanwhile we prepped Autosub for another dive (to collect the missing multibeam and do a camera test at the 100k site). Autosub JC257_108 was deployed shortly before midnight and we then proceeded back to the Fish Trap site to collect that.

Saturday 9 Mar - Day 32

Following the Autosub deployment, we moved back to the Fish Trap site (JC257_095) to recover it. The fish trap was brought along the starboard side of the vessel and grappled as normal. Unfortunately during recovery unexpected currents moved it first against the ship, then the lines got around the propellors and the line to the trap was severed as the crew attempted to move it around to the aft A-frame, with the trap and the release unit lost. Two of the floats were subsequently recovered with the starboard knuckle crane. With no possibility to recover the fish trap we moved on to the next site and took a good boxcore (JC257_109), followed by the last megacore sample of JC257. We then carried on coring with a good box core (JC257_111) that had a large bamboo coral on the surface, and clear topwater. We recovered Autosub in the afternoon from the multibeam survey at the 100km site, which went well, and then took the last box core at the 100k site JC257_112 which was slightly disturbed and slumped but processed as normal. We then commenced the transit back N to the Autosub deployment site at 30km to do a repeat attempt at a photo survey dive.

Sunday 10 Mar - Day 33

Having transited back north to the 30km site, we deployed the Autosub in the early hours (JC257_113) for a long dive to photo survey the 16km and 30km boxes. We then proceeded back to the 1km site to take one final box core, which was a success and brought the total number of cores at the 1km site to 10, and the total number of quantitative box cores to 32, from 40 deployments of the gear. This is an above average success rate for the CCZ, where trapped nodules and issues with sealing the box with the nodules pushed down to the spade can often lead to loss of sample. The box core has worked perfectly throughout the cruise, the only issues have been caused by bad luck for example the wire becoming entangled at the seabed or hitting large nodules that prevented sealing. The entire science party have assisted with processing the large volume of mud from the box cores, an arduous task, and all were relieved to finally complete this part of the science programme. We then proceeded to deploy the ROV on Dive445 (JC257_115) to locate, image and collect from 3 bone and wood colonisation packages put down by Prof Craig Smith formerly of the University of Hawaii, the PSO and others on a cruise to the CCZ on RV Melville in October 2013, 10.5 years ago. We had emplaced these experiments to search for novel species that colonise organic-enriched environments in the CCZ, which like anywhere else in the ocean are likely to harbour a significant portion of the biodiversity but are very difficult to sample - the chances of coming across one being slim on our brief visits to the seabed. The dive proceeded well, and the sonar markers (simple blocks of syntactic foam and bucket lids) showed up well on the Isis scanning sonar and we located 2 of the experiments in the short dive. Both experiments had 3 rib bones of whale and 1 block of wood, and most of the wood and bone had been consumed. However, the sites were still active, with abundant squat lobsters visible in the imagery as well as several polychaetes. We were interested to search for the enigmatic ‘bone-eating worm’ *Osedax* which had never been recorded from abyssal Pacific but we could not see anything obvious on the video. We recovered several bone fragments and pieces of wood to the ROV bio box, took slurp samples and a series of push cores and returned to the surface. The ROV was recovered to the ship at 2200 local. On closer examination we discovered several new species of *Osedax* on the bones (at least 3 species) plus a surprisingly diverse and abundant community of organic-enrichment specialists. Following the ROV dive, we proceeded to the deep CTD site close to the long mooring to take a Yo-Yo CTD for hydrographic measurements of bottom currents which continued through the night.

Monday 11 Mar - Day 34

James Cook continued work at the UK1 ‘0km’ site, with the Yo-Yo CTD in the deep hole near to the Long Mooring. The deployment went well, and we then moved to recover Autosub from the 16/30K photo survey, then back north for the final ROV dive (JC257_117) on the site we called the Sponge Garden, which lies in the OMS contract area about 8 miles north of the UK-1 0km site. Having discovered this location earlier in the cruise (during the recovery of the rock colonisation experiment), with abundant large sponges and corals on a rocky outcrop we decided to return and complete a photogrammetry 3D survey as well as collect more imagery and any fauna that we had missed on the first dive, which was very limited in time. The site is characterised by manganese-encrusted pillow basalts, sedimented slopes and some large mysterious holes in the sediment. There are also regions of exposed manganese crust that has the appearance of fossilised reef, but could be biogenic structures that have become coated in manganese over millions of years. Some very large ‘cauliflower’ sponges are present at the top of the mound, some over 1m in height, as well as some large bamboo coral and many smaller sponge species. The site

has some resemblance to the 'Forest of the Weird' location surveyed by a NOAA vessel in 2017, although is much deeper. The dive went very well, and at 1900 local time we finished the dive, the giant sponges and corals slowly disappearing from view as (piloted by the PSO) the ROV Isis left the abyssal seafloor here in the Pacific for the last time on the cruise, with most of the science party crammed in the van for these final views. The ROV has performed flawlessly throughout the whole expedition. We then proceeded to deploy the Autosub on its last dive to photo survey the 0km and 1km sites (JC257_118).

Tuesday 12 Mar - Day 35

During the last night at the UK1 site, we deployed the Gravity core (JC257_119) to take a sample for the British Geological Survey and BOSCORF. An excellent 4.6m core was returned on the first attempt. We then proceeded to the Long Mooring site (deployed on JC241), this took some time to get confirmation of release and ascent rate but was on the surface and being recovered by mid morning. Recovery went well of all instrumentation and the 2 sediment traps. We then moved to recover the Bathysnap cameras. The first one was recovered successfully, but we then heard that Autosub had aborted to the surface. We recovered the first Bathysnap and then proceeded to the Autosub location. Autosub was recovered to the deck at approximately 1340 local time. A strong smell suggested a battery fire (or overheating) on recovery. Inspection revealed a battery fire had taken place in one of the failed batteries (the reason for the abort). The battery compartment showed some signs of damage and the battery was removed, potentially still on fire, and for safety of the ship reasons was quickly disposed of at sea by the ships crew. The remaining batteries were inspected and deemed safe although were closely monitored. Remarkably, despite the battery fire, the Autosub had aborted the dive, surfaced, and continued to operate the camera thus returning a large volume of good imagery and data. We then moved to recover the last Bathysnap, which went well and this completed over the side deployments at UK-1. With a few hours remaining, we completed some multibeam survey in the UK-1 area then started the transit home, with planned stops for 2 CTDs en route to collect data on mesoscale eddies in the water column for the BAS team.

Wednesday 13 Mar - Day 36

After 27 days at the UK-1 site working more or less non-stop, the James Cook started the long passage home. We followed our previous multibeam track stopping to take two final CTDs (JC257_121 and 122) in the morning and afternoon respectively. Deployment JC257_122 marked the last over the side deployment of JC257. All were relieved to complete this and enjoyed some time on the deck in the sunshine, calm weather and gentle swell from the NW as we transited. No science meeting was held.

Thursday 14 Mar - Day 37

We continued the passage East in fine weather. The science team worked on sample packing, equipment cleaning, data management and cruise report. Late in the evening we passed over a deep hole and undertook a multibeam survey of this depression just to the west of the Mathemetician Ridge - an extinct spreading centre that lies to the N of Clipperton Island. We recorded a maximum depth of 6025m, making this a hadal zone within an abyssal province. Steeply rising to the E of this depression, the Archimedes Seamount rises to a depth of ~1400m, making it (measured from the bottom of the depression), a seamount 4600m high, almost the height of Mont Blanc.

Friday 15 Mar - Day 38

We continued passage East in fine weather. Counter currents slowed the ship and we used 3 engines to maintain SOG over 11 knots in order to ensure arrival on time. The science team cleaned labs and continued to work on data and cruise report. The PSO did an interview with Justin Rowlatt from BBC News, not about JC257 but rather on the subject of deep-sea mining to coincide with the meeting of the ISA Council on 18 March.

Saturday 16 Mar - Day 39

We continued the passage with fine weather, most of the science party busy packing samples and writing up the cruise report.

Sunday 17 Mar - Day 40

We continued the passage in fine weather. The entire ships company enjoyed a BBQ on the back deck at sunset.

Monday 18 Mar - Day 41

We continued the passage in fine weather.

Tuesday 19 Mar - Day 42

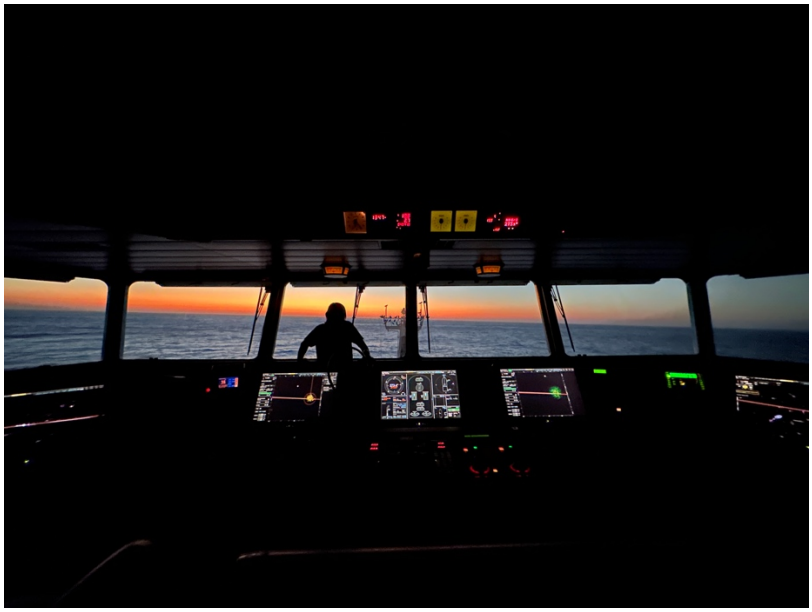
We continued the passage in fine weather. A photograph of all the science and technical team was taken on the ships bow.

Wednesday 20 Mar - Day 43

We continued the passage in fine weather. Many dolphins surrounded the ship during the day, and we observed rays leaping out of the water. An extremely beautiful final sunset, with dolphins around the ship.

Thursday 21 Mar - Day 44

We made passage slowly up the Gulf of Nicoya in the early hours, past numerous small fishing boats, picking up the Pilot before sunrise. We were berthed in Puntarenas at dawn. Thus was the end of JC257.



Sunset on the bridge of the RRS James Cook on JC257. Image by Adrian Glover.

2. Equipment Protocols, Deployments and Summary Reports

2.1. ROV

2.1.1. Technical specification

The ROV Isis is a 6500m deep Remotely Operated Vehicle (ROV) designed for scientific research (Figure 2.1.1). Further details are provided in the Appendix 3 to this report.

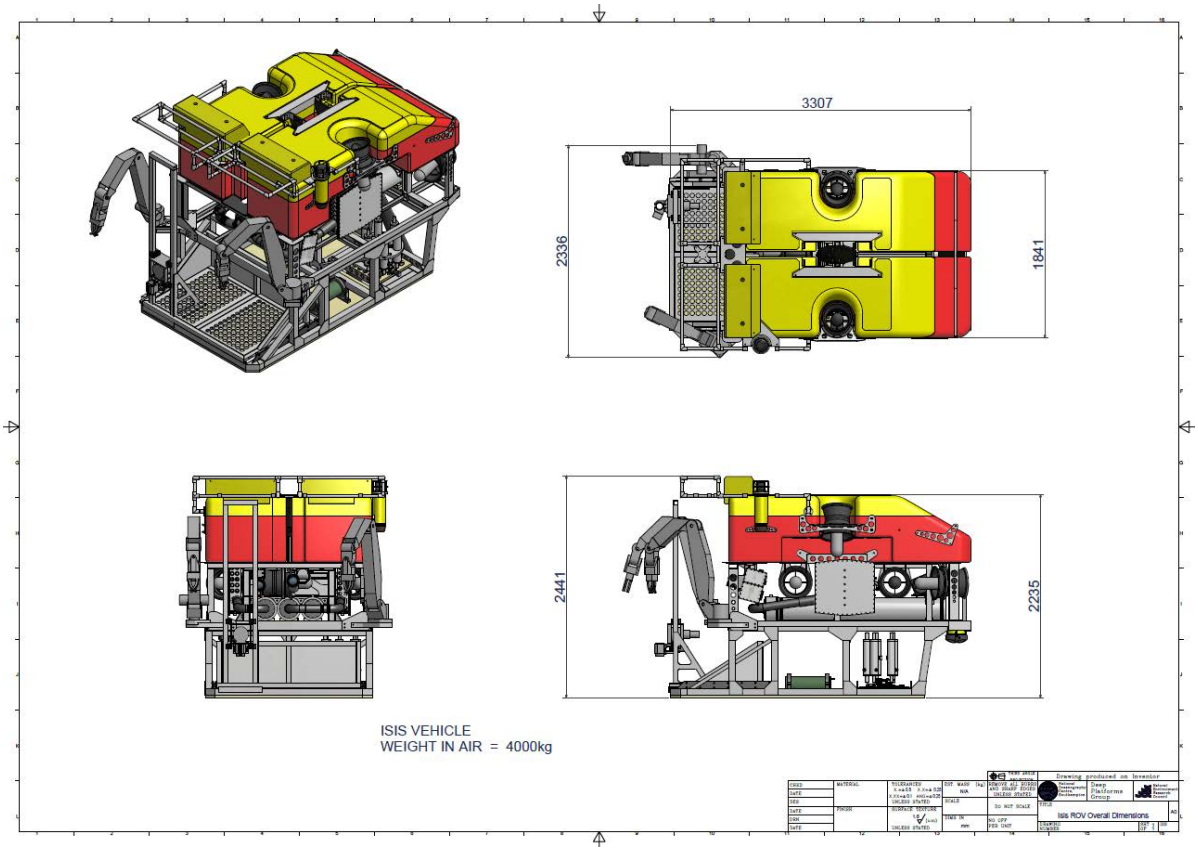


Figure 2.1.1. ROV Isis technical drawing.

Specifications

Dimensions	2.7 m (L)×2.0 m (H)×1.5 m (W)
Weight	~3400 kg (approx.) in air
Max Working Depth	6500 m
Propulsion	6×brushless DC electric thrusters 3.7 kW (2×vertical, 2×lateral and 2×fore/aft)
Maximum forward speed	0.75 ms-1 (1.5 kt)
Autopilot Functions	Auto depth (to ±1 m) Auto altitude (to ±1 m) Auto heading (to ±1 m) Close-loop control using bottom lock with Doppler
Hydraulic power unit	3.7 kW
Manipulators	1 x 7-function Kraft Predator II (6 degrees of freedom, force feedback) 1 x 7-function Schilling Robotics Titan 4 Manipulator

Vehicle Sensors	Altitude	Kongsberg 1007 Series Altimeter 200 kHz
	Pitch and Roll	IXSEA Octans Fibre Optic Gyro (FOG)
	Depth	ParascientificDigiquartz pressure sensor
	Heading	IXSEA Octans Fibre Optic Gyro (FOG)
	Sonar	Forward-looking Tritech Super Seaking Scanning Sonar Head 675 kHz
Acoustic Sensors		RDI Navigator 300 kHz bottom tracking Doppler Velocity Log (DVL)
		Sonardyne Ranger 2 Ultra Short Baseline (USBL) navigation
Oceanographic sensors		Seabird CTD (Conductivity, Temperature, Depth)
Video cameras		1 x High Definition (HD) Insite Pacific Mini Zeus (1080i x 50) “Pilot”
		1 x Kongsberg Imenco OE14-522 High Definition PATZ Colour Camera (1080i x 50) “Science”
Stills Cameras		1 x Insite Pacific Scorpio Digital Stills Camera (3.34 megapixel) “Scorpio”
Lighting		4 x CathX Aphos 16 LED lampheads, 28,000 lumens each 2 x DeepSea Power & Light Multi SeaLite LED lights (smaller) 1 x small light on Schilling Titan 4 Manipulator
Scaling		2 x NOC bespoke laser scale modules, 0.1 m between parallel lasers

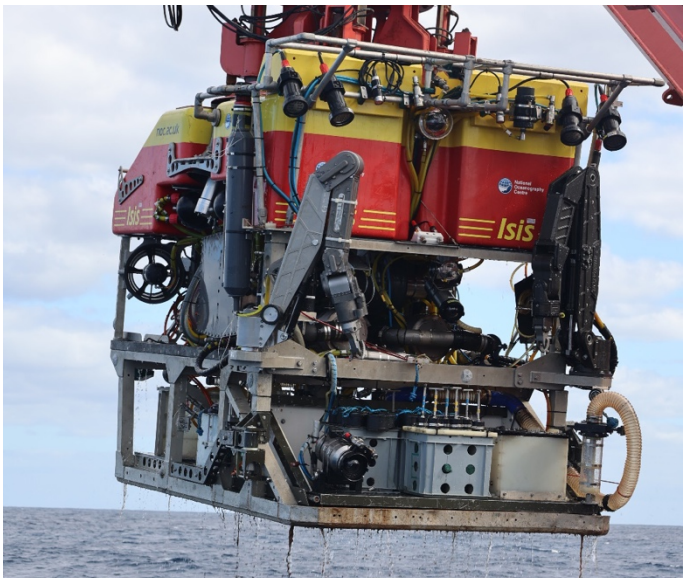


Figure 2.1.2 ROV Isis coming back after dive on JC257.

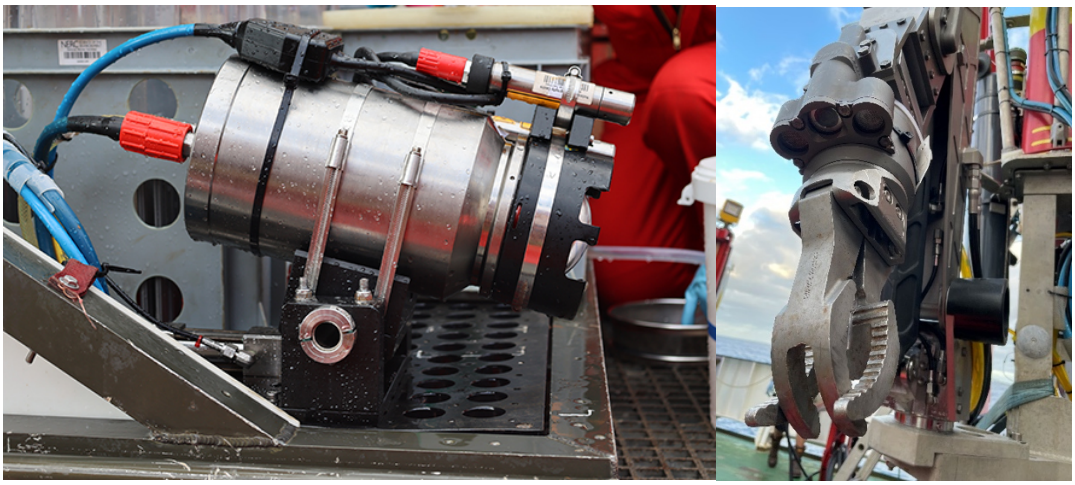


Figure 2.1.3 Left: Scorpio camera mounted on tool tray with parallel lasers above. Right: camera and lights mounted on the Schilling Titan 4 Manipulator.

2.1.2. Sampling configurations

The ROV tool tray was configured in a variety of ways for different purposes on JC257.

Megafaunal collecting

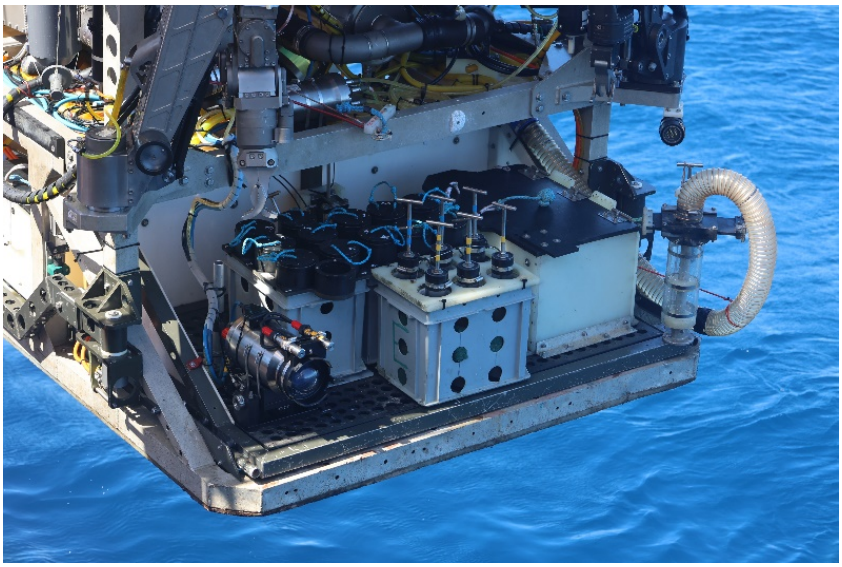


Figure 2.1.4 A typical setup of the ROV for megafaunal collecting used 1 large biobox, 6 push cores, 12 magpots and the SCORPIO camera mounted at the front of the tool tray.

Cube deployment and recovery

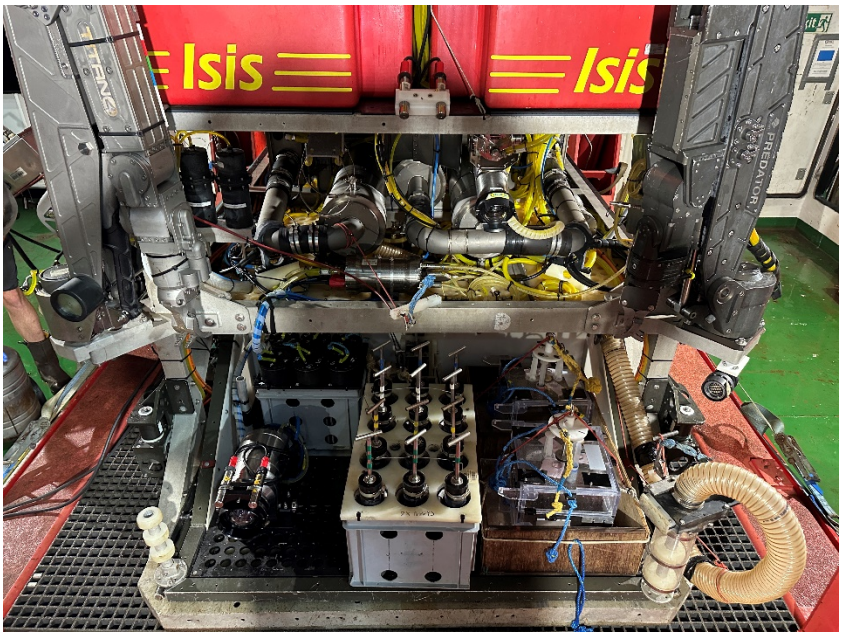


Figure 2.1.5 The ROV setup for recovering and deploying cube experiments.

Other configurations

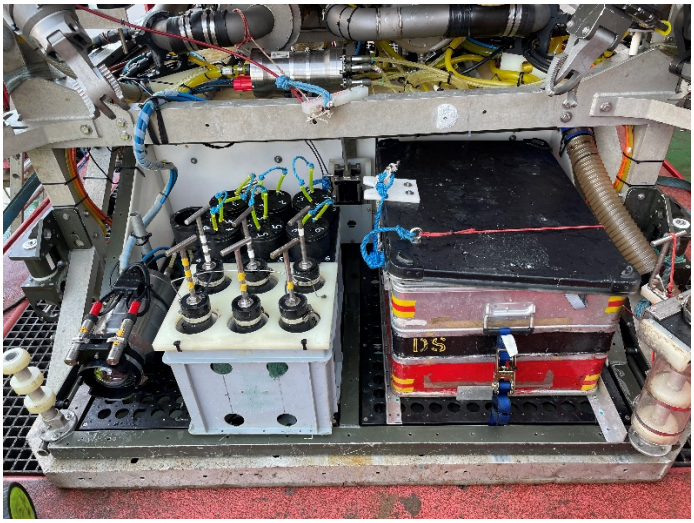


Figure 2.1.6 The ROV tool tray setup for collecting the large colonisation trays and the bone/wood experiment.

2.1.3. Imagery and video recording system

Imagery data are recorded from the three primary camera systems on ISIS. These are:

High Definition (HD) Insite Pacific Mini Zeus (1080i x 50) “Pilot”

Kongsberg Imenco OE14-522 High Definition PATZ Colour Camera (1080i x 50) “Science”

Insite Pacific Scorpio Digital Stills Camera (3.34 megapixel) “Scorpio”

All the cameras record video files in High Definition (1080i). In addition, the Scorpio records still photographs when they are taken by the graphical user interface in the ROV van. This gives users the option to zoom in and out, take photographs and set an intervalometer to record photographs at fixed time periods.

The Pilot and Science cameras are on pan and tilt units, operated from the ROV van. The Scorpio camera is generally fixed. It was fixed in one of two positions on any given dive, on the vehicle (for Cubes dives) and on the tool tray (for collections dives). A hydraulic tilt unit was constructed for the tray-mounted configuration, which enabled the camera to be moved up and down. Side-to-side movement (pan) was only possible by moving the ROV. This low (tray) positioning of the Scorpio (Figure 2.1.2) is what enabled the high-quality images and video

to be obtained. It reduced the amount of water between the subject and the camera sensor. The lighting for the Scorpio camera was provided by the ROV lights, there is no flash system. To obtain the low light images, we used the lights mounted to the Schilling Titan 4 Manipulator to illuminate a small area of seafloor around the subject (Figure 2.1.3). The position of the lighting was controlled by moving the manipulator arm. Stills photographs are recorded on the internal hard drive of the Scorpio camera. They are downloaded when the vehicle is on the surface (they can be downloaded during the dive but this stopped the recording of CTD data).

Video is recorded on the surface after transmission at full resolution via the fibre-optic cable in the umbilical. As the video input arrives into the Blackmagic HyperDeck Studio 4K Pro, it is recorded onto Samsung 870 EVO SSD drives that is slotted in the device in Apple ProRes format. Every two hours, to minimise the risk of any data loss in case of an unfortunate technical fault, the video footage on these drives are copied onto SanDisk G-RAID Shuttle 4 drives (1 Master & 1 Back-up, identical mirror of each other) using the Blackmagic MultiDock 10G and the ROV team Apple mini Mac computer. SanDisk G-RAID Shuttle 4 drives are configured as RAID 5 to maximise the safety against data loss.

2.1.4. Annotation and data logging

The OFOP software system was used for annotation and data logging, alongside paper records.

Cruise : JC257
Station : 105
Remarks :

Task	PC Date and Time	UTC Time	UTC Date	SHIP Latitude	SHIP Longitude	SUB_1 Latitude	SUB_1 Longitude	Water Depth
In the Water	07/03/2024 03:57:31	03:57:30	07/03/2024 03:35:07	13:04.2805	-116:07.4490	0:00.0000	0:00.0000	4044
At the Bottom	07/03/2024 06:27:26	06:27:25	07/03/2024 03:35:07	13:04.1639	-116:07.3862	13:04.2134	-116:07.4559	4311
Off the Bottom	07/03/2024 13:08:17	13:08:16	07/03/2024 03:35:07	13:04.4154	-116:07.3233	13:04.4133	-116:07.3666	0
On Deck	07/03/2024 16:13:15	16:13:15	07/03/2024 03:35:07	13:04.4519	-116:07.3052	13:04.4455	-116:07.3731	0
Gear deployed	:	:						

#Date	Time	PC_Time	SHIP_Lon	SHIP_Lat	SHIP_SOG	SHIP_COG	SHIP_Hdg	Water_Depth	SUB1_Lon	SUB1_Lat	SUB1_Depth	SUB1_Altitude	Elapsed video Time	Observations/Comments Image-Video Path
03/07/2024	03:52:42	07/03/2024 03:52:42	-116.124162	13.069999	0	0	27.3	0	0 0	0 0	00:00:00		[-86] Start video recording: Tape 1	
03/07/2024	03:57:22	07/03/2024 03:57:23	-116.124151	13.07001	0	0	27.3	0	0 0	0 0	00:04:38		[-81] IN THE WATER	
03/07/2024	04:50:25	07/03/2024 04:50:26	-116.12412	13.070092	0	0	28.7	0	-116.1251827	13.0703895	0	0	00:57:11	[-87] Stop video recording: Tape 1
03/07/2024	04:51:39	07/03/2024 04:51:40	-116.124103	13.070101	0	0	26.7	0	-116.1251563	13.0704187	0	0	00:57:11	[-2] really interesting siphonophore
03/07/2024	05:00:02	07/03/2024 05:00:02	-116.124116	13.070083	0	0	29	0	-116.1252098	13.0700383	0	0	00:57:11	[-2] didn't keep midwater recording
03/07/2024	06:27:09	07/03/2024 06:27:10	-116.123098	13.069399	0	0	29.9	0	-116.124265	13.070224	0	0	00:57:11	[-82] AT THE BOTTOM
03/07/2024	06:34:38	07/03/2024 06:34:39	-116.123083	13.069397	0	0	29.3	0	-116.124319	13.0699955	0	0	00:57:11	[-2] doppler reset
03/07/2024	06:44:08	07/03/2024 06:44:09	-116.123079	13.069423	0	0	29.2	0	-116.1242037	13.0697312	0	0	00:57:11	[-2] amazing swimming amperima
03/07/2024	06:51:28	07/03/2024 06:51:29	-116.123019	13.069457	0	0	27.5	0	-116.1238428	13.0697592	0	0	00:57:11	[-88] Changed video tape to: Tape 2
03/07/2024	06:51:29	07/03/2024 06:51:29	-116.123017	13.069456	0	0	27.4	0	-116.1238428	13.0697592	0	0	00:00:00	[-86] Start video recording: Tape 1
03/07/2024	07:09:40	07/03/2024 07:09:40	-116.122698	13.06968	0	0	27.3	0	-116.1234618	13.070102	0	0	00:18:01	[-2] sponge +brittle star in magtube4
03/07/2024	07:17:54	07/03/2024 07:17:54	-116.122711	13.069691	0	0	27.9	0	-116.1234758	13.0700005	0	0	00:26:11	[-2] magtube 1 possible holasvus
03/07/2024	07:18:08	07/03/2024 07:18:09	-116.12271	13.069688	0	0	28.1	0	-116.1234732	13.0700147	0	0	00:26:25	[-2] nice swimming isopod in scorpio
03/07/2024	07:21:08	07/03/2024 07:21:09	-116.122661	13.06971	0	0	29.5	0	-116.123431	13.0700837	0	0	00:29:24	[-2] doppler reset
03/07/2024	07:31:09	07/03/2024 07:31:09	-116.122368	13.069988	0	0	28.4	0	-116.1232625	13.0702172	0	0	00:39:19	[-2] my vindication amperima
03/07/2024	07:37:35	07/03/2024 07:37:36	-116.122375	13.069999	0	0	29.5	0	-116.1233163	13.0702262	0	0	00:45:42	[-2] starboard biobox pink amperima
03/07/2024	07:50:21	07/03/2024 07:50:22	-116.122365	13.070003	0	0	27.3	0	-116.1233095	13.0702327	0	0	00:58:21	[-2] Holascus magtube 6 bit mashed
03/07/2024	08:05:51	07/03/2024 08:05:51	-116.122376	13.070042	0	0	26.5	0	-116.1232145	13.0702617	0	0	01:13:43	[-2] slurp 2 pi 23 synallactes

Figure 2.1.7 Example of the OFOP data logger output file.

2.1.5. ROV Deployments

Table 2.1.1 ROV deployments on JC257. NOTE: coordinates and depth are at dive start.

Station No.	Site	Date	Time (on bottom)	Time (leave bottom)	Latitude (dec min)	Longitude (dec min)	Depth (m)	Dive Aims/Collections
JC257_015	UK1_1km	16/02/2024	16:39:00	02:57:00	13 55.489	-116 31.768	4100	Deployed cubes (A.Sweetman), megafauna collection (NHM), 6 x PC and 3 x Niskin (S.Evans/DEEPEND).
JC257_019	UK1_1km	17/02/2024	18:33:00	13:46:00	13 55.549	-116 32.163	4153	Megafauna collection (NHM), 6 x PC and 3 x Niskin (S.Evans/DEEPEND).
JC257_033	UK1_1km	20/02/2024	17:13:00	23:36:00	13 56.000	-116 32.000	4095	4 x cube collection and 11 x PC (A.Sweetman), megafauna collection (NHM).
JC257_042	UK1_0km	22/02/2024	04:48:00	15:12:00	13 55.971	-116 33.016	4051	Megafauna collection (NHM), 3 x PC (DEEPEND).
JC257_049	UK1_0km	23/02/2024	17:28:00	02:49:00	13 56.000	-116 32.000	4078	Megafauna collection (NHM), 6 x PC and 2 x

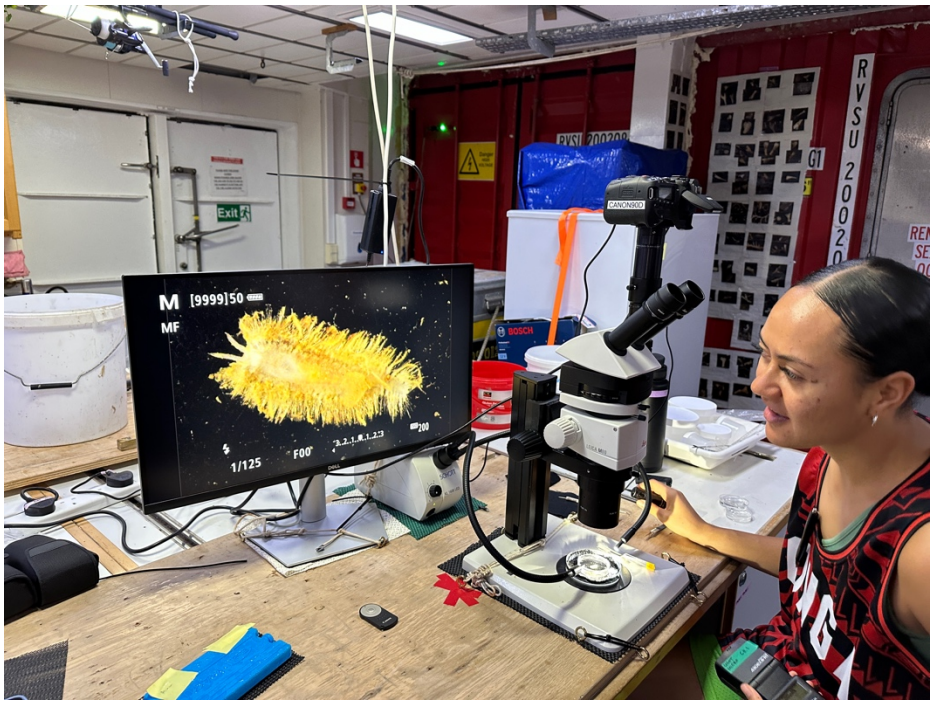
										Niskin (S.Evans/DEEPEND).
JC257_052	UK1_0km	23/02/2024	16:50:00	20:08:00	13	56.001	-116	32.000	4092	4 x cube deployment (A.Sweetman).
JC257_057	OMS - colonisation experiment	24/02/2024	11:11:00	03:17:00	13	56.210	-116	32.520	4083	Retreived ABYSSLINE colonisation experiment deployed in 2013 and collected, 3 x PC inside, 3 x PC next to the site and megafauna collection (NHM).
JC257_063	West UK_0km - colonisation experiment	25/02/2024	04:34:00	14:48:00	13	55.945	-116	30.687	4149	Retrieved colonisation experiment from RC01 deployed in 2020, 3 x PC 0m, 3 x PC 1 m from experiment, mega faunal collection (NHM).
JC257_071	UK1_0km	27/02/2024	16:57:00	20:53:00	13	55.219	-116	32.343	4084	4 x cube collection and 12 x PC (A.Sweetman).
JC257_078	UK1_16km	28/02/2024	04:17:00	14:24:00	13	49.951	-116	29.485	4110	Megafaunal collection (NHM).
JC257_091	UK1_16km	01/03/2024	06:40:00	14:09:00	13	49.590	-116	29.480	4108	4 x cube collection and 12 x PC (A.Sweetman), megafauna collection (NHM).
JC257_094	UK1_100km	04/03/2024	04:26:00	14:31:00	13	6.388	-116	5.137	4125	Megafuna collection (NHM, 6 x PC and 1 x Niskin (S.Evans/DEEPEND).
JC257_105	UK1_100km	05/03/2024	03:57:00	13:08:00	13	6.532	-116	4.964	4044	Megafauna collection (NHM), 5 x PC and 2 x Niskin (S.Evans/DEEPEND).
JC257_107	UK1_100km	07/03/2024	11:59:00	15:25:00	13	4.445	-116	7.373	4127	4 x cube recovery (A.Sweetman)
JC257_115	UK1_0km	08/03/2024	19:30:00	02:09:00	13	43.568	-116	39.900	4127	Bone colonisation experiment and megafauna collection (NHM).
JC257_117	OMS - sponge garden	10/03/2024	16:46:00	02:59:00	13	43.607	-116	39.896	4080	Sponge Garden megafauna collection (NHM).

Table 2.1.2. ROV dive numbers and JC257 station numbers. Date refers to dive start.

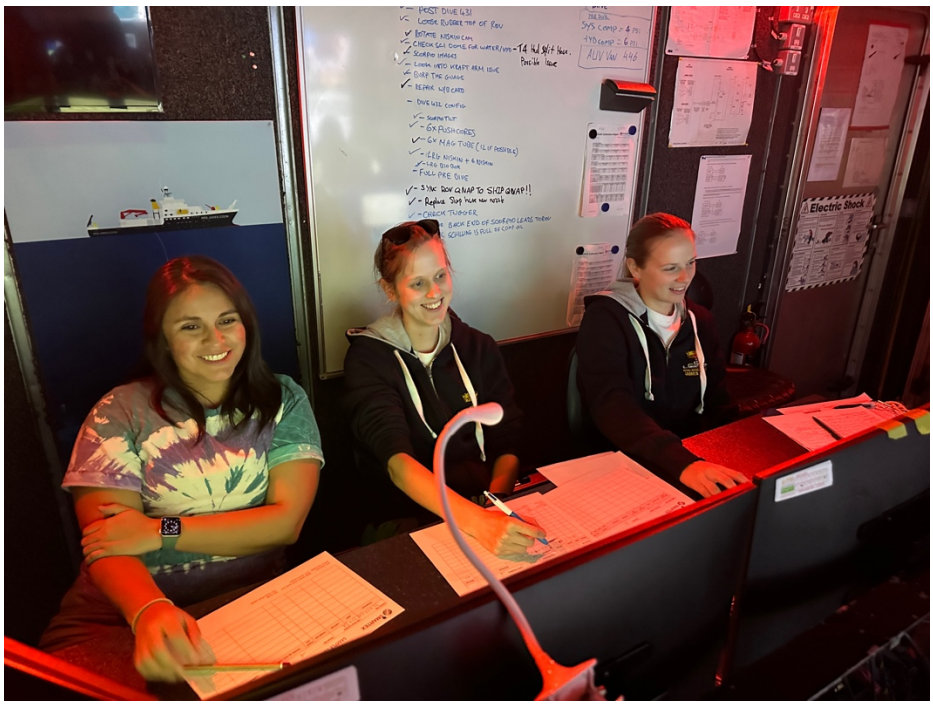
Station No.	ISIS Dive Number	Site	Date
JC257_015	431	UK1_1km	16/02/2024
JC257_019	432	UK1_1km	17/02/2024
JC257_033	433	UK1_1km	20/02/2024
JC257_042	434	UK1_0km	22/02/2024
JC257_049	435	UK1_0km	23/02/2024
JC257_052	436	UK1_0km	23/02/2024
JC257_057	437	OMS - colonisation experiment	24/02/2024
JC257_063	438	West UK_0km - colonisation experiment	25/02/2024
JC257_071	439	UK1_0km	27/02/2024

JC257_078	440	UK1_16km	28/02/2024
JC257_091	441	UK1_16km	01/03/2024
JC257_094	442	UK1_100km	04/03/2024
JC257_105	443	UK1_100km	05/03/2024
JC257_107	444	UK1_100km	07/03/2024
JC257_115	445	UK1_0km	08/03/2024
JC257_117	446	OMS - sponge garden	10/03/2024

The JC257 biology sampling team watch the ROV Isis return with their samples from 4100m. Image by Daniel Jones.



JC257 Trainee Tanga Morris imaging a polychaete worm on JC257 using a photomicroscope



Natural History Museum scientists (l-r) Belen Arias and Regan Drennan with University of Southampton Trainee Lucy Harris (r) logging data in the ROV van on JC257

2.2. AUTOSUB

Loïc Van Audenhaege, Catherine Wardell, Susan Evans, Bethany Fleming, Daniel Jones

2.2.1. General description of vehicle

Autosub5 is a 5.8m long high-power short-range autonomous underwater vehicle (AUV) that generates lift through forward motion. The vehicle consists of three sections: the free flooded nose, the central section and the free flooded tail. The flooded nose houses the forward control tube, Seabird 9+ CTD, Norbit WBMS-bathy multibeam echosounder (MBES), AESA camera system, Flash, Norbit Obstacle Avoidance Sonar (OAS) and the Robotic Cartridge Sampling Instrument (RoCSI). The central section houses syntactic foam encasing pressure tolerant lithium polymer battery packs. The central section also includes the transducers for an Edgetech 2205 Side Scan Sonar (SSS) and Sub Bottom Profiler (SBP). The free flooded tail houses the power tube, the second control tube, Sonardyne Sprint Nav 700 combining 600 KHz DVL and INS system, Sonardyne AvTrack acoustic modem, actuators and control planes in a cross configuration and twin thrusters for forwards propulsion. The position of the sensors (Figure 2.2.1), their relative positions and offsets (Figure 2.2.2 and 2.2.3) are documented.

During this expedition the primary use of the *Autosub5* AUV is to carry out:

1. seabed mapping using the multibeam, side-scan sonar and sub-bottom profiler
2. seafloor imaging using the AESA camera and flash
3. collect eDNA samples using the Robotic Cartridge Sampling Instrument (RoCSI)
4. collect information on the water column properties using the CTD

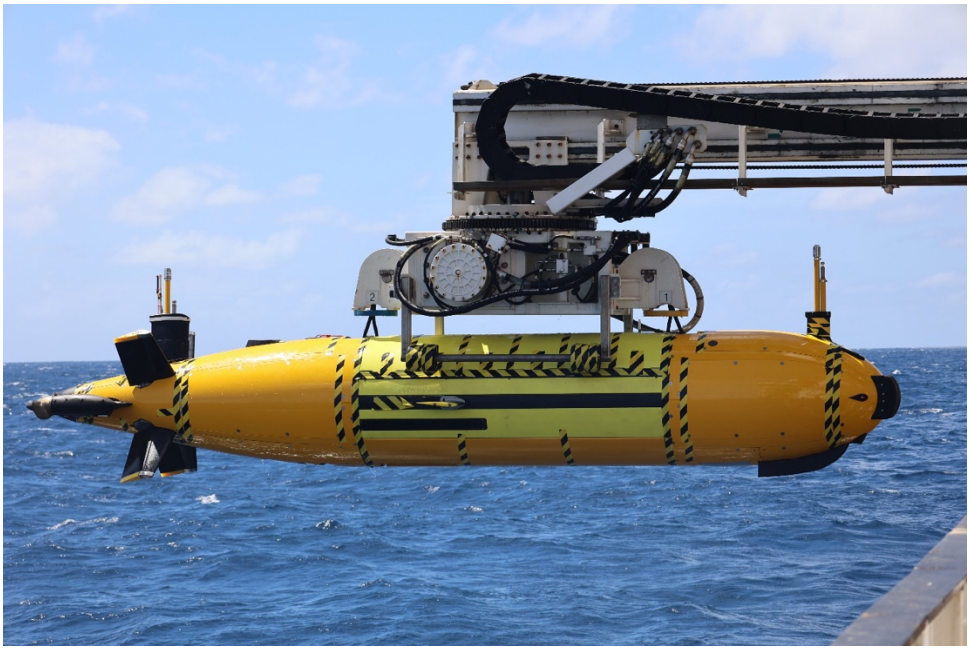


Figure 2.2.1 Autosub5 being deployed during JC257.

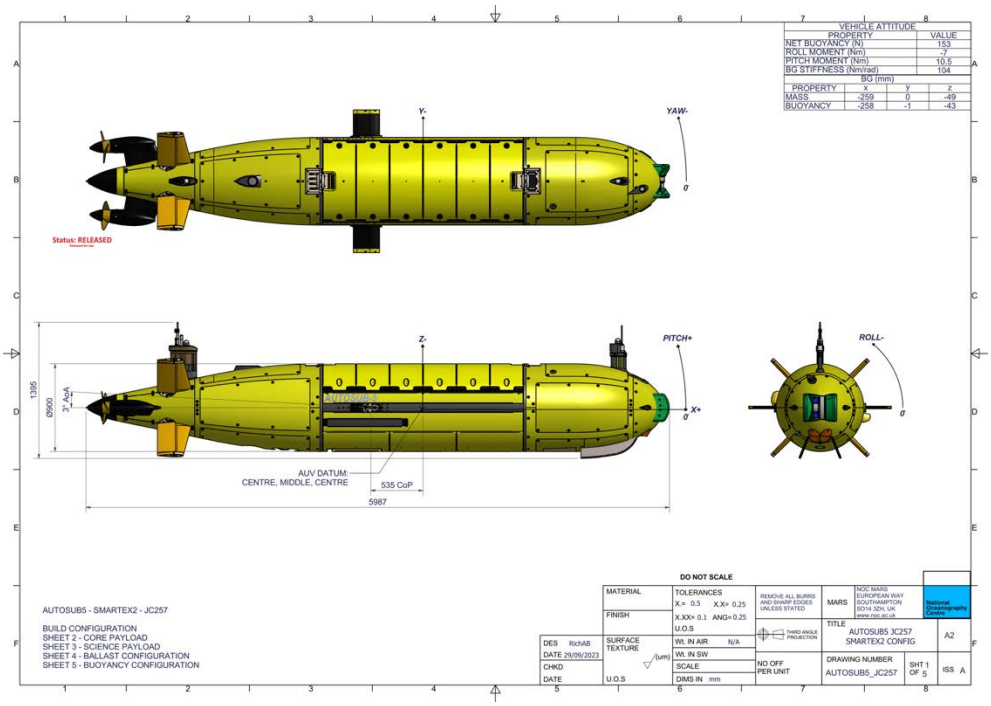


Figure 2.2.2 Autosub5 datum.

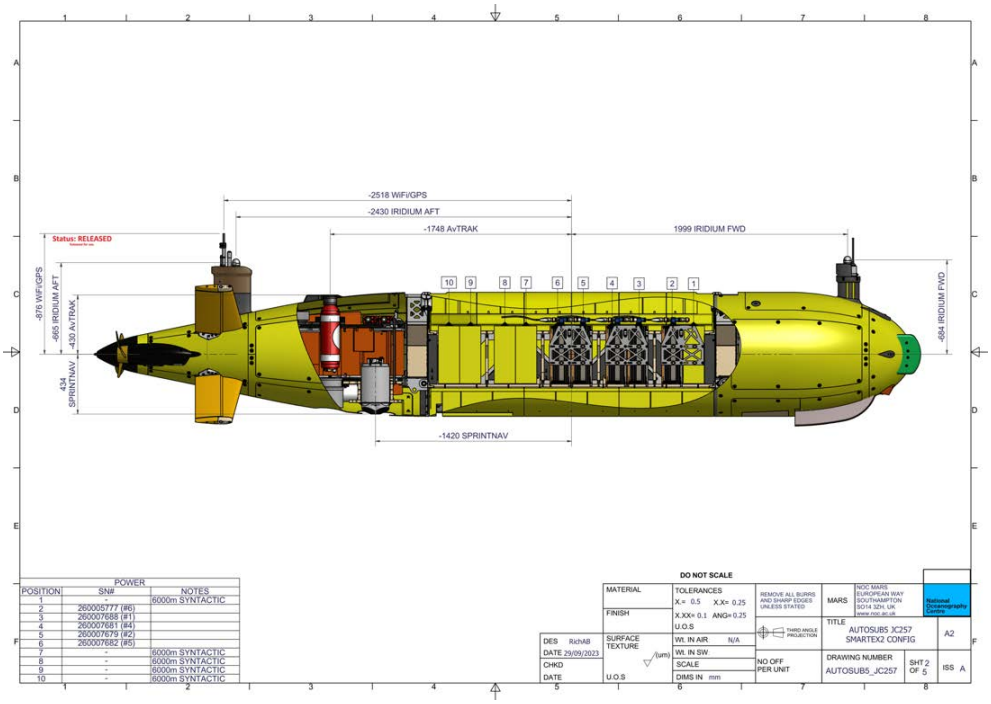


Figure 2.2.3 Lever arms among navigation operation systems.

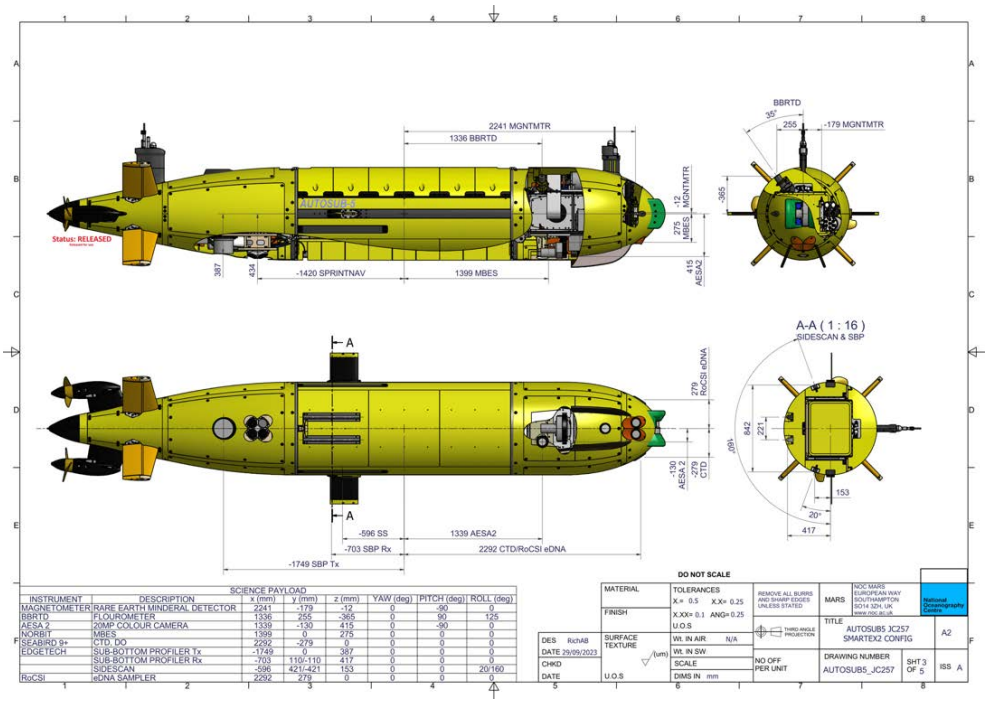


Figure 2.2.4 Offsets among scientific sensors.

2.2.2. AUV Deployments on JC257

Table 2.2.1 Missions of Autosub5.

Station Site	AUV mission	Primary Sensor	Deployment Date, Time (UTC)	Deployment position	Recovery Date, Time (UTC)	Recovery position	Status
JC257_003 Transit	AS5M083	MBES	10/02/2024 12:40	11°3.205'N 98°14.769'W 4005 m	10/02/2024 23:51	11°2.531'N 98°14.467'W 4060 m	Test of MBES successful
JC257-013 UK1:0-1km	AS5M084	MBES (AESA2)	16/02/2024 12:34	13˚54.94'N 116˚31.48'W 4102 m	17/02/2024 16:50	13˚54.89'N 116˚31.92'W	MBES collection successful Imaging test successful
JC257_020 UK1: 1km	AS5M085	MBES SSS	18/02/2024 18:03	13°55.717'N - 116°31.621'W 4091 m	19/02/2024 23:42	13°55.628'N 116°31.89'W 4095 m	MBES collection successful, missing snippets SSS collection successful
JC257-041 UK1:0-1km	AS5M086	AESA2	22/02/2024 02:20	13˚55.616'N 116˚31.73'W 4105 m	22/02/2024 02:47	13°55.73'N 116°31.80'W 4105 m	Aborted at surface
JC257-047 UK1:0-1km & timeseries	AS5M087	AESA2 SSS	23/02/2024 12:06	13˚56.14'N 116˚31.81'W 4105 m	25/02/2024 00:13	13°56.95'N 116°34.62'W 4110 m	Aborted after partial success of imaging (AESA2 failure at timeseries) Partial success of SSS
JC257_056 UK1: 16 & 30km	AS5M088	MBES	25/02/2024 14:51	13°48.814'N 116°29.116'W 4110 m	25/02/2024 16:52	13°49.159'N 116°29.01'W 4117 m	Aborted on descent
JC257_064 UK1: 16 & 30km	AS5M089	MBES	27/02/2024 18:43	13°48.054'N 116°27.994'W 4112 m	29/02/2024 01:07	13°48.653'N 116°28.36'W 4120 m	MBES collection successful

JC257-076 UK1:16-30km	AS5M090	AESA2	29/02/2024 23:54	13°48.04'N 116°28.27'W 4112 m	01/03/2024 18:03	13°48.39'N 116°28.27'W 4112 m	Aborted when imaging SSS collection unsuccessful
JC257-081 UK1:0km & timeseries	AS5M091	AESA1 SSS	02/03/2024 05:24	13°56.14'N 116°31.86'W 4103 m	02/03/2024 04:38	13°55.69'N 116°31.59'W 4098 m	Imaging successful but image settings incorrect SSS collection successful
JC257-090 UK1:100km	AS5M092	MBES AESA1 test	03/03/2024 22:43	13°04.89'N 116°05.74'W 4101 m	05/03/2024 03:22	13°06.81'N 116°06.13'W 4100 m	MBES settings incorrect, unsuccessful Aborted, no images of seabed recovered
JC257-096 UK1:100km	AS5M093	AESA1 MBES SSS	05/03/2024 19:42	13°05.01'N 116°05.68'W 4111 m	6/03/2024 16:06	13°5.764'N 116°6.772'W 4115 m	Failed after 1.2 photo transects MBES and SSS successful over smaller area
JC257-103 UK1:100km	AS5M094	AESA1 MBES	06/03/2024 22:30	13°4.920'N 116°5.660'W 4009 m	07/03/2024 17:05	13°4.802'N 116°6.278'W 4009 m	Successful imaging mission Small area of MBES, positioning offset required
JC257-106 UK1:30 & 16km	AS5M095	AESA1 SSS	08/03/2024 03:45	13°41.500'N 116°24.400'W 4112 m	09/03/2024 01:19	13°49.150'N 116°27.76'W 4127m	AUV successfully completed mission. Camera failed after 30 mins at seabed. SSS collected successfully before abort
JC257-108 UK1:100km	AS5M096	MBES SSS (AESA1)	09/03/2024 08:25	13°4.948'N 116°5.700' 4098 m	10/03/2024 00:17	13°5.150'N 116°7.770 4153 m	Successful MBES and SSS-positioning offset required Opportunistic imaging during SSS and at end of transects. Images of rocks.
JC257-113 UK1:30 & 16km	AS5M097	AESA1	10/03/2024 09:18	13°41.515'N 116°24.528'W 4090 m	11/03/2024 14:57	13°53.965'N 116°28.02'W 4134 m	Successful imaging mission
JC257-118 UK1: 0 & 1km	AS5M098	AESA1	12/03/2024 06:53	13°55.66'N 116°31.56'W 4093 m			

2.2.3. Mission planning

Missions were designed in GIS software (ArcMap and QGIS), using existing shipboard or AUV bathymetry to determine line heading, spacing, AUV altitude and avoidance of terrain not suited to the AUV. MBES missions were conducted at 1.1 m s^{-1} with no fairing on the vehicle. During SSS and photography missions, the fairing was mounted and the speed of the AUV was increased slightly to 1.2 m s^{-1}. The interval between pictures was set at 2 seconds for AESA2 and at 1 second for AESA1 to avoid overlap in images. The speed was increased during optical imaging missions to increase water flow over the fins, providing more altitude control while maintaining suitable image overlap.

Missions were imported to the Oceanids C2 project web-based piloting management system. For ship-based applications, such as this expedition, a stand-alone version of the C2 was used that does not need a connection to the internet and provides the capability to operate the sub via WiFi and/or via Acoustics.

2.2.4. Multibeam Echosounder

The Autosub5 has a NORBIT Multibeam system, this system has operating frequencies of 200, 400 and 700kHz. Throughout the cruise the 400kHz frequency was used at an altitude of 50m. One exception to this was an 800m section of 700 kHz, during a camera test, at an altitude of 3m (AS5M092). Line spacing began at 175m to ensure full coverage as shipboard MBES data was at a 100m resolution, this spacing was increased to 210m for the southern site once initial MBES data was collected.

Where time allowed, the ship would remain in the area and send the AUV a final USBL position once the AUV had reached the seabed. Navigation data was sent to the NORBIT system from the AUV SprintNav throughout the dive. Following the dive, improved position and motion navigation was generated using the final position of the AUV for forwards and backwards post processing in Janus software.

Table 2.2.2 MBES Settings for Autosub5 Missions.

Mission	Frequency (kHz)	Altitude (m)	Line spacing (m)	Beam angle (°)	Comments
AS5M083	400	75	175	70/70	Test for AUV
AS5M084	400	50	175	70/70	0km site part 1
AS5M085	400	50	175	70/70	0km site part 2
AS5M089	400	50	175	70/70	16 + 30km MBES
AS5M092	400	50	175	70/70	100km – wrong settings
	700	3	-	70/70	Camera Test
AS5M093	400	50	210	70/70	100km- small area
AS5M094	400	50	210	70/70	100km- small area- wrong starting position
AS5M096	400	50	210	70/70	100km- small area- wrong starting position

2.2.5. Sidescan sonar

High-frequency sidescan sonar data were acquired using the EdgeTech 2205 Sidescan Sonar, mounted on the Autosub5 AUV. High frequency (410kHz) sidescan was collected at 15m altitude. The navigation data was injected to the Edgetech system from the AUV localiser throughout the dive.

Dedicated SSS survey data was collected at 15m, whilst opportunistic SSS was also collected during the 3m altitude camera surveys. Line spacing was set at 300m for full coverage at 15m altitude, with 180m range. Details in Table 2.2.3.

Table 2.2.3 Sidescan Sonar and Sub Bottom Profiler Settings for Autosub5 Missions.

Mission	SSS 15m	SSS 3m	SBP 15m	SBP 3m	Location	Status
AS5M085	✓	x	✓	x	0km site part 1	Full overage SSS
AS5M087	✓	✓	✓	✓	0km	Last 4 lines 15m missing 3m TimeSeries (2-13kHz sweep at 16ms)
AS5M090	x	x	x	x	16km	LF sidescan at 16km
AS5M091	✓	✓	✓	✓		Last 4 lines of M087 at 15m

						TimeSeries at 3m 2-15kHz sweep at 5ms
AS5M093	✓	✓	x	x	100km- small area	Full overage SSS
AS5M095	x	✓	x	✓	16+30km	Opportunistic 3m SSS during camera survey (2-13kHz sweep at 16ms)
AS5M096	✓	x	✓	x	Western 100km	Coverage of cliff
AS5M097	x	✓	x	✓	16+30km	Final lines of M095

2.2.6. Sub bottom profiler

SBP data were recorded using the EdgeTech2205 Sub Bottom Profiler into .jsf files with navigation incorporated. Due to AUV battery limitations for longer dives, SBP data was collected only throughout selected survey tracks. A 2-13kHz sweep at 16ms was used for the 15m altitude data collection. For the 3m altitude camera surveys, data was collected at 2-13kHz sweep at 16ms and 2-15kHz sweep at 5ms to determine optimum settings for this site. Upon review, the 2-13kHz sweep at 16ms was preferable. Details in Table 2.2.2.

2.2.7. Imagery

Camera systems

Autosub5 is equipped with a camera logger, one gigabit Ethernet machine vision cameras and two custom flash modules. Both flashes are slaved to the camera through the logger tube. The photographic camera is positioned downward facing (Figure 2.2.5). Note that the forward-facing camera used in previous expeditions was not used during JC257.

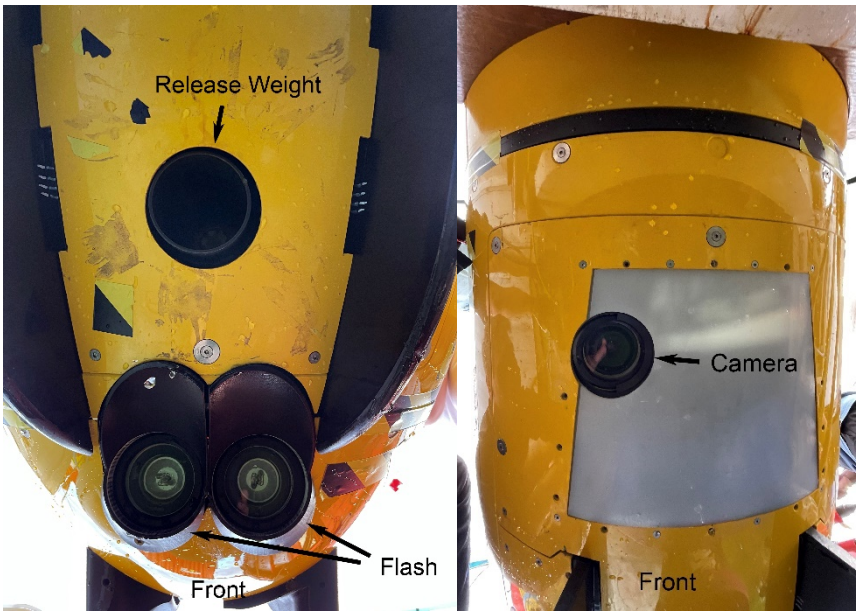


Figure 2.2.5 AUV imagery system taken looking up at underside of AUV. Left: Paired flash systems at the front of the AUV (note release weight already removed). Right: Camera system in the front section of the AUV but behind the flashes. The grey panel is an acoustic window that was used as hydrodynamic fairing to increase endurance during camera missions (compared to multibeam missions).

There were two different camera systems used for different missions, known as AESA 1 and AESA 2. AESA refers to a previous NERC-funded project (Autonomous Ecological Surveying of the Abyss NE/H021787/1), which provided funding for the camera systems. The AESA 2 camera was used initially as it obtained better images (higher resolution and larger seafloor footprint). However, the AESA 2 camera suffered a recurring fault that stopped image acquisition. We decided to replace the AESA 2 camera in the vehicle with the older AESA 1 camera, which appeared to be more reliable. We had some initial problems with the image settings on AS5M091, which meant that images obtained were very dark (likely because of a Gamma correction and/or insufficient gain). The image settings were resolved on AS5M092 and good quality images of suitable exposure were obtained.

The AESA1 camera system is a Teledyne FLIR Grasshopper2 Gig-E camera (Table 2.2.4).

Table 2.2.4 Optical details of the AESA1 camera.

Serial number	11370385

Camera model	Grasshopper2 GS2-GE-50S5C
Sensor	Sony ICX625AQ
Resolution	2448x2048
Image pixel format	RAW8
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 (in air)	2/3”
Horizontal	38.8˚
Vertical	29.6˚
Focal range	2.28m

The AESA2 camera system is a Teledyne FLIR ‘Blackfly’ with a V1624-MPZ lens (Table 2.2.5, Figure 2.2.6). This camera system is the same model as the Bathysnap camera.

Table 2.2.5 Optical details of the AESA2 camera

Camera model	BFS_PGE_200S6C
Sensor	Sony IMX183
Resolution	5472x3648
Image pixel format	RAW16
Lens model	V1624-MPZ
Focus range	100 mm to Infinity
Aperture	F2.4-16.0
Lens Focal Length	16 mm
Angle of view (in air)	1”
Horizontal	43.8˚
Vertical	33.6˚

Table 2.2.6 Settings used in camera deployments.

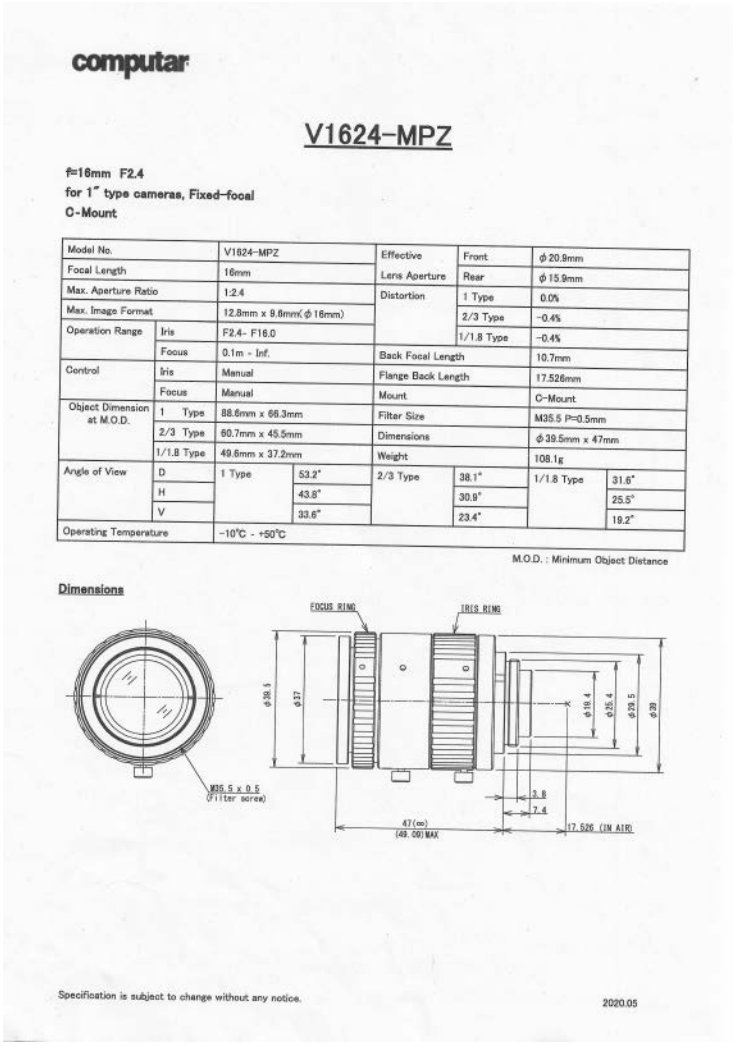
Mission	**AS5M91**	**AS5M92**	**AS5M93**	**AS5M94**	**AS5M95**	**AS5M96**	**AS5M97**	**AS5M98**
Brightness (%)	1.312	1.312	1.312	1.312	1.312	1.312	1.312	1.312
Exposure (EV)	Off	Off	Off	Off	Off	Off	Off	Off
Sharpness	1024	1024	1024	1024	1024	1024	1024	1024
Hue (deg)	0	0	0	0	0	0	0	0
Saturation (%)	1000	1000	1000	1000	1000	1000	1000	1000
Gamma	2.18	2.18	Off	Off	Off	Off	Off	Off
Shutter (ms)	1	2	3	3	3	3	3	3
Gain (dB)	-3.378	2.464	4.012	4.012	4.012	4.012	4.012	4.012
FrameRate (fps)	Off	Off	Off	Off	Off	Off	Off	Off
W.B. (Red)	900	900	900	900	900	900	900	900
W.b.(Blue)	624	624	624	624	624	624	624	624
Trigger Delay	0.0012	0.0012	0.0012	0.0012	0.0012	0.0012	0.0012	0.0012
GPIO1 Polarity	Low	Low	Low	Low	Low	Low	Low	Low
GPIO1 Duration	10	10	10	10	10	10	10	10
Notes	Too dark	Too dark	Good	Good	Good	Good	Good	Good

Both the AESA 1 and AESA 2 cameras were linked via ethernet to loggers. The AESA1 logger used a Commell LP-170G computer. AESA 2 used a Kontron Com Express m(4)AL10 (E2). The logger sent a request to the camera to take a picture. The camera sent a hardware pulse to the flash to trigger it. The flashes are Xenon flashes of around 50 joules energy.

Basic metadata are encoded in the image name. For example the image: AS5M093_AESA_1_13331848_13354193226626.raw has the following characteristics.

- AS5 (Vehicle: Autosub5)
- M093 (Mission 93)
- AESA_1 (camera type)
- 13331848 (Serial number of camera)
- 13354193226626 (milliseconds since 01/01/1601 as used for image files)

This is consistent amongst both Autosub camera systems.



computar

V1624-MPZ

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

Model No.		V1624-MPZ		Effective Lens Aperture	Front	φ20.8mm		
Focal Length		16mm			Rear	φ15.9mm		
Max. Aperture Ratio		1:2.4		Distortion	1 Type	0.0%		
Max. Image Format		12.8mm x 9.6mm(φ16mm)			2/3 Type	-0.4%		
Operation Range	Iris	F2.4- F16.0			1/1.8 Type	-0.4%		
	Focus	0.1m - Inf.		Back Focal Length		10.7mm		
Control	Iris	Manual		Flange Back Length		17.526mm		
	Focus	Manual		Mount		C-Mount		
Object Dimension at M.O.D.	1 Type	88.6mm x 66.3mm		Filter Size		M35.5 P=0.5mm		
	2/3 Type	60.7mm x 45.5mm		Dimensions		φ39.5mm x 47mm		
	1/1.8 Type	49.6mm x 37.2mm		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℃ - +50℃						

M.O.D. : Minimum Object Distance

Dimensions

Specification is subject to change without any notice.

2020.05

Figure 2.2.6 Manufacturer's technical details of the V1264-MPZ lens used in AESA 2. Note angles of view are in air.

For the AESA 1 camera images were recovered as 8-bit .raw images and opened in IrfanView (Figure 2.2.7).

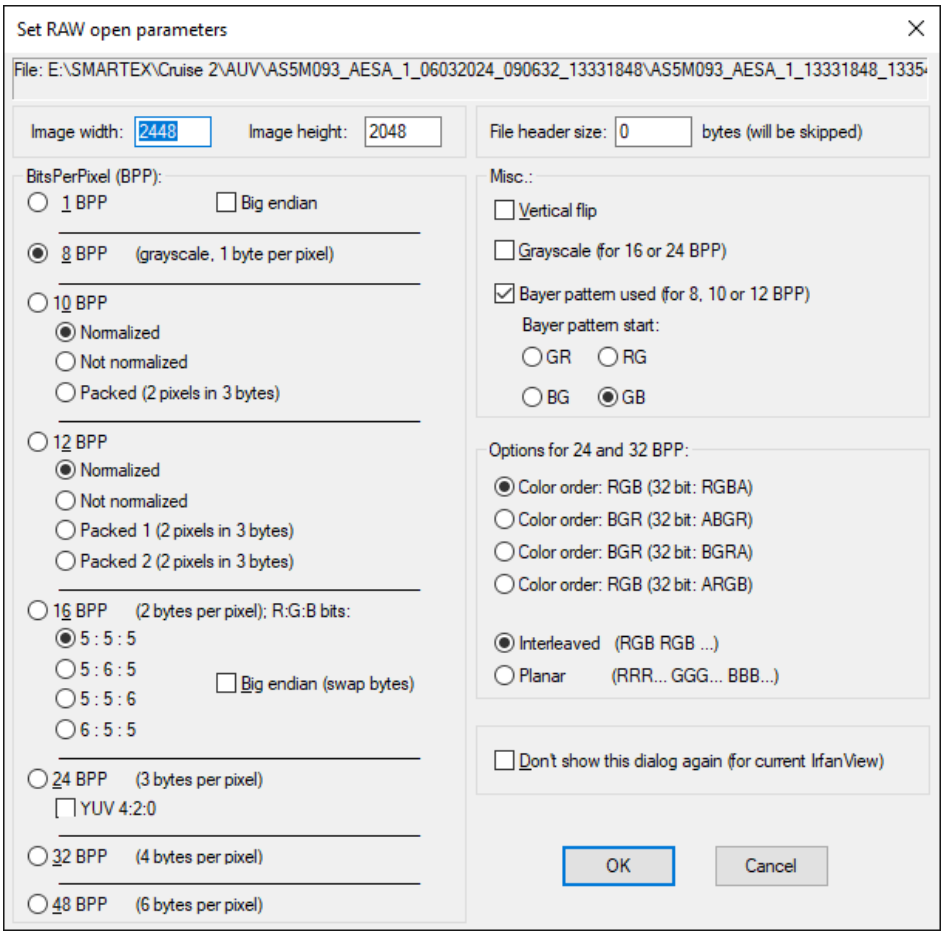


Figure 2.2.7 IrfanView settings to open AESA1 images.

For the AESA 2 camera images were recovered as 16-bit .raw images and converted in .dng images in darktable, which is capable of handling 16-bit images. Correction history was stored in a .xmp file and can be reset in darktable. Each camera recorded a .txt log file called 'bathysnap' and .yaml listing the camera settings. Time of image acquisition was encoded in the image name following: "X_YYYY-MM-DD_hh-mm-ss.dng".

Imaging Deployments

Autosub5 carried out 12 missions that involved seabed imaging (Table 2.2.1). Mission planning was performed in QGIS using existing high-resolution AUV bathymetry. If no bathymetry was available, it was acquired before any imaging mission. Mission planning was then uploaded on C2. The AUV was set to target an altitude of 3 m and a speed of 1.2 $m.s^{-1}$. Images were acquired every 1 second for AESA1 and every 2 seconds for AESA2, due to its larger footprint.

2.2.8. RoCSI

The Robotic Cartridge Sampling Instrument (RoCSI) developed at the NOC, enables the in-situ sampling and preservation of eDNA at depths up to 5000 m in parallel with the image and multibeam surveys. During JC257, RoCSI (v1) was mounted into the nose of Autosub5 and secured into the payload using 2 jubilee clips (Figure 2.2.8). The sample inlet consisted of a 1 m hose at the front right of the nose and the samples collected were all >1 L. During all the AUV missions where RoCSI was switched on, RoCSI received 12V from the Autosub5 and was programmed in the RoCSI node in OCS. RoCSI was programmed using the GUI version: Benchmaps_2_3_13 with firmware Benchmaps_2_3_14.

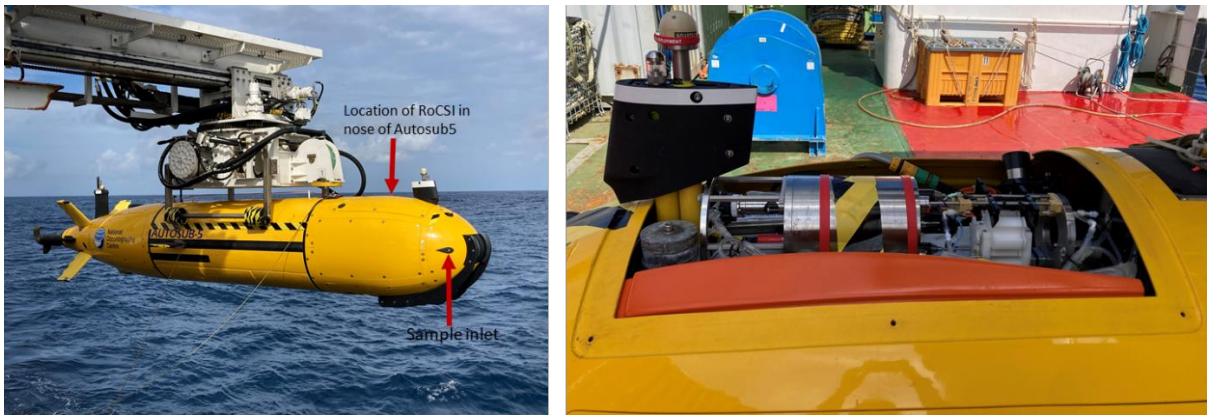


Figure 2.2.8 Location of RoCSI in the nose of Autosub5

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 AUV. 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. The plumbing was checked for leaks and RoCSI was pre-programmed using a GUI. After the AUV mission, the samples were removed from RoCSI, the cartridge units were disassembled, and the Sterivex units sealed. All samples were then immediately transferred to the - 80°C freezer.

Using the grappling lines to secure the Autosub on JC257. Photo by Daniel Jones.

2.3. Box core

2.3.1. Box core equipment

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 2.3.1). 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). The dimensions of the sample are 50x50cm, the box height is 60cm and typically returns a core of ~40cm in height in abyssal sediments. The spade closure includes a rubber seal that prevents water egress thus the box core returns with top water intact. The box core was maintained and deployed by the NOC technical team (Howard King and Billy Platt) and supported by the ship's crew in operation of the winch and safe deployment and recovery.

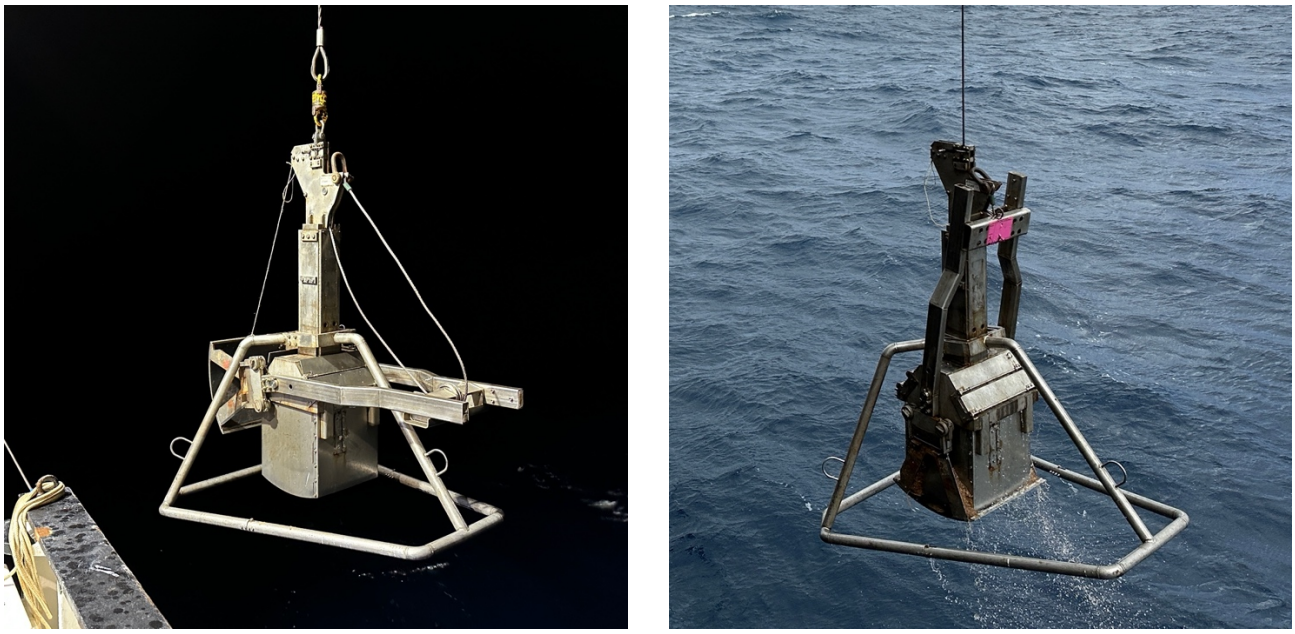


Figure 2.3.1 USNEL spade box core design used on JC257. Left - box core being deployed with spade arm in cocked position. Right - box core on recovery with a successful sample and top water intact. Photos AG Glover.

2.3.2. Box core deployment protocol

In all cases the box core was deployed from the starboard gantry of the James Cook. Up to deployment JC257_075 the 3x25 core wire was used, after an issue with the sheave, we switched to using the trawl wire. The deployment procedure in all cases 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
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
5. Veer 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 15m/min until touchdown was recorded on the tensiometer (Figure 2.3.2)
8. Additional 10m of wire paid out after touchdown
9. All stop at max wire out, with position of ship 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 45m/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 science team
15. The box core safety pins are placed in the box core and the corer lowered to the deck and secured.

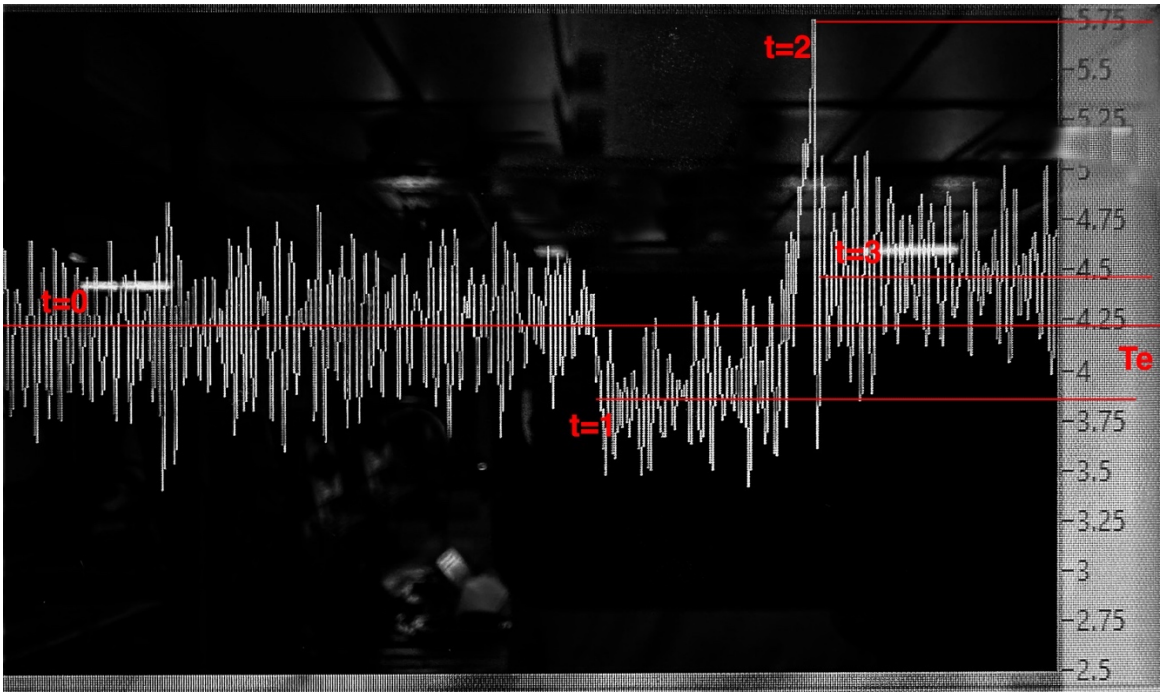


Figure 2.3.2 Graphical read out from the James Cook winch tensiometer during box core deployment on the seafloor. t=0 the box core is being lowered at 10m/min; t=1 the box core has touched down and ~0.5 ton of weight has come off the wire; t=2 after hauling at 10m/min the box core spade has closed and tension has peaked at 5.75 ton; 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 2.3.3 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. Photos: AG Glover.

2.3.3. 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 JC257, 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, starboard side (Figure 2.3.4). 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).

Figure 2.3.4. 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.

2.3.4. 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 2.3.5).

Figure 2.3.5 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.

2.3.5. Box core deployments

All deployments of the box core are summarised in Table 2.3.1. A total of 39 deployments were made, recovering samples from 34 cores, of which 32 were deemed quantitative samples.

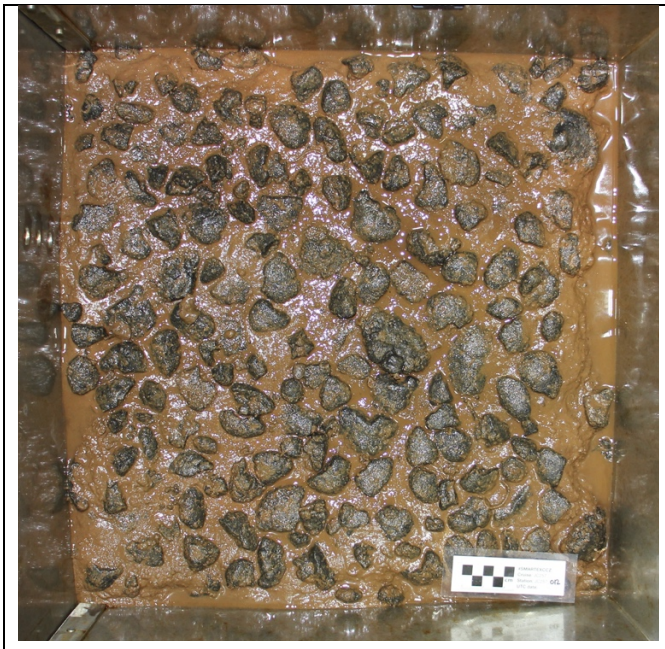
Table 2.3.1 Box core deployments on JC257. Box core number (BC_###), date, time, station number (JC257_###), position, depth and notes (Q: quantitative, NQ: non-quantitative, F: fail).

Deployment Number	Site	Date	Time (UTC: gear on bottom)	Latitude (dec min, on bottom)		Longitude (dec min, on bottom)		Depth (m)	Notes
JC257_012	UK1_1km	16/02/2024	07:26:00	13	55.65	-116	31.669	4098	NQ: winch stuck at 600m for 40 min, only nodules, live sort 0-2 and 2-5.
JC257_021	UK1_1km	18/02/2024	21:05:00	13	55.595	-116	31.6838	4080	Q
JC257_024	UK1_1km	19/02/2024	06:35:00	13	55.714	-116	31.6917	4096	Q
JC257_027	UK1_1km	19/02/2024	16:01:00	13	55.62	-116	31.691	4099	F: Did not trigger (no photo)
JC257_028	UK1_1km	19/02/2024	21:25:00	13	55.62	-116	31.6982	4098	Q
JC257_030	UK1_1km	20/02/2024	06:14:00	13	55.608	-116	31.582	4098	Q
JC257_032	UK1_1km	20/02/2024	14:50:00	13	55.688	-116	31.6422	4155	Q: slanted
JC257_035	UK1_1km	21/02/2024	08:58:00	13	55.589	-116	31.648	4099	F: Did not trigger (no photo)
JC257_038	UK1_1km	21/02/2024	17:57:00	13	55.547	-116	31.649	4100	Q
JC257_040	UK1_1km	22/02/2024	00:14:00	13	55.587	-116	31.6210	4098	Q
JC257_043	UK1_1km	22/02/2024	21:27:00	13	55.653	-116	31.611	4119	Q
JC257_045	UK1_0km	23/02/2024	06:49:00	13	56.114	-116	31.862	4103	Q
JC257_048	UK1_0km	23/02/2024	14:36:00	13	56.134	-116	31.811	4095	Q
JC257_051	UK1_1km	24/02/2024	11:31:00	13	55.676	-116	31.636	4093	Q
JC257_053	UK1_0km	25/02/2024	02:44:00	13	56.012	-116	31.926	4093	Q
JC257_055	UK1_0km	25/02/2024	09:30:00	13	56.178	-116	31.832	4105	F: Hole in base
JC257_058	UK1_0km	26/02/2024	08:37:00	13	56.109	-116	31.788	4092	Q
JC257_059	UK1_0km	26/02/2024	13:02:00	13	56.186	-116	31.875	4090	Q
JC257_060	UK1_0km	26/02/2024	17:42:00	13	56.012	-116	31.925	4102	F: Did not trigger (no photo)
JC257_061	UK1_0km	26/02/2024	21:32:00	13	56.174	-116	31.835	4102	Q
JC257_067	UK1_16km	29/02/2024	02:15:00	13	48.038	-116	27.86	4112	Q
JC257_069	UK1_16km	28/02/2024	10:05:00	13	48.074	-116	27.921	4082	Q
JC257_072	UK1_16km	29/02/2024	03:17:00	13	47.958	-116	27.964	4115	Q
JC257_074	UK1_16km	29/02/2024	11:15:00	13	48.05	-116	27.955	4119	Q
JC257_075	UK1_16km	29/02/2024	16:22:00	13	47.979	-116	27.939	3773	F: (sheave issue)
JC257_080	UK1_0km	02/03/2024	02:55:00	13	56.103	-116	31.826	4107	Q
JC257_082	UK1_0km	02/03/2024	09:05:00	13	56.041	-116	31.961	4101	Q
JC257_083	UK1_0km	02/03/2024	13:21:00	13	56.17	-116	31.944	4095	Q
JC257_085	UK1_0km	02/03/2024	20:00:00	13	56.086	-116	32.0010	4180	Q
JC257_086	UK1_16km	03/03/2024	02:56:00	13	47.976	-116	27.897	4118	NQ: Empty (no photo)
JC257_087	UK1_16km	03/03/2024	06:27:00	13	47.897	-116	27.471	4122	Q
JC257_089	UK1_16km	03/03/2024	13:49:00	13	48.045	-116	27.889	4118	Q: slanted
JC257_097	UK1_100km	05/03/2024	22:08:00	13	4.965	-116	5.648	4104	Q
JC257_098	UK1_100km	06/03/2024	02:19:00	13	4.942	-116	5.71	4107	Q
JC257_100	UK1_100km	06/03/2024	10:18:00	13	4.856	-116	5.72	4105	Q

JC257_109	UK1_100km	09/03/2024	12:41:00	13	4.892	-116	5.693	4100	Q
JC257_111	UK1_100km	09/03/2024	20:35:00	13	4.94	-116	5.61	4102	Q
JC257_112	UK1_100km	10/03/2024	02:33:00	13	4.848	-116	5.7860	4113	Q: slumped
JC257_114	UK1_1km	10/03/2024	15:14:00	13	55.690	-116	31.6890	4106	Q

2.3.6. Box core topshots

Each box core that returned a sample was photographed after topwater was drained (part of the Biology team protocol - see section 3). These are summarised in Figure 2.3.6.



JC257_012 (Site: 1km)

JC257_021 (Site: 1km)

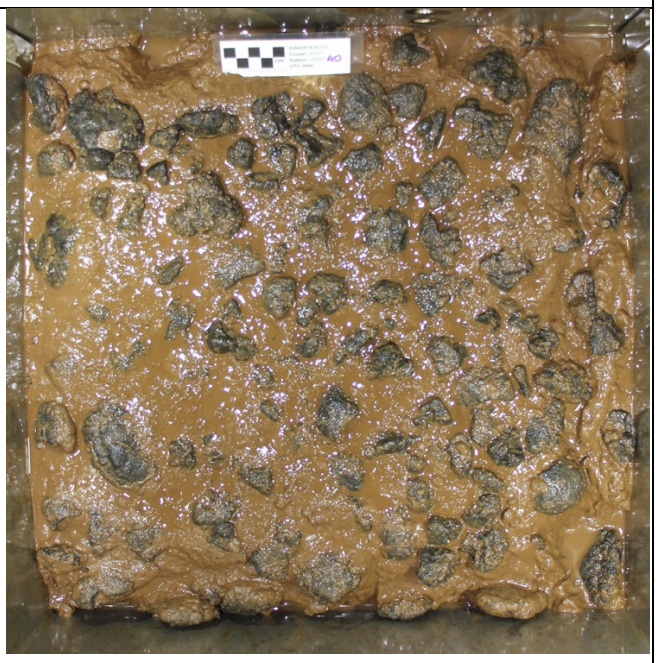
JC257_024 (Site: 1km)

JC257_028 (Site: 1km)

JC257_030 (Site: 1km)

JC257_032 (Site: 1km)

JC257_038 (Site: 1km)

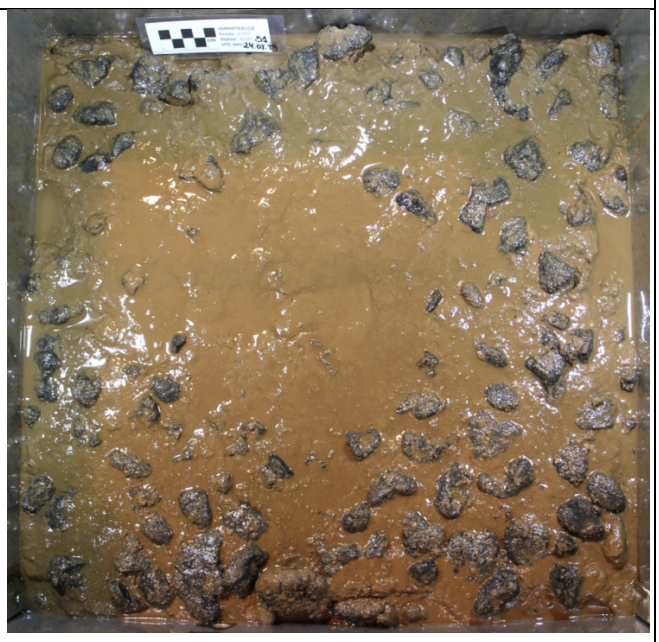


JC257_040 (Site: 1km)

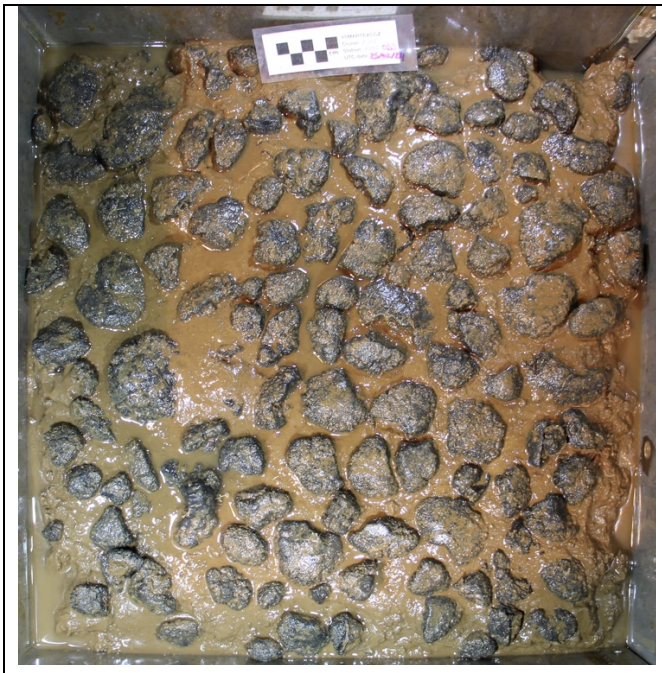
JC257_043 (Site: 1km)

JC257_045 (Site: 0km)

JC257_048 (Site: 0 km)

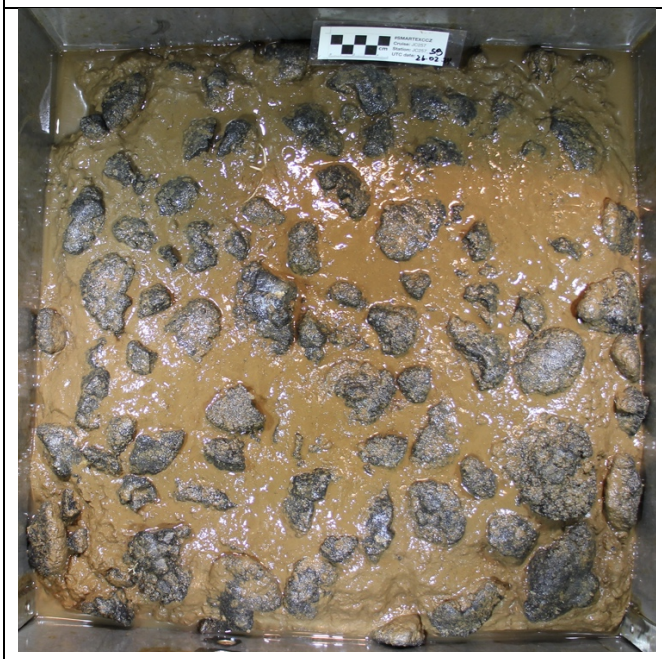


JC257_51 (Site: 1km)

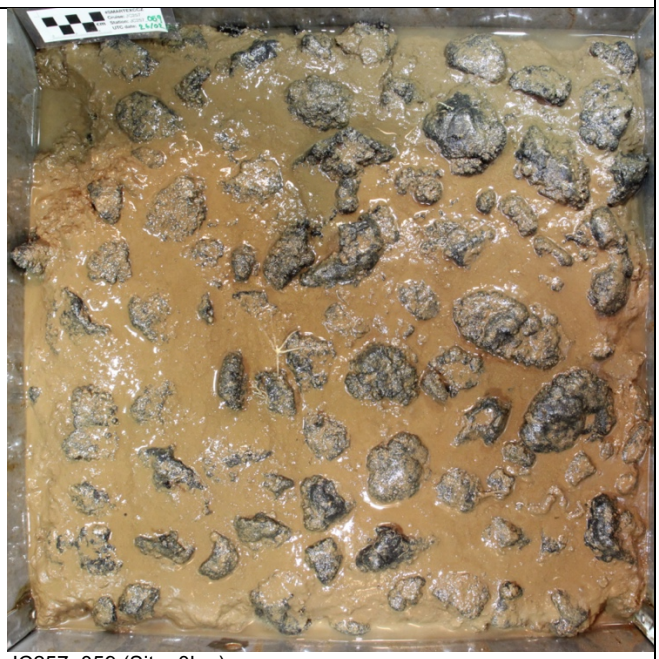


JC257_053 (Site: 0km)

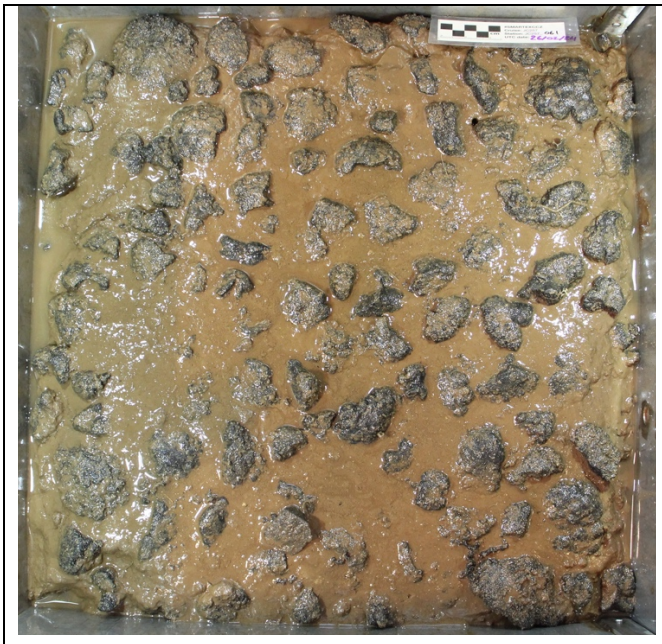
JC257_055 (Site: 0km)



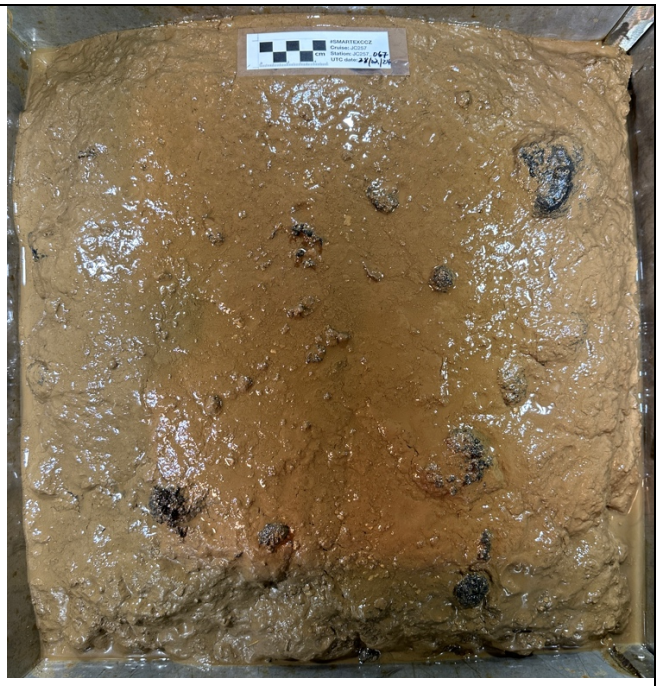
JC257_058 (Site: 0km)



JC257_059 (Site: 0km)

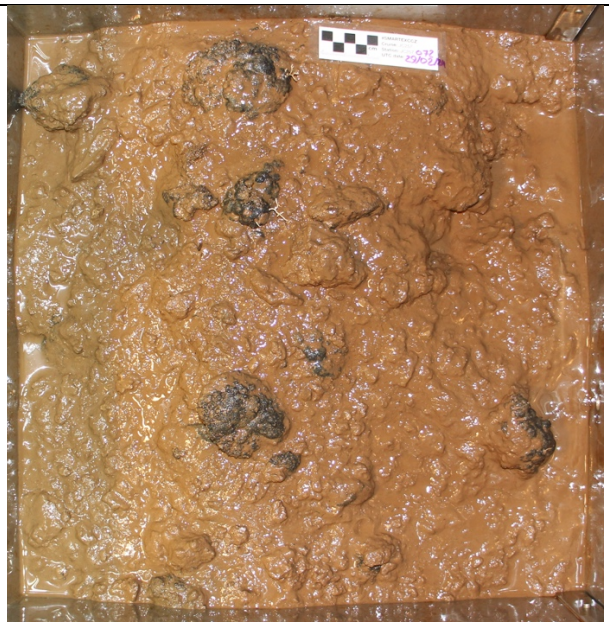


JC257_061 (Site: 0km)

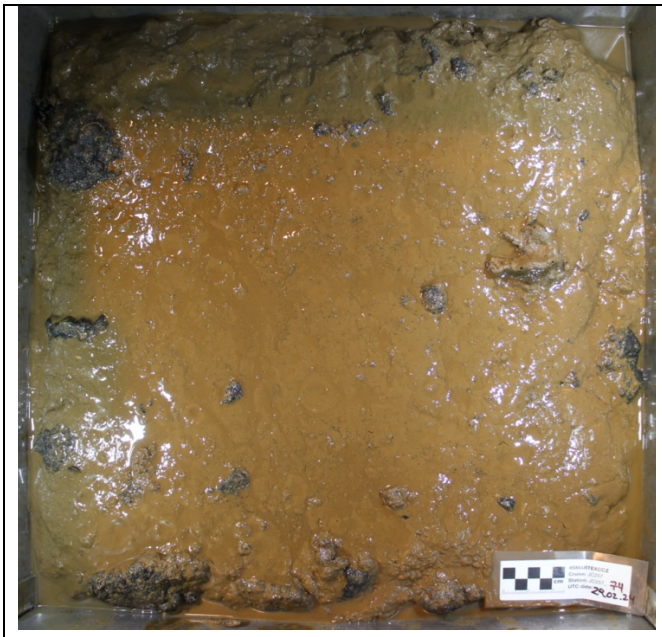


JC257_067 (Site: 16km)

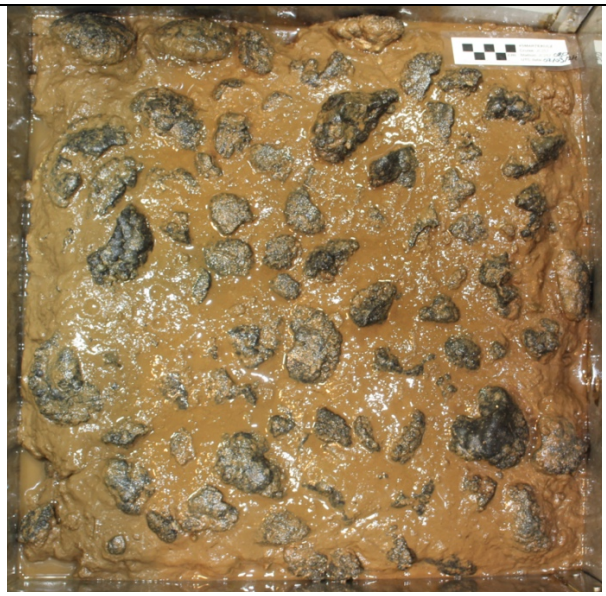
JC257_069 (Site: 16km)



JC257_072 (Site: 16km)



JC257_074 (Site: 16km)

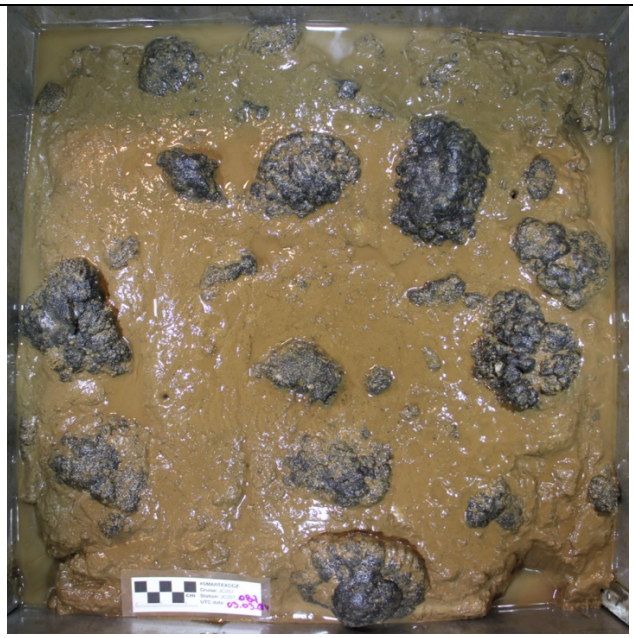


JC257_080 (Site: 0km)

JC257_082 (Site: 0km)

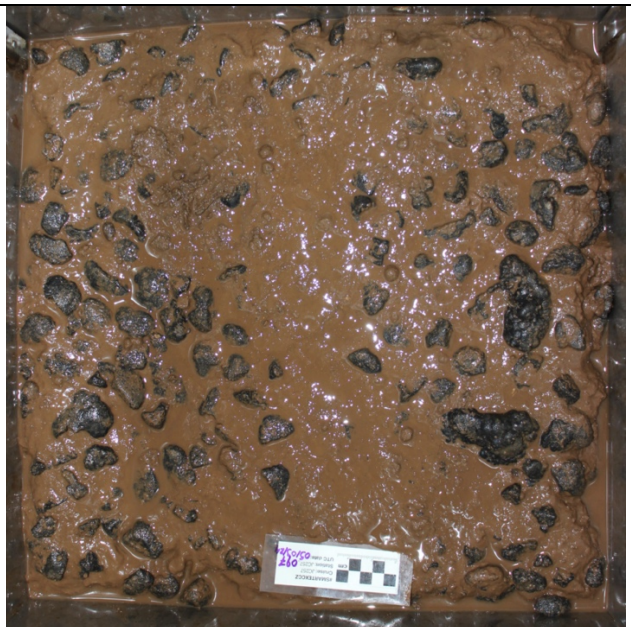
JC257_083 (Site: 0km)

JC257_085 (Site: 0km)



JC257_087 (Site: 16km)

JC257_089 (Site: 16km)



JC257_097 (Site: 100km)

JC257_098 (Site: 100km)

JC257_100 (Site: 100km)

JC257_109 (Site: 100km)

JC257_111 (Site: 100km)

JC257_112 (Site: 100km)

JC257_114 (Site: 0km) (*mistakenly 116 on label*)

Figure 2.3.6 Box core topshots on JC257-SMARTEX. All box core top shots including station number (JC257_###) and sample site. Note the change in colour is due to varied lighting and cameras. Note: except for JC257_055, failed box cores are not included.

An oceanic white-tip shark circles the ship during coring operations on JC257. Photo by Daniel Jones.

2.4. Megacore

2.4.1. Equipment

The NOC DeepSeas Group Megacorer was used during JC257 (Fig 2.4.1). For each deployment, the equipment was fitted with eight 10 cm internal diameter core tubes and the standard ballast load. For one deployment at each site, bleach-cleaned core tubes were fitted for the collection of material for eDNA sampling. The equipment was maintained, prepared for deployment and deployed by NMF technicians, Howard King and Billy Platt. Deployments and recoveries, including winch operations, were supported by the ship's crew.

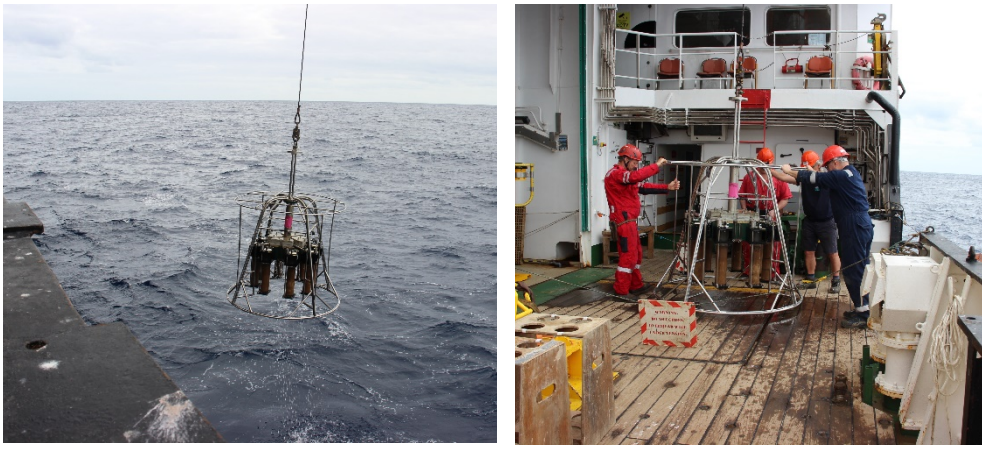


Figure 2.4.1. Recovery of Deep Sea Group megacorer during JC257.

2.4.2. Megacore deployments and protocol

Four replicate deployments were made at each UK1 location, at 0 kn, 1 km, 16 km and 100 km (Fig. 2.4.2) The equipment was deployed from the starboard gantry using the same winches and wires that were used for the box core (Section 2.3). Descent of the megacore equipment was conducted at 40 to 50 m/min with a speed reduction to 15 m/min over the last 100 m above seabed. Touchdown at the seabed was observed and recorded via the tensiometer. Station data were recorded for the moment of bottom contact: time (UTC), ship's position, sounding, and metres of wire deployed. An additional 10 m of wire was paid out prior to recovery. Recovery from the seabed was at 15m/min until the maximum haul tension was recorded, thereafter the equipment was recovered at 40 to 50 m/min. Once on-deck, cores were photographed, quality assessed using criteria listed on a core assessment sheet produced by Bryan O'Malley for JC241, 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: **N**o, **S**light, **M**oderate, **E**xtreme

• Nodule drag - ANS: **N**one, **S**light, **M**oderate, **E**xtreme

• 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: tick or cross

• Sample designation – ANS: eDNA, biogeochemistry, foraminifera/meiofauna, oxygen production.

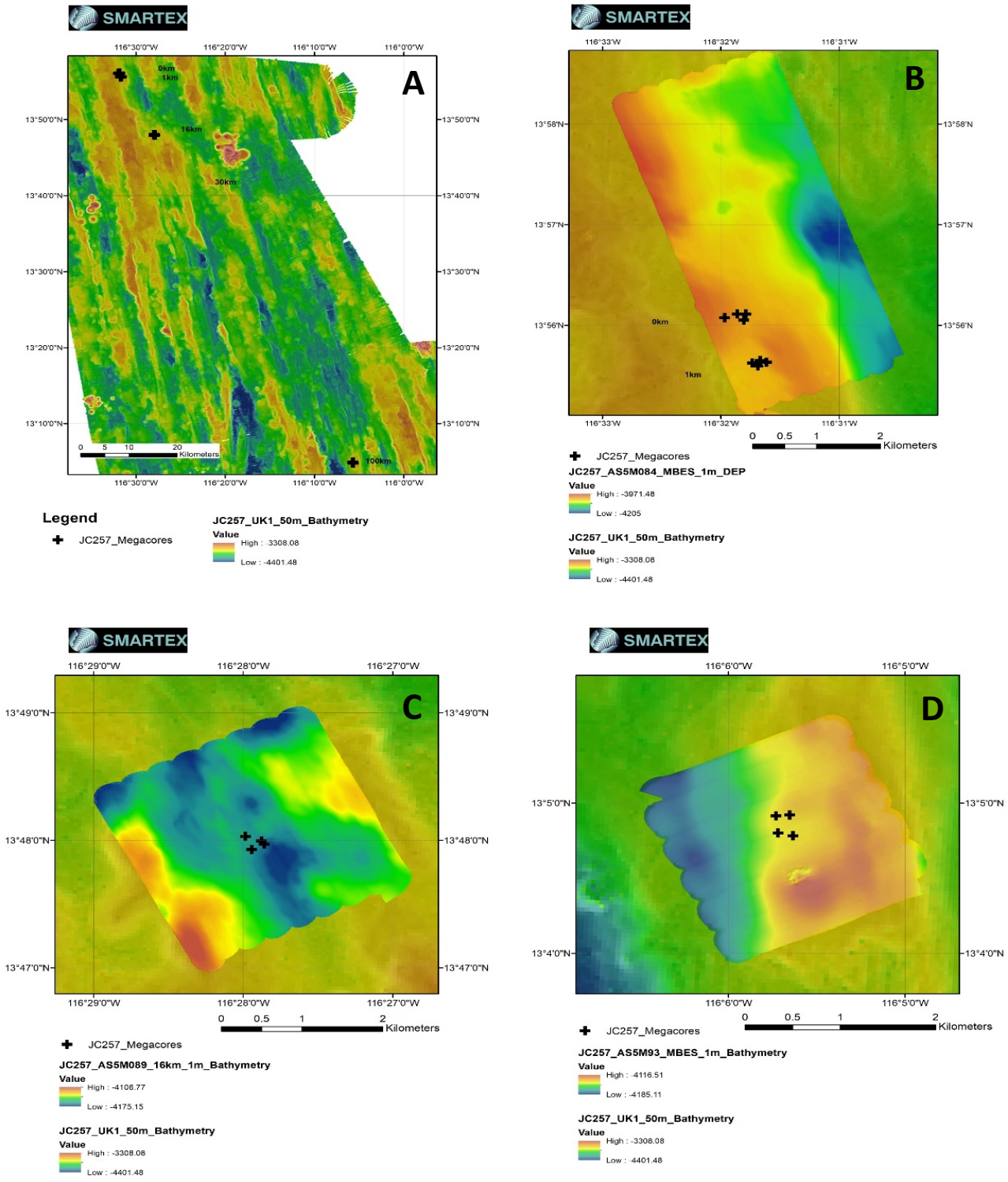


*Figure 2.4.2 Locations and bathymetery of megacore deploments during JC257. **A** – All UK1 sites; **B** – UK1-0 km and UK1–1 km; **C** – UK1-16 km; **D** – UK1-100 km. Maps provided by Catherine Wardell.*

2.4.3. Deployments of the Megacore

In total, the Megacorer was deployed 16 times for the sampling of sediments for biology (foraminifera/meiofauna), eDNA, biogeochemical analysis and oxygen production experiments. Overall, the equipment performed well, with four occasions where a shutter did not close and a core was not recovered. Generally, five to seven cores were recovered from the eight available that were acceptable (based on acceptability criteria) for the sampling required. Despite the abundance of nodules at all but one location (Stn JC257_094), recovered core length was excellent overall with an average successful core recovery size of 43.5 cm, except for Stn JC257_104 that produced much shorter cores, at between 14 cm and 25 cm. These cores were still acceptable as many were required for surface

and sub-surface (to 5 or 10cm) sampling and the integrity of the cores was still good. Ninety nine successful cores were recovered from the eight coring units over the 16 deployments. This equates to 80% recovery. The primary reasons for cores being rejected upon recovery were due to extreme nodule drag heavily disturbing the stratigraphy, or cracks and air bubbles that would likely compromise the integrity of the core. The degree of severe cracking or air pockets that allowed for water ingress appeared to be highly dependent on sediment type. Where a deeper soft surface layer with little transition into a more clay-like layer was observed, this resulted in a higher number of cracked cores, although, in most cases, the integrity of the core was not affected and they were usable.

Table 2.4.1 Megacore station deployments during JC257.

Station	Location	Date (2024)	Time (UTC: gear on bottom)	Latitude (N) (dec min, on bottom)		Longitude (W) (dec min, on bottom)		Depth (m)	Core comments
JC257_011	UK1 – 1 km	16/02	03:08:00	13˚	55.65	-116˚	31.670	4097	8 of 8 accepted
JC258_017	UK1 – 1 km	17/02	09:12:00	13˚	55.593	-116˚	31.683	4101	Tube 8 empty. 7 of 7 cores accepted
JC257_025	UK1 – 1 km	19/02	10:39:00	13˚	55.626	-116˚	31.730	4101	Tube 8 empty. 6 of 7 cores accepted
JC257_029	UK1 – 1 km	20/02	01:58:00	13˚	55.634	-116˚	31.615	4097	8 of 8 cores accepted
JC257_031	UK1 – 0 km	20/02	10:15:00	13˚	56.052	-116˚	31.808	4097	Tube 1 empty. 5 of 7 cores accepted
JC257_034	UK1 – 0 km	21/02	04:46:00	13˚	56.113	-116˚	31.864	4092	Tube 6 empty. 5 of 7 cores accepted
JC257_046	UK1 – 0 km	23/02	10:01:00	13˚	56.077	-116˚	31.972	4090	5 of 8 cores accepted
JC257_050	UK1 – 0 km	24/02	07:22:00	13˚	56.109	-116˚	31.786	4098	5 of 8 cores accepted
JC257_068	UK1 – 16 km	28/02	06:03:00	13˚	47.994	-116˚	27.875	4122	5 of 8 cores accepted
JC257_073	UK1 – 16 km	29/02	07:07:00	13˚	48.027	-116˚	27.982	4120	5 of 8 cores accepted
JC257_088	UK1 – 16 km	03/03	10:00:00	13˚	47.927	-116˚	27.941	4118	7 of 8 cores accepted
JC257_092	UK1 – 16 km	04/03	19:37:00	13˚	47.969	-116˚	27.855	4120	7 of 8 cores accepted
JC257_099	UK1 – 100 km	06/03	06:17:00	13˚	04.914	-116˚	05.722	4106	7 of 8 cores accepted
JC257_101	UK1 – 100 km	06/03	13:48:00	13˚	04.785	-116˚	05.630	4102	6 of 8 cores accepted
JC257_104	UK1 – 100 km	06/03	00:49:00	13˚	04.919	-116˚	05.650	4102	Tube 6 empty. 7 of 7 cores accepted
JC257_110	UK1 – 100 km	09/03	16:14:00	13˚	04.798	-116˚	05.717	4109	5 of 8 cores accepted

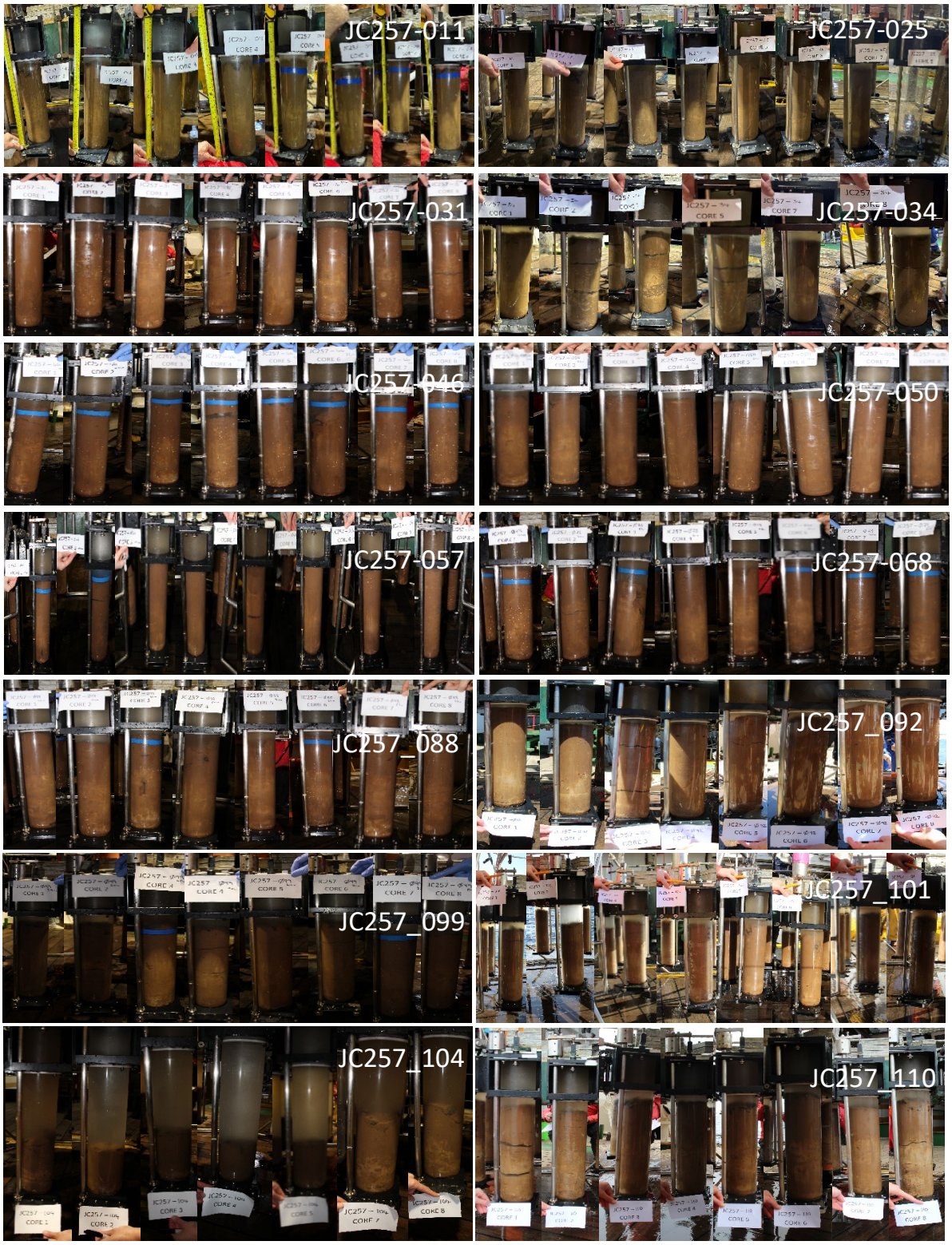


Figure 2.4.3. Examples of recovered cores from megacore deployments during JC257.

Louisa Norman, Bethany Fleming and Catherine Wardell (l-r) processing the megacore samples on JC257

2.5. CTD

2.5.1. CTD Equipment

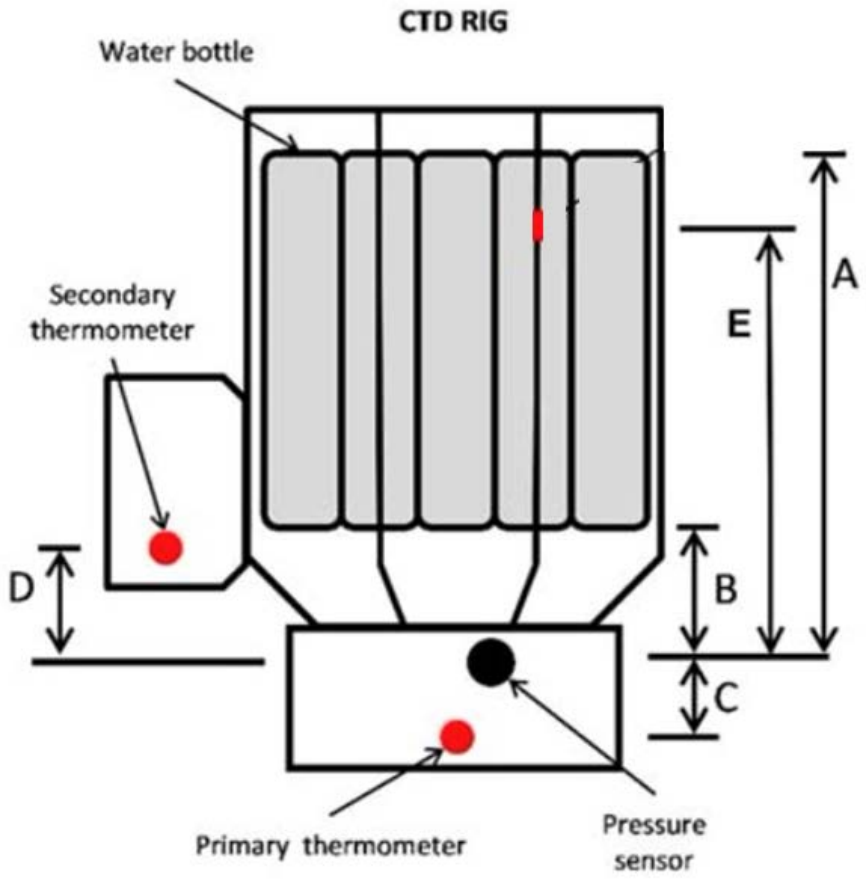


Figure 2.5.1 CTD system geometry. Vertical distance from pressure sensor (m positive-up):A 1.2 (Top of water samplers), B 0.34 (Bottom of water samplers) C -0.075 (Primary T mounted on 9p), D 0.085 (Secondary T mounted on Vane E SBE35 DOST not fitted

2.5.2. CTD Deployments

CTD and LADCP deployments on a rosette frame undertaken in JC257 (Table 2.5.1) were purposed primarily:

a) to evaluate spatial-temporal variability of physical oceanographic conditions near the seabed in the Areas of Interest at distances 0, 1, 16 and 100 km from the test site in the northern part of the UK1 licence area of CCZ. That objective also includes seawater samples collection for EDNA and Nutrient and drift evaluation of Salinity (conductivity) sensors.

b) to utilise CTD records for pre- and post-recovery calibration tests and comparison of instruments mounted on three moorings and deployed in March 2023 during the JC241 cruise and successfully recovered in March 2024 in JC257.

c) to perform (5) CTD casts within the Eddy during the transition to the UK1 site and on a return way and supposed supplement intercomparison with the T, S profiles derived from Argo Floats and deep Sea Glider.

d) Several CTD casts allow testing of various acoustic releases, cables and clamps for AUV and other equipment before deployment in a controlled environment.

Table 2.5.1 Physical oceanographic stations in JC257: mornings (3) recovery and CTD/LADCP casts collected. Light green cells indicate successful CTD casts with samples for salinity calibration collected.

Event-Label	UTC Date	UTC-time (Start)	Latitude (N) Deg	Min	Longitude (W) Deg	Min	Depth (m)	CTD depth (m)	CTD cast #	Comments
JC257_001	08/02/2024	12:35:00	9	35.6300	-89	12.2400	3500			U-way ADCP
JC257_005	13/02/2024	20:54:00	13	23.9900	-110	0.0000	4475			Glider
JC257_006	13/02/2024	21:27:00	13	24.0900	-110	0.0900	4471	2240	1	Eddy E
JC257_007	14/02/2024	00:20:00	13	23.2000	-110	0.0000	4355	1387	2	Eddy E

JC257_008	14/02/2024	14:56:00	12	40.1800	-112	20.1500	3962	430	3	Eddy S
JC257_014	16/02/2024	13:24:00	13	55.1071	-116	31.4774	4102	570	4	UK1, 1 km
JC257_018	17/02/2024	12:08:00	13	54.6200	-116	29.3410	4228	448	5	1 km
JC257_026	19/02/2024	13:13:00	13	54.6200	-116	29.3400	4220	392	6	1 km
JC257_036	21/02/2024	11:16:00	13	54.6212	-116	29.3411	4239	524	7	SE AOI2
JC257_037	21/02/2024	12:32:00	13	54.6193	-116	29.3416	4239	4203	8	SE AOI3
JC257_044	23/02/2024	00:16:00	13	52.8010	-116	28.8710	4252	4250	9	1km
JC257_054	25/02/2024	05:07:00	13	56.0760	-116	31.9700	4101	3118	10	1 km
JC257_062	27/02/2024	00:36:00	13	56.0763	-116	31.9730	4090	4083	11	1 km
JC257_065	27/02/2024	19:53:00	13	48.0211	-116	27.9390	4113	4105	12	16 km
JC257_077	01/03/2024	01:32:00	13	47.9770	-116	27.9370	4113	1000	13	16 km
JC257_079	01/03/2024	21:45:00	13	52.3400	-116	33.1600	4040			SM 3
JC257_084	02/03/2024	17:57:00	13	56.4050	-116	30.4090	4075			SM 2
JC257_102	06/03/2024	17:20:00	13	4.2270	-116	7.7460	4343	4341	16	100 km
JC257_116	11/03/2024	07:01:00	13	52.8000	-116	28.8720	4296	4252	17	Yo-Yo 1km
JC257_120	12/03/2024	13:50:00	13	53.4050	-116	39.3840	4233			LM 1
JC257_121	13/03/2024	17:31:00	13	17.2929	-115	45.4999	4076	4060	18	Eddy W
JC257_122	14/03/2024	02:31:00	13	15.5700	-114	45.0000	4012	3996	19	Eddy C

Nineteen CTD casts were undertaken with an NMF 24-way Stainless Steel CTD frame with 23 of 10L Niskin water samplers. 16 bottles were fitted leaving 8 bottle positions free for fitting three SBE37SMs recovered from three Moorings and were utilised for calibration cast at station JC257_121 (CTD18).

Dual SBE 43 dissolved oxygen sensors were used. The primary temperature, conductivity and dissolved oxygen sensors were fitted to the SBE9 plus with the secondary sensors mounted on the vane. Standard CTD wire (11.8 mm) with the inner armour was used for signal return. The Hydro boom was designed to deploy and recover the CTD from the sampling annex using the CTD wire.

The winch system Active Heave Compensation was used on all casts showing significant improvements in package stability and reducing spiking in wire tension. After each cast the primary and secondary sensors were flushed three times with MilliQ.

There were several issues with the Stainless Steel CTD suite during the cruise that led to multiple replacements of main wire cable termination, switching to the second (reserved) cable as the leak in the main cable up to 600 m was identified, disconnection (bypassing) titanium Swivel rotation compensation unit, testing the 2nd swivel set. All that required a huge amount of effort. Finally, Billy Pratt identified the source of troubles after series of sensors (Pressure, Oxigen & transmissometers) replacements and disconnections. Transmissometer CST-1759TR was plugged (cast 1-7,14) and then disconnected and replaced with CST-1718TR (cast 15,18-19), as the suspected point of failure was finally found with one of two legs of a Y-shape cable, connecting the transmissometers with SBE. After cast 9 it was noticed that one of Niskin bottles (10) was leaking, which was handled for the next station. A summary of changes is given in Table 2.5.2.

2.5.3. CTD Configuration

One CTD system was prepared with frame geometry and CTD sensor locations shown in Figure 2.5.1. The water sampling arrangement was a 24-way stainless steel frame system fitted with 23 off 10 Liters Ocean Test Equipment (OTE) Niskin bottles (except CTD18) and MDS titanium CTD swivel. Sensor information and serial numbers for all underwater components are given in Table 2.5.2

Table 2.5.2 CTD instruments and setup details from Billy Platt, alterations are highlighted.

Instrument / Sensor	Manufacturer / Model	Serial Number	Channel	Casts Used
Primary CTD deck unit	SBE 11plus	11P-24680 0589	n/a	All casts
CTD Underwater Unit	SBE 9plus	09P-71442-1142	n/a	Casts 1-4
CTD Underwater Unit	SBE 9plus	09P-87077-1257	n/a	Cast 5 onwards
Stainless steel 24-way CTD frame	NOCS	SBE CTD10	n/a	All casts
Primary Temperature Sensor	SBE 3P	03P-4814	F0	All casts
Primary Conductivity Sensor	SBE 4C	04C-4139	F1	All casts
Digiquartz Pressure sensor	Paroscientific	124216	F2	Casts 1-4
Digiquartz Pressure sensor	Paroscientific	134949	F2	Cast 5 onwards
Secondary Temperature Sensor	SBE 3P	03P-5700	F3	All casts
Secondary Conductivity Sensor	SBE 4C	04C-4140	F4	All casts
Primary Pump	SBE 5T	05T-7516	n/a	All casts
Secondary Pump	SBE 5T	05T-7517	n/a	All casts

24-way Carousel	SBE 32	32-31240-0423	n/a	All casts
Primary Dissolved Oxygen Sensor	SBE 43	43-2818	V0	All casts
Secondary Dissolved Oxygen Sensor	SBE 43	43-2575	V1	Cast 1-10
Secondary Dissolved Oxygen Sensor	SBE 43	43-3836	V1	Cast 11 onwards
Altimeter	Valeport VA500	81632	V3	All casts
Transmissometer	CST	1759TR	V4	Cast 1-7, 14
Transmissometer	CST	1718TR	V4	Cast 15,18-19
Fluorometer	Chelsea	088-195	V5	All casts
LADCP Down looking	TRDI 300KHz	4275	n/a	All casts
LADCP battery pack	NOC	WH007	n/a	All casts
LADCP Up looking	TRDI 300KHz	12369	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	Cast 1, 13-15, 17
Titanium EM CTD Swivel	MDS V2_2	1267-1	n/a	Cast 2 – 7, 19
DOST	SBE 35	35-34173-0048	n/a	Casts 1-6
Autosal	Guideline 8400B	68426	n/a	n/a

2.5.4. SBE 9 plus data setup and processing

The configuration files (Table 2.5.3) used for preliminary CTD raw data conversion into 1-meter and 1-second subsample profiles were performed with the SeaBird Electronics SBE Data Processing software v7.26.7. Row CTD data were derived from the mounted network share folder ”V:/Sensors_and_Moorings/CTD Data/Raw/” .The empty space in folder and file names were replaced with “_” at the scientists local drive.

Table 2.5.3 Configuration report for SBE 911plus/917plus from Stations CTD01 and CTD19 with all alterations and replacements highlighted.

CTD 01	CTD19
1) Frequency 0, Temperature	1) Frequency 0, Temperature
Serial number : 03P-4814	Serial number : 03P-4814
Calibrated on : 28-Sep-22	Calibrated on : 28-Sep-22
G : 4.30069850e-003	G : 4.30069850e-003
H : 6.23883681e-004	H : 6.23883681e-004
I : 1.80290856e-005	I : 1.80290856e-005
J : 1.16187537e-006	J : 1.16187537e-006
F0 : 1000.000	F0 : 1000.000
Slope : 1.00000000	Slope : 1.00000000
Offset : 0.0000	Offset : 0.0000
2) Frequency 1, Conductivity	2) Frequency 1, Conductivity
Serial number : 04C-4139	Serial number : 04C-4139
Calibrated on : 06-Oct-22	Calibrated on : 06-Oct-22
G : -9.91538545e+000	G : -9.91538545e+000
H : 1.46721674e+000	H : 1.46721674e+000
I : -2.58242793e-003	I : -2.58242793e-003
J : 2.86634814e-004	J : 2.86634814e-004
CTcor : 3.2500e-006	CTcor : 3.2500e-006
CPcor : -9.57000000e-008	CPcor : -9.57000000e-008
Slope : 1.00000000	Slope : 1.00000000
Offset : 0.00000	Offset : 0.00000
3) Frequency 2, Pressure, Digiquartz with TC	3) Frequency 2, Pressure, Digiquartz with TC
Serial number : 124216	Serial number : 134949
Calibrated on : 24-Jan-2020	Calibrated on : 10-March-22
C1 : -6.193577e+004	C1 : -3.695717e+004
C2 : -2.149353e-001	C2 : -2.691791e-001
C3 : 1.865100e-002	C3 : 1.143300e-002
D1 : 2.627600e-002	D1 : 3.349300e-002
D2 : 0.000000e+000	D2 : 0.000000e+000
T1 : 3.027244e+001	T1 : 3.049225e+001
T2 : -3.411760e-004	T2 : -3.372510e-004
T3 : 4.320610e-006	T3 : 3.990980e-006
T4 : 0.000000e+000	T4 : 3.875890e-009
T5 : 0.000000e+000	T5 : 0.000000e+000
Slope : 1.00012000	Slope : 1.00008000
Offset : -1.40690	Offset : -1.82150
AD590M : 1.279600e-002	AD590M : 1.280330e-002
AD590B : -9.557250e+000	AD590B : -9.092840e+000
4) Frequency 3, Temperature, 2	4) Frequency 3, Temperature, 2
Serial number : 03P-5700	Serial number : 03P-5700
Calibrated on : 21-June-22	Calibrated on : 21-June-22
G : 4.34158372e-003	G : 4.34158372e-003
H : 6.28513273e-004	H : 6.28513273e-004

I : 1.87110510e-005
J : 1.15270720e-006
F0 : 1000.000
Slope : 1.00000000
Offset : 0.0000

5) Frequency 4, Conductivity, 2

Serial number : 04C-4140
Calibrated on : 29-Sept-22
G : -9.84969874e+000
H : 1.48780469e+000
I : -3.09340442e-003
J : 3.24715516e-004
CTcor : 3.2500e-006
CPcor : -9.57000000e-008
Slope : 1.00000000
Offset : 0.00000

6) A/D voltage 0, Oxygen, SBE 43

Serial number : 43-2818
Calibrated on : 7th-Feb-23
Equation : Sea-Bird
Soc : 4.79700e-001
Offset : -4.94400e-001
A : -4.67690e-003
B : 2.11720e-004
C : -3.19730e-006
E : 3.60000e-002
Tau20 : 1.24000e+000
D1 : 1.92634e-004
D2 : -4.64803e-002
H1 : -3.30000e-002
H2 : 5.00000e+003
H3 : 1.45000e+003

7) A/D voltage 1, Oxygen, SBE 43, 2

Serial number : 43-2575
Calibrated on : 23-Sept-22
Equation : Sea-Bird
Soc : 4.31200e-001
Offset : -4.73900e-001
A : -4.57280e-003
B : 2.08010e-004
C : -2.82230e-006
E : 3.60000e-002
Tau20 : 1.20000e+000
D1 : 1.92634e-004
D2 : -4.64803e-002
H1 : -3.30000e-002
H2 : 5.00000e+003
H3 : 1.45000e+003

8) A/D voltage 2, Free

9) A/D voltage 3, Altimeter

Serial number : 81632
Calibrated on : 09-June-22
Scale factor : 15.000
Offset : 0.000

10) A/D voltage 4, Transmissometer, WET Labs C-Star

Serial number : 1759TR
Calibrated on : 28th July 2021
M : 21.2755
B : -0.1808
Path length : 25.000

11) A/D voltage 5, Fluorometer, Chelsea Aqua 3

Serial number : 088-195
Calibrated on : 7th Sept2022
VB : 0.140976
V1 : 1.958590
Vacetone : 0.657600
Scale factor : 1.000000
Slope : 1.000000
Offset : 0.000000

12) A/D voltage 6, Free
13) A/D voltage 7, Free
scan length : 45

I : 1.87110510e-005
J : 1.15270720e-006
F0 : 1000.000
Slope : 1.00000000
Offset : 0.0000

5) Frequency 4, Conductivity, 2

Serial number : 04C-4140
Calibrated on : 29-Sept-22
G : -9.84969874e+000
H : 1.48780469e+000
I : -3.09340442e-003
J : 3.24715516e-004
CTcor : 3.2500e-006
CPcor : -9.57000000e-008
Slope : 1.00000000
Offset : 0.00000

6) A/D voltage 0, Oxygen, SBE 43

Serial number : 43-2818
Calibrated on : 7th-Feb-23
Equation : Sea-Bird
Soc : 4.79700e-001
Offset : -4.94400e-001
A : -4.67690e-003
B : 2.11720e-004
C : -3.19730e-006
E : 3.60000e-002
Tau20 : 1.24000e+000
D1 : 1.92634e-004
D2 : -4.64803e-002
H1 : -3.30000e-002
H2 : 5.00000e+003
H3 : 1.45000e+003

7) A/D voltage 1, Oxygen, SBE 43, 2

Serial number : 43-3836
Calibrated on : 27-Sept-23
Equation : Sea-Bird
Soc : 4.29100e-001
Offset : -4.95800e-001
A : -4.94990e-003
B : 2.25240e-004
C : -3.10150e-006
E : 3.60000e-002
Tau20 : 1.14000e+000
D1 : 1.92634e-004
D2 : -4.64803e-002
H1 : -3.30000e-002
H2 : 5.00000e+003
H3 : 1.45000e+003

8) A/D voltage 2, Free
9) A/D voltage 3, Altimeter

Serial number : 81632
Calibrated on : 09-June-22
Scale factor : 15.000
Offset : 0.000

10) A/D voltage 4, Transmissometer, WET Labs C-Star

Serial number : 1718TR
Calibrated on : 19th September 2022
M : 21.3458
B : -0.0789
Path length : 0.250

11) A/D voltage 5, Fluorometer, Chelsea Aqua 3

Serial number : 088-195
Calibrated on : 7th Sept2022
VB : 0.140976
V1 : 1.958590
Vacetone : 0.657600
Scale factor : 1.000000
Slope : 1.000000
Offset : 0.000000

12) A/D voltage 6, Free
13) A/D voltage 7, Free
Scan length : 45

2.5.5. Salinometry

A Guildline Autosal 8400B, s/n 68426, was installed in the Electronics Workshop as the main instrument for salinity analysis. The Autosal temperature set point was 21.47°C and the temperature of the laboratory was kept around 21°C. The salinometer was standardised earlier in the cruise. Once standardised the Autosal was not adjusted.

Salinity sampling

Salinity samples from the CTD were collected from several Niskin bottles fired at each successful deep station (8,9,11,12,16,17,18,19). The procedure was usually to rinse the sample bottle three times with water from the Niskin with cap on, fill the bottle, insert a clean plastic stopper, wipe the bottleneck and inside of the cap (to avoid the formation of salt deposits) and put the bottle cap on. Salinity samples also were taken from the ship's underway system three to four times a day (nominally at 08:00, 12:00, 16:00 and 20:00) throughout the cruise, following the protocol as above. Samples were not taken while the ship was on station due to poor data quality at those times.

Salinity Analysis

The salinometer was standardised once at the start of the cruise, and then bottles of standard seawater (OSIL batch P165, K15 = 0.99986) were analysed throughout the salinometer runs to monitor instrument drift. The samples were stored in crates equilibrated to the temperature-controlled laboratory for at least 24 hours before analysis.

For CTD samples, SSW was analysed at the start and end of each crate processing (4 to 8 bottles from each deep CTD cast). For underway samples, SSW was run at the start and end of each crate (24 bottles). In total 2 CTD crates (#30 and #33) were run and several SG crates. Thus, for each crate of up to 24 salinity samples, three SSWs were used and each CTD station was tied to a start and end SSW at the end of the cruise.

2.5.6. Lowered ADCP Configuration and deployment

The CTD rosette was equipped with two 300-kHz Teledyne RDI Workhorse Monitor LADCPs. One, the down-looker (Master, SN:4275), was installed in a downward-looking orientation while the other, the up-looker (Slave, SN: 12369), was installed in an upward-looking configuration. The LADCPs collected data in beam coordinates with 25 x 8 m bins later converted to earth coordinates during processing. The deployment of the LADCP was usually done by the NMF CTD technician. Checks for the faults in the LADCP were done before every cast with pre-deployment and deployment scripts to set ping, bin, and transformation parameters, and to start the LADCP heads pinging. To reduce interference between the two LADCPs, the Slave LADCP is set to ping in response to the Master LADCP.

Post CTD recovery, both LADCPs were connected to a laptop in the deck lab for charging, data downloading and initial quality checking, carried out by the NMF technician. The files were saved with names of the form JC257_CTD_0XXM.000 and JC257_CTD_0XXS.000 for master and slave respectively, where XX is the CTD cast number, and copied from the networked Sensors and Moorings drive directory to the data processing workstation in the shared network folder *"V:/Sensors_and_Moorings/LADCP Data/Data/"* for processing.

2.5.7. LADCP Data processing

The data processing for each successful CTD cast station was performed using the latest Lamont-Doherty Earth Observatory LDEO-IX v.14, published on 29, 2021. This MATLAB package was developed at Lamont-Doherty Earth Observatory (LDEO) by Martin Visbeck and maintained by Andreas Thurnherr. The software is based on an inverse method for calculating velocity profiles from the LADCP data using different constraints such as:

Ship navigation and position data from the GPS (and stored with the VM-ADCP files)

Bottom tracking (BT) velocities

The change in the profiles as a result of adding the different constraints is checked by adding them one by one at a time in succession. This is firstly done for the two LADCPs, DL and UL, separately before combining them as DLUL for the different constraints below:

Ship navigation (DLUL_GPS)

Ship navigation and bottom tracking (DLUL_GPS_BT)

To automate the running of the processing under different constraints, a wrapper version for the LDEO IX scripts ('process_cast.m', 'set_cast_params.m') had been adjusted to the length of the header of relevant JC257_CTD%stn%_derived_1s.cnv file and updated to accept input arguments determining which constraints would be used, and then invoked from 'A1_run_me_process_cast.m'. Therefore, MATLAB processing steps for each cast (stn) were running the following lines of code:

% selection of VM-ADCP and GPS source in the 'set_cast_params.m'

```
   cruise='JC257'; osXX='os75'; …%  cruise='JC257'; osXX='os150';
% cycle throughout the station list inside the “A1_run_me….m”, % nst=19;
for ist=1:nst
   stn=ist;
   [~,~,~]=mkdir(['../../../LADCP_data/processed/sta_' num2str(stn,'%3.3d') ]);
   process_cast ;  close all ;
end
```

Processing warnings for the LADCP casts

The processing of the LADCP cast files triggered a few typical processing warnings. The casts 7, 14, 15 were not processed due to the instrument not reaching sufficient depth (~100m) before being recovered and redeployed without restarting the LADCP files. Out of overall 19 casts performed, the LDEO software detected errors for 14 casts listed:

```
Cast # errors :
 1 ** found 34 (0.5% of total) velocity measurements > 2.5 m/s
 3 ** found 16 (0.5% of total) velocity measurements > 2.5 m/s
 4 ** found 153 (5.5% of total) velocity measurements > 2.5 m/s
 5 ** found 28 (0.4% of total) velocity measurements > 2.5 m/s
 6 ** found 84 (3.1% of total) velocity measurements > 2.5 m/s
 8 ** found 335 (1.1% of total) velocity measurements > 2.5 m/s
 9** found 50 (0.6% of total) velocity measurements > 2.5 m/s
10** found 15 (0.3% of total) velocity measurements > 2.5 m/s
11 ** found 115 (1.4% of total) velocity measurements > 2.5 m/s
12 ** found 50 (0.6% of total) velocity measurements > 2.5 m/s
13 ** found 136 (2.7% of total) velocity measurements > 2.5 m/s
16 ** found 181 (1.5% of total) velocity measurements > 2.5 m/s
17 ** found 16 (0.1% of total) velocity measurements > 2.5 m/s, Yo-Yo   station
18 ** found 37 (0.4% of total) velocity measurements > 2.5 m/s
19 ** found 110 (1.4% of total) velocity measurements > 2.5 m/s
```

Deploying equipment on JC257. Photo by Daniel Jones.

2.6. Gravity core

2.6.1. Gravity Core Equipment

A NOC system Gravity core with a 5 m barrel was deployed once on JC257. It was deployed at the UK-1 central 1km area.

2.6.2. Deployments of the Gravity Core

Station: JC257_119

Date: 12 March 2024 JDay: 24072

Time in water: 07:35 (UTC) Time on seabed 08:57 (UTC) Time on deck: 10:10 (UTC)

Location: 13°55.652'N 116°31.56'W Depth: 4092m

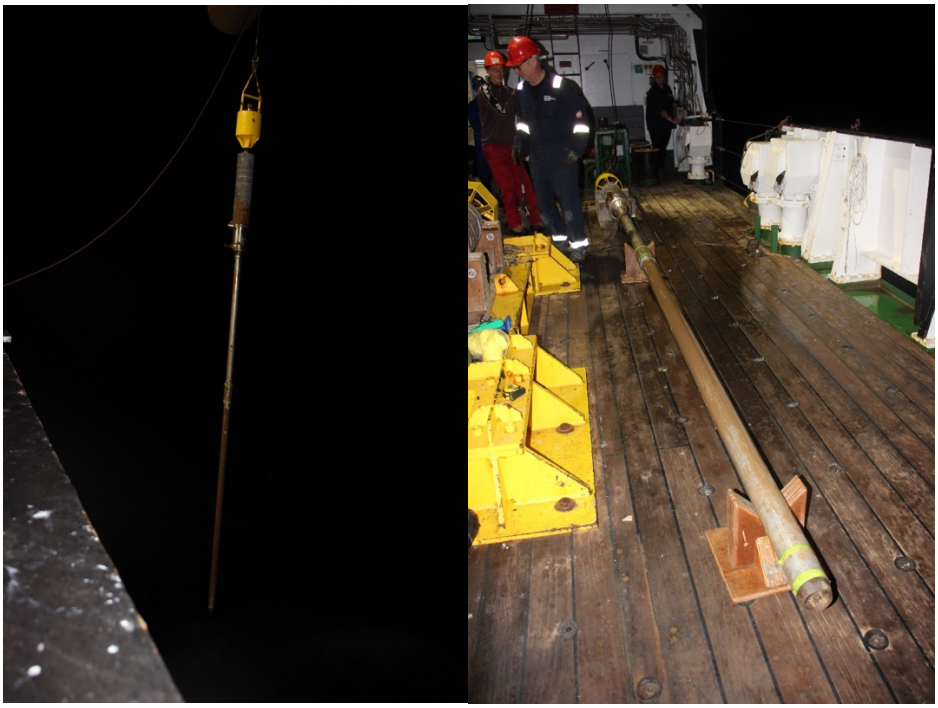


Figure 2.6.1 Images of gravity core deployment on JC257.

Figure 2.6.2 Sections from gravity core deployment on JC257. Station JC257_119. The deepest section (A) is at the top of the photograph and the top of the core (E) is at the bottom of the photo. In all cores the base (deepest) part is on the right.

The core was sectioned as into 5 sections. It totalled 4.63 m in length. The core appeared to be very well preserved and the sediment surface was intact. The core was put into the refrigerated container for shipment back to Southampton. It will be analysed at BOSCORF.

Table 2.6.1 Details of gravity core sections.

Section	Length, m	Notes
JC257_119_A	1	Deepest part of core
JC257_119_B	1.08	
JC257_119_C	1	
JC257_119_D	1	
JC257_119_E	0.55	Includes surface of core. Well preserved surface with nodule.

Daily science meeting on JC257. Image by Adrian Glover.

2.7. Glider

2.7.1. Glider mission details

The research campaign included the deployment of one deep (M6) sea glider in the Eastern Tropical Pacific as part of the SMARTEX project and was funded by the Natural Environment Research Council (NERC). Deep Sea glider (sg042) mission (ID 621) was designed to evaluate the internal hydro-physical structure of the typical mesoscale eddy that enables us to trace its footprint on a seabed several km below. The planned mission includes a series of vertical 'zigzag' dives between the surface and increasing depth levels: 0-30-0-90-0-300-0-900-0-4000 (or near seabed)-0 within 24 hours cycle daily over 4-5 weeks along a line across the eddy centre from east to west (Fig. 2.7.1). Such a combination of layers and deviation of the glider surfacing location from the assigned waypoint along a major axis of the mesoscale eddy was expected to provide sufficient data to evaluate glider drift, representative of averaged ocean currents within a layer between the surface and each selected layers depths.

The optimal location for deep glider deployment was chosen at the eastern edge of the mesoscale eddy, approximately halfway from Port Caldera to the main site of JC257 research activity in the UK1 licence area of CCZ. Mesoscale eddy boundary was detected approximately using a relatively low resolution (1/4 ° or ~27 km) available Satellite Altimetry products (such as Sea Level Anomaly relative multiyear averaged state, Fig. 2.7.2). Eddy's presence was confirmed by the records of both (075 and 150 kHz) Vessel mounted ADCP currents profilers (&2.10), which show an increase of ocean currents speed in the surface layers and their southward direction on the approach to the target position. Deep glider deployment over starboard was performed at Station JC257_005, 13°24' N 110°00' W, 13th February 2024 at 20:55 UTC. Communication with the instrument was lost after the first short dive to 30m. Glider re-surfaced from a 4 km depth at nearly the same site a week later and drifted 100 km south, and communication was finally lost on the 3rd of March 2024.

The ship's return track was altered to pass through both drift points provided by SAMS and NOC modelling teams with a higher chance of encountering on 15th of March 2024, but the area being dark and foggy diminished the probability of finding the glider. It was not found.

Station:	JC257_005	JDay:	Glider failed to communicate by 23:00, tried a visual search but was not able to locate it. Glider regained contact a week later.
Date:	13 February 2024	24044	
Time in water:	20:55 (UTC)	Depth:	
Location:	13°23.990'N,110°00.00'W	4475m	

Metadata and more detailed instrument inventory of the sensors mounted on Deep Glider sg042 for mission ID 621 in SMARTEX Research Cruise JC257 are available at the BODC web page
https://gliders.bodc.ac.uk/inventory/metadata-viewer/?DeploymentId=621
and summarised in Table 2.7.1 below.

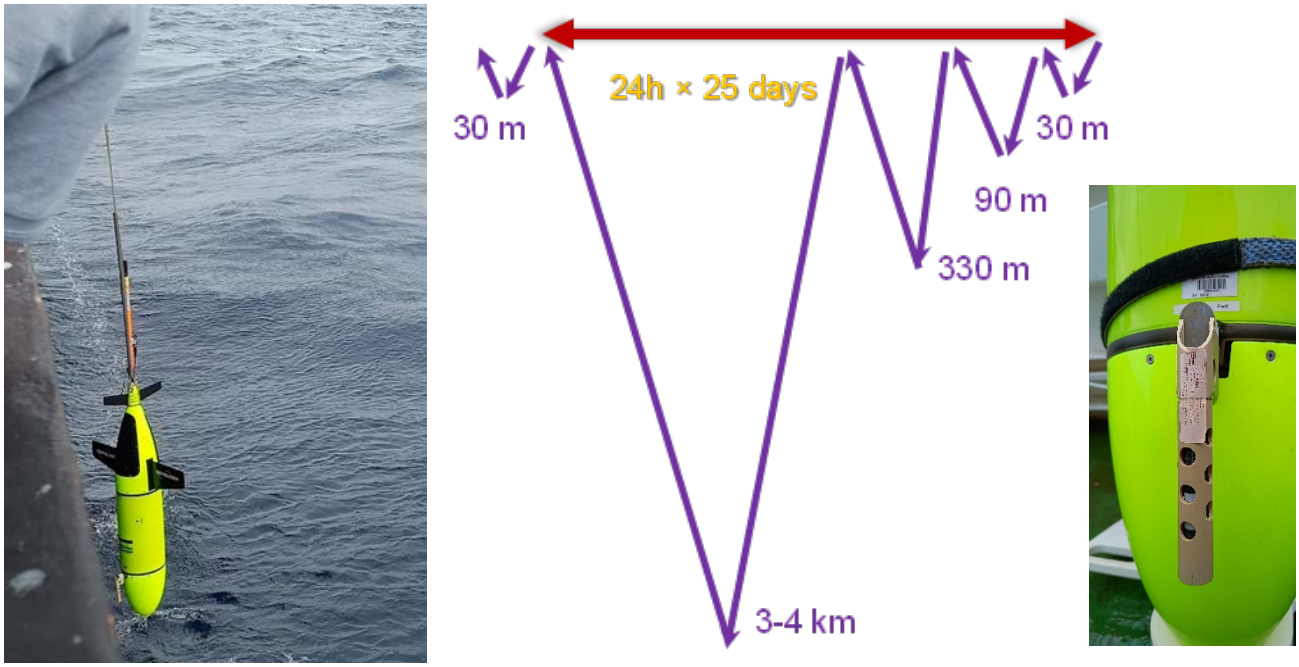


Figure 2.7.1 Darth Glider sg042 deployment photo and mission vertical motion design.

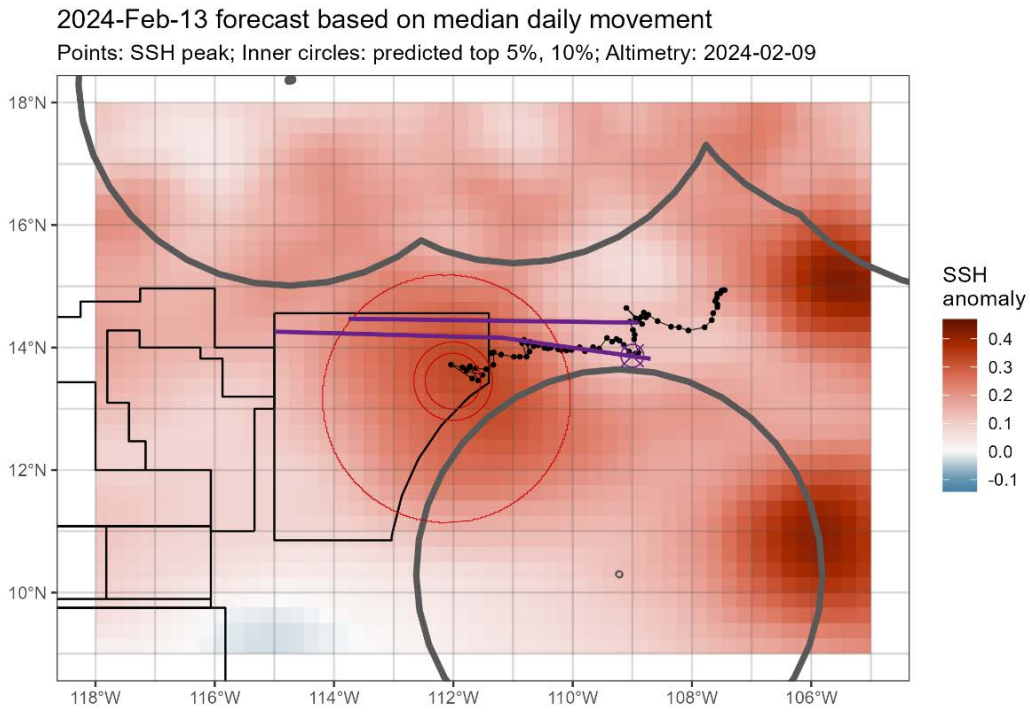


Figure 2.7.2 Forecast of the Sea Surface Height Anomaly (in respect to twenty-year 1993-2012) mean for glider deployment date 13.02.2024. Dots show the eddy centre track in the past 2 months. Source: Global Ocean Gridded L4 Sea Surface Heights and Derived Variables NRT, Product ID: sealevel_glo_phy_l4_nrt_008_046, https://doi.org/10.48670/moi-00149 Image courtesy Dr Tim Szewczyk.

Table 2.7.1 Metadata of Deep Sea Glider sg042 for mission ID 621.

Platform Model information	Value
BODC Platform Model ID	291
Platform Model ID	921
NVS Platform ID	B7600030
Platform Type	seaglider
Platform Manufacturer	University of Washington
Platform Model Name	M6
Platform instance information	
BODC Platform ID	1566
BODC Platform Type ID	1112
Platform Name	Darth_Glider
Platform Serial Number	sg042
Platform Owner	NOCS
Platform Family	open ocean glider
WMO Platform Code	6801560
Data Type	EGO glider time-series data
Platform sensors	There are 4 sensors on the platform
1.Seaglider M6 data logger sg042	
BODC Sensor Model ID	941
BODC Sensor Model Version/Registry ID	292
Instrument Type	data loggers
Sensor Manufacturer	University of Washington
Sensor Model	University of Washington Seaglider M6 Deepglider data logger
Model Name	University of Washington Seaglider M6 Deepglider data logger
BODC Sensor Version/Registry ID	1567
BODC Sensor ID	1113
Sensor Name	Seaglider M6 data logger sg042
Sensor Serial Number	sg042

Parameter Records:	LATITUDE, GLIDER_PITCH, LONGITUDE_GPS, LONGITUDE, GLIDER_DEPTH

2. Seaglider CT sail 0292

BODC Sensor Model ID	446
BODC Sensor Model Version/Registry ID	232
Instrument Type	water temperature sensor
Sensor Manufacturer	Sea-Bird Scientific
Sensor Model	Unpumped CT sail CTD
Model Name	Sea-Bird Scientific
Unpumped	CT sail CTD
BODC Sensor Version/Registry ID	1568
BODC Sensor ID	1114
Sensor Name	Seaglider CT sail 0292
Sensor Serial Number	0292
Parameter Records:	PRES, POTDENS_SURFACE THETA , SIGMA_,T, CNDC, PSAL,

3. WETLabs ECO 6KFLBBCD 4746

BODC Sensor Model ID	625
BODC Sensor Model Version/Registry ID	262
Instrument Type	radiometers
Sensor Manufacturer	WET Labs
Sensor Model	WET Labs {Sea-Bird WETLabs}
Sensor	ECO Triplet 6KFLBBCD scattering fluorescence sensor
Model Name	WETLabs ECO Triplet 6KFLBBCD scattering fluorescence sensor
BODC Sensor Version/Registry ID	1569
BODC Sensor ID	1115
Sensor Name	WETLabs ECO 6KFLBBCD 4746
Sensor Serial Number	FLBBCD6K-4746
Records:	TEMP_CPU_CHLA, FLUORESCENCE_CDOM, BETA_BACKSCATTERING700 FLUORESCENCE_CHLA

4. Aanderaa 4831F oxygen optode 453

BODC Sensor Model ID	961
BODC Sensor Model Version/Registry ID	293
Instrument Type	dissolved gas sensors
Sensor Manufacturer	Aanderaa
Sensor Model	Aanderaa 4831F oxygen optode
Model Name	Aanderaa 4831F oxygen optode
BODC Sensor Version/Registry ID	1570
BODC Sensor ID	1116
Sensor Name	Aanderaa 4831F oxygen optode 453
Sensor Serial Number	453
Parameter Records:	DPHASE_DOXY, MOLAR_DOXY, TPHASE_DOXY, TEMP_DOXY, OXYSAT_DOXY

ROV Isis returns from another mission in the night to the James Cook after diving to 4100m on JC257. Photo by Adrian Glover.

Loïc Van Audenhaege on the bridge of the James Cook on JC257. Photo by Daniel Jones.

2.8. Fish Traps

2.8.1. Fish Trap Equipment

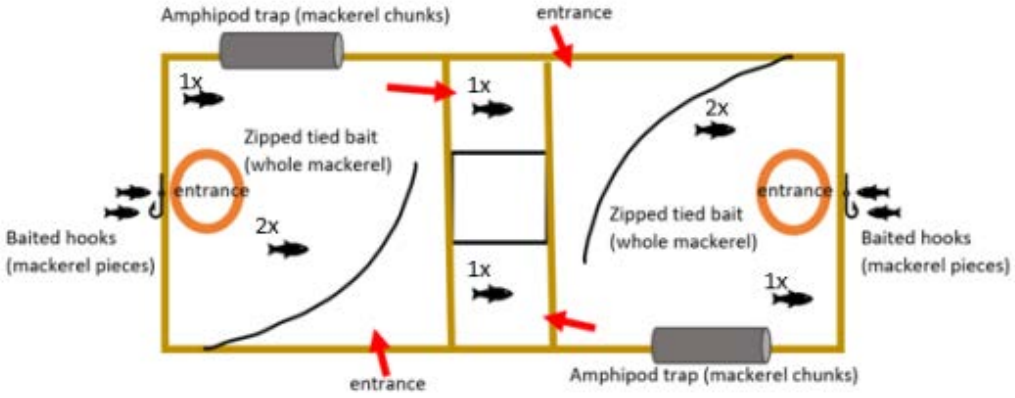


Figure 2.8.1 Bird's eye diagram of the fish trap deployed on JC241.

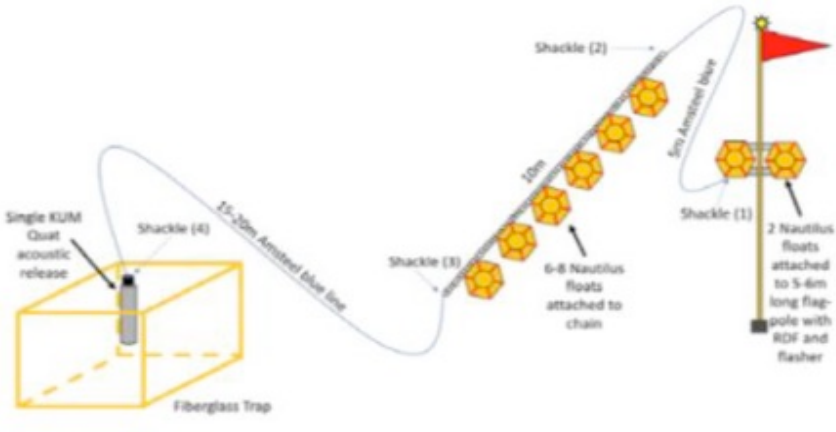


Figure 2.8.2 Diagrammatic depiction of the fish trap and its typical mooring configuration. Only three Nautilus floats were used on JC241 deployments.

The trap was modified to include a sliding trap door to be operated by the ROV in order to be able to carry out *in situ* sediment exposure experiments with fish retained inside (Figure 2.8.2).

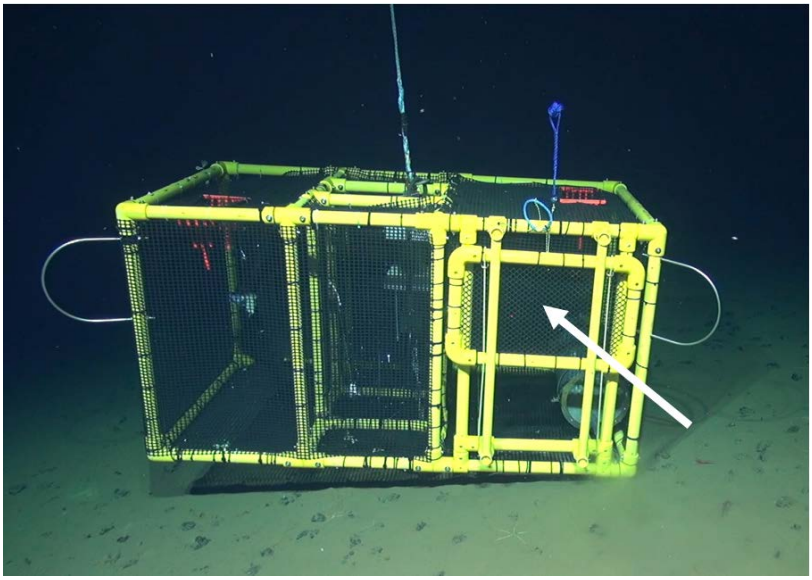


Figure 2.8.3 Trap on the seafloor showing the open trap door (white arrow)

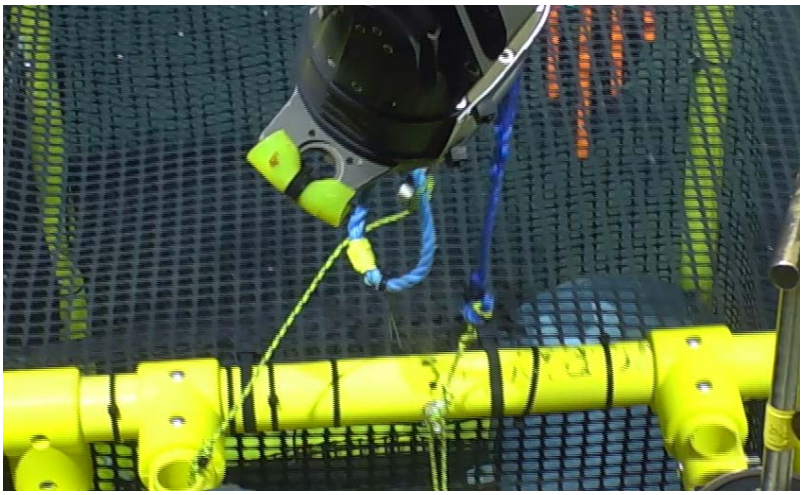


Figure 2.8.4 ROV manipulator removing the safety pin

The trap also carried a USBL device (Marker 6, Type 8226-000-7511) to aid location on the seafloor.

2.8.2. Fish Trap Deployments

Table 2.8.1 List of deployments during JC257.

StationID	Reference	Region	Deployment date/time (Ship_time)	Deployment date/time (UTC)	Recovery date/time (UTC)	Position Lat/Long		Depth (m)
JC257-16	AKS354	UK1	16.02.24_06:44	16.02.24_14:44	18.02.24_02:00	13º56.44N	116º33.30W	4,056
JC257-39	AKS355	UK1	21.02.24_20:44	22.2.24_02:44	24.03.24_11:05	13º49.38N	116º29.62W	4,105
JC257_66	AKS357	UK1	27.02.24_15:46	27.02.24_23:46	01.03.24_18:00	13º49.38N	116º29.62W	4,095
JC241_95	AKS360	UK1	05.03.24_09:50	05.03.24_17:50	09.03.24_09:08	13º06.64N	116º05.03W	4,141

Andrew Sweetman and Mark Hartl monitoring the fish trap. Photo by Daniel Jones.

2.9. Moorings - Long Term

2.9.1. Moorings - Equipment

Three moorings were deployed at UK-1 to the south of Area of interest 2 (AOI-2) during James Cook Expedition JC241 (last year) (Figure 2.9.1). 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 (see Figures 2.9.2 to 2.9.5).

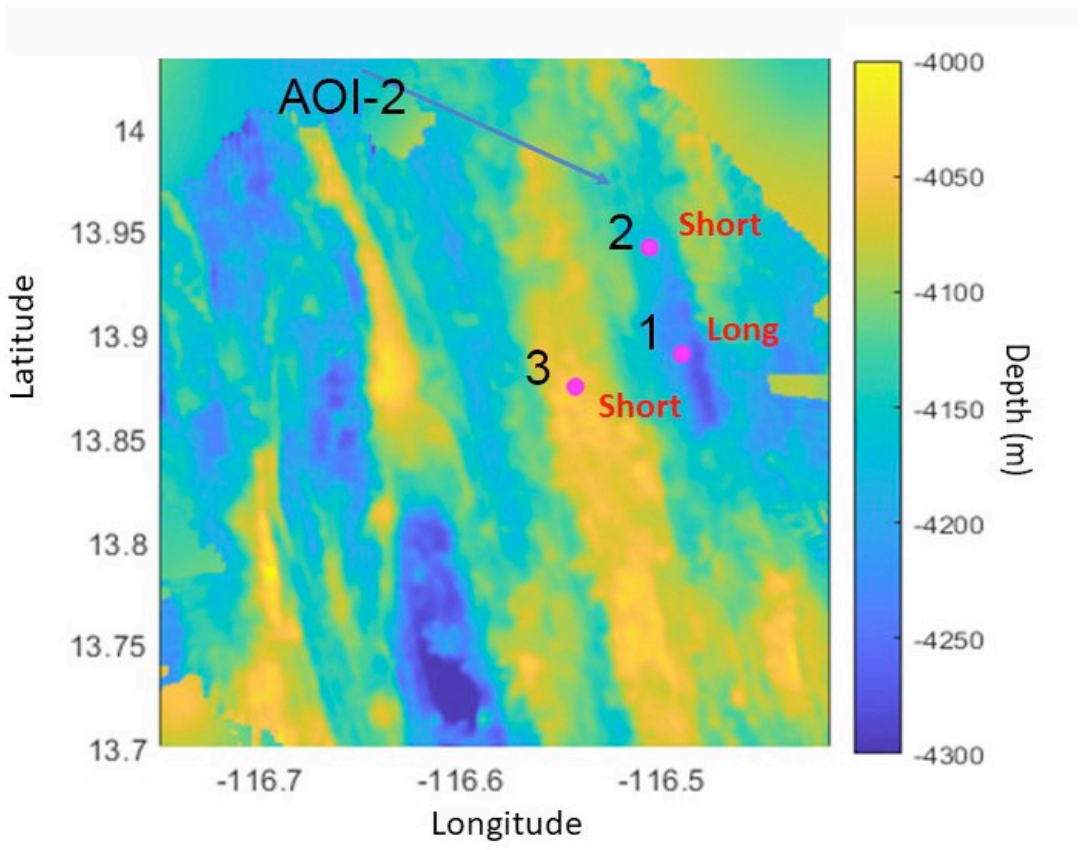


Figure 2.9.1 Location of moorings.

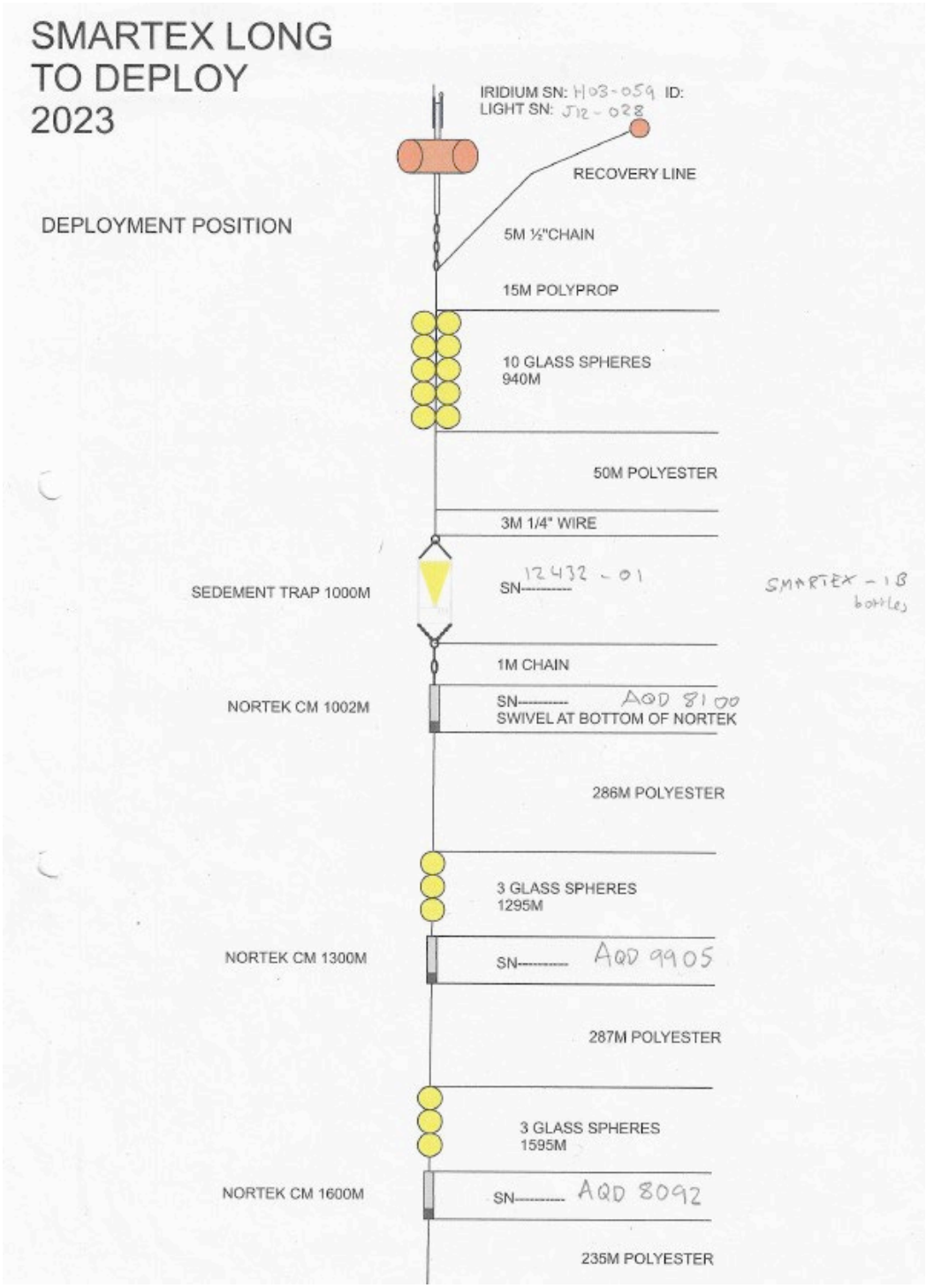


Figure 2.9.2 Diagram of Mooring 1 (long) top half.

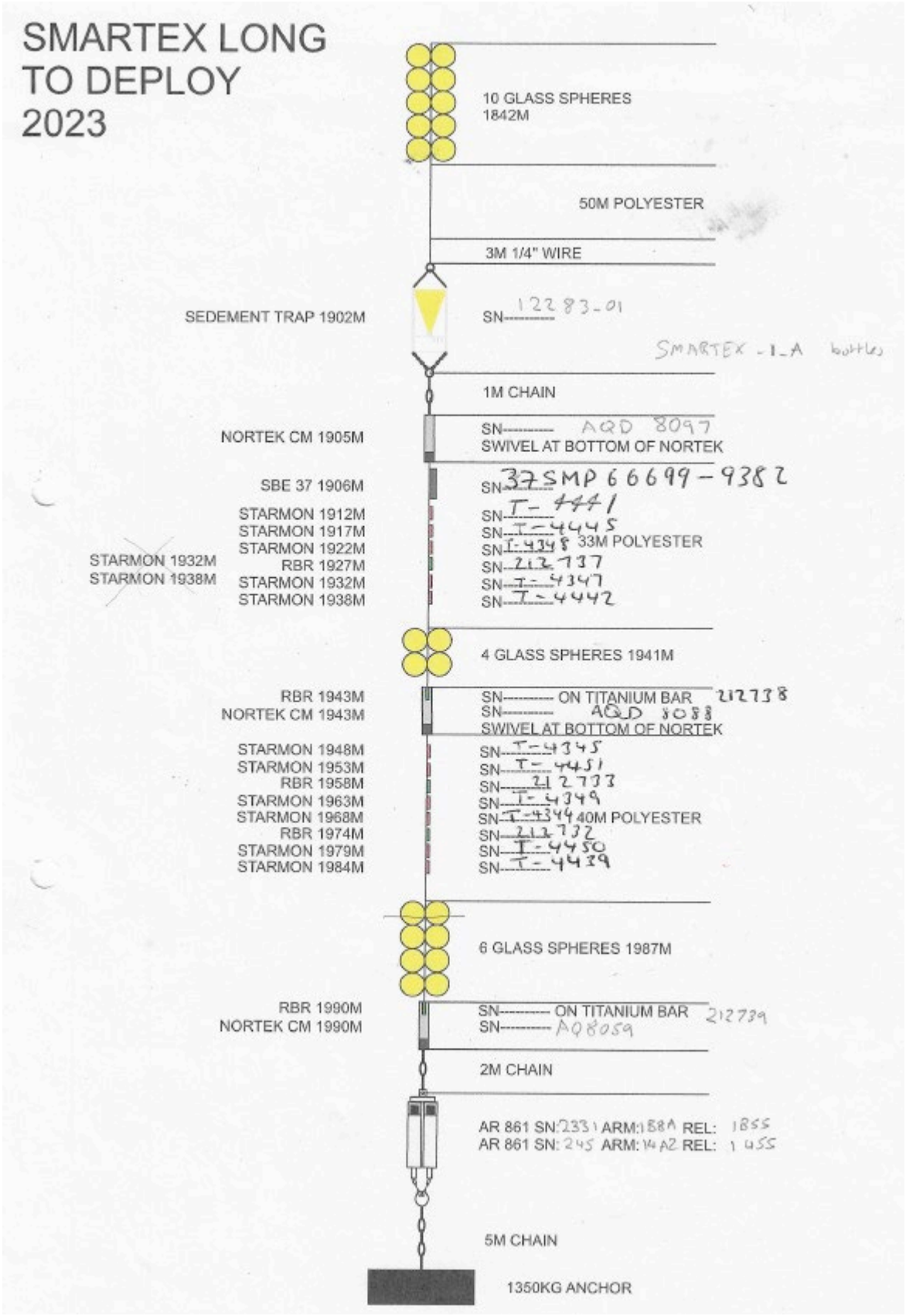


Figure 2.9.3 Diagram of Mooring 1 (long) bottom half.

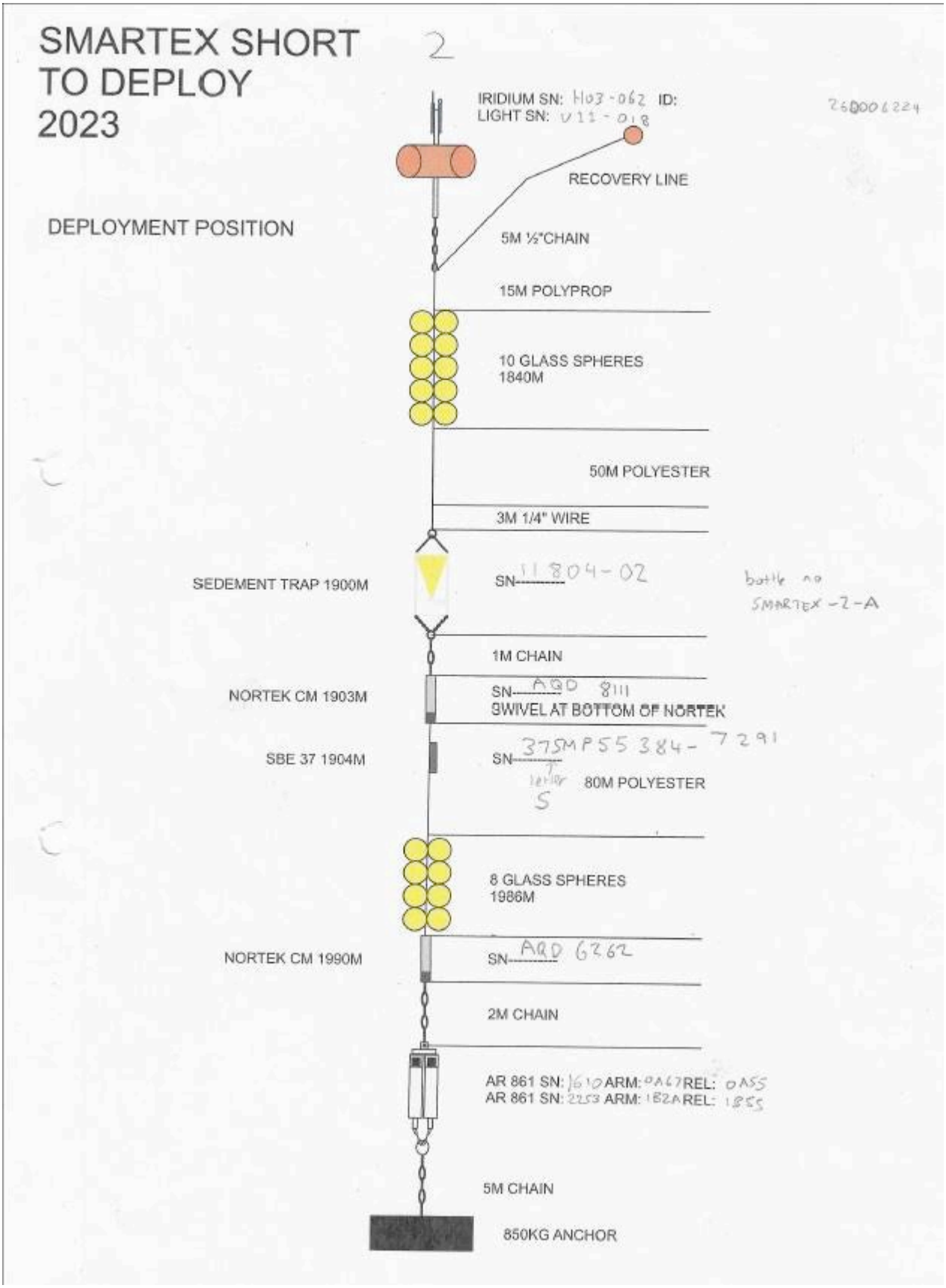


Figure 2.9.4 Diagram of Mooring 2 (short).

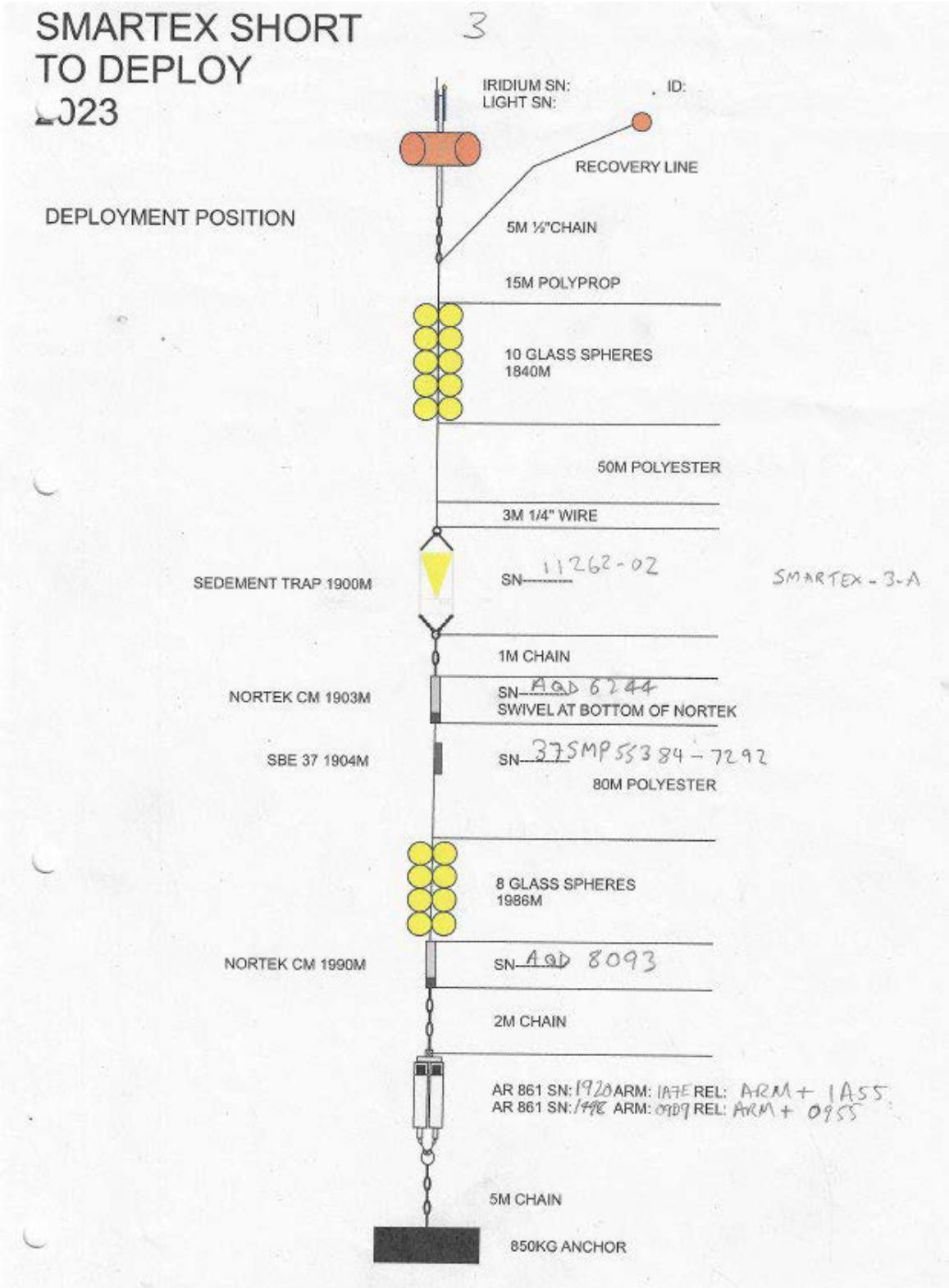


Figure 2.9.5 Diagram of Mooring 3 (short).

2.9.2. Sediment traps on moorings

Table 2.9.1 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 intervals. 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 2.9.2 Deployment details for all sediment traps on JC241. Trap A used as an example for the numbering system.

Number	Programmed 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
The final move to open the hole	11/03/24

2.9.3. Mooring Deployment and Recovery

Table 2.9.3 Deployment details for moorings.

Mooring	Location (decimal degrees)	Depth, m	Deployment Date and Time (UTC)	Recovery Date and Time (UTC)
SMARTEX 1 (Long)	116.49000 W 13.89000 N	4222	15/03/2023 19:30:00	12/03/2024 18:08:00
SMARTEX 2 (Short)	116.50626 W 13.94164 N	4187	15/03/2023 15:51:00	02/03/2024 22:57:00
SMARTEX 3 (Short)	116.54320 W 13.87421 N	4051	15/03/2023 21:39:00	02/03/2024 00:08:00

Mooring	JC241 Station Number	JC257 Station Number
SMARTEX 1 (Long)	JC241_093	JC257_120
SMARTEX 2 (Short)	JC241_092	JC257_084
SMARTEX 3 (Short)	JC241_094	JC257_079

Moorings were given station numbers for deployment and recovery (Table 2.9.3) these are different numbers (to make sure they were logged correctly on both expeditions) but refer to the same moorings.

Mooring recovery

Both short moorings were recovered untangled and as expected.

The long mooring had some tangling in the upper sections (visible in Figures below).

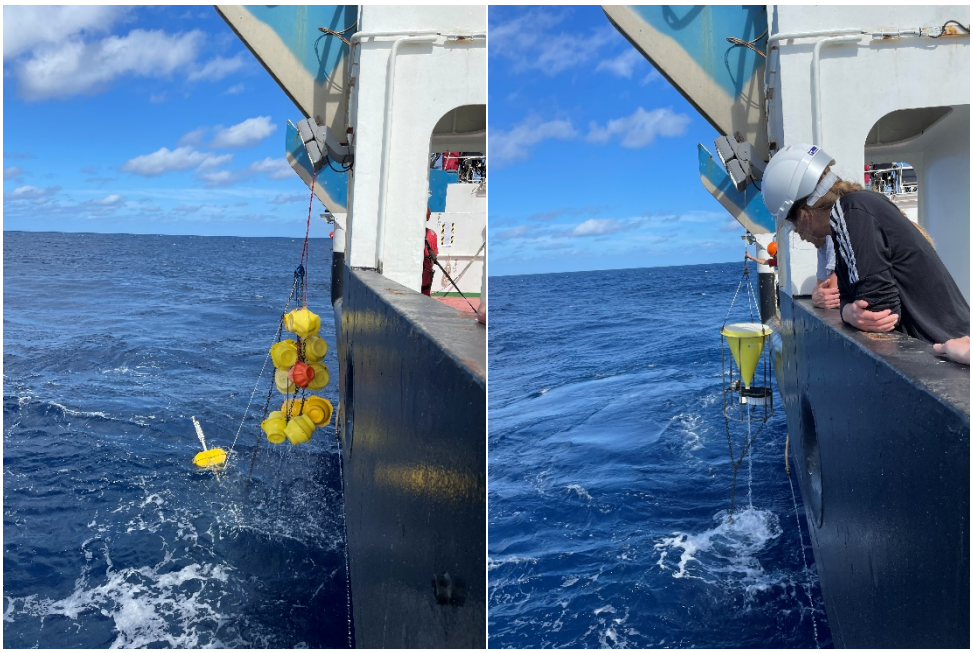


Figure 2.9.6 Photographs of recovery of upper section of JC257_120. Left: Photo taken on 12/03/2024 09:04 showing tangled top section buoyancy (10 glass spheres), recovery line, pellet buoy and flag. Right: Photo taken on 12/03/2024 09:14 of 1000 m sediment trap (SN 12432_01) with water pouring from open hole between bottles, showing bottle rotation completed. The sediment trap itself was not tangled in other equipment.

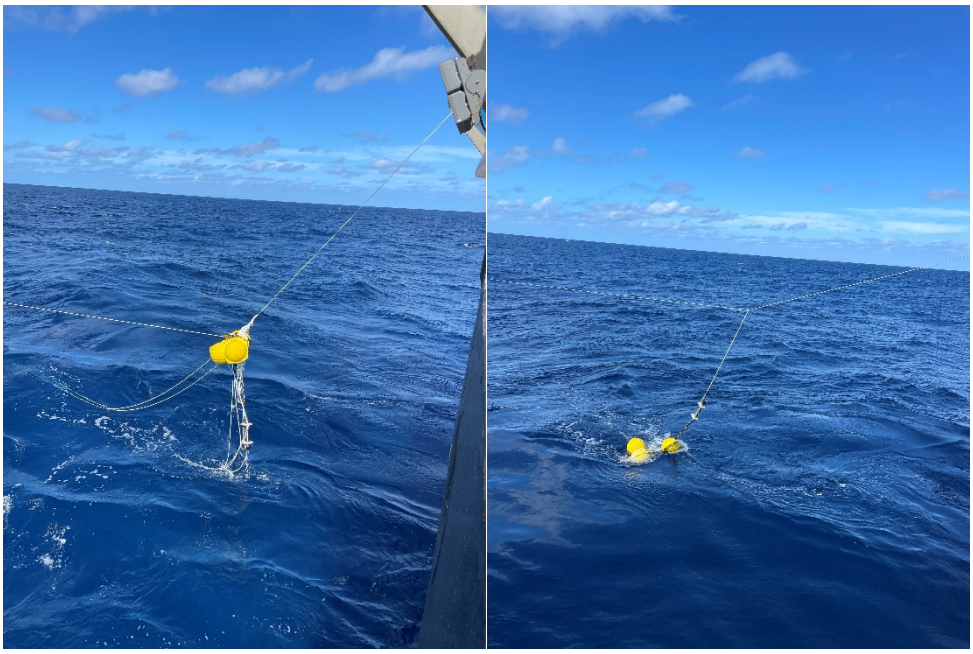


Figure 2.9.7 Photographs of recovery of mid section of JC257_120. Left: Photo taken on 12/03/2024 09:25 showing tangled mid section buoyancy (3 spheres below 1000 m sediment trap – these are the upper 3 spheres) and Nortek current meter (SN AQD9905). Right: Photo taken on 12/03/2024 09:37 showing lower mid section buoyancy (lower 3 glass spheres) and nortex current meter (SN AQD 8092) with twist in rope, likely generated on recovery.

Figure 2.9.8 Photographs of recovery of mid section of JC257_120. Left: Photo taken on 12/03/2024 09:49 showing untangled lower section of 10 glass spheres (above 100 m sediment trap). Right: Photo taken on 12/03/2024 09:52 showing 100 m sediment trap (SN 12283_01) with water pouring from open hole between bottles, showing bottle rotation completed. All the sections below the 100 m sediment trap were not tangled.

2.9.4. Physical Oceanographic equipment mounted on three moorings

Table 2.9.4 Instrument types, Serial Numbers and position on a mooring rope.

Instrument sequential No and type	Instrument Inventory / Model Number	S/N	Height, m above seabed	Comments after recovery
Long Mooring 1				JC257_120
1. Sediment Trap B	SN 12432-01		956	SMARTEX-1-B

2. AquaDopp 2MHz DW	NORTEK CM 1002M	8100	954	
3. AquaDopp 2MHz DW	NORTEK CM 1300M	9905	667	Rope partially entangled below glass spheres
4. Aquadopp 2MHz DW	NORTEK CM 1600M	8092	379	3m of the rope twisted
5. Sediment Trap B 1902M	12283-01		88	SMARTEX-1-A
6. AquaDopp 2MHz DW	NORTEK CM 1905M		87	Currents magnitude exceeds 5 times in comparison to other instruments, while signal shape preserved
7. SBE37SM-RS232-1906M	55384-9382	9382	86	Pressure sensor drift +50 db was detected when SBE37S had been mounted on a rosette 1m apart of SBE9+ at station CTD-18, JC257_121.Averaged depth = 3953 m (instead of expected 4136 m)
8. STARMON 1906M	SN T-4441		80	
9. STARMON 1912M	SN T-4445		75	
10. STARMON 1917M	SN T-4348		70	
11. RBR 1927M	SN T-212737		65	
12. STARMON 1932M	SN T-4347		60	
13. STARMON 1938M	SN T-4442		55	
14. RBR 1943M	SN T-212738		51	
15. AquaDopp 2MHz DW	NORTEK CM 1943M		50	Large vertical velocity
16. STARMON 1948M	SN T-4345		45	
17. STARMON 1953M	SN T-4451		40	
18. RBR 1958M	SN T-212733		35	
19. STARMON 1963M	SN T-4349		30	
20. STARMON 1968M	SN T-4344		25	
21. RBR 1974M	SN T-212732		20	
22. STARMON 1979M	SN T-4450		15	
23. STARMON 1984M	SN T-4439		10	
24. RBR 1990M	SN T-212739		9	
25. AquaDopp 2MHz DW	NORTEK CM 1943M	8059	8	
Short Mooring 2				JC257_084
1. Sediment Trap 1900M	11804-02		100	SMARTEX-2-A
2. AquaDopp 2MHz DW	NORTEK CM 1903M		92	Too high currents magnitude (horizontal and vertical)
3. SBE37SM-RS232-1904M	55384-7291	7291	91	Minor (~3db) drift of pressure sensor since deployment, averaged depth = 4084 m
4. AquaDopp 2MHz DW	NORTEK CM 1990M	6262	8	
Short Mooring 3				JC257_079
1. Sediment Trap 1900M	11262-02		100	SMARTEX-3-A
2. AquaDopp 2MHz DW	NORTEK CM 1903M	6244	92	
3. SBE37SM-RS232-1904M	55384-7292	7292	91	averaged depth = 4074 m
4. AquaDopp 2MHz DW	NORTEK CM 1990M	8093	8	

The sample interval for Seabird SBE37SM systems was set equal to 600 seconds. The latest calibration details for each sensor of all three SBE37SM- RS232 instruments (S/N 55384-7292, 55384-7291, and 55384-9382) are recorded in the internal configuration files (such as SBE37SM-RS232_03707291_2024_03_03.xmlcon), that are required for conversion of raw output using SBE Data processing V.7.26.7 software. Certificates of calibration are included in supplementary materials (JC257_Cruise_Report_Appendix_2.9.1).

Figure 2.9.9 Instruments from Mooring 3 (short): two AquaDopp 2MHz DW NORTEK CM # 6244, 8093 and SBE37SM-RS232-1904M 55384-7292 after recovery.

Figure 2.9.10 Instruments from Mooring 1 (Long): Starmon mini Star ODDI (left) and RBR Solo Tdeep (right) temperature recorders after recovery. Photo by Thomas G Dahlgren.

Billy Platt and Mark Squibb recovering mooring equipment on JC257. Photo by Daniel Jones.

2.10. Moorings - Bathysnaps

2.10.1. Bathysnap equipment

Bathysnap is composed of a pressure case containing the computer (Jetson nano 2GB) and the battery. 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 2.10.1). The computer operates a flashlight with an adjustable pitch and a 'Blackfly' camera, being the same model as the 'AESA2' camera of the *Autosub*5. The same process for image post-processing was applied as for AUV surveys (section 2.2.3).

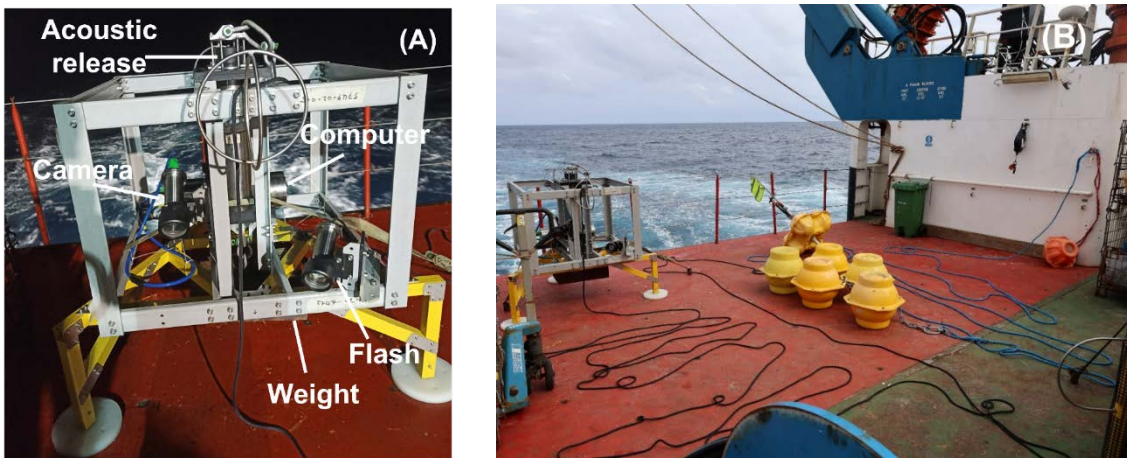


Figure 2.10.1 (A) Close-up picture of the Bathysnap deployed on 12 February 2023, (B) Picture of the Bathysnap mooring ready to be deployed on 12 February 2023.

The bathysnap systems both used FLIR Blackfly S BFS-PGE-200S6C cameras with Computar V1224-MPZ 16mm lenses. The aperture (F-stop: F8) and focus was set mechanically on the lens. The shutter speed/exposure time was set in the control software to 3ms. This is the shortest period of time allowed by the control software. The Camera Settings in the software were BayerRG16, ADC 12bit, 20MP, 17.03dB Gain.

The pitch of the camera was set to 30˚ (Figure 2.10.2). The flash was deployed horizontally.

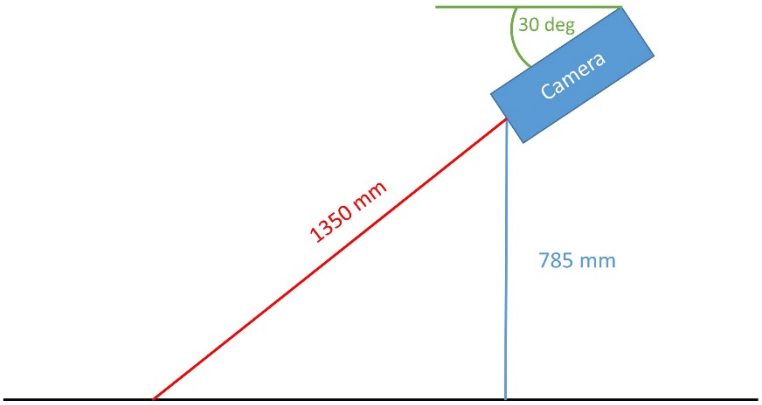


Figure 2.10.2 Offset and angle of the Bathysnap camera.

Four recoveries and two deployments were carried out. Firstly, they were deployed during JC241 and recovered during JC257, to acquire images every day for a year (Table 2.10.1). For JC257-009, twelve minutes were needed to effectively communicate the release ping (19:44 UTC). JC257-010 immediately released the mooring (24:52 UTC). Ascent rates ranged from 45 to 55 $m.min^{-1}$. Forty-five minutes were needed for recovery after surfacing. Batteries were changed and data retrieved before another deployment. Four days later, both Bathysnap landers were deployed once again to acquire seabed images every 15 minutes till the end of JC257.

Table 2.10.1 Details of Bathysnap deployment/recovery on JC257.

Station	Date, Time (UTC)	Deployment position	Deployment sounding	Date, Time (UTC)	Recovery position	Recovery sounding	Imaging period
JC241-100 JC257-009	18/03/2023 22:00	13˚54.86'N 116˚31.26'W	4100 m	15/02/2024 21:44	13°53.50'N 116°30.52'W	4185 m	1 day
JC241-099 JC257-010	18/03/2023 22:36	13˚53.27'N 116˚30.59'W	4146 m	16/02/2024 00:26	13°54.85'N 116°31.51'W	4095 m	1 day
JC257-022	19/02/2024 03:11	13°54.87'N 116°31.22'W	4106 m	12/03/2024 20:34	13°55.08'N 116°31.38'W	4094 m	15 min
JC257-023	19/02/2024 03:53	13°53.26'N 116°30.60'W	4150 m	13/03/2024 00:57	13°53.92'N 116°30.77'W	4158 m	15 min

2.11. Underway Instrumentation

2.11.1. Vessel-mounted Acoustic Doppler Current Profiler (VM-ADCP)

The James Cook features two Teledyne RD Instruments Ocean Surveyor VM-ADCPs mounted on the port drop keel and operating at 75 kHz and 150 kHz respectively (transducers s/n 671883 and 34). Both instruments were configured to ping in narrowband (nb) mode starting at 12:35:15 UTC on 2024/02/08. Separate deck units (1587 and 1815) were used exclusively for UHDAS data acquisition systems and processing operations for each instrument separately. The data acquisition program was based on the latest UHDAS+CODAS software (*Common Oceanographic Data Access System)*. Obtained ADCP data were processed in real-time with Python 3.8 and C scripts, which run with the following parameters, summarised in Table 2.11.1. The 'pycurrents' and other necessary software packages and detailed documentation are available at the web site https://currents.soest.hawaii.edu/docs/adcp_doc/codas_doc/index.html.

Table 2.11.1 VM-ADCP UHDAS acquisition and processing settings for both sonars.

	VM-ADCP PARAMETERS	os150	os75
BT	bottom track mode (on or off)	off	off
SI	sampling interval or averaging period for ensemble (sec)	300	300
NB	number of bins	50	50
BL	bin length (m)	8	16
TD	transducer depth (m)	6	6
BK	blanking length (m)	4	8
HO	heading offset applied by DAS (deg)	0.33	-6.27
HB	heading bias (deg)	0.00	0.00
GRPH	compensation for roll-pitch-heading, 1 is on	0001	0001
dx	xducer_dx (starboard) meters from GPS	0	0
dy	xducer_dy (fwd) -"-	10	5

Binary raw VM-ADCP data products were scattered throughout the UHDAS cruise directory, and useful for scientist data were in the individual sonar processing directories, were accessible at mounted shared drive ..\cruise_data\Ship_Systems\Data\Acoustics\ADCP\
in a folder /JC257/proc/os75nb:

VM-ADCP data	files to use
every 5 min averaged bin profile (matlab)	contour/allbins_*.mat
coarse resolution averaged ocean velocity	vector/vect*.mat
moderate resolution averaged ocean velocity	contour/cont*.mat
every bin profile (netCDF)	contour/*.nc
every bin and profile (CODAS database)	adcpdb/a*.blk
daily figures from the cruise	png_archive/*.png

Data return quality was generally good, with ranges of 300 m (for the os150) to 400-500 m (for the os75) as expected in the Eastern Tropical Pacific area, while manual editing was not required. The 'gyro' was assigned as the main source for heading data, which is more accurate than 'posmv', which derives gyro between GPS readings. Nominal heading alignment angles did not change and remained 0.33º for os150 since May 2021 and -6.27º for os75, which was replaced in Jan 2022.

Bottom tracking data were not acquired either during the first and the last parts of the cruise on the transits to and from the port as the Central America shelf is very narrow and remains entirely within regional EEZ. Due to the absence of bottom track records, a water column offset calibration track was performed at the beginning of the cruise. On 2024/02/08 from 15:00 till 17:00 UTC we collected VM-ADCP data statistics for misalignment corrections and verification of the acquired data validity. Dogleg manoeuvre results demonstrate general agreement between currents derived along and across ship tracks in both 075 and 150 kHz records (Fig 2.11.1)

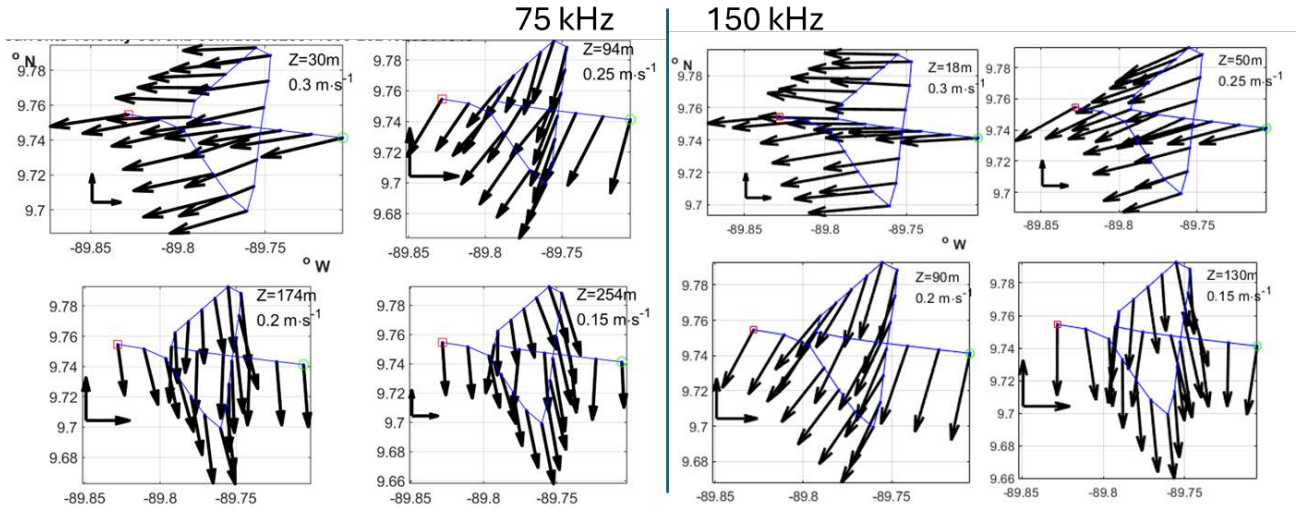


Figure 2.11.1 Averaged (5 minutes) currents strength and direction at different layers derived from Teledyne RDI os75(left) and os150 kHz (right) VM-ADCP during the experiment, which was designed to evaluate currents along and across perpendicular ship passages (10 km each) on 8th February 2024, 15:00-16:45 UTC. Note that average bin depths and scale arrows are different at each panel.

Multiple deployments of acoustically highly reflective instruments such as CTD, BC or ROV, and stable ship dynamic positioning enable water track sampling collection for heading corrections all over the cruise (Fig.2.11.3).

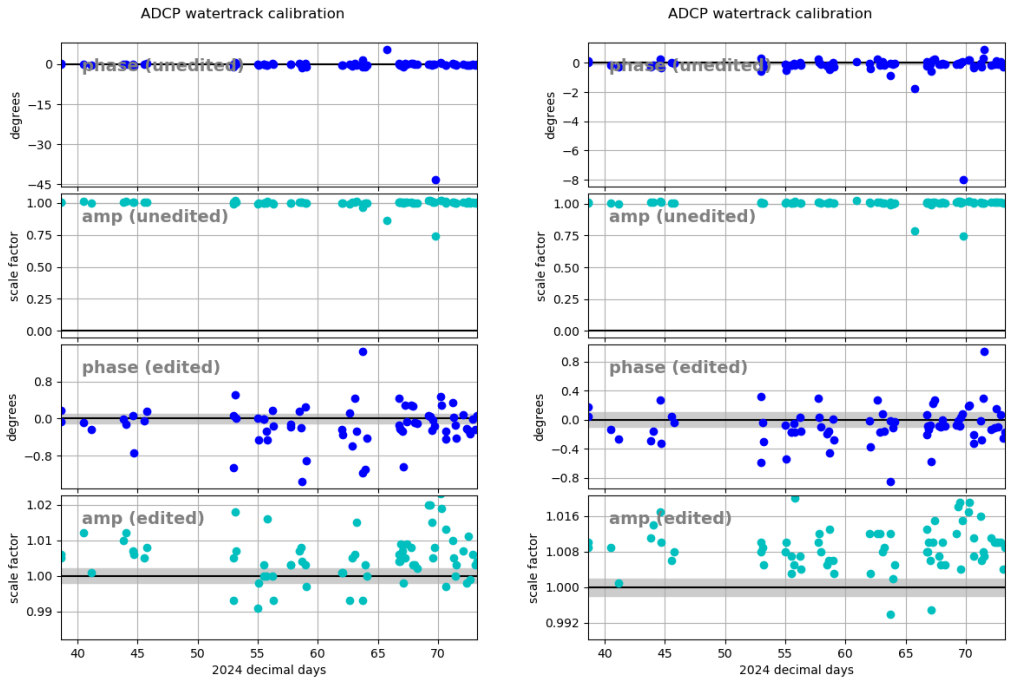


Figure 2.11.2 VM-ADCP water track calibration statistics for os150 (left), and os75(right) during the JC257 cruise.

Underway measurements of the atmospheric (wind, air temperature etc) and the upper-layer oceanographic parameters (Temperature, Salinity) were also collected during the JC257 cruise with the instruments listed in Table 2.11.2.

Calibration of underway surfmet Thermo-Salino Graph TSG (SBE45, s/n 0233) data was performed on 16th of March at 15:45-20:18 local time (UTC-7) with bath Temperature 21°C and using Autosal Salinometer 8400B S/N 68426. All 3 crates had 24 salinity samples in each: crate #10, samples TSG200-TSG223, #11, TSG225-TSG247 and crate #4, tsg73-tsg89. Sea water samples were collected daily during the JC257 cruise. The offset in conductivity was found equal 0.000017 mS/cm in all tests.

Table 2.11.2 Underway atmospheric and oceanographic sensors deployed on RRS James Cook for JC257 cruise.

Niche	Manufacturer	Sensor	Serial Number	Cal.	Last Cal Date	Cal Due Date
Wind Speed & Dir, Port (NMEA)	Gill	Windsonic	064537		Tested 25 Jan 2021	N/A
Wind Speed & Dir, Starboard (Analog to SM)	Gill	Windsonic	064538		Tested 17 Jan 2021	N/A
Air Temp & Humidity	Vaisala	HMP155	N1211118	No	14 Jun 2023	13 Jun 2024
Air Pressure	Vaisala	PTB110	J0710002	No	14 Jun 2023	13 Jun 2024
Surf Fluorometer	WetLabs	WS3S	WS3S-117	No	10 May 2023	09 May 2024
Surf Transmissometer	WetLabs	CST	CST-1132PR	No	09 Jun 2022	08 Jun 2024
Surf Temperature	SeaBird	SBE38	0490	Yes	14 Jul 2023	13 Jul 2024
Surf Temperature (Drop Keel)	SeaBird	SBE38	0476	Yes	14 Jul 2023	13 Jul 2024
Surf TSG	SeaBird	SBE45	0233	Yes	21 Jun 2022	20 Jun 2024

Additional Underway data collection was performed with two extra CTD units deployed on a Remote Operating Vehicle ISIS ROV and AutoSubmarine. Calibration and configuration details of the sensors of SBE 49 Fastcat CTD unit mounted on ISIS ROV (left) and SBE 911plus/917plus CTD mounted on AutoSub in JC257 are summarised below in Table 2.11.3. Raw data from both CTDs and the coordinates and time stamps of the vehicles motion underwater were kindly provided by members of engineering teams (Emre and Eoin) in a separate files.

Table 2.11.3 Underway atmospheric and oceanographic sensors deployed on RRS James Cook for JC257 cruise.

ISIS ROV CTD
SBE 49 Fastcat CTD
Dive431\240213_SBE49_0279.xmlcon

Scans to average : 1
NMEA position data added : No
NMEA depth data added : No
NMEA time added : No
Surface PAR voltage added: No
Scan time added : Yes

1) Count, Temperature

Serial number : 0279
Calibrated on : 31-Aug-2023
A0 : 1.14042100e-003
A1 : 2.04050900e-004
A2 : 5.24201600e-006
A3 : -4.65998800e-008
Slope : 1.00000000
Offset : 0.0000

2) Frequency 0, Conductivity

Serial number : 0279
Calibrated on : 31-Aug-2023
G : -9.79706700e-001
H : 1.51039900e-001
I : -4.31462800e-004
J : 5.66439500e-005
CTcor : 3.2500e-006
CPcor : -9.57000000e-008
Slope : 1.00000000
Offset : 0.00000

3) Count, Pressure, Strain Gauge

Serial number : 0279
Calibrated on : 31-Aug-2023
PA0 : 5.86441200e-001
PA1 : 2.97824300e-002
PA2 : 1.77056700e-009
PTEMPA0 : -8.57405700e+001
PTEMPA1 : 4.22068500e+001
PTEMPA2 : 9.04039300e-001
PTCA0 : 5.24474700e+005
PTCA1 : -6.15409200e+000
PTCA2 : 2.51413800e-001

AutoSub CTD
SBE 911plus/917plus
AS5M084-ctd\dy166v002.xmlcon

Frequency channels suppressed : 0
Voltage words suppressed : 0
Computer interface : RS-232C
Deck unit : None
Scans to average : 1
NMEA position data added : Yes
NMEA depth data added : No
NMEA time added : Yes
NMEA device connected to : PC
Surface PAR voltage added : No
Scan time added : No

1) Frequency 0, Temperature
Serial number : 5009
Calibrated on : 2022-07-20
G : 4.35574440e-003
H : 6.39067870e-004
I : 2.24783510e-005
J : 2.08593650e-006
F0 : 1000.000
Slope : 1.00000000
Offset : 0.0000

2) Frequency 1, Conductivity
Serial number : 2179
Calibrated on : 2022-04-01
G : -1.03941500e+001
H : 1.41501400e+000
I : 1.47915200e-003
J : -1.48247500e-005
CTcor : 3.2500e-006
CPcor : -9.57000000e-008
Slope : 1.00000000
Offset : 0.00000

3) Frequency 2, Pressure, Digiquartz with TC
Serial number : 0930
Calibrated on : 2022-10-17
C1 : -4.228838e+004
C2 : -1.066698e-001
C3 : 1.254770e-002
D1 : 3.442700e-002
D2 : 0.000000e+000
T1 : 2.995984e+001

4) Frequency 3, Temperature, 2
Serial number : 4458
Calibrated on : 2022-04-01
G : 4.34210980e-003
H : 6.36656150e-004
I : 1.93264620e-005
J : 1.36346750e-006
F0 : 1000.000
Slope : 1.00000000
Offset : 0.0000

5) Frequency 4, Conductivity, 2
Serial number : 4308
Calibrated on : 2022-04-01
G : -9.77320700e+000
H : 1.26046700e+000
I : 1.25114500e-003
J : 2.69536500e-006
CTcor : 3.2500e-006
CPcor : -9.57000000e-008
Slope : 1.00000000
Offset : 0.00000

6) A/D voltage 0, Free
7) A/D voltage 1, Free

8) A/D voltage 2, Oxygen, SBE 43
Serial number : 2451
Calibrated on : 20-Jul-16
Equation : Sea-Bird
Soc : 5.81000e-001
Offset : -5.15100e-001
A : -3.62520e-003
B : 1.63160e-004
C : -2.51920e-006
E : 3.60000e-002
Tau20 : 1.70000e+000
D1 : 1.92634e-004
D2 : -4.64803e-002
H1 : -3.30000e-002
H2 : 5.00000e+003
H3 : 1.45000e+003

9) A/D voltage 3, Free ;

PTCB0 : 1.02795800e+002 PTCB1 : -8.95855000e-003 PTCB2 : 0.00000000e+000 Offset : 0.000000 Scan length : 30	T2 : -3.101915e-004 T3 : 3.530880e-006 T4 : 5.370900e-009 T5 : 0.000000e+000 Slope : 1.00009630 Offset : -4.14166 AD590M : 1.281300e-002 AD590B : -9.235760e+000	10) A/D voltage 4, Free 11) A/D voltage 5, Free ; 12) A/D voltage 6, Free 13) A/D voltage 7, Free Scan length : 41

2.11.2. Shipboard multibeam

The RRS James Cook has a Kongsberg EM122 hull mounted system for deep water mapping. It is a 12kHz system with 512 beams, a 150° swath width was used with equidistant beam sampling. Datafiles were divided into 2 hour passes. Sound velocity profiles were applied from CTD casts. The parameters for JC257 were:

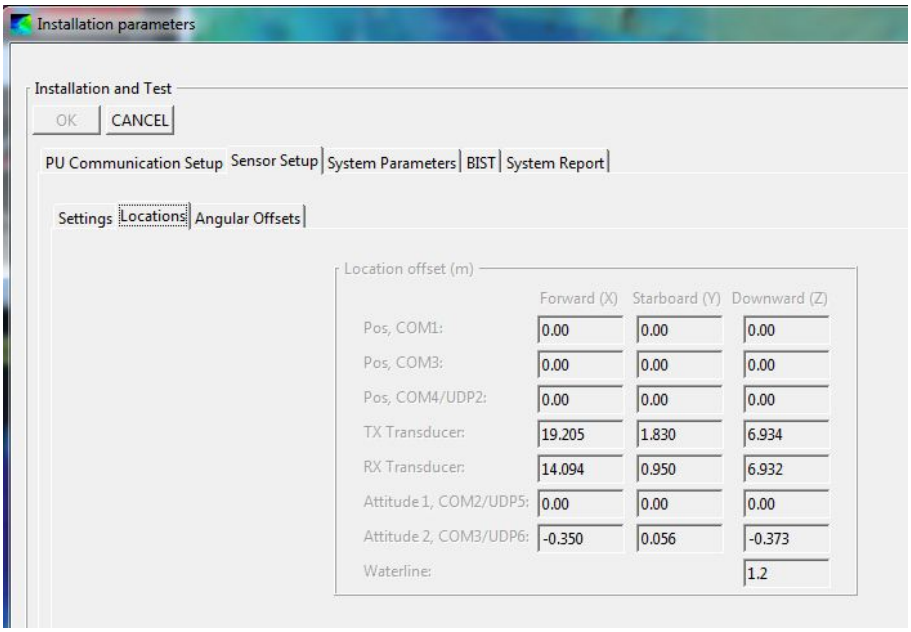


Figure 2.11.3 Kongsberg EM122 Configuration

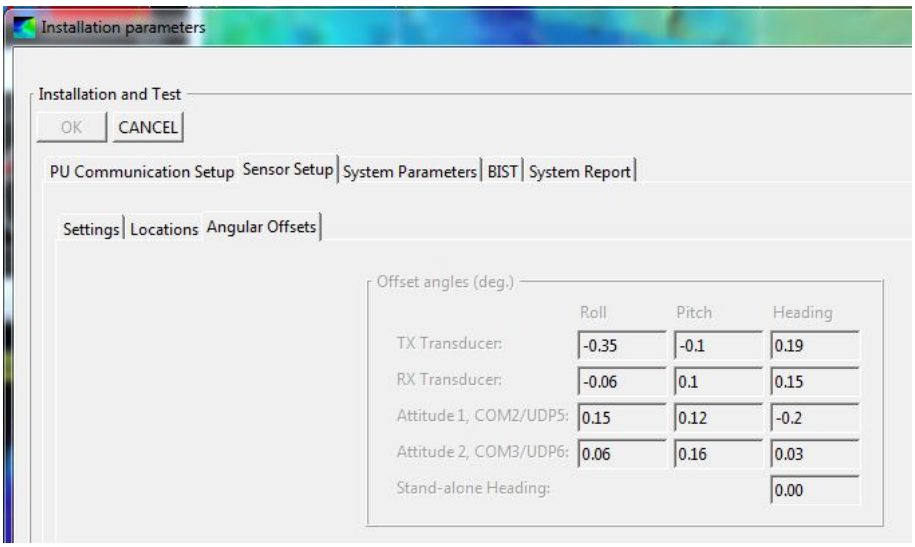


Figure 2.11.4 Kongsberg EM122 Angular Offsets

3. Science Team Protocols and Summary Reports

3.1. National Oceanography Centre - Geophysical Survey

3.1.1. Shipboard Multibeam

Multibeam data acquisition began once we left the Costa Rican EEZ. A marine mammal (MMO) watch was done before soft starting the multibeam system over 20 minutes (see Section 2.11). The system remained on throughout the cruise, excluding mooring recovery, where the system was switched off to prevent interference with the beacon communication. Data was only logged when the vessel was at speeds of more than 2 knots. Transits were done at 10-11knots and whenever timing allowed, additional multibeam lines were planned to compliment existing bathymetry data (Figure 3.1.1). Routes to and from Costa Rica were designed to run parallel to JC241 tracks, except where Glider/ CTD deployment or ship time limitations did not allow.

The multibeam and backscatter data was recorded in the Kongsberg ".all" format. Caris Hips and Sips v10.4 was used for processing the data, with a zero tide file. Backscatter was collected and viewed in Caris Hips and Sips v11.4. Data quality was dependent on weather and heading.

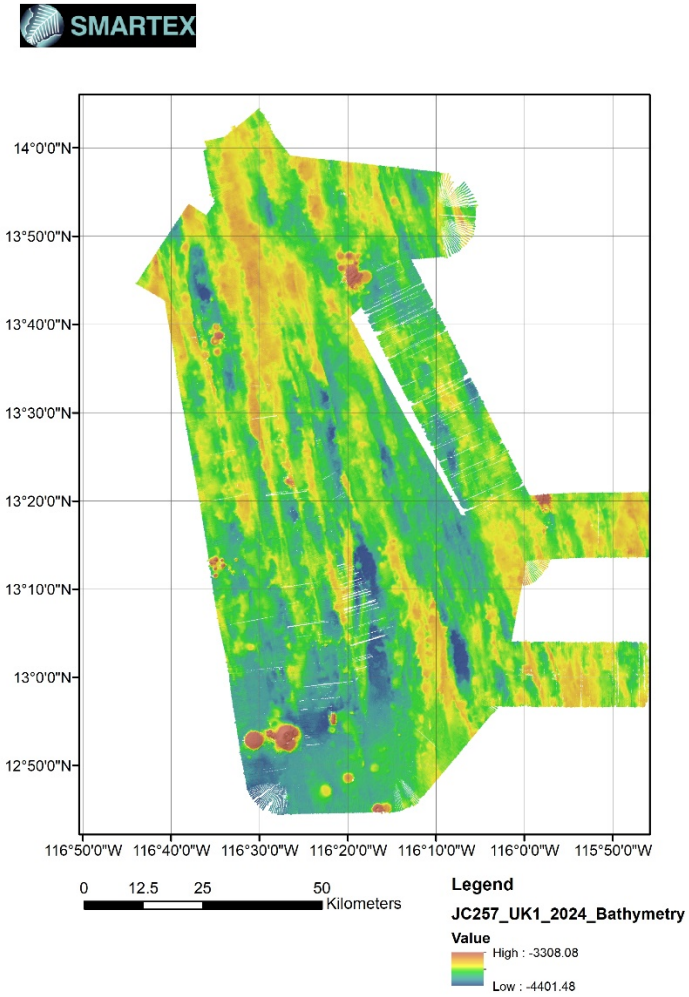


Figure 3.1.1 2024 Shipboard EM122 multibeam bathymetry collected during JC257

3.1.2. AUV Multibeam and Backscatter

Each site (0, 16, 30 and 100km) was mapped using the AUV Norbit system. Navigation data was sent to the NORBIT system from the AUV SprintNav throughout the dive. Where time allowed, the ship would remain in the area of the AUV descent and send a final USBL position once the AUV had reached the seabed. Following the dive, improved position and motion navigation was generated using the final position of the AUV for forwards and backwards post processing in Janus software.

The data .log files were exported from the Norbit GUI as a .s7k file. Due to the size of the .s7k files, files containing only MBES data were exported separately, and files containing MBES and backscatter snippets were exported in sections. The improved navigation was then injected to the .s7k files using a python script. These files were imported to SonarWiz, where the pressure depth was applied and merged. Files were split to remove AUV descents and ascents. Noise was minimal and so little cleaning was required. Tide was applied (see section 3.2. Moorings for tide data generation process) and bathymetry grids of 1, 3 and 5m resolution were created and exported to GIS software to assist planning of subsequent deployments (AUV camera, ROV and coring locations). Backscatter data was exported in 50cm grids.

AUV bathymetry for the 0-1km site existed from cruise RC01 on the Pacific Constructor (Ocean Infinity). This dataset, alongside the JC257 AUV dataset will be used to assess change in the UK1 site. Therefore, 2 missions (M084 and M085) were dedicated to mapping a large area of this (3.2 x 6km). Snippet data was collected alongside each MBES dive, however, M085 had an error, causing the loss of snippet data. Sites 16km and 30km were mapped to support AUV camera missions.

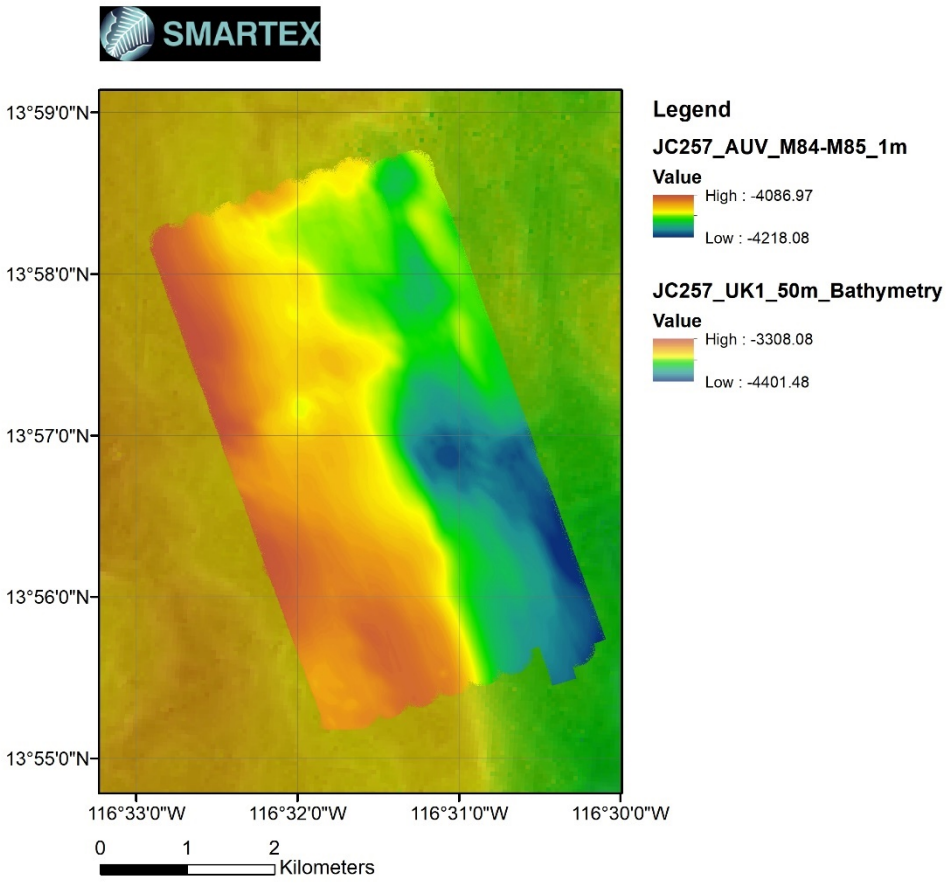


Figure 3.1.2 AUV Norbit bathymetry from AS5M084 and AS5M085 at UK1 0km site.

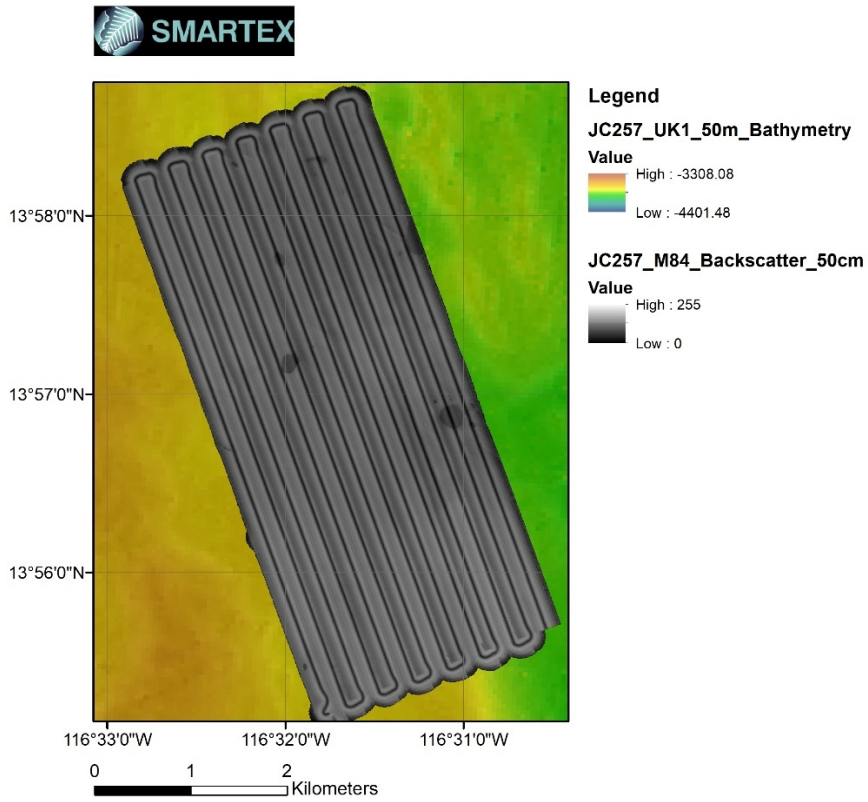


Figure 3.1.3 AUV Norbit backscatter from AS5M084 at UK1 0km site.

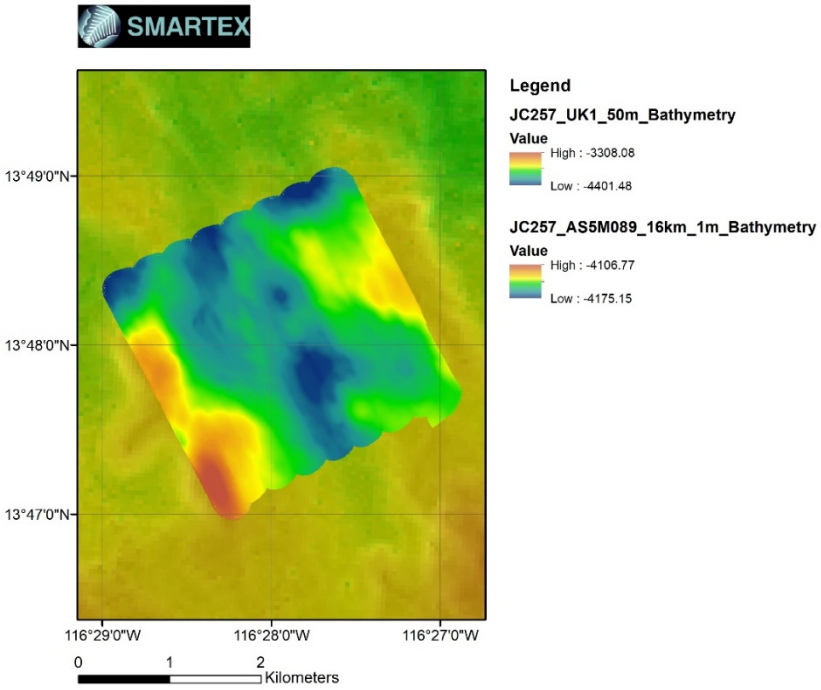


Figure 3.1.4 AUV Norbit bathymetry from AS5M089 at UK1 16km site.

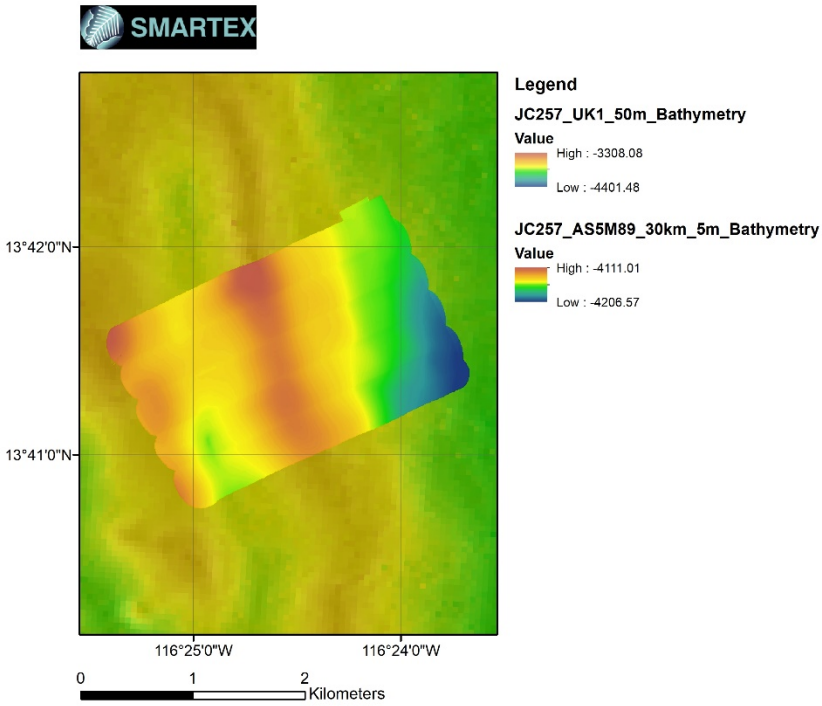


Figure 3.1.5 AUV Norbit bathymetry from AS5M089 at UK1 30km site.

The 100km site is made up of four missions: M092, M093, M094 and M096. M092 had an error during the mission that caused the MBES settings to be set at 100m swath range and auto ping mode. This meant there were data gaps between each line. Due to tight timing constraints it was not possible to send M094 and M096 a final USBL position once the AUV had reached the seabed and so an offset had to be applied to these datasets. M093 was used as the baseline navigation, as this had the highest navigation confidence. Depth contours were created for M093, M094 and M096 and used to tie together the missions.

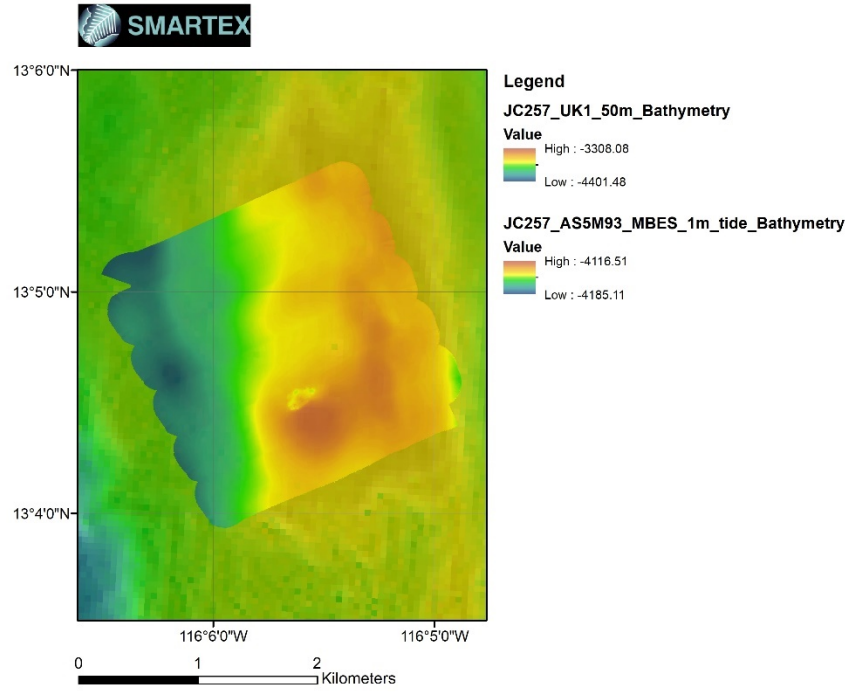


Figure 3.1.6 AUV Norbit bathymetry from AS5M093 at UK1 100km site.

3.1.3. AUV Sidescan Sonar

High- frequency AUV sidescan sonar data was collected as .jsf files. The.jsf files were opened in SonarWiz where bottom tracking and an empirical gain normalisation (EGN) were applied. Grids were exported as 8bit .tifs at 0.5m. An altitude of 15m was used at both the 0 and 100km sites. To assess the robustness of our AI-based habitat mapping techniques, we repeated the 15m altitude SSS mapping at the 0km site (M085 and M087+M091). Due

to battery limitations, M087 aborted before the area was completed and so the final lines were collected on M091. Opportunistic SSS was collected throughout the 3m altitude camera dives at each site.

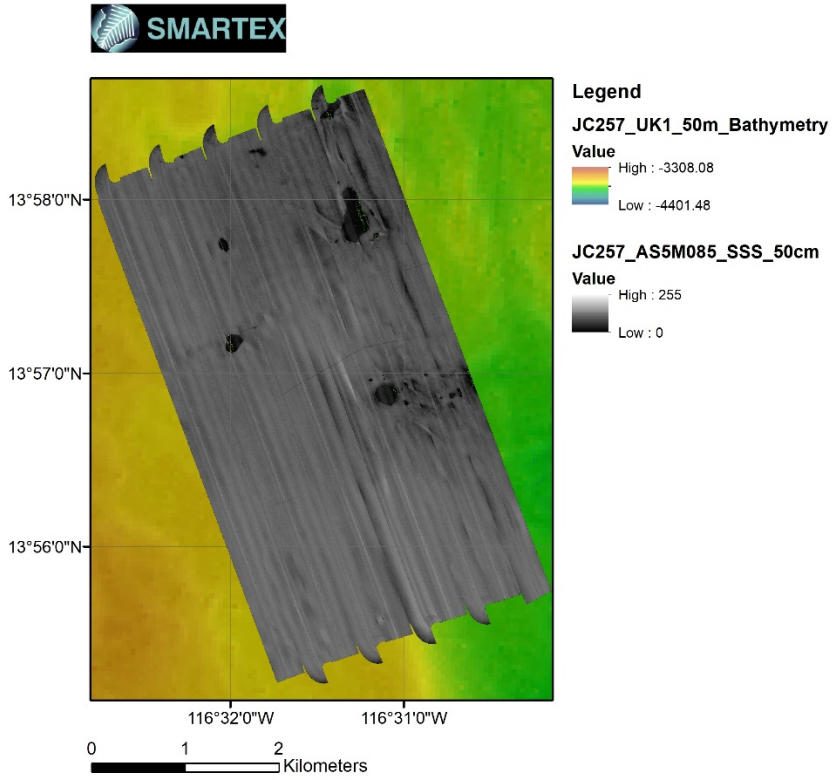


Figure 3.1.7 AUV SSS from AS5M085 at UK1 0km site, 15m altitude.

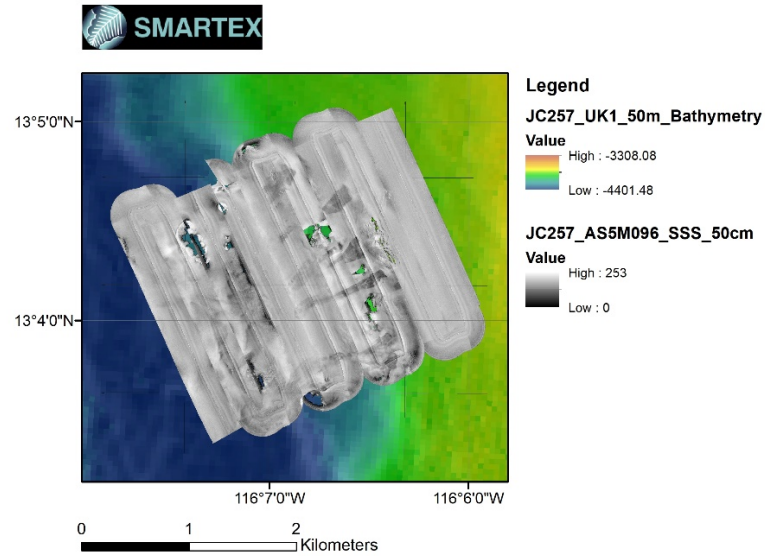


Figure 3.1.8 AUV SSS from AS5M096 at UK1 100km site, 15m altitude.

3.1.4. AUV Sub Bottom Profiler

SBP data were recorded using the EdgeTech2205 Sub Bottom Profiler into .jsf files with navigation incorporated. The produced .jsf files were opened in SonarWiz for a brief check whilst offshore and will be further investigated on return to land.

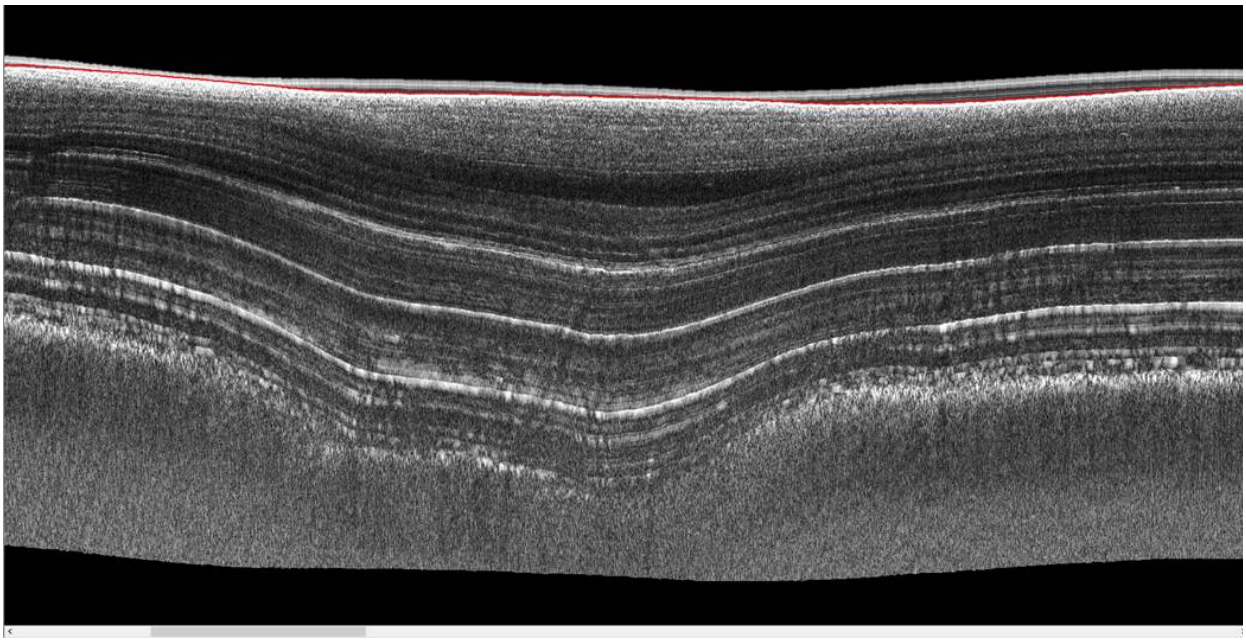


Figure 3.1.9 AUV SBP from AS5M087 at UK1 0km site. 3m altitude with 2-13kHz sweep at 16ms.

3.2. Scottish Association of Marine Science - Physical Oceanography

Dmitry Aleynik

3.2.1. Preliminary overview of the measurements in JC257

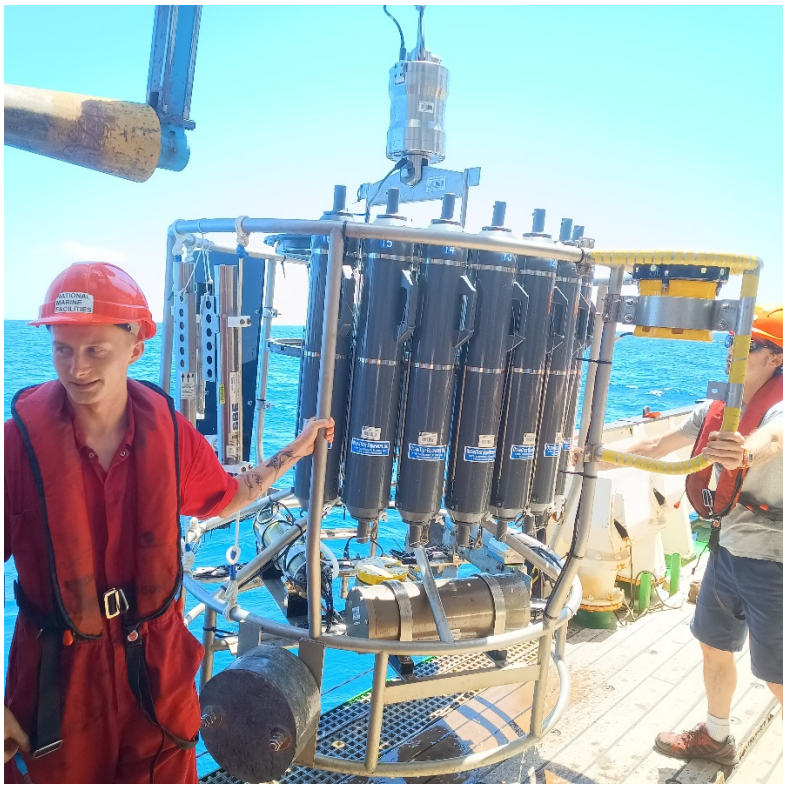


Figure 3.2.1. Ryan Paris and Billy Platt deploying Inter-calibration CTD station 18 with three SBE37SM, recovered from Moorings and attached to the rosette, JC257_120, 13th March 2024. Photo by Dmitry Aleynik.

The main objective of physical oceanographic studies in the JC257 research expedition was to collate a wide range of hydro-physical measurements at the northern part of the UK1 licence area of CCZ, which are required for the following-up development of localised high-resolution 3D ocean circulation model and plume dispersal studies. These data and multibeam bathymetry will provide the initial state of the deep ocean conditions and

boundary forcing to trigger model runs and will be used for model calibration and validation exercises. Chosen measurements strategy and modelling enable us to investigate in detail aperiodic natural and artificial enhancement of the near seabed currents, which affect either positively (via widening larvae spreading, re-colonisation) or negatively (increased burden with plumes of resuspended sediments) every component of unique yet un-impacted benthic communities, starting from microbial, symbiotic organisms which reside on top of Fe-Mn nodules and abyssal clays and up to the very top of the trophic chain and regional megafauna.

Most of the obtained results are considered successful (except the loss of the deep-sea glider), while a set of corrections is expected to be applied to CTD records based on salinity samples analysis and post-cruise calibration upon the ship's return to Southampton, UK. Similarly, post-cruise calibration and intercomparison are expected with thermistors and current meters recovered from moorings.

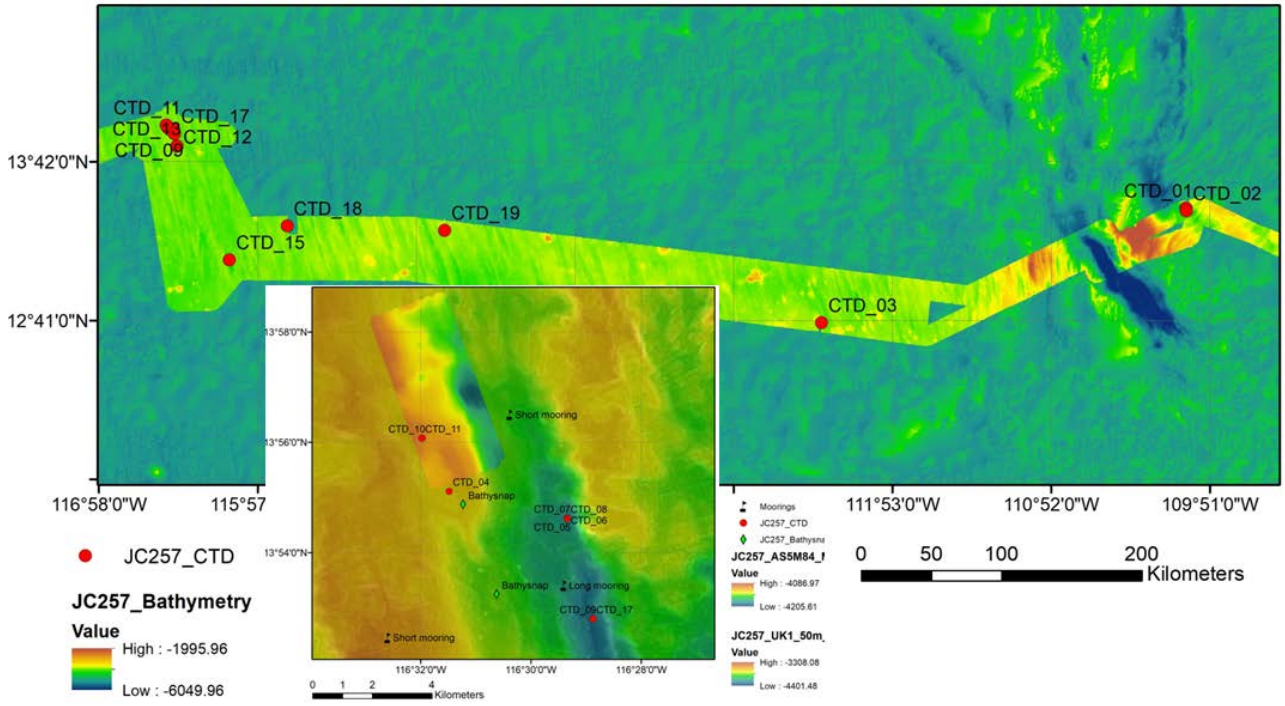


Figure 3.2.2. The position of all CTD stations and moorings in the JC257 cruise is shown overlayed with high-resolution multibeam maps on a transit to and from UK1 study area, also zoomed.

The source of the current enhancement in the near seabed bed is likely linked to Mesoscale eddies passing by the area (*Aleynik et all, 2017*). Perfect candidate eddy for the survey with VM-ADCP, CTD, LADCP and deep seaglider was selected with remote-sense Satellite altimetry data when it approached a tall underwater mountain chain between Euclide (12.7ºN, 110.5ºW), Archimedes (13.3ºN, 110.4ºW), and Fourier Seamounts (14ºN,111ºW). Mesoscale eddy boundary was detected approximately using a relatively low resolution (1/4º or ~27 km grid) products available from the Satellite Altimetry Level 4 resources, such as Sea Level Anomaly relative multiyear (1993-2012) averaged state. Eddy's presence was confirmed by the records of both 75 and 150 kHz Vessel mounted ADCP currents profilers, which show an increase of ocean currents speed in the surface layers and their consistent southward direction near the launch target position, as shown in Fig. 3.2.3.

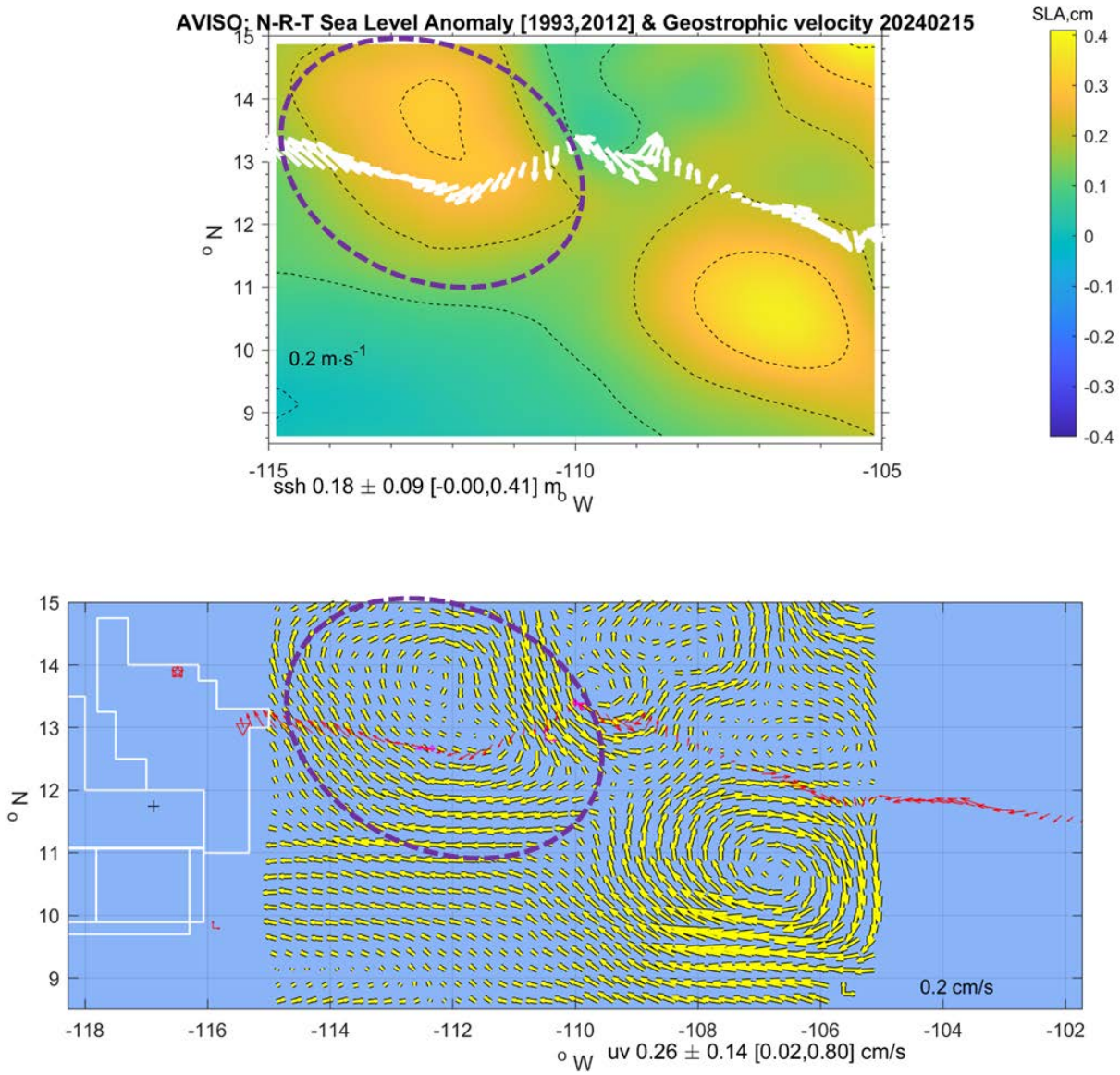


Figure 3.2.3. Nea real-real-time Sea Level Anomaly (top) and sea surface currents (bottom) on 15th Feb 2024, derived from Global Ocean Gridded L4 product SEALEVEL_GLO_PHY_L4_NRT_008_046, https://doi.org/10.48670/moi-00149. white and red arrows show hourly averaged currents derived from VM-ADCP 75kHz measured on 13-15th February during JC257 transit to UK1 area.

Vertical profiles of horizontal velocities obtained by the Up- and Down- looking 300kHz ADCPs mounted on a rosette match closely with currents measured by Vessel Mounted ADCPs (os75nb and os150nb) both in the direction and the strength in the upper ocean layer on a transect across the Eddy periphery on 13-15th February 2024.

Horizontal current distribution within the same eddy at different bin depths was revealed on maps with the arrows from both VM-ADCPs in February as shown in Fig. 3.2.4 and in March 2024, Fig 3.2.5.

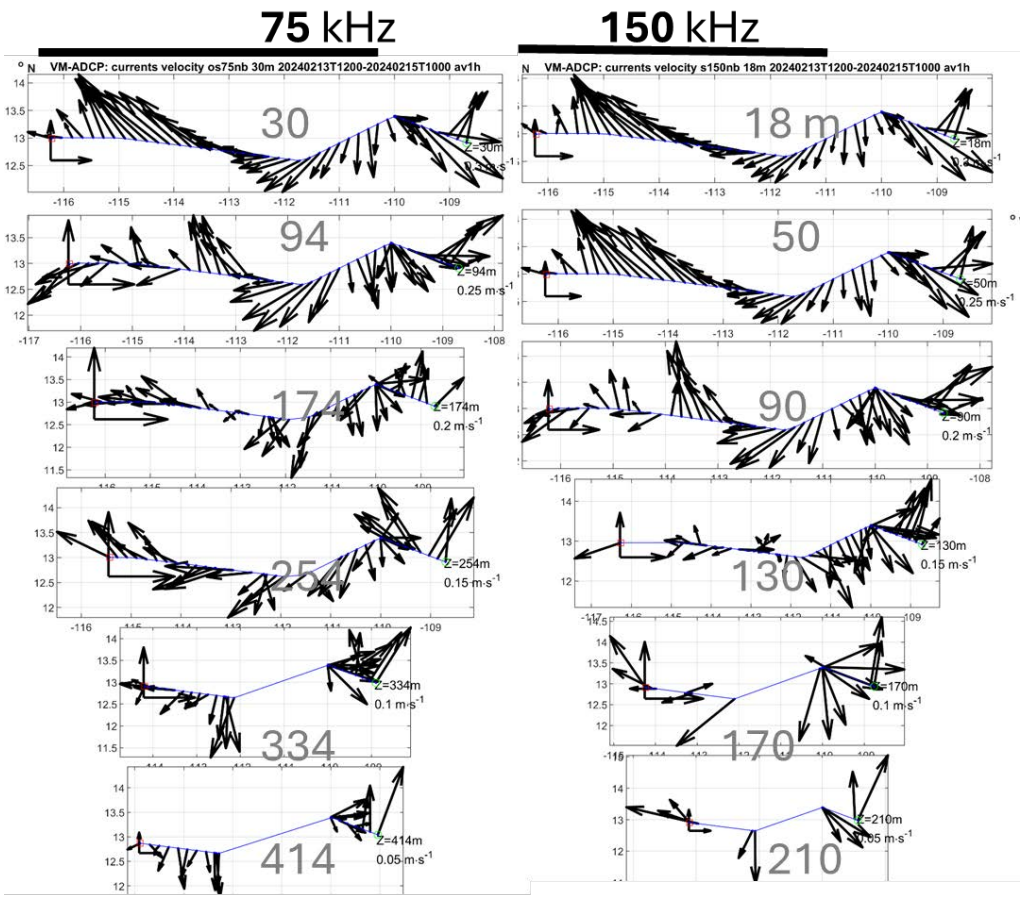


Figure 3.2.4. Hourly-averaged horizontal currents at different bin layers derived from VM-ADCP during the transect across the eddy 13-15th Feb 2024, on JC257 transit to UK1 area. Note that scale varies at each panel.

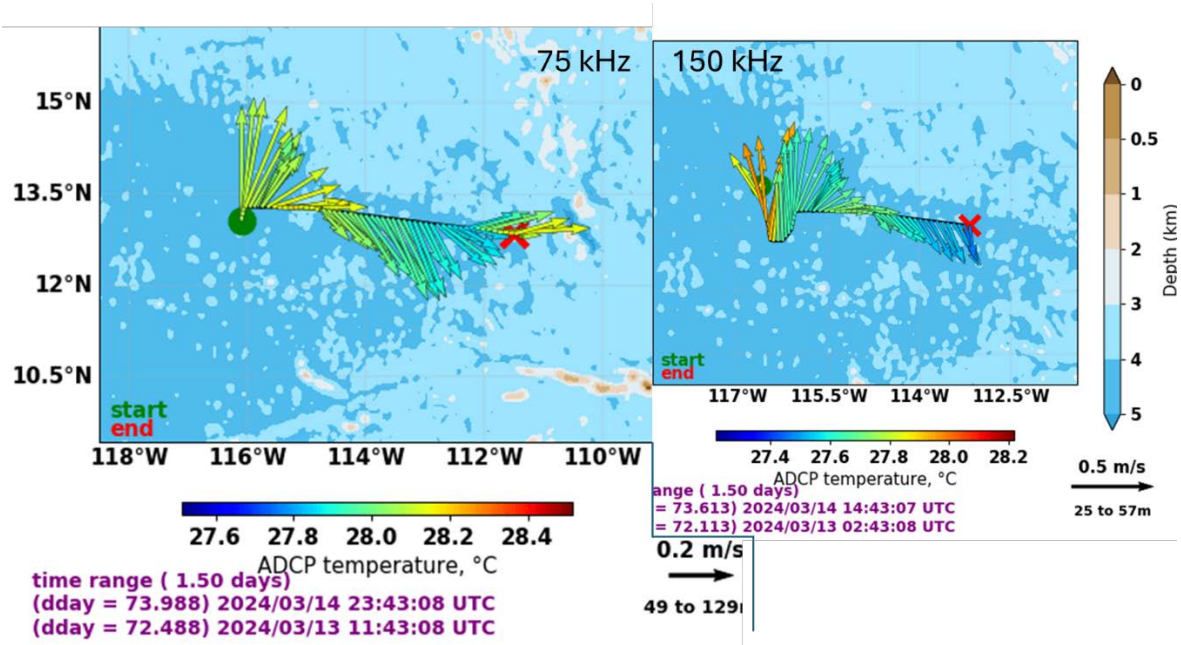


Figure 3.2.5. A subset of averaged horizontal currents at different bin layers derived from VM-ADCPs during the transit across the eddy 13-15th March 2024 (49-129m and 25-57m for 75 and 150kHz instruments respectively). Currents switched direction near the eddy centre -114.5ºW from NE to SE. Note that scale varies at each panel.

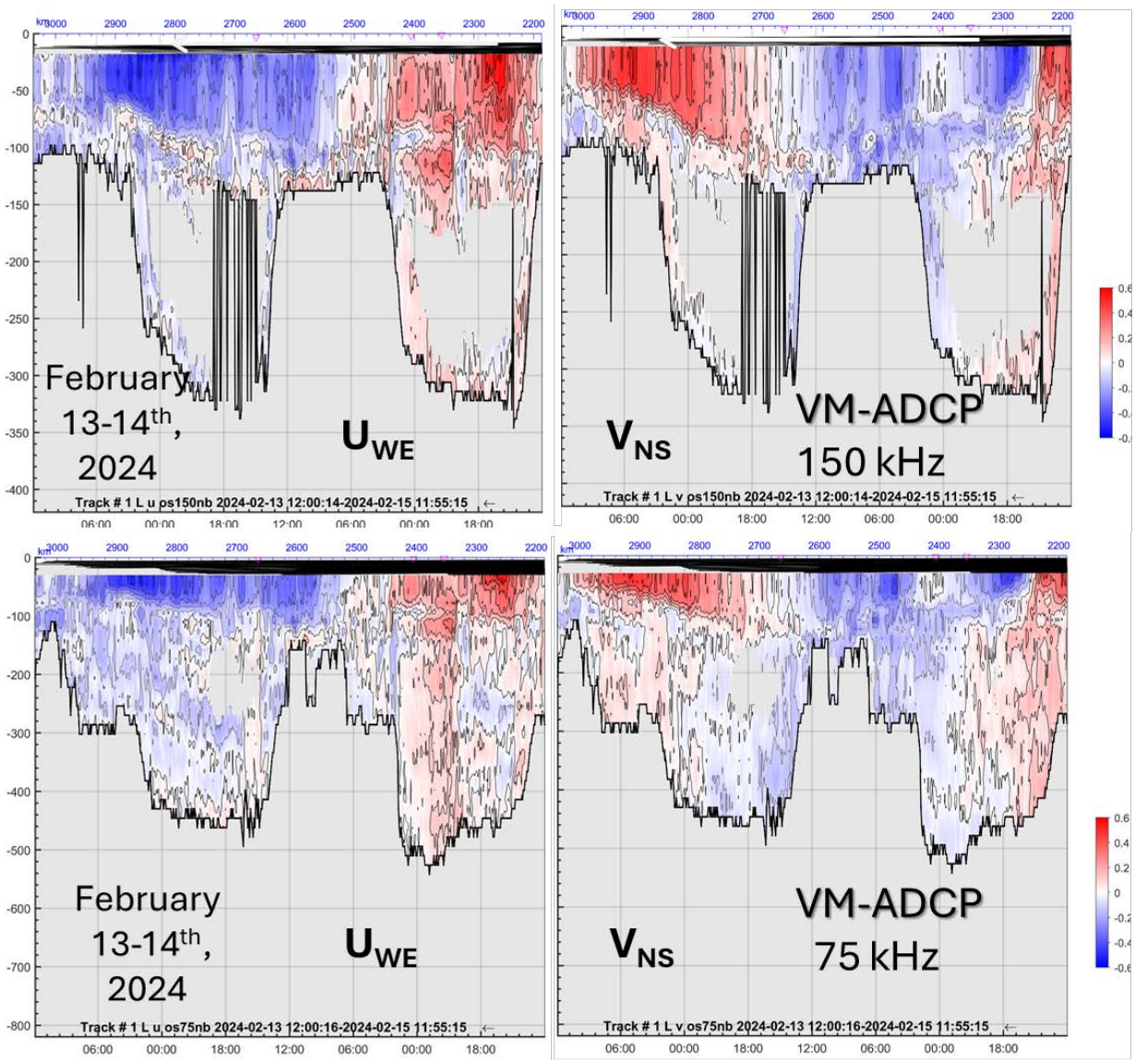


Figure 3.2.6. Vertical transect of 5-min averaged zonal (U) and meridional (V) currents components under the ship measured by VM-ADCPs during the transit across the eddy 13-15th February 2024 from east to west. X-axis is reversed, and 150 kHz shown on top and 75 kHz below, Times scale is shown in UTC. Note the signal strength varies with the day-night migration of acoustic reflective particles (plankton).

Lowered LADCP profiles were taken in the western edge of the same eddy on its approach to the study area UK1 1km, cast #17, and close to the maximum horizontal velocity within the eddy, cast #18 (Fig. 3.2.7). Good agreement is evident between U zonal and V meridional components of the current speed measured with LADCP (red, green lines) and VM-ADCP (blue, yellow lines respectively) in the upper 150-250m layers in North-East direction. RDI-bottom track records enable reliable current speed estimates in BBL and up to 8 mab and show a small increase at cast 18.

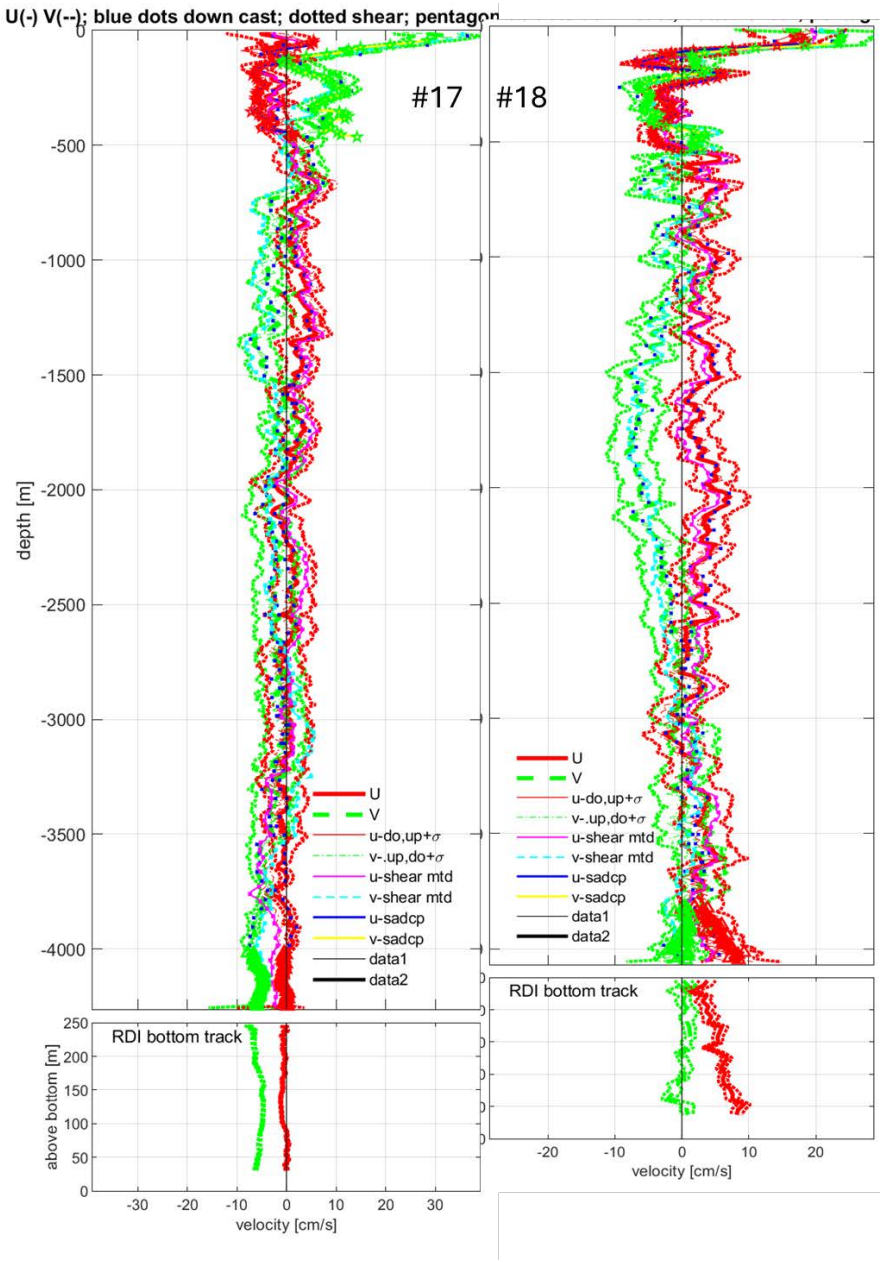


Figure 3.2.7 Example of two LADCP records performed with CTD casts 17 and 18 (11 and 13 March 2024) and processed with LDEO_IX MATLAB loobox. Zonal (U) and meridional (V) components of the horizontal current speed were measured with LADCP and shown wit red and green lines respectively, while blue and yellow lines show currents derived from simultaneous VM-ADCP os75nb records.

The vertical and horizontal structure of the Eddy was also revealed in a deepening of the pycnocline from 70-72 m at the eastern edge to 90-92 m closer to 1/3 distance from its centre according to collected CTD profiles. The vertical structure of the eddy and its evolution over time was detected again in March 2024 by deepening the upper mixed layer approximately 25-30 m close to its centre (St 3, 19) in comparison to the eddy's periphery as it's shown with profiles of the Conservative Temperature (°C), Absolute Salinity ($g \cdot kg^{-1}$), potential, density $\sigma_{\theta 0}$ ($kg \cdot m^{-3}$) (TEOS-10, 2010), Chl ($Ug \cdot l^{-1}$) and O_2 ($Umol \cdot kg^{-1}$, %Sat) taken on the 13-14th of February and on a return way 13-14th of March 2024 (Fig. 3.2.8). These results consistently match in the shape while approximately 10 meters deeper than on the T,S profiles transmitted by Argo profiler R4902333 in its dives on 20th Feb and 1st March 2024, when this float was trapped within a distance 10-15 miles north of this eddy centre (not shown).

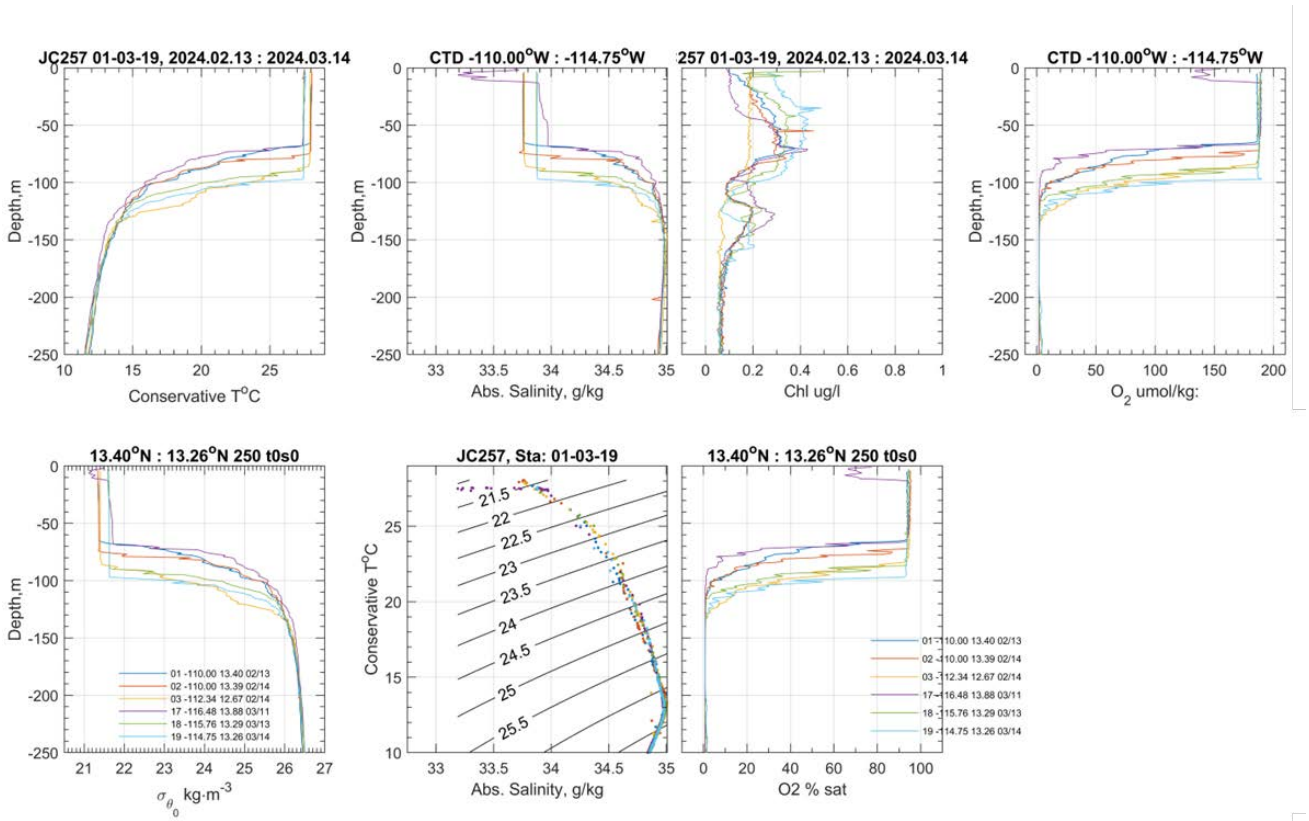


Figure 3.2.8. Vertical profiles in the eddy periphery (CTD 1, 17) and closer to its centre (3, 18,19) taken on 13-14th February 2024 and a month late in JC257 cruise: Conservative Temperature (°C), Abs. Salinity (g·kg^{-1}), potential, density $\sigma_{\theta 0}$ (kg·m^{-3}), Chl (Ug·l^{-1}) and O_2 (Umol·kg^{-1}, %Sat) and T,S diagram of the upper 250m are included.

Back-tracking of this eddy centre to its initial location revealed that its origin place was to the west off Ismith of Tehuantepec (95°W,15°N) and happened under the strong Gap Wind event on 6-9th June 2023. Three weeks later this meander of Costa Rican Coastal Current detached from the continental shelf and was essentially steered up by the first hurricane of the season named Adrian (Category 2, with a maximum wind speed 105 mph and the lowest pressure 970 hPa (https://www.nhc.noaa.gov/). Transit of Hurricane Beatriz (C1, 85mph, 992 hPa, *ibid*) north-westward from the Gulf of Tehuantepec along the Mexican Pacific coastline at the end of June 2023 triggered the emerging of the second eddy near 99°W,15.5°N. Both eddies merged on the 2nd of November 2023 above the East Pacific Rise (106°W, 14.5°N), which happened soon after the upper ocean currents were enhanced by the passage of major Hurricane Norma (C4 with 130 mph winds, last week in October 2023). Then the merged eddy drifted westward to 116.5°W arriving in the first week of March 2024 at the main study site of the UK1 area (Fig.3.2.9)

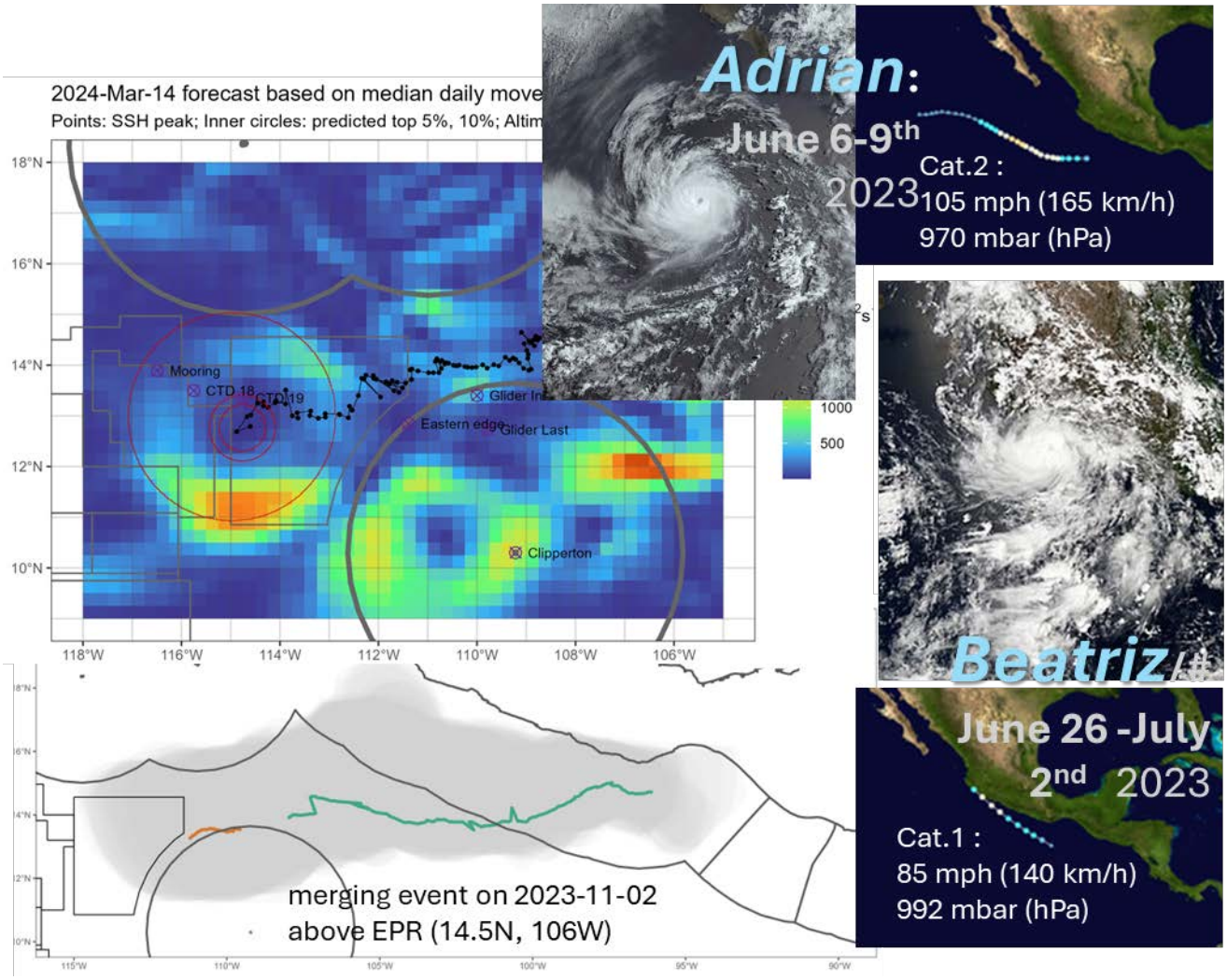


Figure 3.2.9 Eddy history and backtracking to its origin site (courtesy Tim, Szewczyk at SAMS) shown with (black dots on a top of Eddy kinetic energy EKE (cm^2s^{-1}) distribution calculated with AVISO SLA and surface currents product (Gridded L4 product SEALEVEL_GLO_PHY_L4_NRT_008_046, https://doi.org/10.48670/moi-00149). Snapshots of Adrian and Beariz Hurricanes clouds and tracks are shown in an accord to US National Hurricane Center NOAA https://www.nhc.noaa.gov/.

Eddy's presence was detected by VM-ADCP currents profiles on both-way transits and the response on its propagation in the water column was noticed right before the recovery by several (all) Nortek currents meters and SBE37 CTD deployed at the Long Mooring. Vectors of de-tided residual currents from all 3 moorings are shown on Fig *3.2.10 a,b.*

Spectral analysis of moorings currents is shown for M1 as an example in Fig 3.2.11, as the results for moorings M2 and M3 are similar). In a water column between 1000 m and 8 m above the seabed Power Spectral Density distribution across frequencies (periods) was the following: 28-33% belong to the tidal range, 7-24% were in the inertial band (2.5 days at 13.9ºN latitude). Finally, the energy fluctuations at the mesoscale range (5-50 days) were counted a 12-16%.

Substantial mesoscale activity is also evident in the timeseries of Temperature, Salinity and calculated density records from SBE37SM CTDs mounted on all 3 moorings (Fig. 3.2.12 a,b), as well as RBR thermistors records (Fig. 3.2.12c). Two eddies were detected in the area: a clockwise rotating Anticyclonic in April and May, and another counter-clockwise Cyclonic eddy detected in July-August 2023.

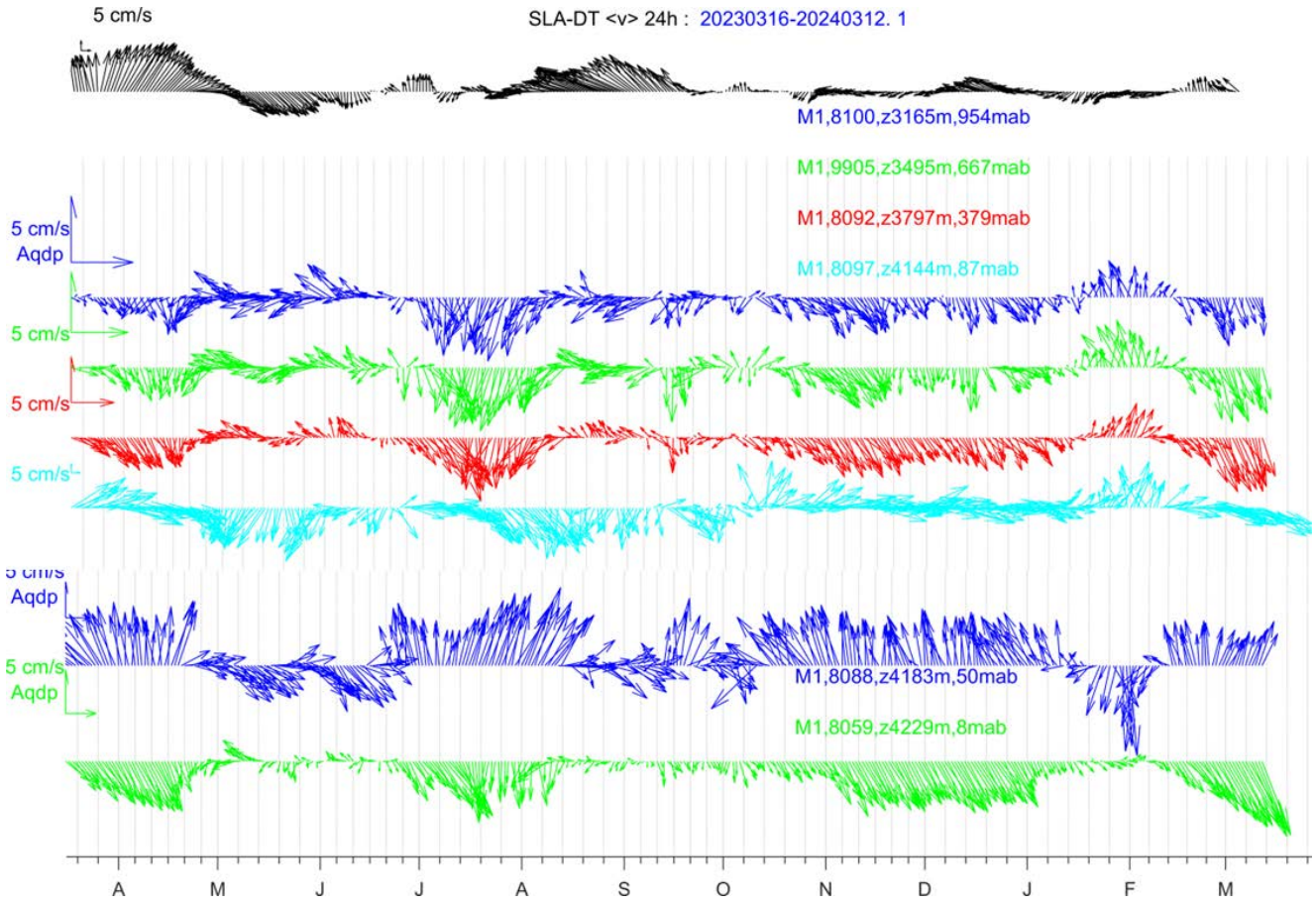


Figure 3.2.10 a. Residual currents (de-tided and averaged over 24h) that were measured at Long Mooring 1 by 6 Nortek AquaDopp 2Hz DW instruments at different depths (954, 667, 379, 87, 50 and 8) meters above the seabed, note arrow scales are different for each depth level over a year (2023.03.16-2024.03.12). The black arrow shows daily sea surface currents above the mooring site derived from AVISO (Gridded L4 product SEALEVEL_GLO_PHY_L4_NRT_008_046, https://doi.org/10.48670/moi-00149)

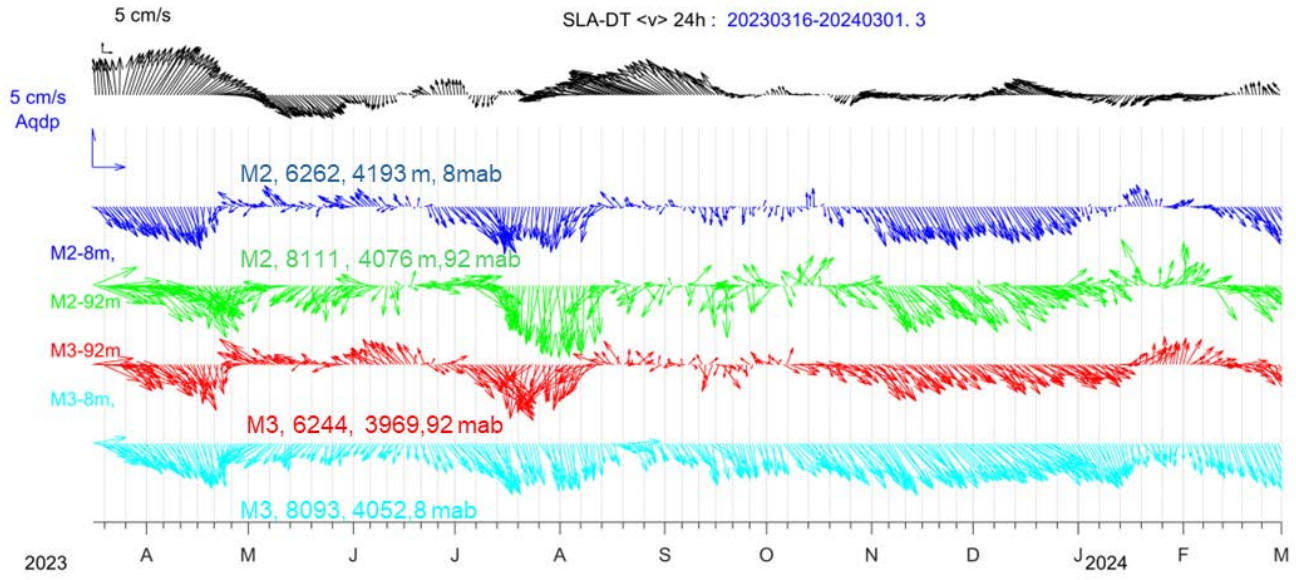


Figure 3.2.10 b. Residual currents (de-tided and averaged over 24h) that were measured at Short Moorings 2 and 3 equipped with 2 Nortek AquaDopp 2Hz DW instruments at 92 and 8 meters above the seabed, note arrow scales are different for each depth level. The black arrow shows daily sea surface currents above the mooring site derived from AVISO (ibid).

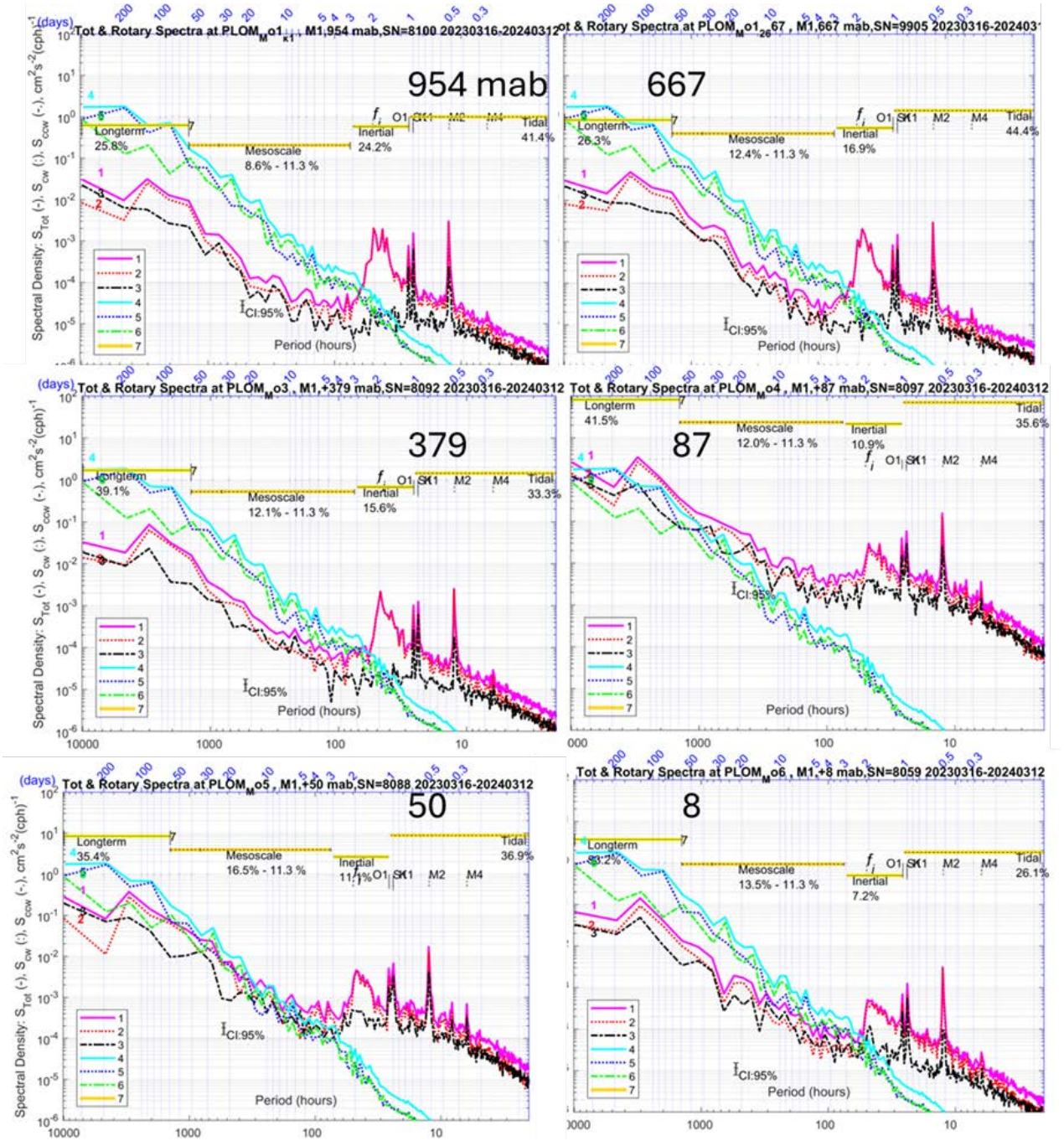


Figure 3.2.11. Total (pink) and Rotary (red is clockwise CW, black is counter-clockwise CCW) Power Spectral Density estimates of the currents recorded on at Long mooring at depths 954, 667, 379, 87,50 and 8 mab. The cyan line is the total PSD for the AVISO sea surface currents above this LM1 site, CW rotary spectra are shown with blue dots, and CCW with a green line. Note that current energy at 87 m seems too energetic, and correction (instrumental calibration) is likely required.

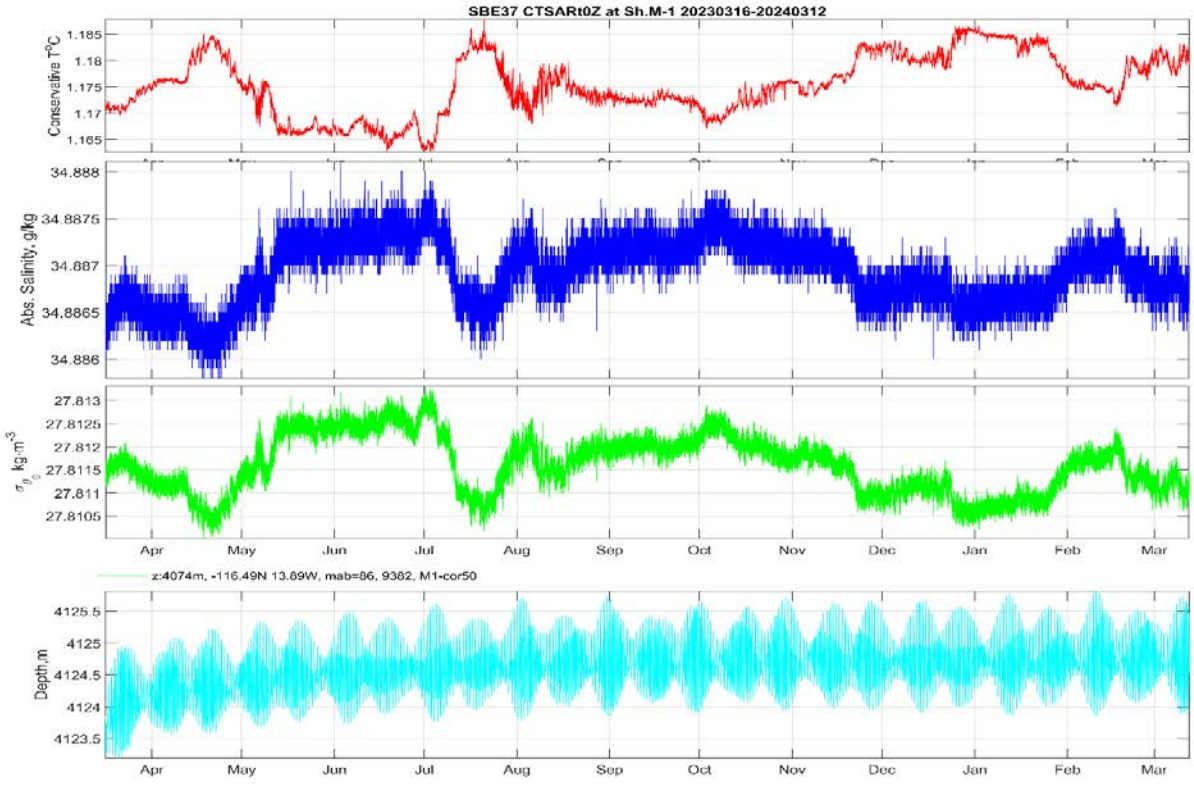


Figure 3.2.12a Conservative Temperature (°C), Abs. Salinity (g·kg^{-1}), potential, density $\sigma_{\theta 0}$ (kg·m^{-3}) and calculated Depth long Mooring 1. SBE SM 9382, 92 mab after pressure correction (+50db) applied.

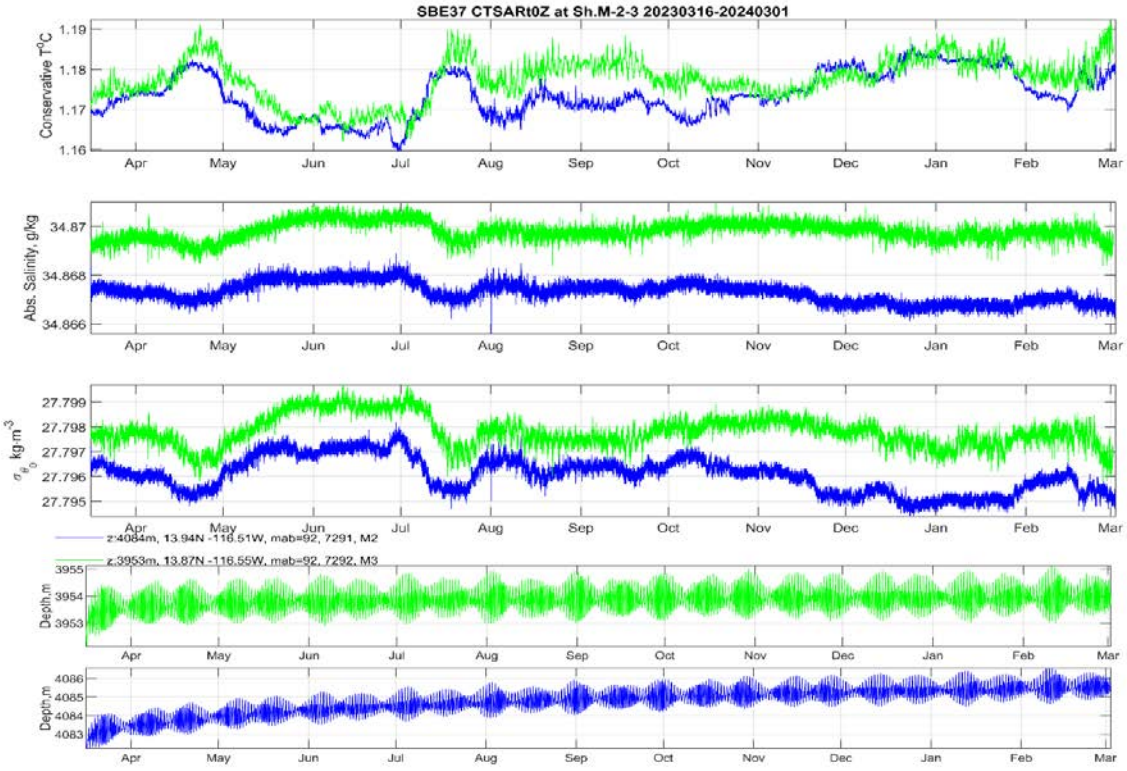


Figure 3.2.12b Conservative Temperature (°C), Abs. Salinity (g·kg^{-1}), potential, density $\sigma_{\theta 0}$ (kg·m^{-3}) and calculated Depth at Short Mooring 2. SBE37SM 7291 and Short Mooring 3, SBE37SM 7291 (blue), 92 mab

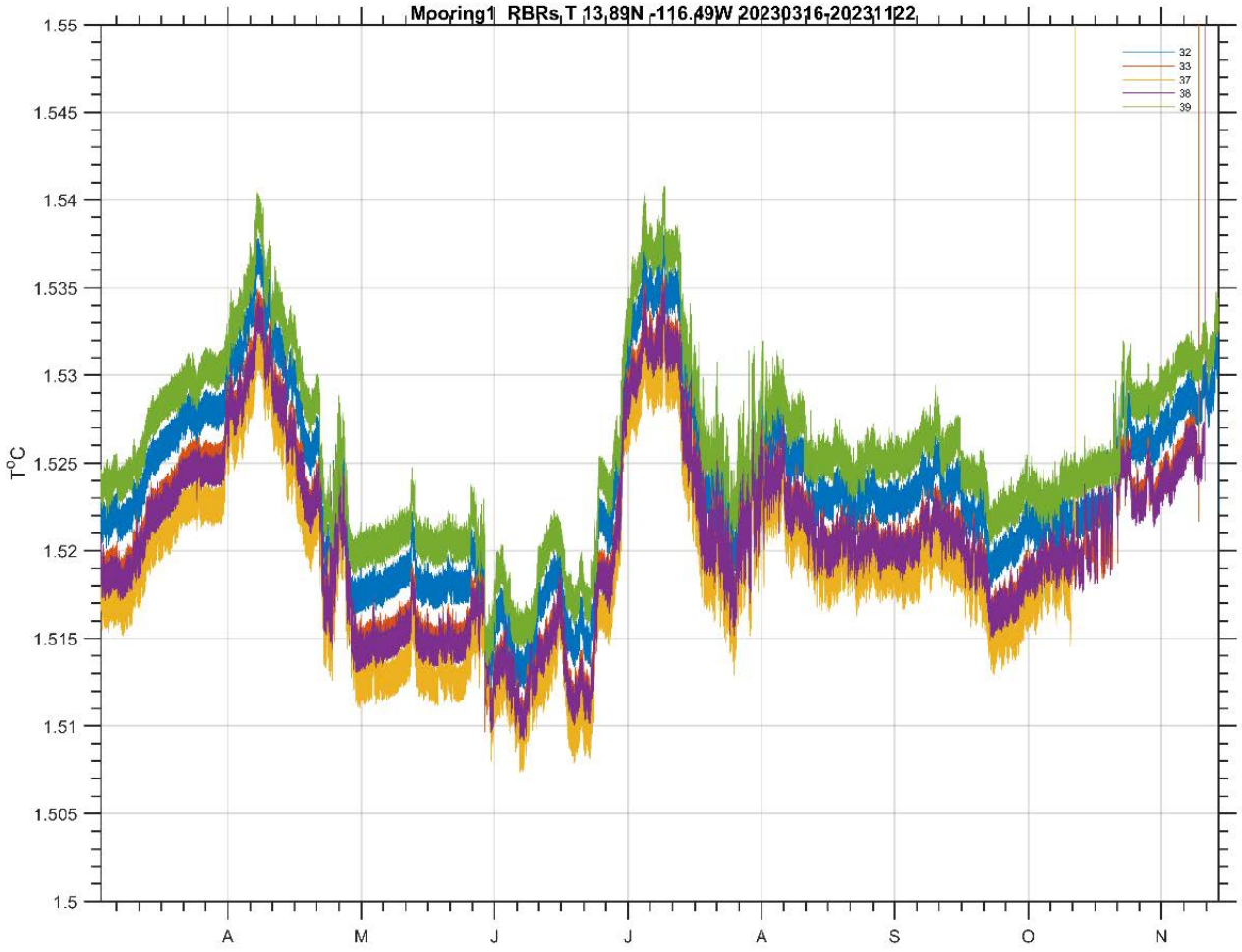


Figure 3.2.13 Temperature records from RBR thermistors mounted on a wire of Long Mooring 1. Eddies presence above mooring site is evident.

Tides and pressure records

Prediction of sea surface elevations relative to the mean sea level (MSL) was calculated at the Long Mooring 1 site (13.9420 ºN, -116.5057 ºW) using TMD2.5 (Egbert, Erofeeva, 2002) and T_tide v1.4 (Pawlowicz, et al 2002) toolboxes, based on Oregon State University TPXO7.2 tidal inverse model with 11 and 54 tidal constituents (Fig. 3.2.14). The averaged biases between predictions and pressure measurements (after the mean pressure was removed) did not exceed 4.4, -2.6 and 5.3 cm at M1, M2 and M3 moorings respectively (<2.5%). The tidal prediction results were used for the correction of multi-beam, side-sonar and echosounder surveys in JC257.

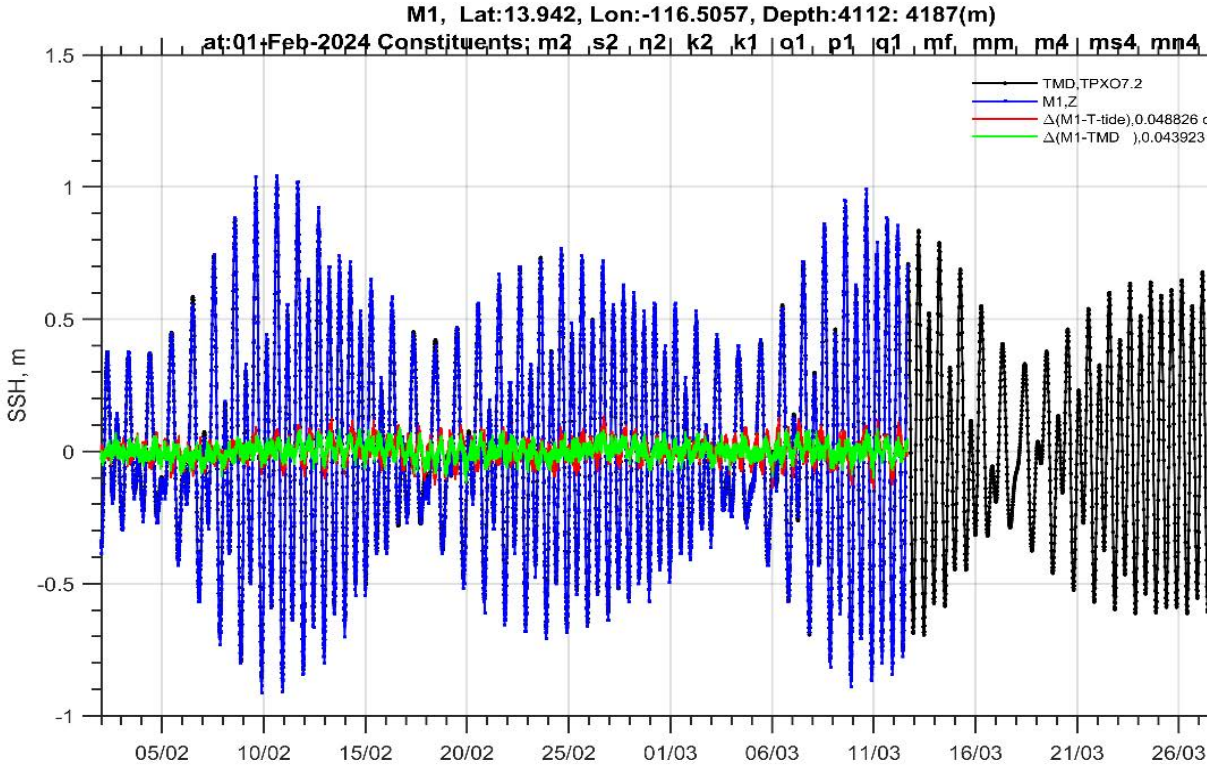


Figure 3.2.14. Measured fluctuation of seabed Pressure (records after mean Pressure was removed, blue line) and compared with Sea Surface Height (SSH, black line) predictions derived from inverse tidal solution based on OSU TPXO7.2 with 11 (TMD) and 54 tidal constituents (t_tide). Biases are shown with red and green colour lines.

The presence of internal waves in the deep layers was detected from full-depth down- and up-cast profiles on every deep CTD station. The fine-scale temperature-salinity structure below 3.5 km depth remained very similar and not being destroyed but vertically displaced by several meters also during a specially designed for that purpose yo-yo profiling station CTD 17 (Fig. 3.2.15). The study of nonlinear internal waves (NIW) dynamics and the estimates of turbulence scales are important for correct diffusivity parametrisation in localised 3D hydrodynamic and plume dispersal models. Yo-yo CTD records could be used alongside the fine-scale (1 sec) records of temperature RBR loggers recovered from the Long Mooring 1 (Fig 3.2.13).

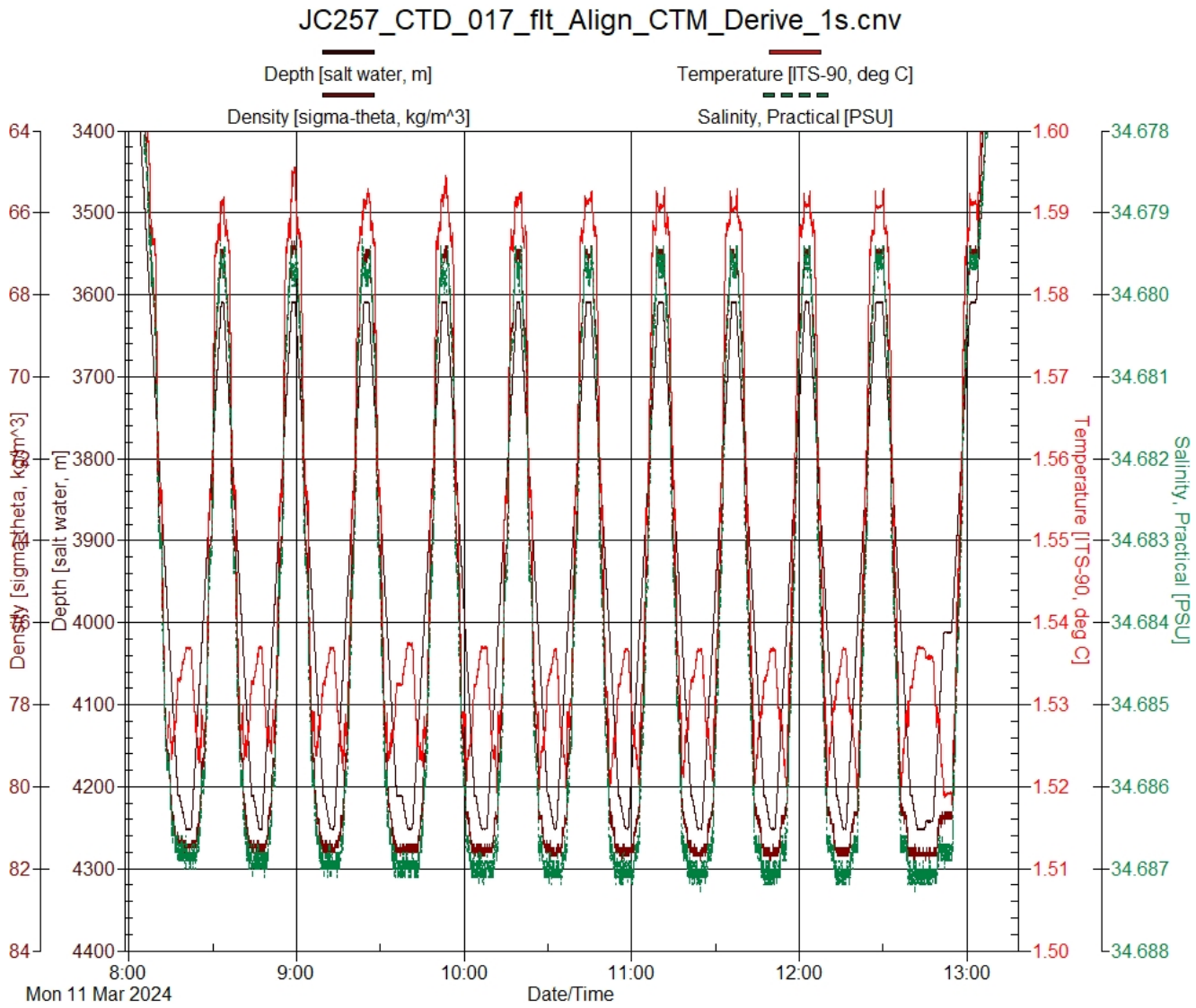


Figure 3.2.15 Yo-Yo vertical profiles of in-situ Temperature (°C), Salinity (psu), potential density $\sigma_{\theta 0}$ (kg·m-^{3}) and sensor depth (m) computed using ITS-90 algorithms and derived from CTD 17 cast 11th March 2024, 1.3 km south of the Long Mooring 1.

Sea water properties

Full depth vertical profiles of Conservative Temperature, Absolute Salinity, and potential density $\sigma_{\theta 0}$ were computed relative to the surface pressure Pr=0 dbar using TEOS-10 (2010) algorithms from the processed and averaged 1m profiles derived from the primary CTDs sensors set (t0, c0). Those profiles were marginally narrower than the variations derived from a secondary sensors set (t1,c1) of SBE 9+ mounted on a rosette. Note that both sets of sensors are subject to post-cruise calibration and Autosal sampling corrections. The shape of the full-depth CTD profiles is typical for the Centre of the Eastern Tropical Pacific (Fig. 3.2.16).

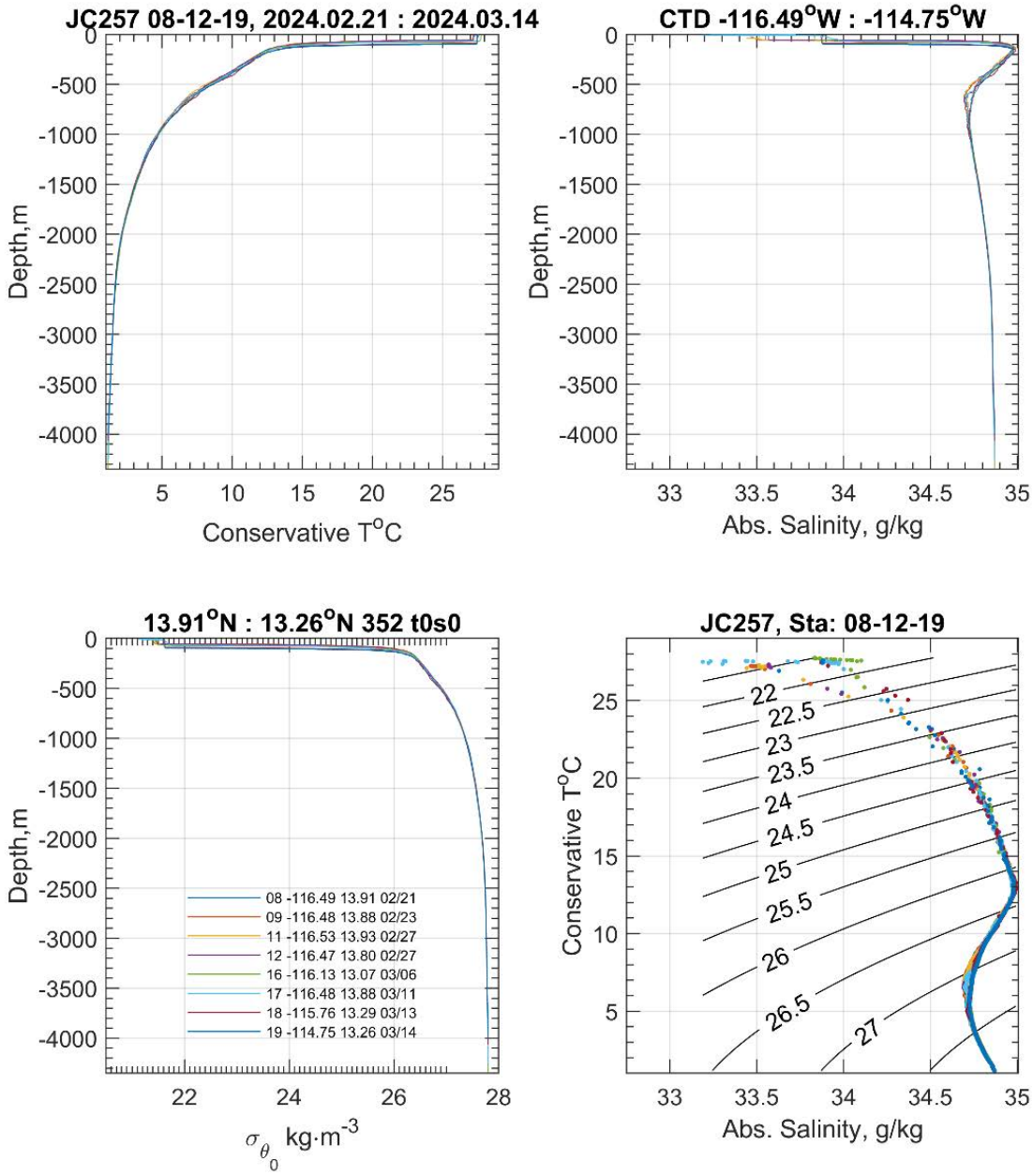


Figure 3.2.16 Full depth vertical profiles of Conservative Temperature (°C), Absolute Salinity (g·kg^{-1}), potential, density $\sigma_{\theta 0}$ (kg·m-^{3}) computed using TEOS-10 (2010) algorithms derived from all deep CTD casts (8,9,11,12,16-19) in JC257 cruise 21st Feb -14th March 2024. Cast coordinates and dates are given in a legend.

Small variations in each parameter were detected in layers between 3400 m and seabed, as shown in Fig. 3.2.16 for all deep CTD casts (8,9,11,12,16-19) in the area between UK1 0 km and 100 km and taken in a period 21st Feb -14th March 2024 in JC257 cruise. The spread of each parameter remains within the range of fluctuations, which were measured previously in this area: at 3 CTD casts in 2013 (Abyssal Bassline, AB01 on October 3 – 27, 2013 R/V Melville SFI-Cruise report, 2013), one CTD station in JC120 cruise in May 2015, and JC241 in March 2023.

Vertical gradients of T, S and potential density reduced below 3600m. Even greater reduction of these gradients was observed in lower and more stable parts of vertical profiles on stations in the bathymetric depressions

(trenches) below 3800-3900m. Typical values at depth 4000 m for Conservative Temperature CT were between 1.176 and 1.199ºC and absolute Salinity AS=34.867÷34.869 g kg^{-1} (Fig. 3.2.17)

Quasi-homogeneous bottom boundary layer (BBL) in the UK1 area was composed of Lower Circumpolar Water (a mixture of the Antarctic Bottom and North Atlantic Deep waters) and extended as high as 300-450 m above the seabed (3800–4350m depth) with a narrow range of *in situ* temperatures (ITS-90) T=1.493–1.524 ˚C, salinity S=34.867–34.869 and a very low buoyancy frequency N=0.24 cph (6.7·10-5 s^{-1}).

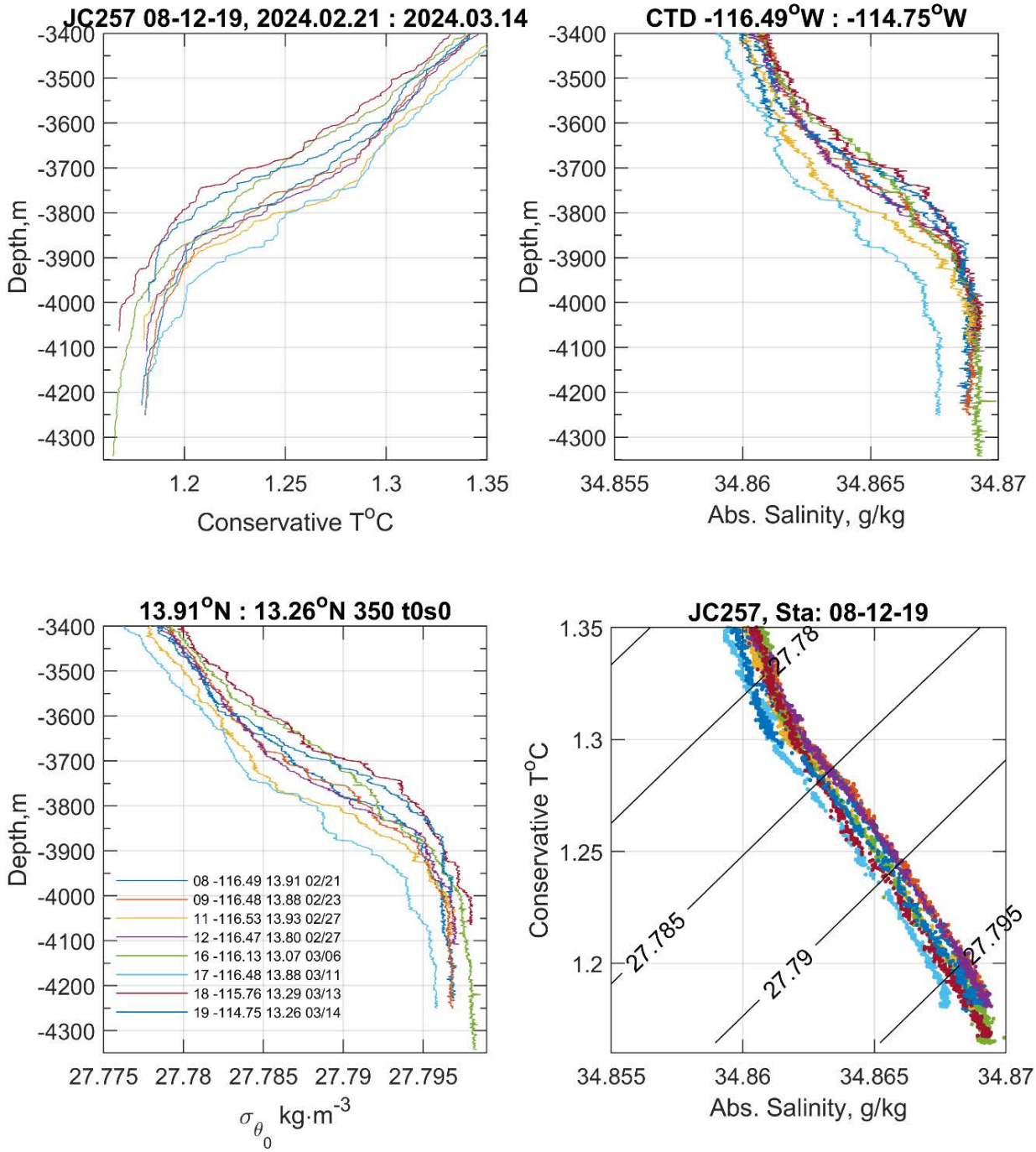


Figure 3.2.17. Vertical profiles of Conservative Temperature (ºC), Absolute Salinity (g·kg^{-1}), and potential density $\sigma_{\theta 0}$ (kg·m-^{3}) computed using TEOS-10 (2010) algorithms in layers between 3400 m and seabed in all deep CTD casts (8,9,11,12,16-19) obtained in JC257 cruise 21st Feb -14th March 2024. The deepest part of the T-S diagram (CT/SA) is also included for given density and depth ranges.

ROV an AUV CTDs

CTD profile measurement with remotely operated vehicle (ROV) and Autonomous Submarine (Autosub) have the advantage of relatively prolonged periods of observations exceeding typically one-two tidal cycle and could be utilised i.e. as (a) moving platform data source for modelling trials or (b) fine-scale dynamics and turbulence

studies. For comparison, data obtained in JC257 during ISIS ROV dives with SBE 49 Fastcat CTD are shown in Figure 3.2.18.

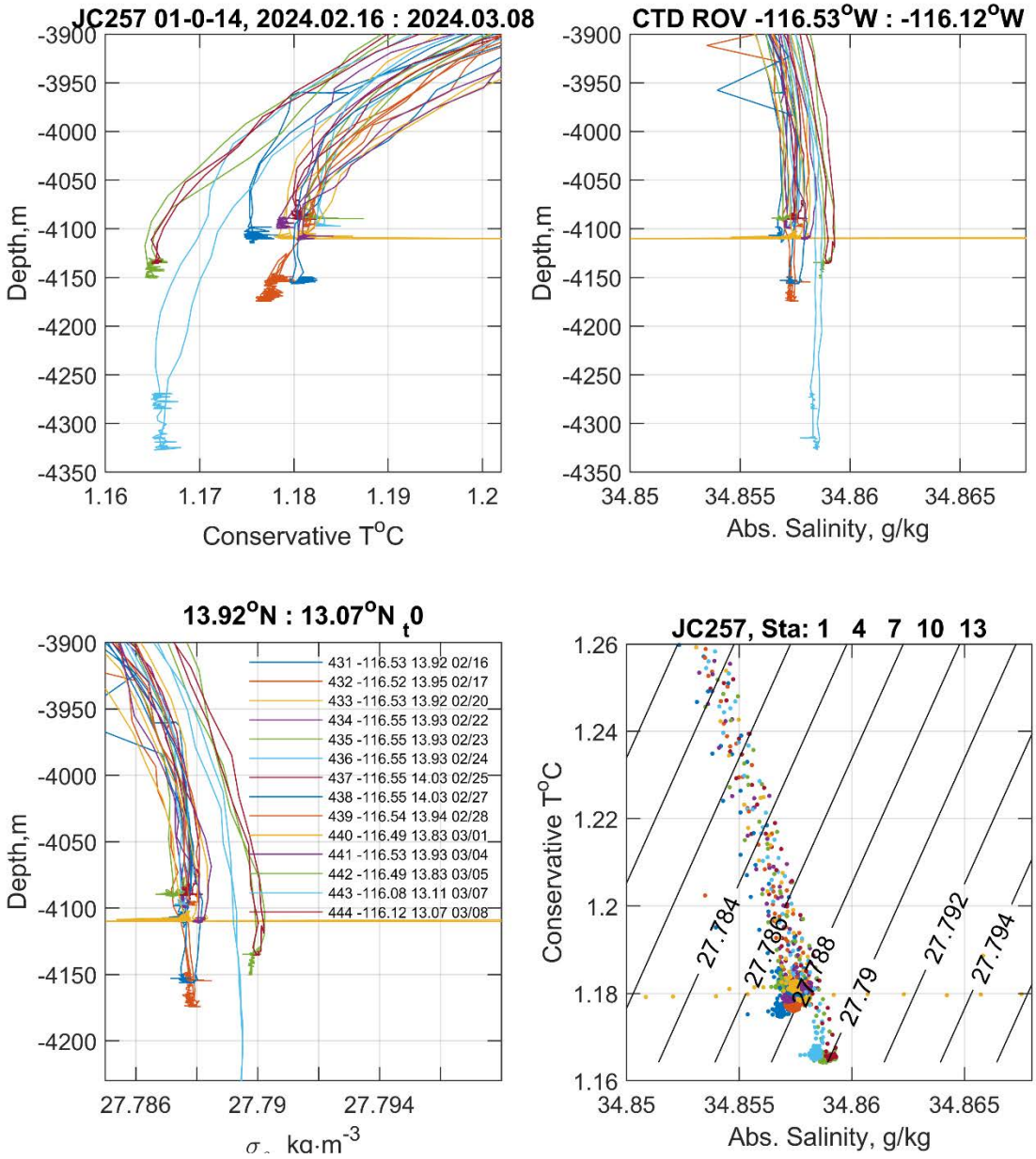


Figure 3.2.18. ROV SBE49 CTD dives 431-444: profiles of Conservative Temperature (°C), Absolute Salinity (g·kg^{-1}), potential, density $\sigma_{\theta 0}$ (kg·m-^{3}) computed using TEOS-10 (2010) algorithms in layers below 3900 m and few cm above seabed in JC257 cruise 16th Feb -8th March 2024. The deepest fragment of the T-S diagram (CT-SA) is also included for a given density and depth range.

Similarly, profiles derived from the SBE 911plus/917plus CTD mounted on Autosub AUV from several autonomous underwater missions are shown in Figure 3.2.19.

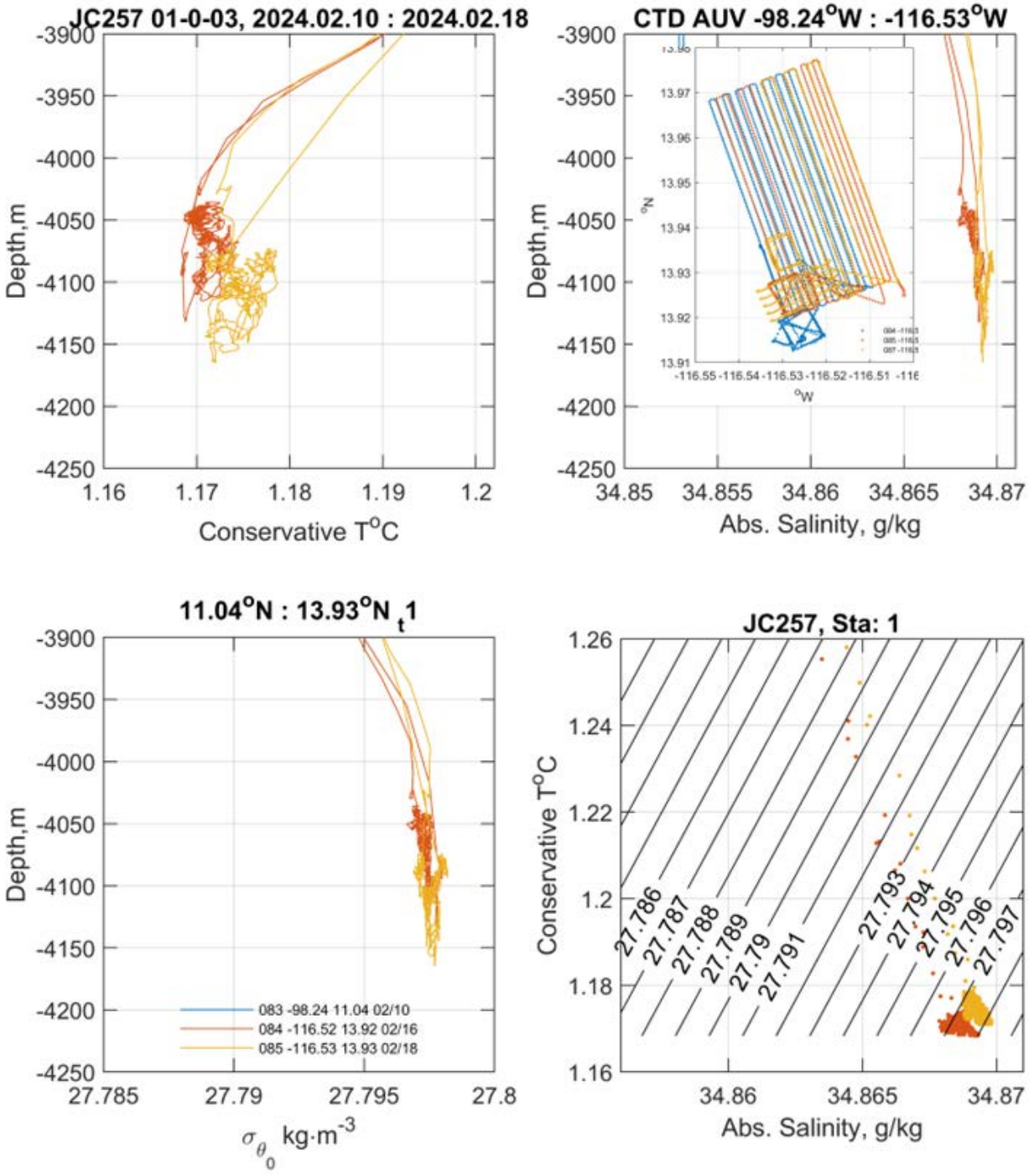


Figure 3.2.19. AUV SBE9/17 plus CTD results from missions 83- 85: profiles of Conservative Temperature (°C), Absolute Salinity (g·kg^{-1}), potential, density $\sigma_{\theta 0}$ (kg·m-^{3}) computed using TEOS-10 (2010) algorithms in layers below 3900 m and few meters above seabed in JC257 cruise 10-18st Feb 2024. The mission geographic layout is shown as in-cut in the top right panel. Note that the vertical motion of the AUV vehicle over uneven bathymetry was in the range of 100-250 mab.

Oxygen concentration in the deepest layers

The deepest layers of the ocean waters in all parts of studied UK1 (0 km-100 km) areas experienced a pronounced increase in dissolved O_2 concentration below 3600 m toward the seabed in all deep CTD casts (8,9,11,12,16-19) in JC257 cruise between 21st Feb -14th March 2024 (Fig. 3.2.20). That fact requires a feasible explanation as usually the abundance of the oxygen consumers seems to increase in this direction, toward the seabed, which should lead intuitively to reduced O_2 concentration.

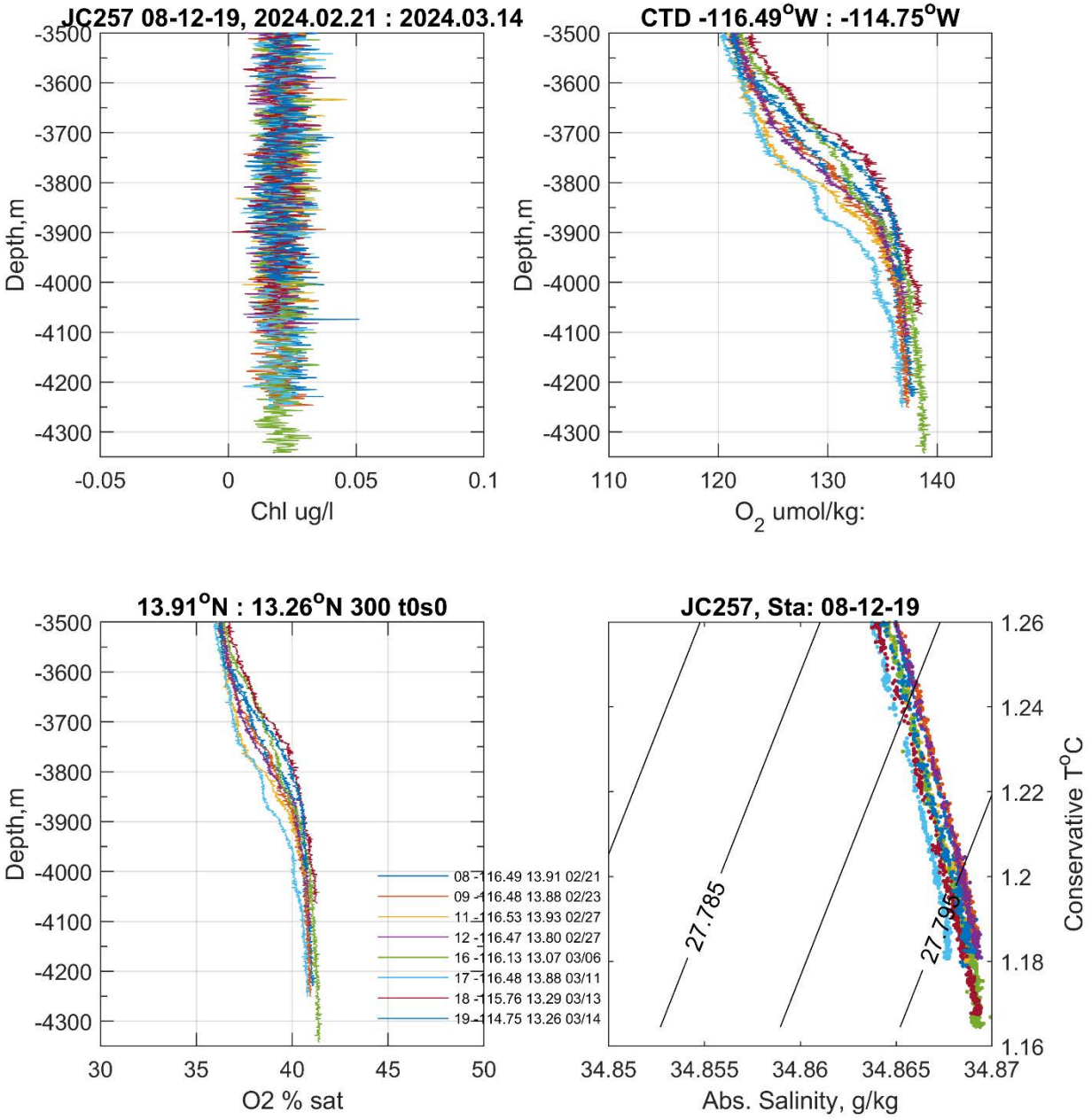


Figure 3.2.20. Vertical profiles of CHla and Oxygen in layers between 3500 m and seabed in all deep CTD casts (8,9,11,12,16-19) obtained in JC257 cruise 21st Feb -14th March 2024: Chlorophyll, Ug·L^{-1}, O_2 Umol kg^{-1} and O_2 % saturation. The deepest part of the T-S diagram (CT/SA) diagram is also included for given depths.

Conclusion remarks

The data successfully collected in a JC257 research cruise substantially increased the knowledge baseline about the temporal and spatial variability of the physical oceanographic environment near the seabed, in the bottom boundary layer and further up in a water column to the surface in the Eastern Tropical Pacific. Three mesoscale eddies passed over the study site during 2023-2024 El Nino cycle with one of three the strongest ENSO index (>2.5°C in CET region) ever recorded in the past 70 years. The disturbing impact of mesoscale eddies passing over the UK1 licence area resulted in a substantial increase of residual current speed detected on the way from

the surface to the seabed, which was confirmed by many (every) instruments deployed in a previous JC241 cruise in March 2023 and recovered in March 2024 in JC257 expedition. During the year of observations 1/6 of the whole time the seabed currents were enhanced in comparison to the average state with a likely sequence for resuspension of the sediments. The evidence collected in JC257 should be considered in the future development of ISA regulations concerning marine protection measures, and mitigation (minimising) potential artificial plumes' impact on benthic communities.

All the success of the physical oceanographic measurements programme in the JC257 expedition was achieved thankfully due to excellent skills, great enthusiasm, and willingness to support this study by Billy Platt, and all the hard work of the engineers and ship deck crew of RRS James Cook.

References

Abyssal Bassline, AB01 on October 3 – 27, 2013 R/V Melville SFI-Cruise report, (2013). BODC.

Aleynik, D., Inall, M. E., Dale, A. & Vink, A. (2017). Impact of remotely generated eddies on plume dispersion at abyssal mining sites in the Pacific. Scientific Reports 7, 16959, https://doi.org/10.1038/s41598-017-16912-2

AVISO Sea level anomaly SLA L4 product SEALEVEL_GLO_PHY_L4_NRT_008_046, https://doi.org/10.48670/moi-00149. Accessed 2024.03.19

Egbert, Gary D., and Svetlana Y. Erofeeva. (2002). "Efficient inverse modelling of barotropic ocean tides." Journal of Atmospheric and Oceanic Technology 19.2 183-204.

Glover, A. G., Källström, B., Smith, C. R., & Dahlgren, T. G. (2005). World-wide whale worms? A new species of Osedax from the shallow north Atlantic. *Proceedings of the Royal Society B: Biological Sciences*, *272*(1581), 2587-2592.

JC120 Cruise report, (2015). BODC.

McDougall, T.J., D.R. Jackett, D.G. Wright and R. Feistel, (2003). Accurate and computationally efficient algorithms for potential temperature and density of seawater. J. Atmosph. Ocean. Tech., 20, pp. 730-741.

National Hurricane Center NOAA, USA, https://www.nhc.noaa.gov/. Accessed 2024.03.19

Oregon State University OSU inverse tidal solution models (global and regional) http://www.coas.oregonstate.edu/research/po/research/tide/index.htm. Accessed 2024.03.19

Pawlowicz, R., Beardsley, B., and S. Lentz. (2002). Classical tidal harmonic analysis including error estimates in MATLAB using T_TIDE", Computers and Geosciences 28, 929-937.

Sherwin, T. J., Aleynik, D., Dumont, E., and Inall, M. E. (2015). Deep drivers of mesoscale circulation in the central Rockall Trough, Ocean Sci., 11, 343–359, https://doi.org/10.5194/os-11-343-2015.

TEOS-10. IOC, SCOR and IAPSO, (2010). The international thermodynamic equation of seawater - 2010: Calculation and use of thermodynamic properties. Intergovernmental Oceanographic Commission, Manuals and Guides No. 56, UNESCO (English), 196 pp. http://www.TEOS-10.org Accessed 2024.03.19

3.3. National Oceanography Centre - Megafaunal Survey

Loïc Van Audenhaege, Bethany Fleming, Daniel Jones

3.3.1. AUV surveys

Goal

We set out to image the seabed to understand spatial variability of megafaunal (organisms > 1cm) communities in similar habitats. We aimed to assess the following study scales: 0, 1, 15, 16, 30, 50, 70, 89, 90, 99 and 100 km; '0 km' being the south of the 'Area of Interest 2' North of UK-1 (Figure 3.3.1). The strict minimum sampling effort was set at sites at 0, 1, 16, 100km with a set of images whose quality should be standardised across sites. The position of the site was constrained to a 'ridge' feature extending SSE. Sites were positioned in areas with as similar environments as possible, specifically a depth of around 4100 m and on the upper part of the ridge system as determined from the shipboard bathymetry. In each site, ten zig-zag transects of 2 km were planned parallel to the slope to be followed by the AUV at 3 m altitude and 1.2 $m.s^{-1}$. Furthermore, we intended to characterise annual variability of benthic communities by redoing the 2-km zigzag transects made by the ROV *Isis* during D413 of JC241 in the 0-1 km site.

All AUV missions were refined to avoid protruding features or slopes > 4%.

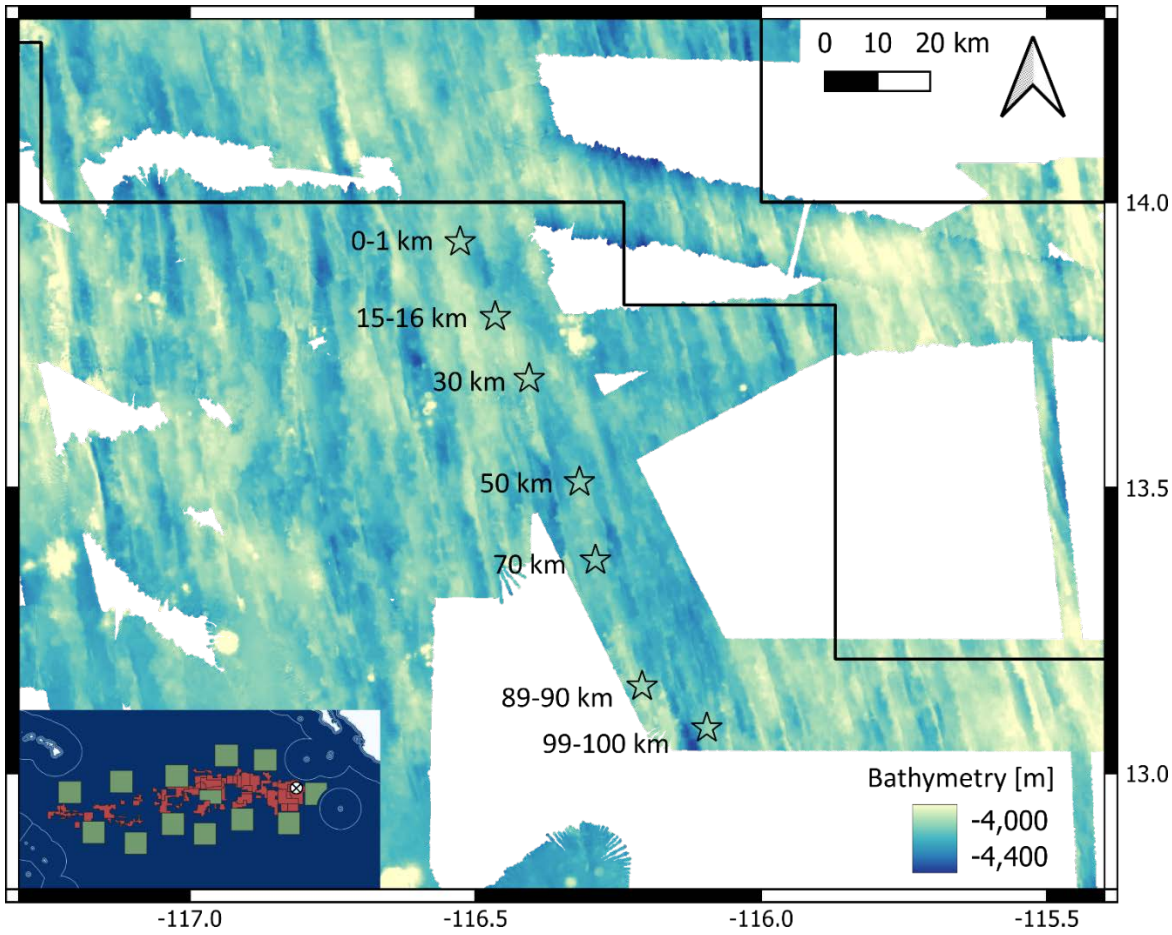


Figure 3.3.1 Spatial arrangement of the sites where AUV imaging investigation was originally intended.

Image quality assessment

Image set quality was assessed to diagnose for any potential lack of compatibility with our study aim (i.e., distinguish benthic morphospecies > 1 cm over at least five transects of 1,320 m^2; see (Simon-Lledo et al. 2019)). Corrected altitude was derived from the altitude as measured in metre by the Doppler Velocity Log, located -1420 (X-Y) and 434 mm (Z) from the AUV datum centre where the pitch is measured in radian (see Figure 2.2.1). The camera was located 1339 (X-Y) and 415 mm (Z) from the datum centre of the AUV.

$$altitude_c = altitude + \sin(pitch) \times \left(\frac{1420 + 1339}{1000}\right) - \left(\frac{415 - 434}{1000}\right)$$

Angles of acceptance in seawater were derived from angles of acceptance known from the manufacturer's specifications (α_v and α_h respectively, see Table X for camera specifications). We applied a seawater refractive index (n_2 = 1.34, see Parrish C. 2020, Oregon State University) compared to the air (n_1 = 1) following the Snell's law.

$$n_1 \sin(\alpha_{air}) = n_2 \sin(\alpha_{water})$$

$$\alpha_{water} = \sin^{-1}\left(\frac{n_1}{n_2}\sin(\alpha_{air})\right)$$

Assuming a flat seabed and negligible roll, the area of the image footprint was determined from the corrected altitude and from vertical and horizontal angles of acceptance corrected for water refraction, $\alpha_{water,v}$ and $\alpha_{water,h}$.

$$area = \left(2 \times \tan(\alpha_{water,v}) \times altitude_c\right) \times \left(2 \times \tan(\alpha_{water,h}) \times altitude_c\right)$$

To verify the use of a refractive index of 1.34, we compared this theoretical relationship between the vehicle altitude and the image area using image measurement ground truthed with lasers during JC241-D413 (Figure 3.3.2). Although area-altitude relationship is supposed to be quadratic, linear regressions modelled correctly the relationship between 2.4 and 2.8 m altitude. Differences between linear regressions remained negligible. For instance, an altitude of 2.6 m represented 1.24 m^2 and 1.27 m^2 based on theoretical and ground truthed linear regressions, respectively.

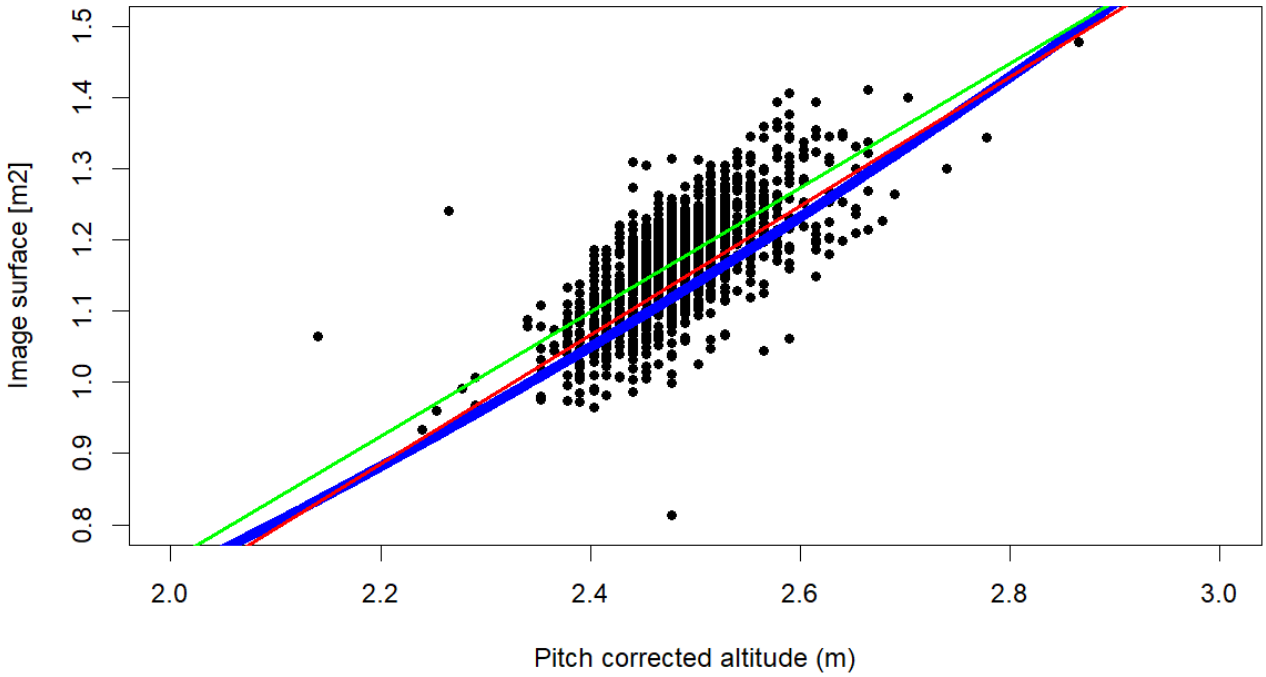


Figure 3.3.2 Relationship between altitude and image area. Black points are measurements made during JC241-D413 with lasers, from which a linear regression (y=–0.9947+0.8721x, R^2=0.48) was drawn in green. Blue points are the theoretical relationship between the image area calculated considering pitch corrected altitude and seawater corrected angles of acceptance. Red line represents the linear regression of this theoretical relationship (y=–1.1057+0.9049x, R^2=0.99).

Diagnosis of image set quality was performed only on images taken at a corrected altitude ranging min. 1.6 and max. 3 m (hereafter, 'at seabed'). Four main criteria were investigated:

- Influence of the altitude on image quality at variable altitude, by binning five images per 0.2 m altitude;
- Overall image quality of the set, by subsampling fifty random images;
- Evenness of spatial distribution of images selected, by graphical visualisation;
- Total area sampled during the mission, divided by the number of transects.

Image quality assessment was made in R (v.4.3.2) immediately after receiving any image set to diagnose for any potential issues that could prevent from comparing benthic communities across sites.

Mission narratives

This section intends to summarise the decisions taken along the failure and success of AUV missions.

During AS5M084, a transect test was performed at the end of the acoustic mission. The goal was to test the camera and to calibrate the settings. The test was successful. As expected, the targeted altitude set at 3.5 m did not provide sufficient image resolution for ecology purposes confirming our request to lower the targeted altitude to 3.0 m.

Shortly after starting her descent for AS5M086, *Autosub5* aborted her dive. The reason for this failure was a faulty cable interfering with the battery status. The cable was tested during a CTD deployment.

AS5M087 was a partial success. Seabed images were taken from the South of the 1km site to the North of the 0km site. However, owing to a failure of the AESA2 camera, the recording of images stopped at half of the 19th transect (Figure 3.3.3). We therefore recommend displaying image-associated navigation as soon as possible, to diagnose any gap of image acquisition in order to react quick for the next mission planning.

AS5M090 was impacted from the failure of the AESA2 camera before reaching the seabed where the mission was aborted shortly after. As a result of the unknown reason for camera failure, we switched our strategy by swapping AESA2 with AESA1, thought to be more reliable.

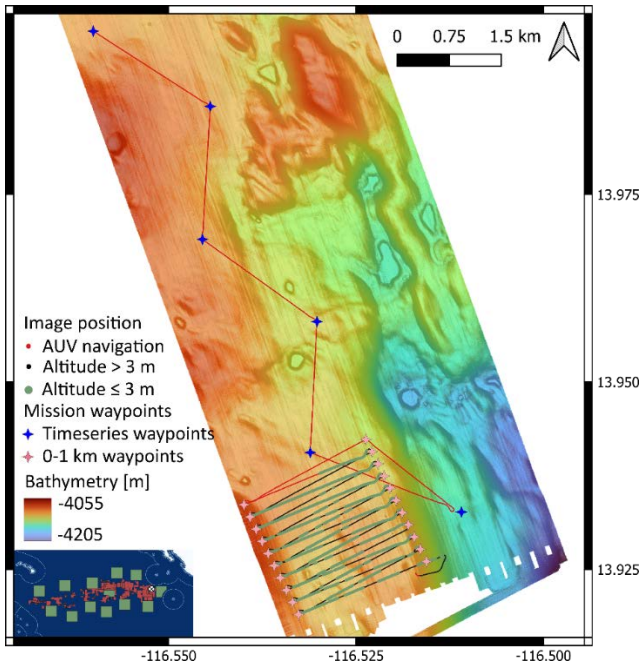


Figure 3.3.3 Imaging transects performed in AS5M087 planned at the 0-1km site and timeseries (WGS84). The bathymetry is underlaid by slope. The camera stopped running at the middle of 19th transect in the 0 km site.

AS5M091 was a success since *Autosub5* carried out all planned imaging transects. However, selection of images at seabed depicted that the vehicle struggled to maintain a target altitude when going down a slope (Figure 3.3.4).

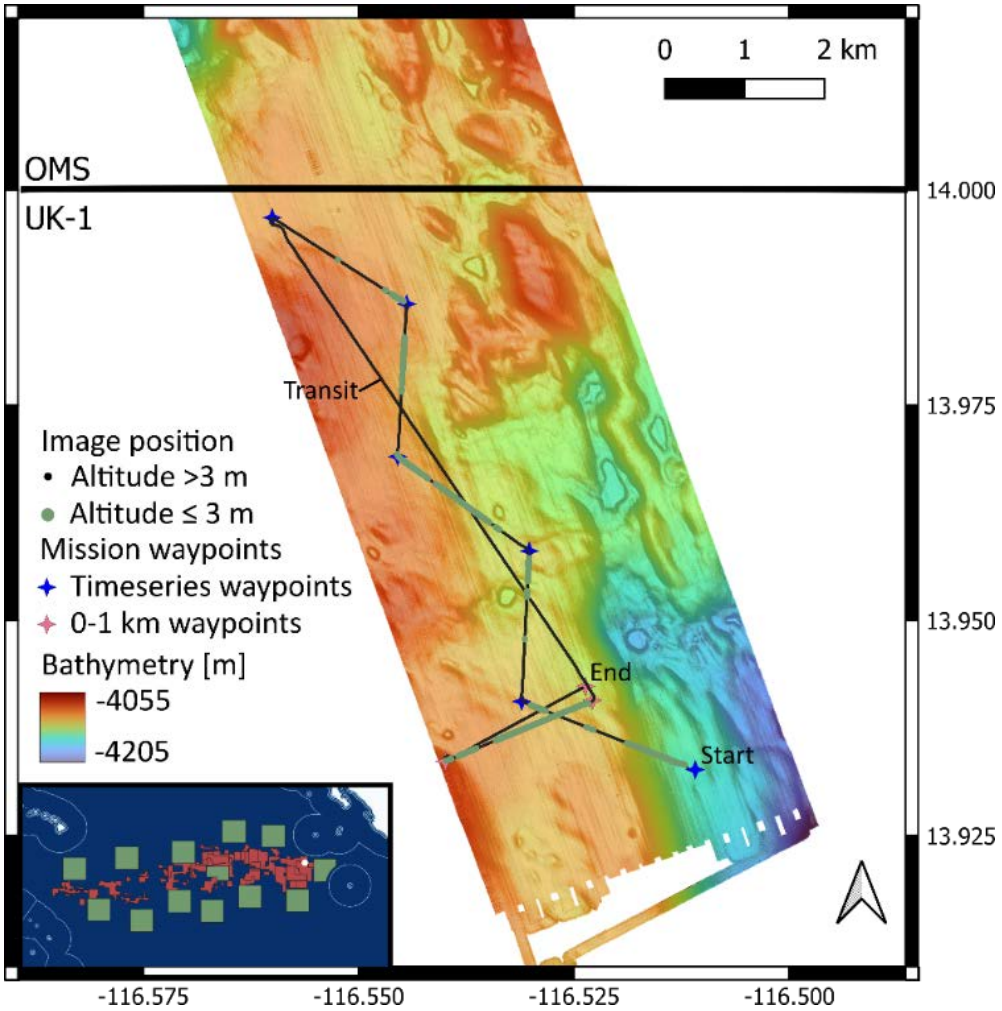


Figure 3.3.4 Imaging transects performed in AS5M091 to finish the AS5M087 planned at the 0-1km site and timeseries (WGS84). The bathymetry is underlaid by slope.

However, gain was set at -3dB in AS5M091. This low value generated dark and, when colour corrected, noisy images (Figure 3.3.5A). Indeed, this is a low value compared to the 2.464dB calibrated at-bottom while mounted on the *Isis* ROV during JC241. As a result of the dark images, we recommend to systematically configure the camera's settings based on previous calibration. Furthermore, we advise to produce a .yaml file recording all camera parameters, as carried out for the AESA2. Following this outcome, an imaging transect of ~1 km was performed in AS5M092 to test the effect of a gain of 2.5dB on image brightness.

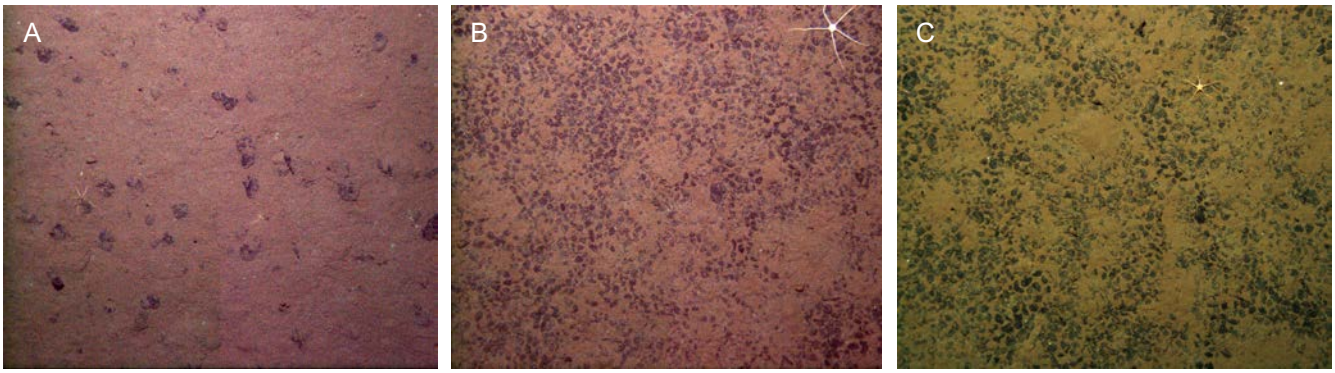


Figure 3.3.5 Imaging retrieved during at 3-m altitude and colour corrected ('autocolor correction') in IrfanView. A. Images taken during AS5M091 clearly showed a split in the image, and speckly noise due to low gain setting. B. AS5M092 images were slightly better but noise was still present, and the white balance was not appropriate as shown by the brightness of the ophiuroid compared to the seabed. C. AS5M093 displayed satisfactory results as shown by the good level of contrast between the ophiuroid and the seabed, and the lack of noise on the seabed. This is a satisfactory threshold for observing small organisms of ~ 1 cm and discerning details to identify organisms at the morphotype level.

AS5M092 imaging test was successful. Image brightness was enhanced compared to AS5M091, but the later was still not satisfactory (Figure 3.3.5B). Therefore, we revised the camera settings for AS5M093 to a gain of 4dB. Furthermore, we interpreted this outcome to arise from a lack of flashlight exposure. Hence, we adapted the shutter speed from 2 ms to 3 ms. Additionally, we switched to no gamma correction as it is not necessary for *.raw* images.

Following this change of settings, AS5M093 provided satisfactory picture quality (Figure 3.3.5C). However, *Autosub5* aborted her mission at the start of the second imaging leg, coinciding with the start of a 5% slope. Furthermore, the first transect was depleted of 3m-altitude images as we noticed that the vehicle was going downhill, along the first transect. The lack of constant altitude in AS5M091 and AS5M093 (also suggested in AS5M087), combined with that latest mission abort, questioned the ability of the sub to undertake imaging missions along the slope. Therefore, we redesigned imaging surveys to minimise tracks along the slope. As a best approach, we flew the AUV perpendicular to the slope, by following contour lines with parallel transects interspaced by 100 m (i.e., turning radius of AUV ranging from 25 to 33 m). When transiting from one transect end point to the next start point, hence along the slope, we flew the AUV at 4 m altitude. Moreover, camera surveys were now systematically initiated from uphill to downhill to prevent from facing the slope when making a turn. Finally, minimum altitude abort criterion was set from 1 m to 0.5 m altitude over 10 seconds.

AS5M094 was a success since *Autosub5* completed her mission and the images collected were of sufficient quality.

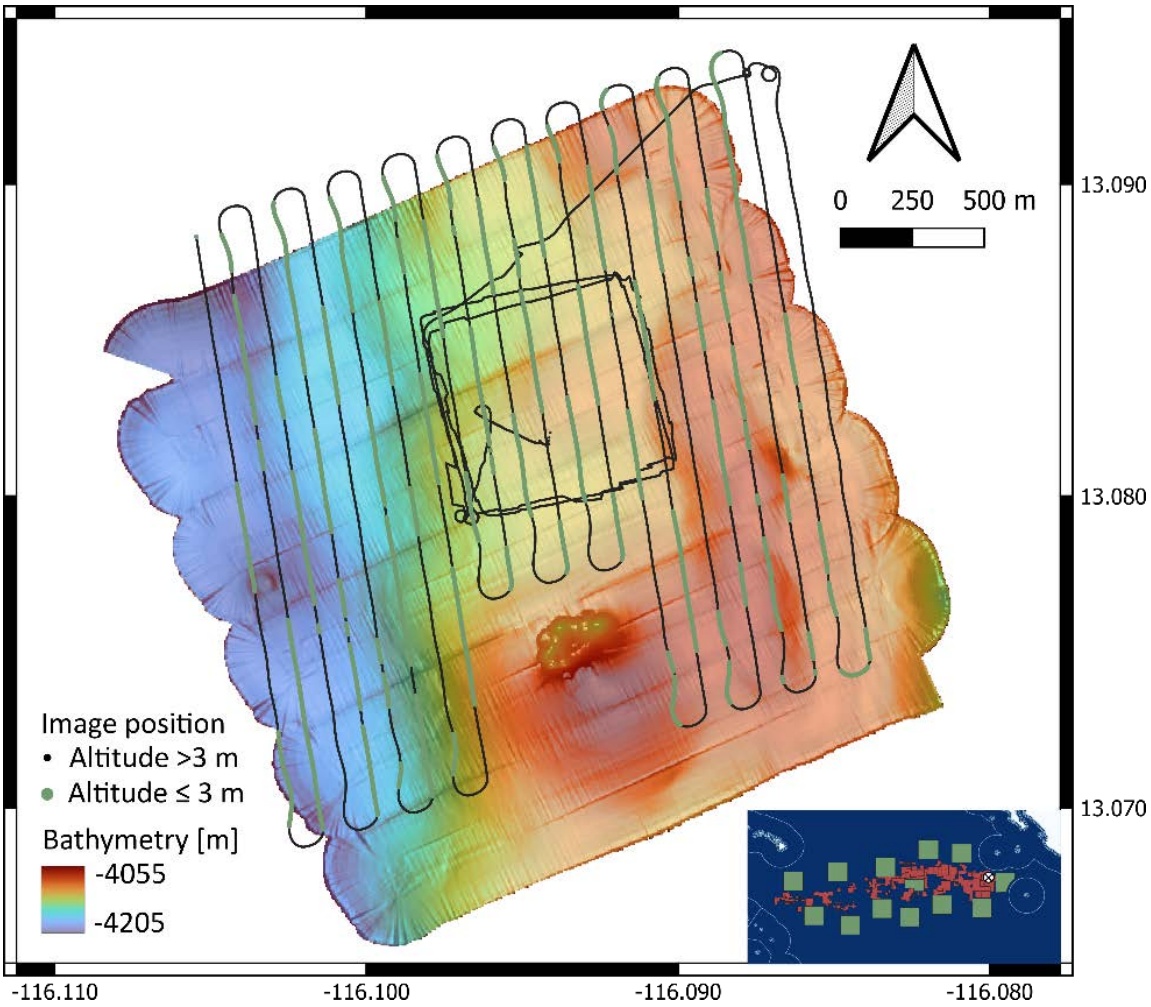
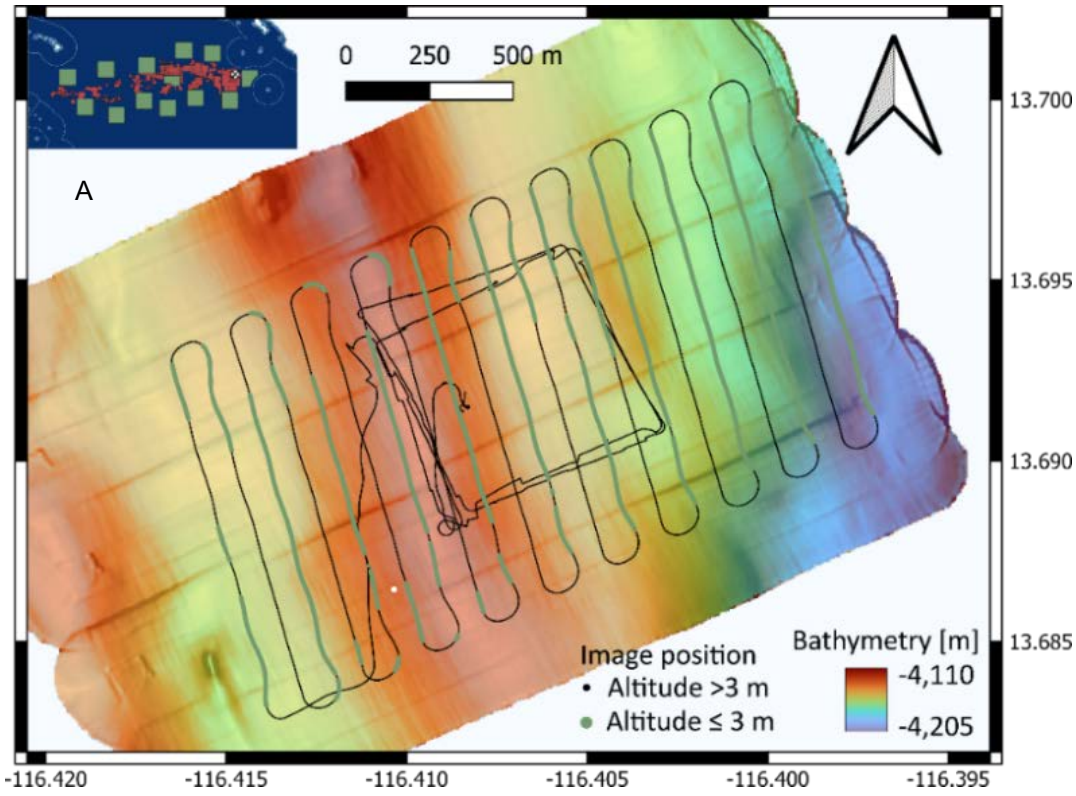


Figure 3.3.6 Imaging transects performed during AS5M094 at the 100-km site (WGS84). The bathymetry is underlaid by slope.

AS5M095 aimed to attempt again seabed imaging that failed at the 16 km site during AS5M090. Since the 16 km site was more complex than the 30 km, the dive was planned to start from the latter. Parallel transects were created along bathymetric lines, as for AS5M094. For the 30-km site, transit over the crest (waypoints 9 to 20) were performed with higher altitude (5 m). The 16-km site exhibited a more complicated topography with no linear contour lines and presence of steeper slopes (max. 4-6%). Based on the AUV team's suggestion, the image survey was extensively redesigned there to avoid slopes >4% and any features where the bathymetry showed a downward slope followed by a sharp upward slope. Where a slope of 2 to 4% was encountered, the new survey design intended to fly the AUV at an altitude was of 14 m followed before returning to 7 m and then to 3 m, once the 'obstacle' was behind. To be as conservative as possible to avoid potential problematic features the final survey design ended up more complicated, but with less likelihood of abort which was prioritised at that stage of the cruise. To deal with terrain displaying no clear slope pattern, we acknowledge the use of slope maps to refine an initial survey design as much as possible. During AS5M095, *Autosub5* performed the transects until the timeout was reached. Unfortunately, the camera did not take photos after 30 mins. The problem arose from the operating system which got corrupted for unknown reason. The operating system was imaged to a new hard drive.

The new operating system was tested on the AS5M096 multibeam survey dive at the 100-km site, as the camera remained on for the whole. AS5M096 successfully provided ground truthing for acoustic data, showing a seabed scattered with rocks. However, the sub hit three times both rocky and nodule seabed. Altitude over seabed averaged 2 m, which suggests *Autosub5* struggled to maintain the target altitude for unknown reason.

AS5M097 was a successful dive since images of sufficient quality and quantity were acquired over enough transects (Figures 3.3.7). Like AS5M091, transects with a downhill orientation were only sparsely imaged.



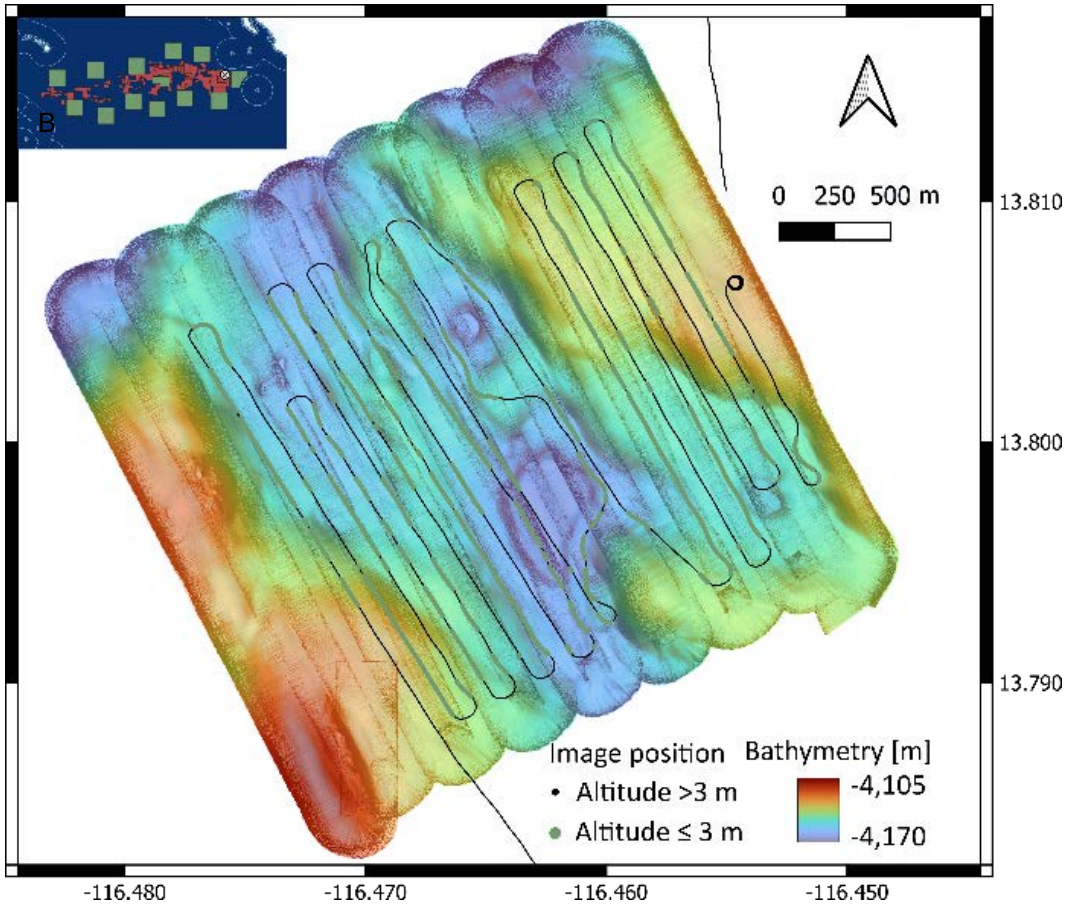


Figure 3.3.7 Imaging transects performed during AS5M097 A. at the 30-km site and B. at the 15-16-km site (WGS84). The bathymetry is underlaid by slope.

AS5M098 was planned to repeat the 0-1-km site and timeseries as it was initially imaged with different settings (i.e., AESA2 for AS5M087 and lower image quality for AS5M091). Additionally, we intended to image a site north of the 0-1km (-7 km site). Seventeen transects were imaged before the AUV prematurely ended her dive, therefore excluding image acquisition at the timeseries and -7-km site (Figure 3.3.8). At recovery, the cause for abort was related to the ignition of one of the batteries. This ended any expectation to redeploy the AUV during that last sampling day at UK-1.

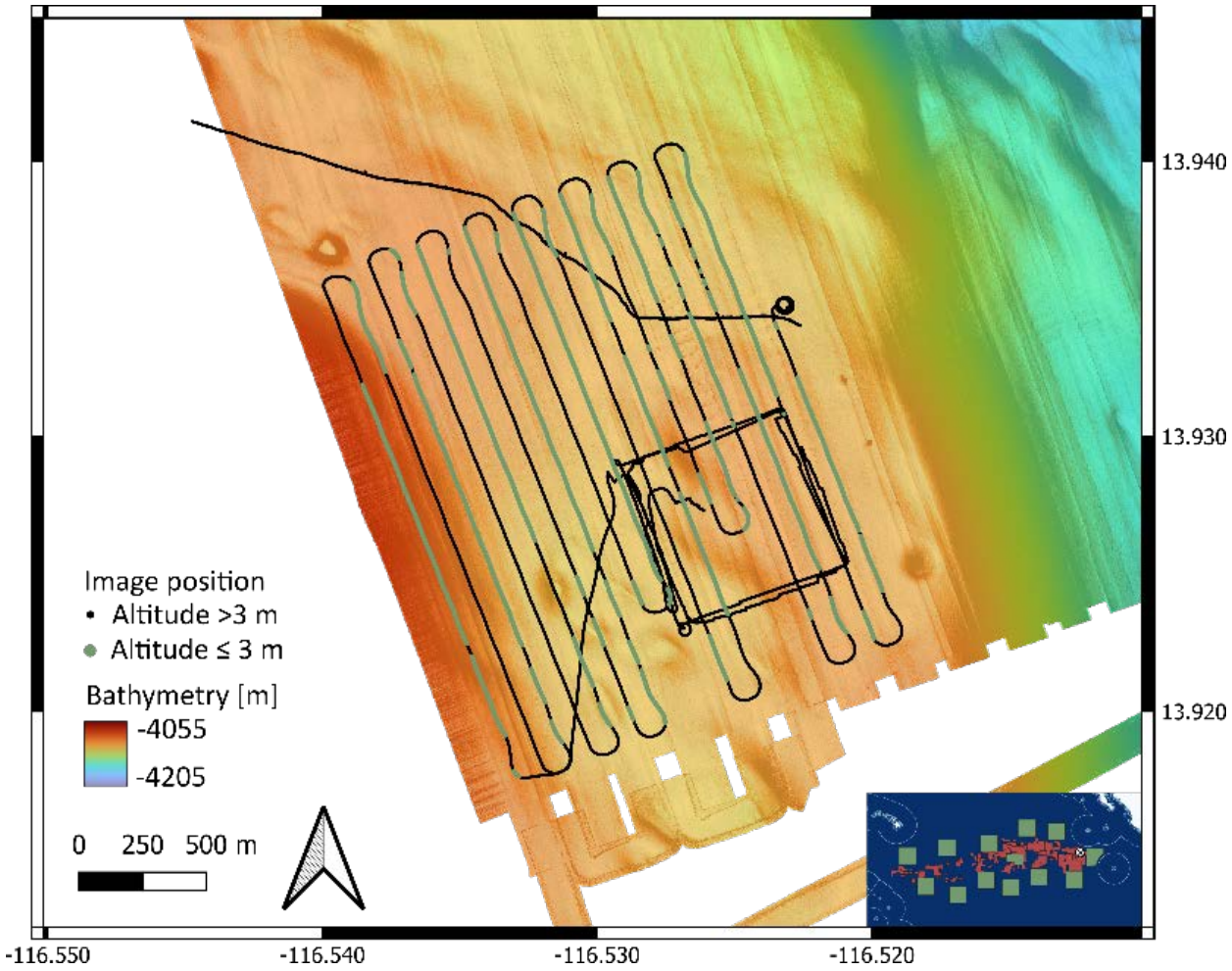


Figure 3.3.8 Imaging transects performed during AS5M098 at the 0-1-km site (WGS84). The mission was aborted at the seventeenth transect due to battery failure. The bathymetry is underlaid by slope.

In overall we are satisfied by the amount of data that was collected, meeting our strict minimum criterion for sampling effort and image set standardisation. However, when putting that in perspective with the duration at seabed we believe that we did not benefit from the full potential of the AUV (e.g., sites at 50, 70 and 90 km were not visited, 21.6 hours of satisfactory image acquisition over a 1.5-month cruise). This statement can be explained by the following reasons:

- Preliminary mission needed for MBES mapping, limited to distance of ~90 km by the battery while imagery acquisition is limited to ~120-km mission.
- Four missions were aborted, including three related to electronical issues.
- Three missions underwent a camera failure, even forcing a swap from AESA2 to AESA1 which threatened the standardisation of the image set. Several tests were needed to calibrate the camera settings.

Table 3.3.1 Autosub5 imaging missions presented along with description of the image set collected. 'at seabed' means images taken at an altitude ranging from 1.6 to 3 m. Time at seabed is calculated from the first to last picture at seabed. Estimated imaging distance is derived from time at seabed and the speed of the vehicle (1.2 $m.s^{-1}$). *AS5M084 had no image taken below 3 m altitude as the later was not targeted.

Station	Site (UK1)	Mission and camera	Survey type	Images taken	Images at seabed	Area imaged [m^2]	2-km transect intended	Time of imaging (hour)	Estimated imaging distance (km)	Problems encountered
JC257-013	0-1km	AS5M084 AESA2	Test	1,363	0*	0*	0	0	0	Targeted altitude too high (3.5 m)
JC257-041	0-1km	AS5M086 AESA2	Zigzag + Timeseries	0	0	0	25	0	0	Aborted
JC257-047	0-1km	AS5M087 AESA2	Zigzag + Timeseries	17,436	8,619	13,088	25	4.8	20.7	Camera failure
JC257-076	16-30km	AS5M090 AESA2	Zigzag	5,022	0	0	30	0	0	Camera failure then aborted
JC257-081	0-1km	AS5M091 AESA1	Zigzag + Timeseries	51,124	6,184	7,736	7	1.7	7.4	Unsatisfactory image quality
JC257-090	100km	AS5M092 AESA1	Test	3,523	320	426	0	0.1	0.4	None
JC257-096	100km	AS5M093 AESA1	Zig-zag	7,560	384	431	20	0.1	0.5	Aborted
JC257-103	100km	AS5M094 AESA1	Parallel	51,026	16,471	21,046	20	4.6	19.8	None
JC257-106	16-30km	AS5M095 AESA1	Parallel	11,112	427	530	30	0.1	0.5	Three resets until camera failure
JC257-108	100km	AS5M096 AESA1	Test	54,950	99	76	0	0.1	0.1	Hit seabed three times
JC257-113	16-30km	AS5M097 AESA1	Parallel	100,300	22,204	27,141	30	6.2	26.6	None
JC257-118	0-1km and -7km	AS5M098 AESA1	Parallel + Timeseries	52,671	14,276	18,996	35	4.0	17.1	Aborted
Total		**12 missions**	**3 tests, 9 surveys**	**356,087**	**68,985**	**89,470**	**227**	**21.6**	**93.1**	**4 aborts 3 cam failures**

3.3.2. Bathysnap survey

The objective for the Bathysnap system was to assess biological processes over (1) a yearly timescale at a daily rate and (2) monthly timescale at an infra-hourly rate (15 min).

Considering yearly timescale first, JC257-009 successfully recorded 338 images until the end of the deployment. JC257-010 stopped recording earlier totalling 268 images. Further investigation in the time series of JC257-010 demonstrated that images were recorded with uneven timestamps. Starting from 02/10/2023 to 15/11/2023 at which images were spread apart of more than a day, and from 15/11/2023 to 16/11/2023 at which images were recovered at decreasing periods. The Bathysnap system has two internal clocks: one to wake up the camera, and the second to encode the image time step. The later froze on the 17/11/2023. Reason for failure is unknown. Therefore, we advise to (1) systematically check for any drift/failure of internal clock immediately after recovery and to (2) remove any images taken after the 02/10/2023, as data quality is uncertain for further analyses.

Table 3.3.2 Dataset acquired with Bathysnap cameras. Note dual station numbers for deployment/recovery over two expeditions.

Station	Folder name	Images taken	Images at seabed	First image at seabed	Last image at seabed
JC241-100 JC257-009	'JC241_Bathysnap01'	338	334	19/03/2023, 00:09:21	15/02/2024, 00:09:07
JC241-099 JC257-010	'JC241_Bathysnap02'	268	252	19/03/2023, 00:58:23	16/11/2023, 20:13:30
JC257-022	'JC257_Bathysnap04'	2,311	2,183	19/02/2024, 05:13:51	12/03/2024, 22:54:50
JC257-023	'JC257_Bathysnap05'	2,226	2,171	19/02/2024, 04:31:25	12/03/2024, 18:46:29

Examples of field of view and image quality are provided in Figure 3.3.9.

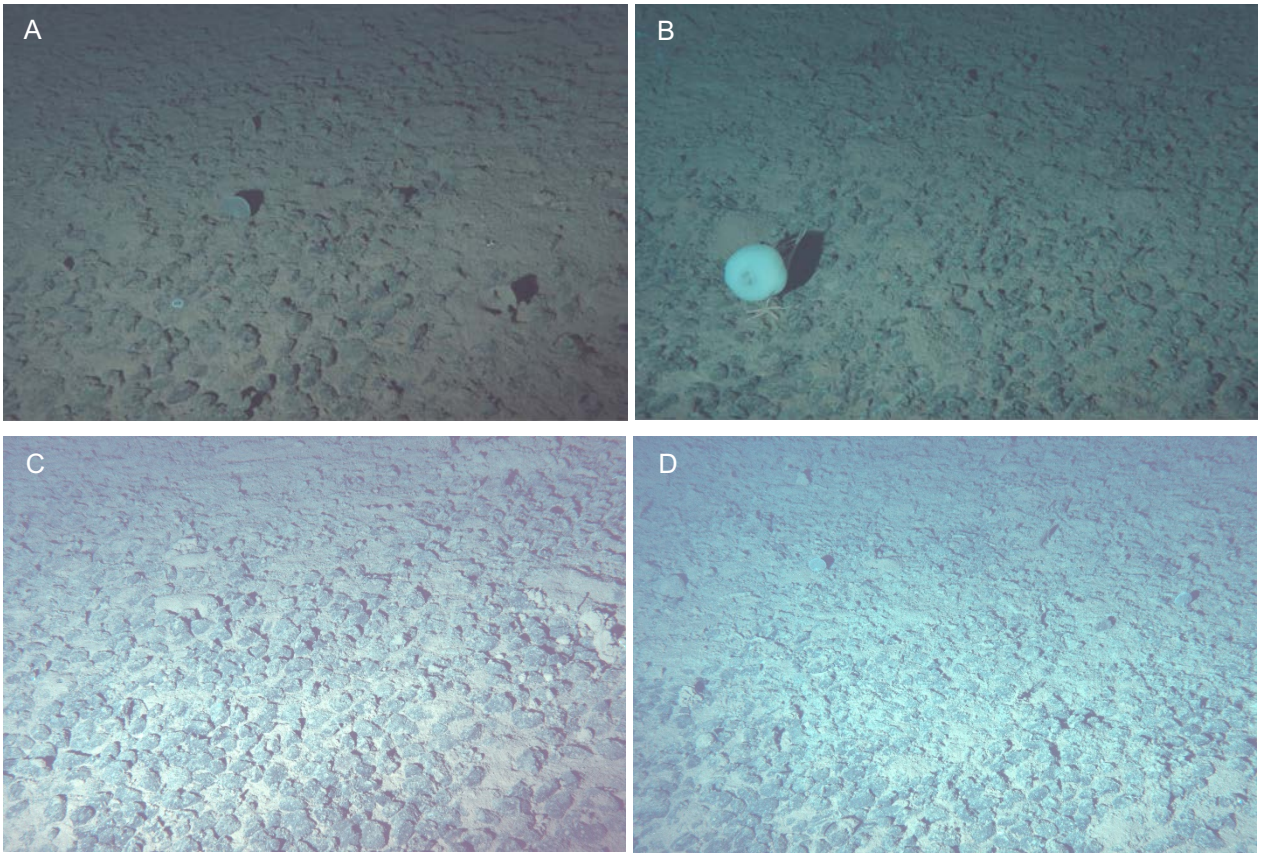


Figure 3.3.9 Field of view of seabed images retrieved by the bathysnaps: A. JC257-009, picturing a Trachymedusae and several Psammina xenophyophores. B. JC257-010, picturing a Hyalonema sponge with a Brisingidae attached. C. JC257-022, high density of nodules captured. D. JC257-023, high density of nodules scattered with several Psammina xenophyophores.

3.3.3. Data storage

Data was stored on two QNAPs connected in parallel as Static/DHCP Server. To access 'Daniello'/'Adriano' we used an ethernet cable from their Port 1 directly to the computer to network them respectively at \\192.168.1.25\JC241 and \\192.168.2.35\jc257.

References:

Simon-Lledo, E., B. J. Bett, V. A. I. Huvenne, T. Schoening, N. M. A. Benoist, R. M. Jeffreys, J. M. Durden, and D. O. B. Jones. 2019. Megafaunal variation in the abyssal landscape of the Clarion Clipperton Zone. Prog Oceanogr **170**:119-133.

Parrish, C. 2020. Index of Refraction of Seawater and Freshwater as a Function of Wavelength and Temperature. Oregon State University. Accessed on 18/03/2024. https://research.engr.oregonstate.edu/parrish/index-refraction-seawater-and-freshwater-function-wavelength-and-temperature

3.4. Natural History Museum - Megafaunal Sampling

3.4.1. Introduction and Objectives ROV sampling

Megafauna sampling was carried out to deliver on SMARTEX Proposal Objective 4 (Life history, Reproduction and Connectivity) and Objective 5 (Biodiversity, community structure and trophic dynamics). Further details of these objectives are provided in the SMARTEX proposal, but in summary, the primary goals were to:

- Collect a wide range of megafauna on the spatial scaling range of 0 km, 1 km, 16 km and 100 km to provide fundamental baseline data on levels of natural biodiversity using the ROV Isis
- Target widespread and abundant species for genomic 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

3.4.2. ROV configuration for megafaunal sampling

The Remotely Operated Vehicle 'ROV Isis' was used for megafauna collection. This ROV possesses two manipulator arms (Kraft Predator and Schilling T4) with DSPL LED mounted under each manipulator arm used during *in situ* imaging for high-resolution captures and one slurp gun for capturing and handling specimens and tools. Section 2.1 describes the ROV sampling configuration per megafauna collection dive. In summary, three bioboxes were available, one at each port and starboard swing arm and a larger one on the front tray. Depending on the dive requirements, we could include between 6 and 12 'mag' tubes (individual tube-shaped containers with magnetic lids) for the collection and storage of fragile and extremely rare specimens during the dives.

Three high-resolution cameras were recording simultaneously during each dive: 1) Mini Zeus HD (hereafter referred to as Pilot Cam), 2) Super Scorpio (hereafter referred to as Scorpio), 3) Konsberg Eye Ball Cam (hereafter referred to as Science Cam). Scorpio has the highest resolution of the three cameras and can take stills at regular intervals. For JC257 megafauna dives this timer was set to capture a still every 45 seconds. To obtain high-resolution images *in situ* from the different specimens, most of the megafauna dives had positioned Scorpio 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 not implemented in Dive 431, Dive 433 and Dive 414 because the dive was shared with cube experiment (details in section 2.1). This camera has a pair of NOC lasers with 10 cm spacing, which were switched on and off depending on specimen collection requirements. The Science Cam is a dome pan and tilt-mounted camera located on top of the ROV Isis to provide a peripheral view ca. 180 degrees, providing enhanced visibility for finding specimens on the seafloor; this camera is largely controlled by the scientists.

The ROV team on board was in charge of maintaining ROV Isis and deploying and recovering all megafauna dives 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 it was off the deck until it was back on deck. Every event was recorded with at least USBL coordinates (from ROV), depth, and UTC time across the different logging methods, i.e. Dive log (dive details), Sample log (samples collected) and Media log (video recordings and back-ups).

Target megafaunal specimens were selected to include a wide range of megafaunal taxa representative of the abyssal biodiversity of the area. Specimens were mostly collected with the manipulator arms and placed in the bioboxes or mag tubes. 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.

The general protocol for ROV Isis dive 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 the manual provided by ROV Isis.
3. Log details of the ROV entering the water and start video recording on Scorpio.
4. When the ROV is almost at the seabed (~100 m above) change the Scorpio video tape and start recording on all three cameras (Pilot and Science Cam as well as Scorpio).
5. Log the ROV reaching the seabed, ensure all cameras are recording, and set Scorpio stills to be taken every 45 seconds.
6. Use Science Cam to locate target megafaunal specimens for collection.
7. Land the ROV on the seabed with the specimen aligned with Scorpio and ensure lasers are on.
8. Take some Scorpio stills, even if it is still set to take photos every 45 seconds.
9. Turn off the lasers and stop automatic stills on Scorpio. Depending on how mobile the specimen is, record at least 40 seconds of video, or anywhere between 10 and 20 minutes if the animal is stationary.
10. Turn off the main lights and turn on the lights on the manipulator arms to take Scorpio stills with lighting from different angles.
11. Turn the lasers back on, resume Scorpio stills, and proceed with collection using either a slurp gun or a manipulator arm. Log the event in the sample log, OFOP, and create a waypoint.
12. Log when the ROV leaves the bottom and stop video recording in all cameras. On the way up, only record the last 1,000 m before reaching the deck with Scorpio.

13. Ensure everything is logged until the ROV is on deck

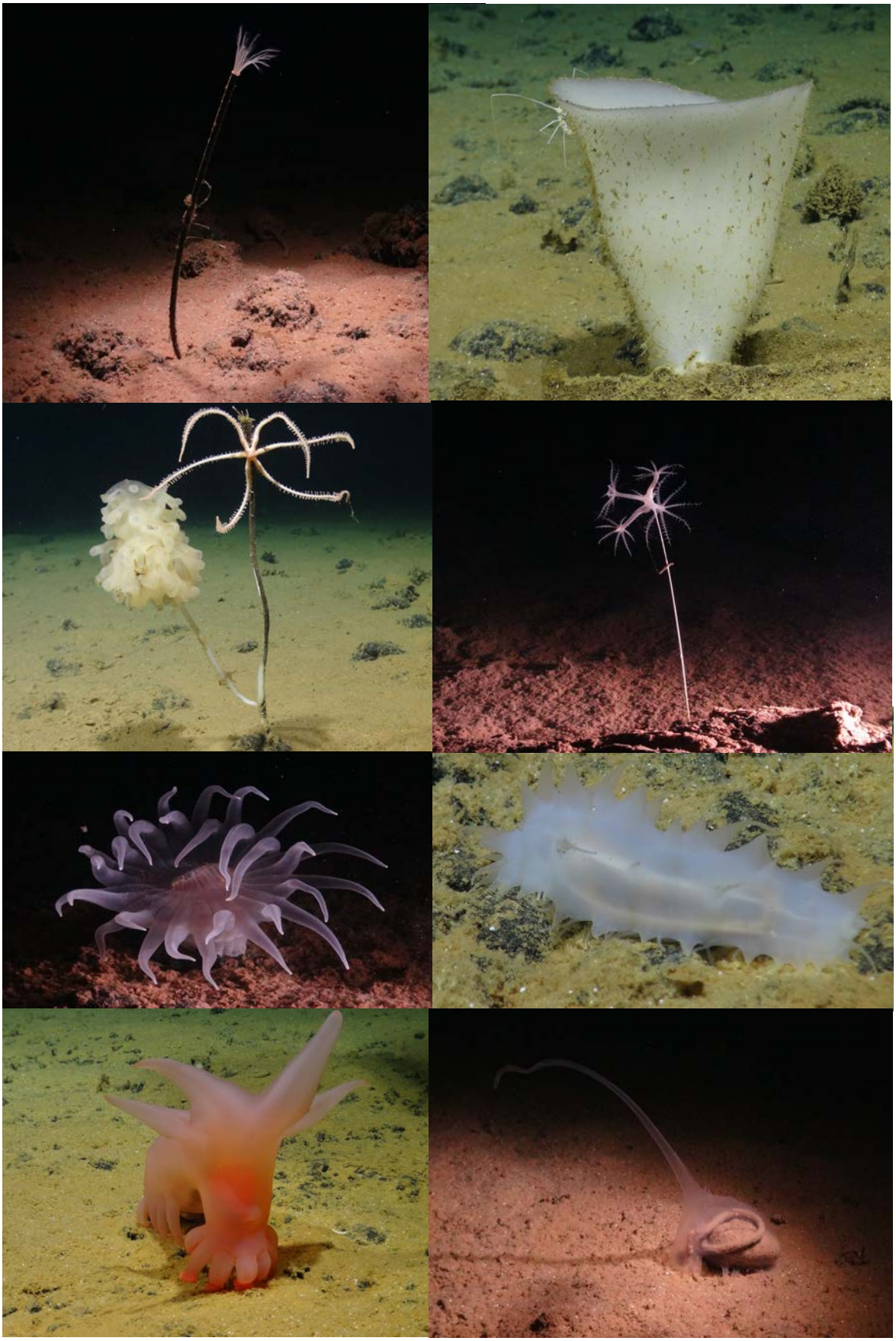


Figure 3.4.1 In situ images of benthic megafauna taken with ROV Isis Scorpio camera.

3.4.3. Preparation before megafauna arrived on deck

Dissection tools, trays, and buckets were bleached in advance by immersing them in a 1% bleach solution for at least 15 minutes, then rinsed three times with Milli-Q water. Disposable gloves were worn throughout cleaning equipment and processing areas and for handling megafauna samples. Areas for dissection were cleaned after each dive with 1% bleach and between specimens with RNaseZap for decontamination and to avoid cross-contamination. Buckets, trays and jars were filled with Cold Filter Seawater (CFSW) when ROV Isis reached the deck.

3.4.4. Megafaunal samples processing upon arrival

As soon as the ROV was securely fastened, all specimens were recovered in individual containers or together based on their collection container e.g. all specimens from the port biobox were placed in the same bucket. The water-sediment mix from the bioboxes was recovered and sieved using 300 µm sieves to assist in the visibility of megafauna and for potential opportunistic macrofauna sampling. Specimens were recovered with gloves and placed in clean buckets filled with CFSW. Sponge specimens received special attention, being kept in individual or other porifera buckets to avoid or reduce contamination from other specimens. All samples were transferred to a 4° C temperature-controlled room as part of the cold-chain process described in Glover et al (2016).

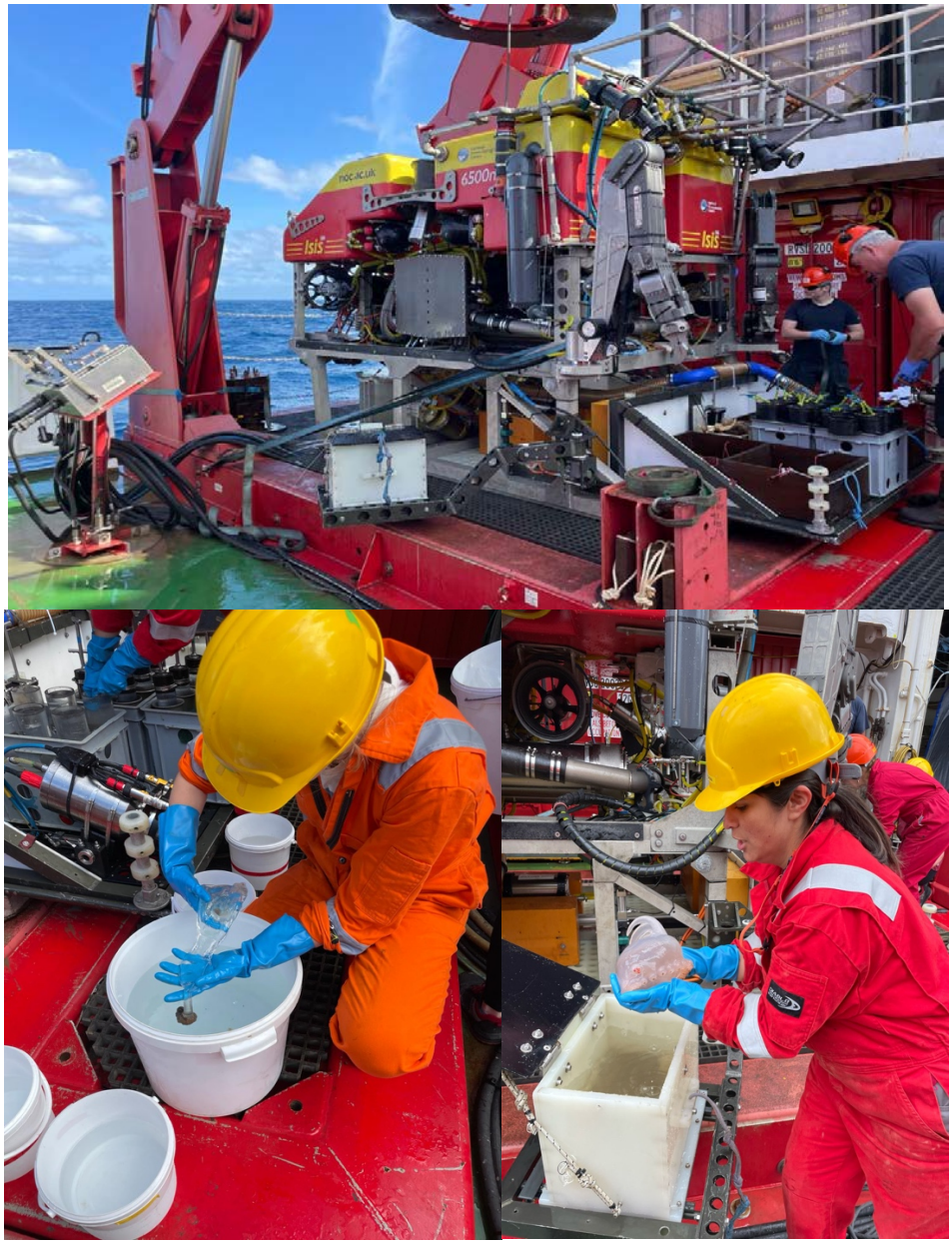


Figure 3.4.2 Megafauna recovery from ROV Isis. Top: The ROV Isis is secured on deck, and the ROV Team prepares swing bioboxes and mag tubes for the recovery and mobilisation of animals to the cold room. Bottom left: Tunicate carefully transferred to a bucket filled with cold-filtered seawater. Bottom right: a sea cucumber is carefully transferred from starboard biobox to a bucket for transportation to the cold room. Credit: Lucy Harris.

3.4.5. Imaging and processing

Megafauna specimens were processed in the cold lab of the *RRS James Cook*, where the temperature fluctuated between 4° C and 6° C. Here, each specimen was kept in an individual bucket with fresh CFSW and prepared for photographs (Figure 3.4.3). Specimens were photographed individually using a Canon EOS850d; depending on the animal size, one of two different-sized glass aquarium tanks was used, each one filled with CFSW. For some specimens, details of taxonomically-informative characteristics were photographed under the Canon EOS5D Mark III with a 100 mm macrolens and/or a Leica MZ9.5 stereo microscope with a trinocular head and Leica phototube attached to a Best Scientific Canon EOS camera adaptor attached to a Canon EOS90d also equipped with 2x Canon 430EXII speedlights. All specimen photographs were taken with a non-reflective black cloth in the background and a scale bar to the side. After a couple of specimens were photographed, the CFSW inside the aquarium was replaced to avoid sample cross-contamination. Image numbers were recorded in a spreadsheet along with unique identifiers and any other relevant information.

Figure 3.4.3 Left: Individualisation of megafauna in fresh cold-filtered seawater in the cold room before photography. Right: The NHM Team setting up the aquarium and the specimen for photography. Credit: Lucy Harris & Adrian Glover

3.4.6. Megafauna subsampling

After photographs were taken, all specimens were subsampled even though they were considered voucher specimens (i.e., the first individual collected of each species). Most voucher specimens were subsampled in a way that maintained their taxonomically informative characteristics, and the tissue was stored in RNAlater for molecular analyses. Additional tissue samples (i.e. mangled or shredded body sections) were preserved in liquid nitrogen (snap-frozen) or 99% ethanol. In addition, some animals were dissected to get specific tissue samples; for example, a section of an arm was dissected from brittle stars, tube feet and arms from seastars, and a section of the body wall from anemones. Sea cucumbers and sponges were the most collected animals thus multiple tissue replicates were taken. In sea cucumbers, the digestive system and longitudinal muscles were dissected, and in sponges tissue collected from different body sections were dissected and for both of these, tissue samples were preserved in 99% ethanol, RNAlater, snap-frozen and DMSO (dimethyl sulfoxide) for molecular purposes, and OCT (Optimal Cutting Temperature) and formalin for histological preparations.

Figure 3.4.4 Left: Subsample table set up including tubes filled with different fixation methods. Right: Subsample dissection from sponge body. Credit: Daniel Jones.

Some specimens presented gonadal tissue that was subsampled and preserved in 10% formalin for life-history trait analyses (details in Table 3.4.1). Each specimen and each subsample was assigned a unique identifier (NHM number), along with all other relevant information (e.g., deployment number, photo number etc.). For subsamples, tissue type and preservation method were recorded along with the NHM number from the specimen from which it was taken. As soon as tissue samples were processed, the snap-frozen and 99% ethanol subsamples were stored at -80° C. Subsamples stored in RNAlater were kept at -80° C after 12 hours, having been kept at 4° C prior to this following manufacturer instructions. For most voucher specimens, their 80% ethanol was changed after 24–48 hours to ensure reliable preservation.

Table 3.4.1 Subsamples collected for life-history trait analysis. All subsamples were preserved in 10% formalin.

Deployment #	NHM #	Taxonomy				Sample preserved
JC257_042	13135	Cnidaria	Actinaria			Eggs
JC257_045	13178	Echinodermata	Ophiuroidea			Gonads
JC257_045	13179	Echinodermata	Ophiuroidea			Eggs
JC257_063	13677	Echinodermata	Echinoidea	Aspidodiadematidae	Plesiodiadema cf. globulosum	Eggs
JC257_063	13693	Echinodermata	Holothuroidea	Synallactidae	Synallactes	Suspected gonads
JC257_063	13698	Annelida	Polychaeta		Polynoidea	Gonads or sperm sacs
JC257_078	13977	Cnidaria	Actinaria			Eggs
JC257_078	13981	Cnidaria	Actinaria			Eggs
JC257_083	14053	Annelida	Amphinomida	Amphinomidae		Eggs
JC257_105	14448	Echinodermata	Holothuroidea	Elpidiidae	Amperima	Gonads
JC257_105	14471	Echinodermata	Holothuroidea	Synallactidae	Synallactes	Gonads

3.4.7. Summary of megafaunal samples collected

ROV Isis carried out a total of 12 megafaunal collection dives. Three dives were carried out in UK1_0km and UK1_1km, while only two dives were carried out in UK1_16km and UK1_100km (Figure 3.4.5). One dive was carried out in the OMS area (Singapore) to collect a colonisation experiment deployed in 2013 as part of the ABYSSLINE project. As a result of the initial mapping in the area, an outcrop was identified nearby and briefly explored during Dive437, identifying an abundance of sponges and corals associated with the rocks; for this reason, this area is denominated Sponge Garden hereafter. This area was explored, and some specimens were collected during a return to the site on Dive 446.

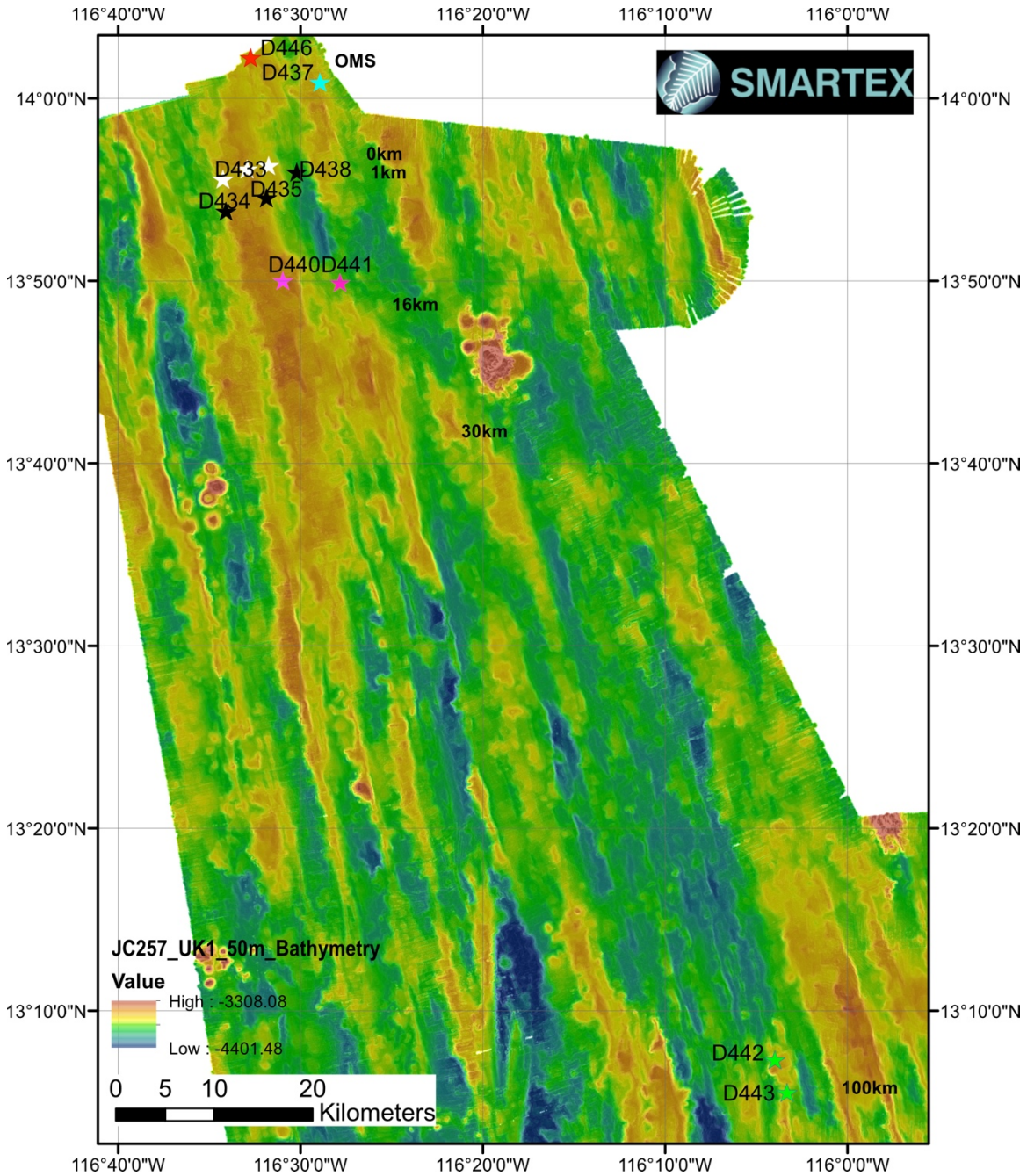


Figure 3.4.5 Map of locations of ROV Isis dives to collect megafauna in the different sampling sites: UK1_0Km (black stars), UK1_1Km (white stars), UK1_16Km (pink stars), UK1_100Km (green stars). Dive 437 (light blue star) was exclusively performed in OMS (Singapore) and Dive 446 (red star) was performed in the Sponge Garden area. Map produce by Catherine Wardell, NOC and modified by JC257 NHM team.

The megafauna dives lasted a total of 151.3 hours, of which 86.23 hours were exclusively bottom time. During this period, we collected 159 samples from the seabed (Table 3.4.2) corresponding to seven different phyla (Figure

3.4.6); all dives were very successful, with an average of 32 minutes per sample collection and few losses during the ascent (five animals).

Table 3.4.2 Number of megafaunal metazoan specimens collected from seabed across the six sampling stations using the ROV.

Phylum	Sampling Station						Total indiv. by phylum
	UK_0Km	UK_1Km	UK_16Km	UK_100Km	OMS	Sponge Garden	
Annelida				1			1
Arthropoda	1		1				2
Bryozoa		1	1		1		3
Chordata		1	1	1		1	4
Cnidaria	7	4	8	2	3	1	25
Echinodermata	21	16	9	12	1	1	60
Porifera	17	15	10	12	6	4	64
Total individual by Sampling Station	**46**	**37**	**30**	**28**	**11**	**7**	**159**

Figure 3.4.6 Diversity representation of megafauna collected with ROV Isis during JC257. Images were obtained in the laboratory upon specimen arrival on deck. Credit: NHM JC257 Team.

Porifera and Echinodermata were the most collected phyla across JC257. In Porifera, 55 Hexactinellida and 5 Demospiagea were obtained (Figure 3.4.7), while in Echinodermata, 28 Holothuroidea (Figure 3.4.8), 13 Asteroidea, and 11 Ophiuroidea specimens were collected exclusively from the seabed.

Figure 3.4.7 Representation of Porifera diversity collected during JC257 with ROV Isis, images were obtained in the laboratory upon specimen arrival on deck. Credit: NHM JC257 Team.

Figure 3.4.8 Diversity representation of Holothurian samples collected during JC257 with ROV Isis. Images were obtained in the laboratory upon specimen arrival on deck. Credit: NHM JC257 Team.

3.4.8. Non- quantitative opportunistic samples

Most of the megafauna collected contained more than one specimen, yielding over 350 specimens from nine phyla. In addition, amphipods were collected from the amphipod traps deployed in fish trap landers JC257_016 and JC257_078 (section 2.8). On deck, the amphipod traps were placed in CFSW, moved to the deck lab and all the specimens in the trap were bulk-fixed in ethanol 80% for sorting and identification at the NHM lab.

3.4.9. Megafaunal samples for molecular identification and genomics analyses

At least one tissue subsample was taken from each collected megafauna specimen using the dissection protocol described above. The tissue subsamples will be a fundamental source for molecular studies such as barcoding/metabarcoding, population genomics, transcriptomics, whole genome sequencing, and microbial assessments. From the 159 megafauna collected in JC257 (Table 3.4.3), we took a total of 516 subsamples from different body sections, preserving them using six different methods: 99% ethanol, RNAlater, snap-frozen, DMSO, OCT, and formalin.

Table 3.4.3 Complete list of megafaunal samples taken during JC257 from the ROV. Dep. N refers to JC257 deployment number.

Taxonomy			Latitude	Longitude	Depth	UTC	Dep. N
Annelida							
	Polychaeta	Sabellidae	13°06.417	-116°05.053	4129	11:24	094
Arthropoda							
	Pycnogonida		13°55.955	-116°30.656	4145	07:37	063
	Pycnogonida		13°49.383	-116°29.80	4094	13:37	078
Bryozoa							
	Cheilostomatida		13°55.536	-116°32.040	4094	01:43	015
			14°02.036	-116°32.792	4075	01:18	057
			13°49.474	-116°29.537	4096	08:32	078
Chordata							

	Ascidiacea	Octacnemidae	Megalodicopia sp?	13°55.455	-116°31.960	4098	20:46	033
	Ascidiacea			13°49.470	-116°29.694	4094	12:25	078
	Ascidiacea			13°04.329	-116°07.361	4263	10:55	105
	Ascidiacea			14°01.960	-116°32.864	4079	00:55	117
Cnidaria								
	Actinaria			13°55.455	-116°31.959	4098	21:02	033
	Actinaria			13°55.638	-116°33.239	4087.8	08:38	042
	Actinaria			13°55.594	-116°33.085	4078.6	13:40	042
	Actinaria			14°01.956	-116°32.854	4077	02:32	057
	Actinaria			13°56.038	-116°30.886	4138	14:43	063
	Actinaria			13°49.392	-116°29.677	4095	11:42	078
	Actinaria			13°49.537	-116°29.471	4099	10:36	091
	Actinaria			14°01.957	-116°32.862	4079	00:53	117
	Antipatharia	Cladopathidae	Abyssopathes lyra	13°56.953	-116°30.870	4151	04:57	019
	Antipatharia	Cladopathidae	Abyssopathes lyra	14°02.094	-116°32.759	4078	00:23	057
	Antipatharia	Schizopathidae	Bathypathes	13°49.474	-116°29.537	4096	08:26	078
	Antipatharia	Schizopathidae	Bathypathes	13°49.551	-116°29.545	4098	09:34	091
	Octocorallia	Primmnoidae	Callozostron bayeri	13°57.209	-116°30.731	4140	09:10	019
	Octocorallia	Primmnoidae	Abyssoprimnoa?	13°55.767	-116°33.054	4078	01:10	049
	Octocorallia	Primnoidae	Calyptrophora	13°55.452	-116°31.961	4098	20:09	033
	Octocorallia	Taiaroidae		13°49.518	-116°29.511	4098.8	11:02	091
	Octocorallia			14°02.096	-116°32.758	4078	00:29	057
	Octocorallia			13°06.429	-116°05.045	4130	12:27	094
	Octocorallia			13°06.388	-116°05.127	4123	08:42	094
	Pennatulacea	Umbellula	Umbellula	13°55.797	-116°33.035	4077	23:27	049
	Pennatulacea	Umbellulidae	Umbellula	13°55.634	-116°33.226	4087.1	09:46	042
	Scleractinia			13°49.606	-116°29.509	4099	11:59	091
	Scleractinia			13°49.625	-116°29.503	4099	12:13	091
	Scleractinia			13°49.634	-116°29.501	4100	12:26	091
				13°55.762	-116°33.056	4078	01:50	049
Echinodermata								
	Asteroidea	Brisingida		13°56.859	-116°31.024	4161	01:02	019
	Asteroidea	Brisingida		13°57.053	-116°30.792	4148	06:05	019
	Asteroidea	Brisingida		13°57.149	-116°30.741	4140	07:15	019
	Asteroidea	Brisingida		13°57.154	-116°30.738	4140	07:43	019
	Asteroidea	Brisingida		13°55.763	-116°33.051	4078	01:29	049
	Asteroidea	Brisingida		13°55.759	-116°33.059	4079	02:15	049
	Asteroidea	Brisingida		13°56.038	-116°30.641	4141	12:20	063
	Asteroidea	Freyellidea	Freyastera cf. benthophila	13°55.436	-116°32.012	4096	00:35	015
	Asteroidea	Freyellidea	Freyastera cf. benthophila	13°55.634	-116°33.374	4082.9	11:53	042
	Asteroidea	Porcellanasteridae		13°49.469	-116°29.697	4094	12:33	078
	Asteroidea	Pterasteridae	Hymenaster	13°55.436	-116°32.012	4096	00:35	015
	Asteroidea	Pterasteridae	Hymenaster	13°55.956	-116°30.787	4144	09:17	063
	Asteroidea	Pterasteridae	Hymenaster	13°49.380	-116°29.657	4096	10:55	078
	Crinoidea			13°56.070	-116°30.618	4141	13:35	063
	Crinoidea			13°49.387	-116°29.804	4094.3	13:51	078
	Crinoidea			13°49.571	-116°29.533	4098	09:54	091
	Crinoidea			14°01.953	-116°32.855	4076	01:51	117
	Echinoidea	Aspidodiadematidae	Plesiodiadema cf. globulosum	13°56.060	-116°30.783	4141	13:09	063

	Echinoidea	Aspidodiadematidae	Plesiodiadema cf. globulosum	13°55.956	-116°30.787	4144	09:02	063
	Echinoidea			13°04.303	-116°07.364	4269	10:00	105
	Echinoidea			13°04.261	-116°07.37	4272	08:56	105
	Holothuroidea	Deimatidae	Oneirophanta	13°03.795	-116°07.173	4258	11:58	105
	Holothuroidea	Elpidiidae	Amperima	13°55.979	-116°30.792	4144	09:49	063
	Holothuroidea	Elpidiidae	Amperima	13°06.387	-116°05.127	4123	08:10	094
	Holothuroidea	Elpidiidae	Amperima	13°04.212	-116°07.396	4103	07:36	105
	Holothuroidea	Elpidiidae	Amperima	13°03.793	-116°07.133	4260	12:26	105
	Holothuroidea	Elpidiidae		13°04.275	-116°07.37	4273	09:29	105
	Holothuroidea	Molpadiodemidae	Molpadiodemas	13°55.535	-116°32.043	4094	01:30	015
	Holothuroidea	Molpadiodemidae	Molpadiodemas	13°56.361	-116°30.991	4159	01:27	019
	Holothuroidea	Molpadiodemidae	Molpadiodemas?	13°55.613	-116°33.136	4081.4	12:25	042
	Holothuroidea	Molpadiodemidae	Molpadiodemas	13°06.398	-116°05.076	4125.6	10:31	094
	Holothuroidea	Pseudostichopodidae	Pseudosthichopus	13°57.090	-116°30.763	4138	06:32	019
	Holothuroidea	Psychropotidae	Benthodytes	13°57.210	-116°30.727	4140	08:59	019
	Holothuroidea	Psychropotidae	Psychropotes	13°56.918	-116°30.903	4155	04:25	019
	Holothuroidea	Psychropotidae	Psychropotes verrucicaudatus	13°57.027	-116°30.814	4146	05:53	019
	Holothuroidea	Psychropotidae	Benthodytes	13°56.970	-116°30.913	4157	03:42	019
	Holothuroidea	Psychropotidae	Benthodytes	14°02.015	-116°32.756	4078	23:43	057
	Holothuroidea	Psychropotidae	Psychropotes	13°56.080	-116°30.842	4140	14:09	063
	Holothuroidea	Psychropotidae	Benthodytes	13°04.270	-116°07.570	4270	09:17	105
	Holothuroidea	Synallactidae	Synallactes?	13°55.484	-116°32.010	4096	00:03	015
	Holothuroidea	Synallactidae	Paelopatides?	13°55.615	-116°33.141	4082	12:12	042
	Holothuroidea	Synallactidae	Synallactes	13°55.990	-116°30.784	4143.2	11:06	063
	Holothuroidea	Synallactidae	Synallactes	13°55.107	-116°30.788	4144	08:45	063
	Holothuroidea	Synallactidae	Synallactes?	13°49.388	-116°29.671	4095	11:29	078
	Holothuroidea	Synallactidae	Synallactes?	13°49.578	-116°29.471	4098	10:28	091
	Holothuroidea	Synallactidae	Synallactes	13°04.215	-116°07.399	4302	07:05	105
	Holothuroidea	Synallactidae	Synallactes	13°04.260	-116°07.372	4272	08:46	105
	Holothuroidea		Peniagone	13°49.599	-116°29.506	4099	11:11	091
	Holothuroidea			13°04.260	-116°07.370	4273	08:49	105
	Ophiuroidea	Ophiurida		13°55.637	-116°33.245	4087.8	08:48	042
	Ophiuroidea			13°55.445	-116°31.983	4097	22:35	015
	Ophiuroidea			13°55.454	-116°31.990	4096	23:16	015
	Ophiuroidea			13°55.634	-116°33.226	4087.2	10:14	042
	Ophiuroidea			13°55.602	-116°33.103	4079.6	12:55	042
	Ophiuroidea			13°55.763	-116°33.051	4078	01:13	049
	Ophiuroidea			13°55.762	-116°33.056	4078	01:50	049
	Ophiuroidea							049
	Ophiuroidea			13°56.001	-116°30.712	4143.2	11:25	063
	Ophiuroidea			13°49.469	-116°29.697	4094	12:36	078
	Ophiuroidea			13°49.571	-116°29.533	4098	09:57	091
Porifera								
	Demospongiae	Cladorhizidae		13°55.597	-116°33.078	4078.3	14:14	042
	Demospongiae	Cladorhizidae		14°02.091	-116°32.760	4078	00:49	057
	Demospongiae	Cladorhizidae		13°56.003	-116°30.778	4143.7	10:19	063
	Demospongiae	Cladorhizidae		13°56.021	-116°30.782	4143.6	10:40	063
	Demospongiae	Cladorhizidae		13°49.571	-116°29.533	4098	09:54	091
	Hexactinellida	Euplectellidae	Holascus euonyx	13°55.444	-116°31.993	4097	22:14	015

Hexactinellida	Euplectellidae	Holascus euonyx	13°55.528	-116°32.065	4095	01:17	015
Hexactinellida	Euplectellidae	Holascus euonyx	13°55.455	-116°32.013	4096	00:23	015
Hexactinellida	Euplectellidae	cf. Holascus					019
Hexactinellida	Euplectellidae	Saccocalyx cf. pedunculatus	13°55.634	-116°33.374	4082.9	11:53	042
Hexactinellida	Euplectellidae		13°55.630	-116°33.226	4087.2	10:04	042
Hexactinellida	Euplectellidae	Holascus	13°55.633	-116°33.195	4085.5	10:42	042
Hexactinellida	Euplectellidae		13°55.602	-116°33.103	4079.6	12:55	042
Hexactinellida	Euplectellidae	Saccocalyx cf. pedunculatus	14°01.951	-116°32.851	4077	02:40	057
Hexactinellida	Euplectellidae	Saccocalyx cf. pedunculatus	14°01.956	-116°32.852	4077	03:02	057
Hexactinellida	Euplectellidae	Holascus spinosus	13°55.956	-116°30.787	4144	09:22	063
Hexactinellida	Euplectellidae	Holascus euonyx	13°56.038	-116°30.641	4141	12:20	063
Hexactinellida	Euplectellidae	Holascus euonyx	13°56.057	-116°30.780	4141	12:52	063
Hexactinellida	Euplectellidae	Holascus euonyx	13°49.438	-116°29.631	4095	11:55	078
Hexactinellida	Euplectellidae	Holascus euonyx	13°49.449	-116°29.761	4094	13:22	078
Hexactinellida	Euplectellidae	Holascus cf. taraxacum	13°06.396	-116°05.115	4123.4	10:02	094
Hexactinellida	Euplectellidae	cf. Holascus	13°04.202	-116°07.406	4307	07:16	105
Hexactinellida	Euplectellidae	cf. Holascus	13°04.212	-116°07.395	4303	07:49	105
Hexactinellida	Euplectellidae	cf. Holascus	13°04.217	-116°07.393	4302	08:14	105
Hexactinellida	Euretidae	Chonelasma?	13°56.001	-116°30.712	4143.2	11:25	063
Hexactinellida	Hyalonematidae		13°55.445	-116°31.990	4097	22:51	015
Hexactinellida	Hyalonematidae		13°55.463	-116°32.004	4096	23:45	015
Hexactinellida	Hyalonematidae		13°56.862	-116°31.067	4161	00:17	019
Hexactinellida	Hyalonematidae		13°49.448	-116°29.754	4094	13:08	078
Hexactinellida	Hyalonematidae		13°49.384	-116°29.807	4094.3	14:06	078
Hexactinellida	Hyalonematidae		13°49.384	-116°29.830	4094	14:23	078
Hexactinellida	Hyalonematidae		13°49.599	-116°29.508	4099	11:24	091
Hexactinellida	Hyalonematidae		13°06.477	-116°04.974	4137	13:27	094
Hexactinellida	Hyalonematidae		13°04.341	-116°07.361	4261	11:18	105
Hexactinellida	Hyalonematidae		14°01.948	-116°32.849	4076	02:36	117
Hexactinellida	Hyalonematidae		14°01.958	-116°32.859	4079	00:56	117
Hexactinellida	Hyalonematidae?		13°57.154	-116°30.738	4140	07:43	019
Hexactinellida	Hyalonematidae?		13°57.190	-116°30.739	4140	08:29	019
Hexactinellida	Rossellidae	Sympagella clippertonae	13°55.536	-116°32.044	4094	01:49	015
Hexactinellida	Rossellidae	Sympagella clippertonae	13°55.456	-116°31.957	4098	20:34	033
Hexactinellida	Rossellidae	Sympagella clippertonae	13°55.456	-116°31.966	4098	21:20	033
Hexactinellida	Rossellidae	Sympagella clippertonae	13°55.453	-116°31.972	4098	21:40	033
Hexactinellida	Rossellidae	Sympagella clippertonae	13°55.453	-116°31.959	4098	20:21	033
Hexactinellida	Rossellidae	Sympagella clippertonae	13°55.309	-116°33.151	4077	14:58	042
Hexactinellida	Rossellidae	Sympagella clippertonae	13°55.765	-116°33.052	4078	00:52	049
Hexactinellida	Rossellidae	Sympagella clippertonae	14°02.036	-116°32.791	4075	01:10	057
Hexactinellida	Rossellidae	Sympagella clippertonae	14°02.097	-116°32.761	4078	00:16	057
Hexactinellida	Rossellidae	Sympagella clippertonae	13°49.380	-116°29.657	4096	10:40	078
Hexactinellida	Rossellidae	Sympagella clippertonae	13°49.535	-116°29.473	4099	10:14	091
Hexactinellida	Rossellidae	Sympagella clippertonae	13°49.571	-116°29.533	4098	09:57	091
Hexactinellida	Rossellidae		13°06.252	-116°05.070	4123.4	09:44	094
Hexactinellida	Rossellidae	Sympagella clippertonae	13°06.426	-116°05.043	4130	12:07	094
Hexactinellida	Rossellidae	Sympagella clippertonae	13°06.485	-116°04.963	4138	13:50	094
Hexactinellida	Rossellidae	Sympagella clippertonae	13°06.172	-116°05.104	4126	10:45	094
Hexactinellida	Rossellidae	Caulophacus	13°03.766	-116°07.214	4258	11:40	105

Hexactinellida	Rossellidae	Sympagella clippertonae	14°01.956	-116°32.856	4078	01:13	117
Hexactinellida	Rossellidae	Sympagella clippertonae	14°01.956	-116°32.858	4078	01:21	117
Hexactinellida			13°55.759	-116°33.059	4079	02:15	049
Hexactinellida			13°55.783	-116°33.034	4077	23:57	049
Hexactinellida			14°01.956	-116°32.854	4077	02:16	057
			13°55.453	-116°31.971	4097	21:32	033
			13°55.589	-116°33.062	4077.3	14:40	042
			13°55.039	-116°30.640	4142	12:32	063
			13°04.203	-116°07.409	4307	07:09	105

3.5. Natural History Museum - Quantitative Macrofaunal Sampling

3.5.1. Box core sampling goals

Spade box core sampling was carried out on JC257 to support two SMARTEX objectives. A total of 39 box core deployments were made, returning 34 cores of which 32 are fully-quantitative samples. Replicated sampling was achieved at several stations in the CCZ.

All samples are being returned to the NHM London for further analysis by the SMARTEX team.

3.5.2. Macrofauna box core sampling

The macrofaunal sampling was designed to collect samples to deliver on the SMARTEX Proposal Objective 4 (Life history, Reproduction and Connectivity) and Objective 5 (Biodiversity, community structure and trophic dynamics). The JC257 sampling did not study the impacts of a seafloor mining test as originally planned in the SMARTEX proposal. Instead it focused on the spatial scaling of biodiversity and natural geochemical drivers of biodiversity to provide fundamental baseline data on levels of biodiversity. In summary the main goals were to:

- Provide images of an intact seafloor surface to enable observation and photography of nodule abundance
- Collect quantitative, replicated 50x50cm USNEL spade box core samples for macrofauna retained on a 300micron sieve using a randomised stratified design in four abyssal areas:
    - 0km site
    - 1km site
    - 16km site
    - 100km site
- 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

3.5.3. Macrofauna box core processing (deck)

Responsible on each watch for box core processing were Adrian Glover (noon - midnight) and Belen Arias (midnight-noon). 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. Due to time constraints and requirements of the BIO sampling team to assist with other deployments, a subsample of the 0-2cm box core layer was not taken for live sort at sea. Topwater and nodule wash were live sorted for most deployments, however during shifts in which multiple back-to-back deployments were being carried out and where BIO sampling team numbers were limited, this sample was not live sorted at sea and directly added instead to the 0-2 quantitative sample.

General box core deck processing protocol (Figure 3.5.1)

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 80% ethanol on ice for initial sample fixation (this is later changed for a new batch of 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. **Put gloves on**
4. **Move box core** to shade and secure
5. **Measure temperature** of Sed-Water interface > add to box core 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 JC257_ station label. Use Box Core Canon 600D. Spare battery in deck lab.
9. **Microbiology / eDNA**. Nodules without fauna & sediment sample. NOC LEAD.
10. **Megafuana / Xenophyophores**. Remove any LARGE motile megafauna / best xenos > Tupperware ice baths. (QUICK)
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 JC257_XX)
12. **Quantitative sample 0-2cm**. Cut 0-2cm layer in 0-2cm bucket > Sieve station
13. **Quantitative sample 2-5cm**. Cut 2-5cm layer in 2-5cm bucket. > Sieve station
14. **Quantitative sample 5-10cm**. Cut 5-10cm layer in 5-10cm bucket. > Sieve station
15. [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]
16. **Sieving**. Sieve LIVE SORT/ NODULE WASH, and 0-2cm, 2-5cm, 5-10cm quantitative nucketsin trays with 300micron sieve underwater in CFSW. If doing back-to-back coring, do not use CFSW for 5-10cm layer, use deck hose.
17. **Buried nodules**. Pass to GEO buckets for 0-2, 2-5, 5-10 >10cm layers. > BGS
18. **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
- Nodules with fauna on for immediate photography, ID and fixation in the lab
- Topwater/nodule wash 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 in 80% 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)

The end result of the deck processing of box cores was a range of samples that were taken into the lab for further processing.

1. Free-living megafauna where present ready for lab photography, ID and fixation
2. Nodules with fauna on for immediate photography, ID and fixation in the lab
3. Sieved topwater/nodule wash samples for live sorting (residue returned to 0-2cm quantitative sample upon completion).
4. 0-2cm, 2-5cm and 5-10cm quantitative sample residues from sieving on 300micron sieves - these are kept cold in 80% ethanol until the live-sort process is finished, then transferred to a new batch of 80% ethanol

Figure 3.5.1 Deck processing of box cores on JC257. Left to right from top row: 1) measuring temperature of clear topwater from recovered box core; 2, 3, 4) draining of top water; 5) box core with top water drained with deployment number and scale bar ready for top down photo; 6) washing nodules into live sort bucket, placing nodules with fauna in cold filtered seawater for lab processing. Photos by AG Glover, TG Dahlgren.

3.5.4. Macrofauna box core processing (lab)

Macrofaunal lab protocol

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 EOS5D Mark III with a 100mm macro lens and 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, and the photograph numbers from any of the cameras were added to the database.

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

- The residue retained on a 300micron sieve from the LIVE SORT / NODULE WASH bucket 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 300 micron sieve from the slicing of the entire box core surface) comprised three samples: 0-2cm, 2-5cm and 5-10cm. After approximately 24 hours these jars (already filled with 80% 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 4°C reefer van on board. Finally, nodules examined for fauna were returned to buckets with the other nodules from the core and passed to the geology team.

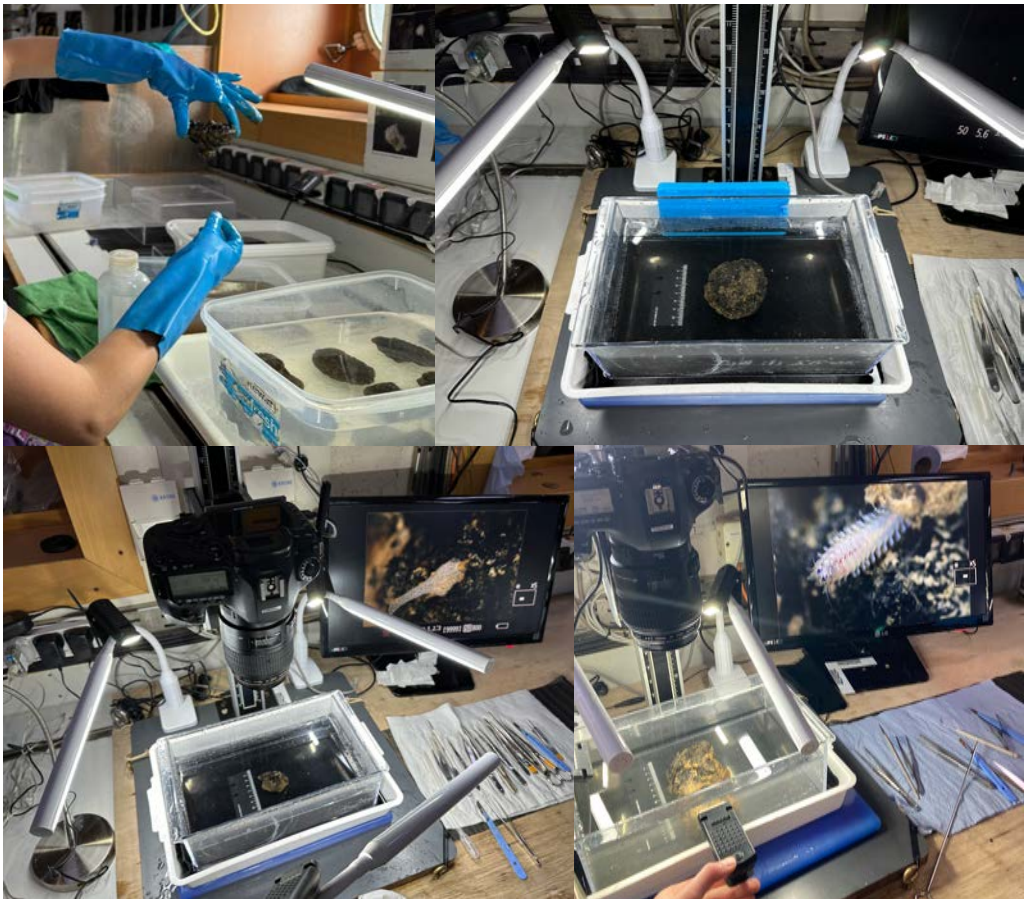


Figure 3.5.2 Laboratory sorting of box core samples on JC257 (1 of 2) from left to right from top left: 1) selecting nodules for photography from basins on ice in cold filtered seawater; 2) camera set up for nodule photography using ice packs to keep

water cool; 3) imaging a nodule-dwelling sponge; 4) imaging the nodule-dwelling nereidid annelid Neanthes goodayi. Photos by AG Glover, TG Dahlgren, R Drennan.

Figure 3.5.3 Laboratory sorting of box core samples on JC257 (2 of 2) from left to right from top left: 1) sorting macrofaunal samples from the live-sort fraction; 2) imaging a dorvilleid annelid from the live sort fraction; 3) specimen database and 2ml microtubes (pre chilled in ice pack); 4) final quantitative samples for the entire box core sample preserved in 80% ethanol. Photos by AG Glover, TG Dahlgren, R Drennan.

3.5.5. Summary of Macrofaunal samples collected

In general the box core operations ran smoothly with excellent samples obtained. The reasons behind the failed box cores are unclear; the triggering failed on three deployments, the box core came up partially or completely empty for two deployments, and for a further two there were issues with the cable that were resolved after those deployments.

Sampling followed a stratified randomised design, with replicate samples taken in each of four areas: 0km (n=10), 1km (n= 10), 16km (n=6) and 100km (n=6) (Table 3.5.1, Figure 3.5.4). At each site, randomised box core locations were taken within a 200m radius to avoid sampling the same location.

An additional deployment at UK1_1km (JC257_012) was not counted as quantitative, however, nodules and two10x10cm sediment subsamples (0-2 and 2-5cm) were processed/live sorted as qualitative samples.

Table 3.5.1 Summary of total number of quantitative box cores taken at each site on JC257.

Site	# Quantitative box cores
0km	10
1km	10
16km	6
100km	6
Grand Total	32

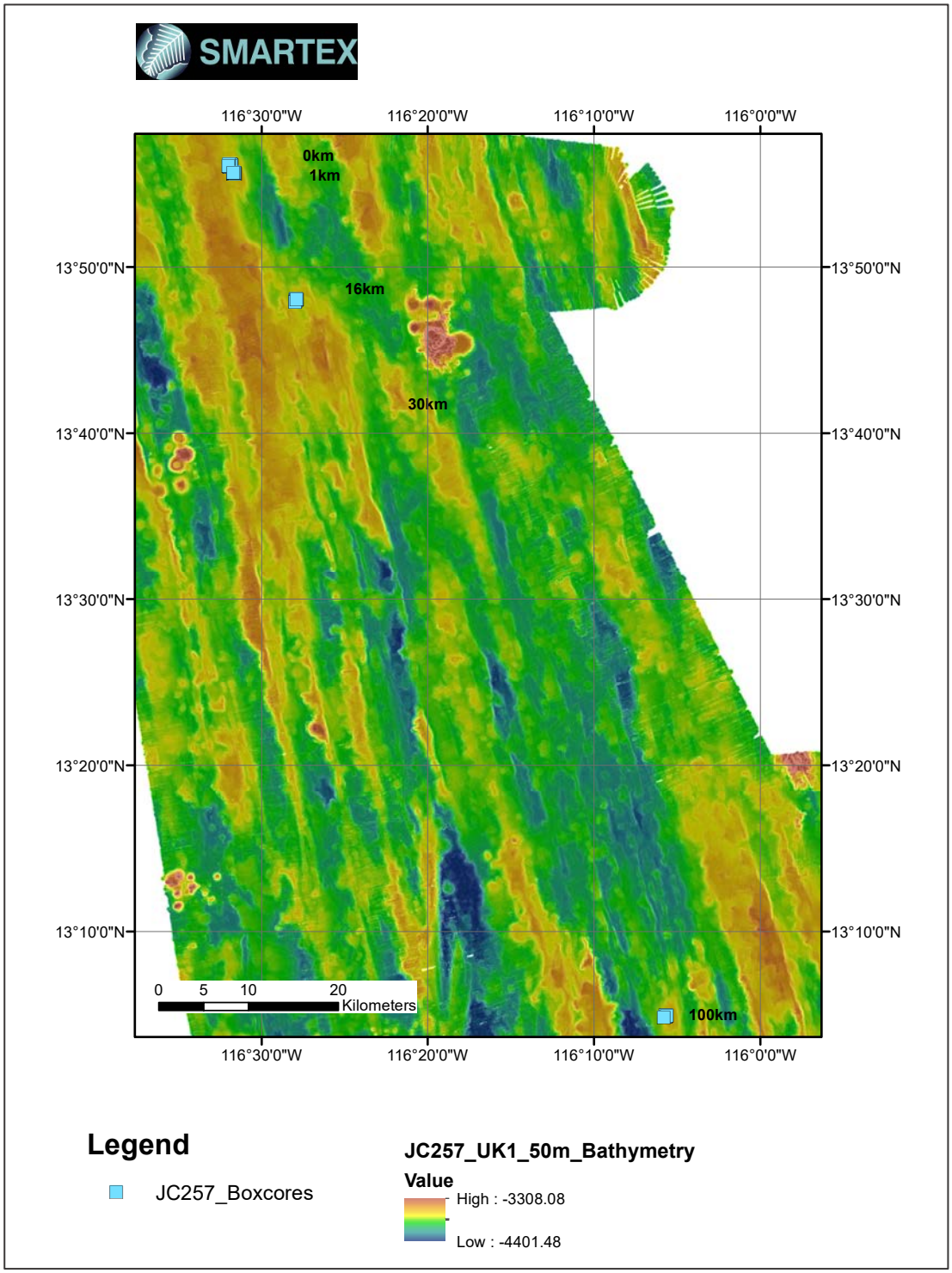


Figure 3.5.4 Map of UK-1 target sampling region, showing box core deployments at 0km, 1km, 16km and 100km sites.

3.5.6. Macrofauna sample analysis and distribution

A total of 1,426 live-sorted metazoan specimens were processed at sea from quantitative and non-quantitative box cores (Table 3.5.2), with each specimen photographed and given a unique individual ID to enable future taxonomic work. This excludes all the specimens in the bulk-fixed residue which will be picked later in the project at the NHM. Results are preliminary, with insight into sediment fauna abundance and diversity limited until quantitative samples are analysed. The live sort/nodule wash fraction is treated as qualitative-only, as it was not taken on all deployments, and included sediment subsamples from non-quantitative box cores.

In terms of nodule fauna, a total of 957 specimens across nine metazoan phyla were processed (Table 3.5.2; Figure 3.5.5 A), Porifera was the dominant phylum by a large margin (n=570 individuals) with the majority of specimens (n=443) comprising of a single taxon, *Plenaster craigi. (*Figure 3.5.5 B; Figure 3.5.6). Cnidaria was the

next most abundant phylum (n=176), primarily comprised of *Nausithoe* spp. jellyfish polyps, (n=156) (Figure 3.5.5 B; Figure 3.5.6). Following this it was Bryozoa (n=133) and non *P. craigi* sponges (n=126). The most abundant Annelid family was Serpulidae (n=17) living directly on nodules. Also notable were observations of Acrocirridae, Amphinomidae and the nereidid *Neanthes goodayi* living within crevices and burrows within nodules.

Table 3.5.2 Total number of individuals sampled in the JC257 macrofaunal box cores broken down by phylum for all box cores processed (quantitative and non-quantitative). These data exclude sediment fauna samples taken in the quantitative analysis, which will contain much larger numbers of individuals once sorting is complete at the end of the project. Live sort/nodule wash fraction is qualitative only.

Phylum	Total box core	Nodule fauna	Live sort/nodule wash
Annelida	206	54	152
Arthropoda	222	2	220
Brachiopoda	3	3	-
Bryozoa	139	133	6
Chaetognatha	10	-	10
Chordata	3	-	3
Cnidaria	183	176	7
Echinodermata	31	9	22
Metazoa	4	4	-
Mollusca	51	6	45
Porifera	574	570	4
Grand Total	**1426**	**957**	**469**

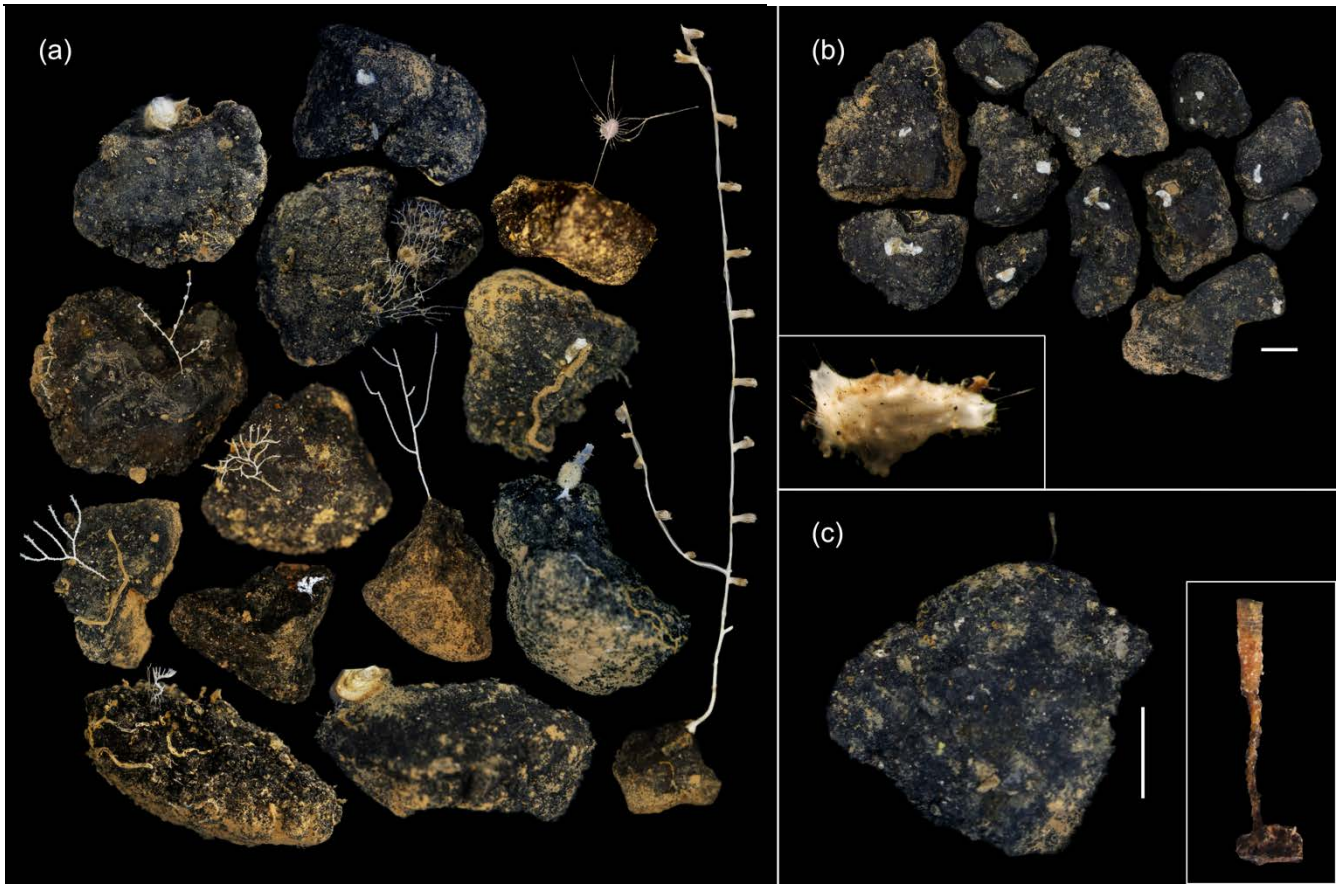


Figure 3.5.5 (a) Nodule fauna highlights (b) The sponge Plenaster craigi on nodules (scale bar 1cm) with detail of individual on microscope camera. (c) Scyphozoan Nausithoe sp. polyps on nodule (scale bar 1cm), with detail of individual on microscope camera.

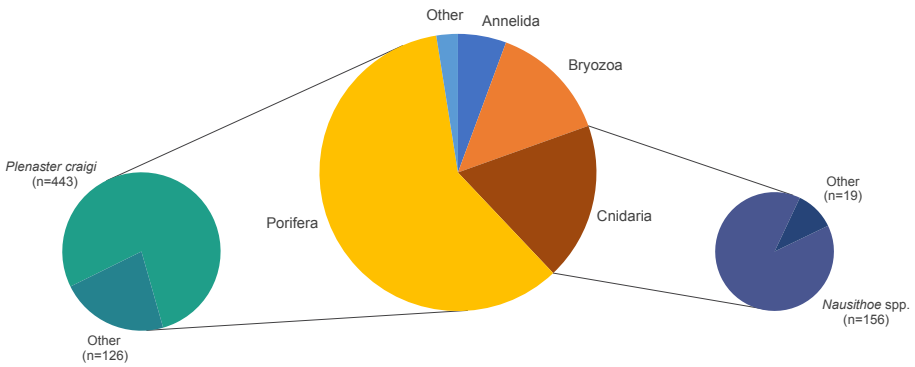


Figure 3.5.6 Breakdown of nodule fauna collected by phylum JC257.

Nodule fauna abundance data across sampling sites for quantitative box cores only is summarised in Table 3.5.3 and Figure 3.5.7. UK1_16km notably appears to have lower abundance relative to other sites, particularly in terms of the phylum Cnidaria, with very few individuals (n=3) relative to other sampling sites (*Nausithoe* spp. polyps). Further taxonomic work will be required to assess true measures of diversity, which will be assessed alongside variables such as nodule coverage and abundance and compared across sites.

Table 3.5.3 Total number of nodule fauna by phylum at each site from the quantitative box cores of JC257. Number of deployments are as follows. 0km (n=10), 1km (n= 10), 16km (n=6) and 100km (n=6).

Phylum	UK1_0km	UK1_1km	UK1_16km	UK1_100km	Grand Total
Annelida	13	15	8	15	51
Arthropoda	2	-	-	-	2
Brachiopoda	-	-	3	-	3
Bryozoa	42	38	10	35	125
Cnidaria	56	66	3	33	158
Echinodermata	3	5	1	-	9
Metazoa	-	1	1	2	4
Mollusca	2	2	1	1	6
Porifera	182	225	52	77	536
Grand Total	**300**	**352**	**79**	**163**	**894**

As live sort was qualitative, interpretation of results are limited until analysis of quantitative samples is taken. The most dominant phyla from the nodule wash fraction were Arthropoda (n= 220) followed by Annelida (n=152) (Table 3.5.2; Figure 3.5.8). Annelida are often the most dominant group in terms of macrofaunal abundance in the CCZ and exceeded Arthropods in numbers in SMARTEX I (JC241). However without a sediment subsample, many sediment-dwelling annelids would not have been live sorted at sea, in addition to a sampling bias towards swimming arthropods (e.g. copepods) in the topwater fraction, which may explain the higher number of Arthropods in JC257.

The box core samples will be returned to NHM London for further sorting and analysis by the SMARTEX team, with the exception of the geological specimens of nodules, which will go to BGS. Highlights of macrofaunal are presented in Figure 3.5.9.

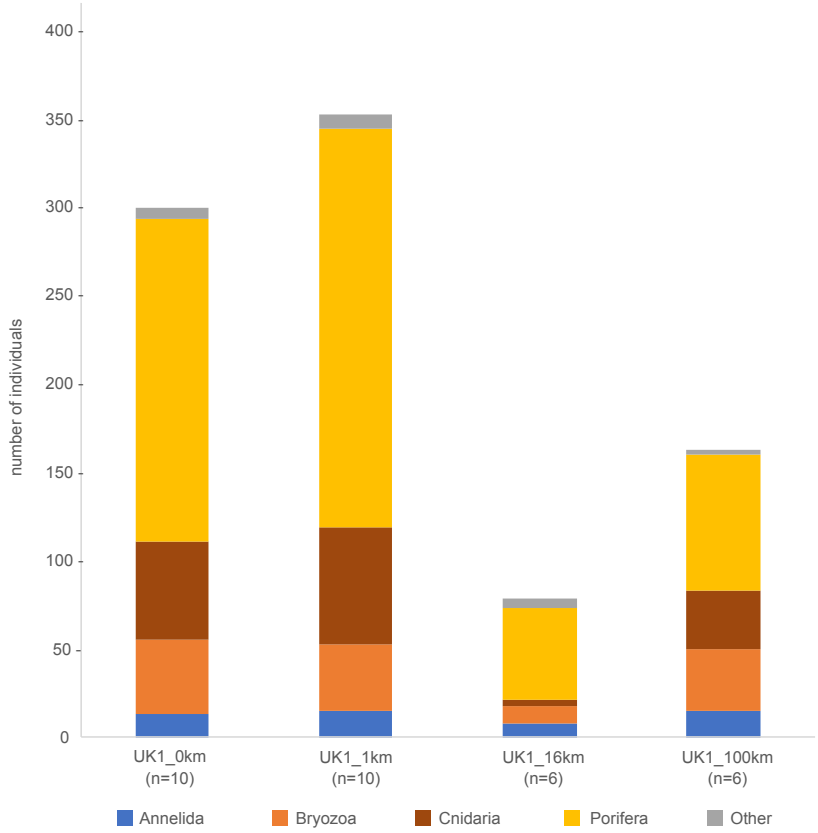


Figure 3.5.7 Nodule fauna abundance by phylum across sampling sites (number of deployments given below each site).

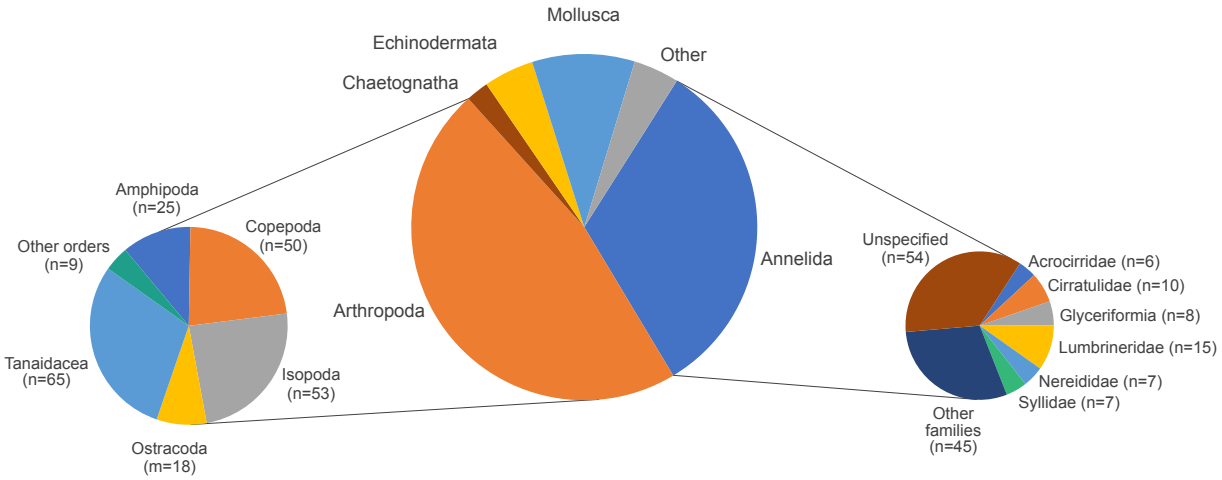


Figure 3.5.8 Breakdown by phylum of the live sort / nodule wash fraction of the box core samples, including nodule and sediment-dwelling fauna, and opportunistic fauna on JC257.

Figure 3.5.9 Highlights of macrofaunal processed at sea from JC257 box core deployments.

3.6. Natural History Museum - Foraminifera and Meiofaunal Sampling

3.6.1. Megacore Deployments

All deployments of the megacore for foram analysis are summarised in Table 3.6.1. A total of 12 deployments were made, recovering foraminifera samples from 20 cores. Upon arrival on deck, the megacores were assessed and allocated for foram analysis if the top ~6-10 cm and top water was undisturbed. The cores were then sliced according to Bryan O'Malley's (JC241) slicing protocol (Figure 3.6.1) as follows:

1. Place tube on extruder.
2. Slide core tube until overlying water is accessible (avoid resuspension as much as possible and siphon off water onto 63-micron sieve (forams).
3. Using clean forceps, pick off the nodules and place them into the 0-1cm 250mL/500mL jar.
4. Collect sieve residue into the 0-1 cm 250/500 mL jar.
5. Place slicing ring onto core tube and push tube down so that the desired 1 cm sediment slice sits within the slicing ring.
6. 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 avoiding mud sticking to the bottom of the slicing plate, resulting in inaccurate sediment horizons).
7. Rinse the core slicing ring and slicing plate into the funnel into the sample jar with a squirt bottle of 70% ethanol.

8. Add 70% ethanol to the sample bottle until the sediment volume is at least doubled. Shake bottle so preservative penetrates the sediment and so there are no sediment chunks.
9. Repeat step 5 – 8 until sliced 5 cm into clearly labelled jars (ethanol-proof pen).

Figure 3.6.1 Foram slicing schematic (A) and processing steps 1 – 2 (B), steps 3 – 4 (C), steps 5 – 6 (D) and step 7 (E).

Table 3.6.1 Details of megacore deployments that resulted in foram slicing.

Station	Location	Date (2024)	Time (UTC: gear on bottom)	Latitude (dec min, on bottom)		Longitude (dec min, on bottom)		Depth (m)	Cores used for foram analysis
JC258_017	UK1_1km	17/02	07:26:00	13˚	55.593	-116˚	31.683	4098	Cores 3, 4 and 7
JC257_025	UK1_1km	19/02	08:47:00	13˚	55.626	-116˚	31.730	4101	Cores 6 and 4
JC257_029	UK1_1km	20/02	00:12:00	13˚	55.634	-116˚	31.616	4097	Core 7
JC257_031	UK1_0km	20/02	08:40:00	13˚	56.052	-116˚	31.808	4095	Core 6 and 8
JC257_034	UK1_0km	21/02	02:53:00	13˚	56.110	-116˚	31.860	4092	Core 1
JC257_050	UK1_0km	24/02	05:42:00	13˚	56.109	-116˚	31.786	4098	Cores 2 and 7
JC257_068	UK_16km	28/02	04:18:00	13˚	47.994	-116˚	27.875	4122	Core 7
JC257_088	UK_16km	03/03	08:21:00	13˚	47.927	-116˚	27.947	4115	Cores 1 and 8
JC257_092	UK_16km	04/03	17:41:00	13˚	47.969	-116˚	27.855	4097	Core 7
JC257_101	UK_100km	06/03	12:12:00	13˚	4.783	-116˚	5.631	4102	Cores 5 and 7
JC257_104	UK_100km	06/03	23:12:00	13˚	4.919	-116˚	5.650	4102	Cores 1 and 2
JC257_110	UK_100km	09/03	14:39:00	13˚	4.748	-116˚	5.717	4106	Core 2

3.6.2. Pushcore Deployments

Using the ROV we also collected pushcores for foraminifera analysis when sampling for the purpose of examining the impact of the colonisation and whale bone experiments. These cores were transferred to the cold temperature lab and sliced and preserved in the same way as the megacore using a smaller slicing ring (Table 3.6.2).

Table 3.6.2 Details of pushcore deployments that resulted in foram slicing.

Station	Location	Date (2024)	Time (UTC)	Latitude (dec min)		Longitude (dec min)		Depth (m)	Cores	Notes
JC258_057	OMS	25/02	23:16:00	14˚	02.100	-116˚	32.755	4078	White 2	

JC258_057	OMS	25/02	23:08:00	14˚	02.103	-116˚	32.756	4078	Yellow 2	
JC258_057	OMS	25/02	23:11:00	14˚	02.100	-116˚	32.754	4078	Yellow 3	
JC257_063	UK1_0km	27/02	08:22:00	13˚	55.955	-116˚	30.660	4144	Blue 2	1m from colonisation experiment
JC257_063	UK1_0km	27/02	08:25:00	13˚	55.954	-116˚	30.660	4144	Blue 3	1m from colonisation experiment
JC257_063	UK1_0km	27/02	08:10:00	13˚	55.955	-116˚	30.660	4144	Green 2	0m from colonisation experiment
JC257_063	UK1_0km	27/02	08:05:00	13˚	55.957	-116˚	30.658	4144	Green 3	0m from colonisation experiment
JC257_115	Bone colonisation	10/03	23:17:00	13˚	43.543	-116˚	39.940	4131	White 1	0m from bones
JC257_115	Bone colonisation	10/03	22:44:00	13˚	43.544	-116˚	39.940	4131	White 3	0m from bones
JC257_115	Bone colonisation	10/03	00:53:00	13˚	43.544	-116˚	39.940	4131	Blue 1	1m from bones
JC257_115	Bone colonisation	10/03	00:49:00	13˚	43.545	-116˚	39.941	4131	Blue 2	1m from bones
JC257_115	Bone colonisation	10/03	00:02:38	13˚	43.575	-116˚	39.946	4132	Yellow 1	>10m from bones
JC257_115	Bone colonisation	10/03	00:02:40	13˚	43.571	-116˚	39.946	4132	Yellow 2	>10m from bones

3.7. National Oceanography Centre – Microbial ecology and eDNA

Susan Evans

3.7.1. Introduction

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 potential 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 JC257, 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 in the UK-1 site.

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).

One specific aim of this work was to demonstrate simultaneous biological sampling using the high-resolution autonomous eDNA sampler, the Robotic Cartridge Sampling Instrument (RoCSI) (Figure 3.7.1) developed at National Oceanography Centre, UK in the nose of the Autosub5, together with image and multibeam surveys at different altitudes from the seabed.

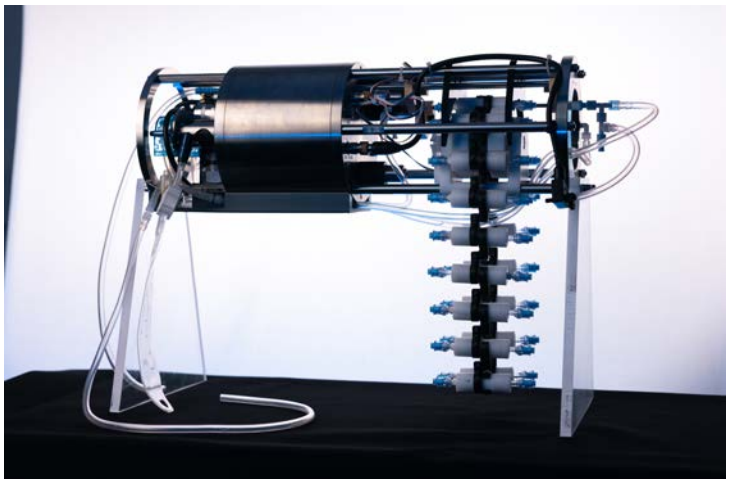


Figure 3.7.1: RoCSI (photo credit:NOC)

3.7.2. 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 Sterivex filter. During JC257, 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 at the UK-1 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, box core and ROV push cores.

3.7.3. RoCSI on Autosub5

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 AUV. 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 a usb cable. After the AUV 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. RoCSI samples were collected during image dives at at 3 m altitude above seabed from 2 Autosub5 missions (87 and 90) (Table 3.7.1) with 3.5 samples taken in total because of technical issues described below. Specific details about the imaging and SSS AUV dives can be found in section 2.2.

Troubleshooting

A low pressure leak occurred inside the pressure housing during one of the first AUV dives and despite extensive cleaning of the PCB there was damage to one of the motor electrical components causing only 2 out of the 3 motors to be functional. The source of the leak was found and the pressure housing remained water tight for the duration of the cruise. The lack of a motor was mitigated by updating the firmware so that the sample motor was functional but the stabliser motor was not enabling a sample to be taken but not preserved with RNAlater. For effective ballasting and especially for the image surveys, RoCSI was required to be in the nose of the AUV for every dive making troubleshooting very difficult and prolonging the amount of time it took to make repairs. An additional problem occurred with a suspected leak in the IE55 connector of the hall effect sensor cable. A new connector was soldered onto the cable between AUV dives but this did not rectify the problem with the hall effect sensor readings and no further RoCSI samples were taken.

Table 3.7.1. Summary of RoCSI samples collected on Autosub5 during JC257.

Date	Station	Mission number	Number of RoCSI samples	Location	AUV dive type
23/02/2024	47	87	2.5	UK-1 0-1km + timeseries	3 m altitude Photography + SSS
01/03/2024	76	90	1	UK-1 16-30 km	3 m altitude Photography

3.7.4. Water Sampling

Water samples were collected from niskin bottles on both the CTD-rosette and on the ROV along a spatial gradient in UK-1 to characterise the water column community composition and function as measured by eDNA and microbial functional genomic profiles along a gradient with potential influence of plume disturbance.

CTD-rosette

Water was collected from the 10L OTE sampling bottles mounted on the CTD-Rosette from a total of 5 casts at 4 of the sites (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) with the exception of station 116 where an additional depth was include above the shoulders of a ridge at 4095 m (to understand how bottom currents influence eDNA dispersal). In total, 79 samples were collected for eDNA and microbial analysis with a total of 257 L of water filtered (Table 3.7.2).

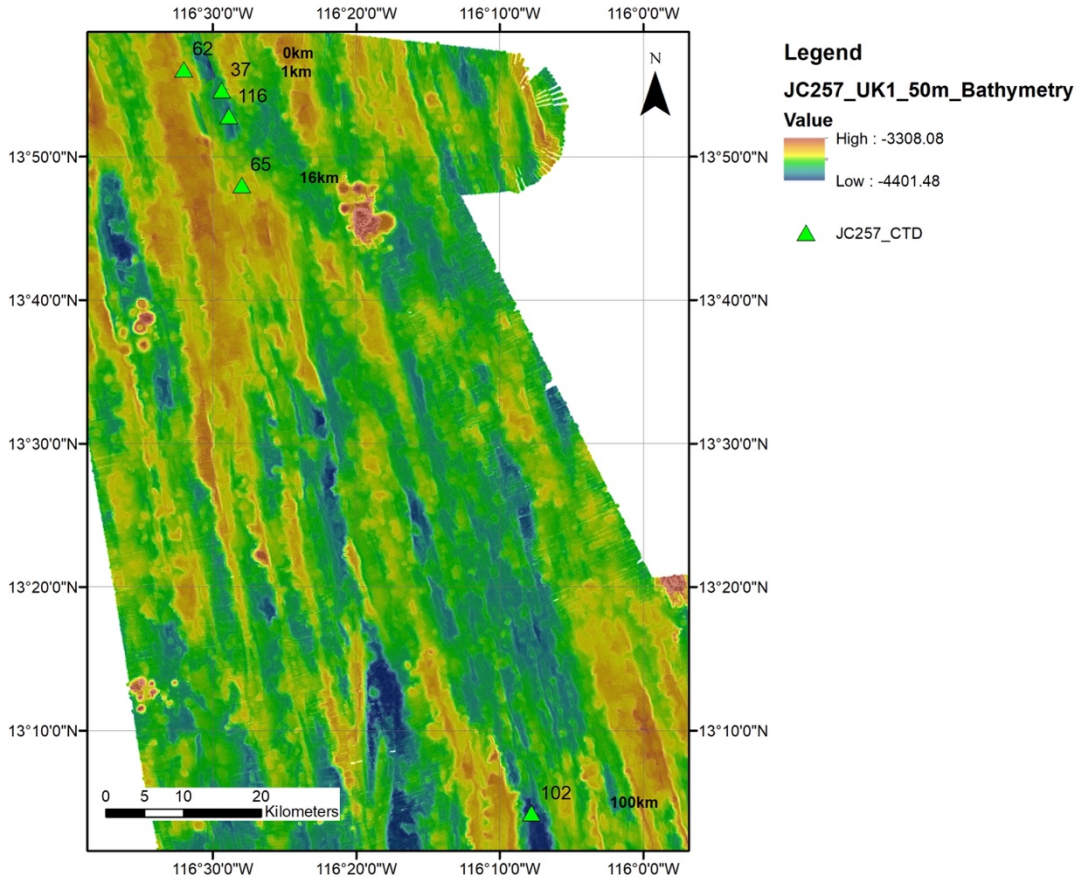


Figure 3.7.2. Map of CTD casts in UK-1 area and respective bathymetry.

Table 3.7.2. Location and number of eDNA samples filtered from CTD-rosette casts during JC257.

Station number	Location	Number of samples	Total volume of water filtered (L)
37	UK-1 1 km	15	50
62	UK-1 0 km	15	48.5
65	UK-1 16 km	15	47.4
102	UK-1 100 km	15	47.9
116	south of UK-1 0 km	19	63.2
		79	257

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 3.7.3) 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 3.7.3. 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 3.7.4).

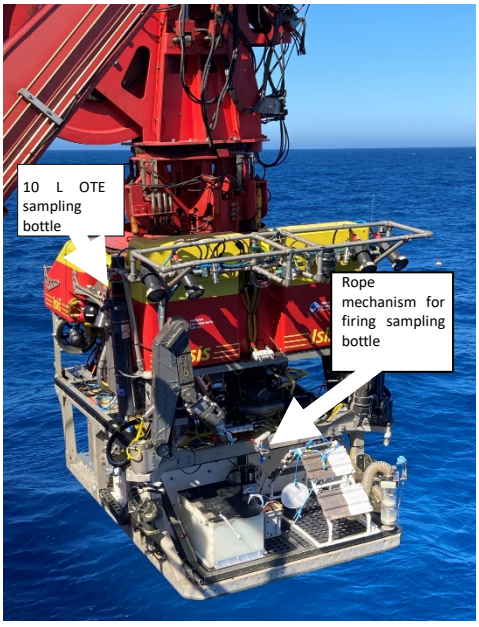


Figure 3.7.4. Location of 10L water sampling bottle on ROV ISIS and the triggering mechanism.

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 but this was not successful during every dive due to sediment carried over from the megafauna collections. In total, 31 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 3.7.3 Location of ROV dives at UK-1 where water was filtered from ROV niskin bottles for eDNA analysis.

Station number	Location	ROV dive	Number of samples	Volume of water filtered (L)
15	UK-1 1km	431	8	15
19	UK-1 1km	432	8	15
49	UK-1 0 km	435	4	14
57	OMS The Hole	437	3	8.4
105	UK-1 100 km	443	4	8.4
117	Sponge Garden	446	4	8
			31	68.8

3.7.5. Sediment sampling

Sediment samples for eDNA analysis and microbial ecology (abundance, biomass, community composition and functional diversity of microbes) were collected from 4 sites in total using box core, megacore and ROV pushcores. Specific details about the sediment sampling methods can be found in section 2.1 of this report.

ROV push core

Sediment for microbial and eDNA were collected using 3 push cores deployed using ROV ISIS at each of the UK-1 sites (Table 3.7.4). The push cores were bleached prior to the dive with 5% sodium hypochlorite and rinsed in Milli-Q. The push cores were typically taken at the end of the ROV dive, directly under the site of where the water sampling bottles were fired and the 3 cores were taken within a 1 m area (Figure 3.7.5). Once back on deck, the push cores were retrieved and sectioned in a 4°C cold room within 1 hour of being back on deck.

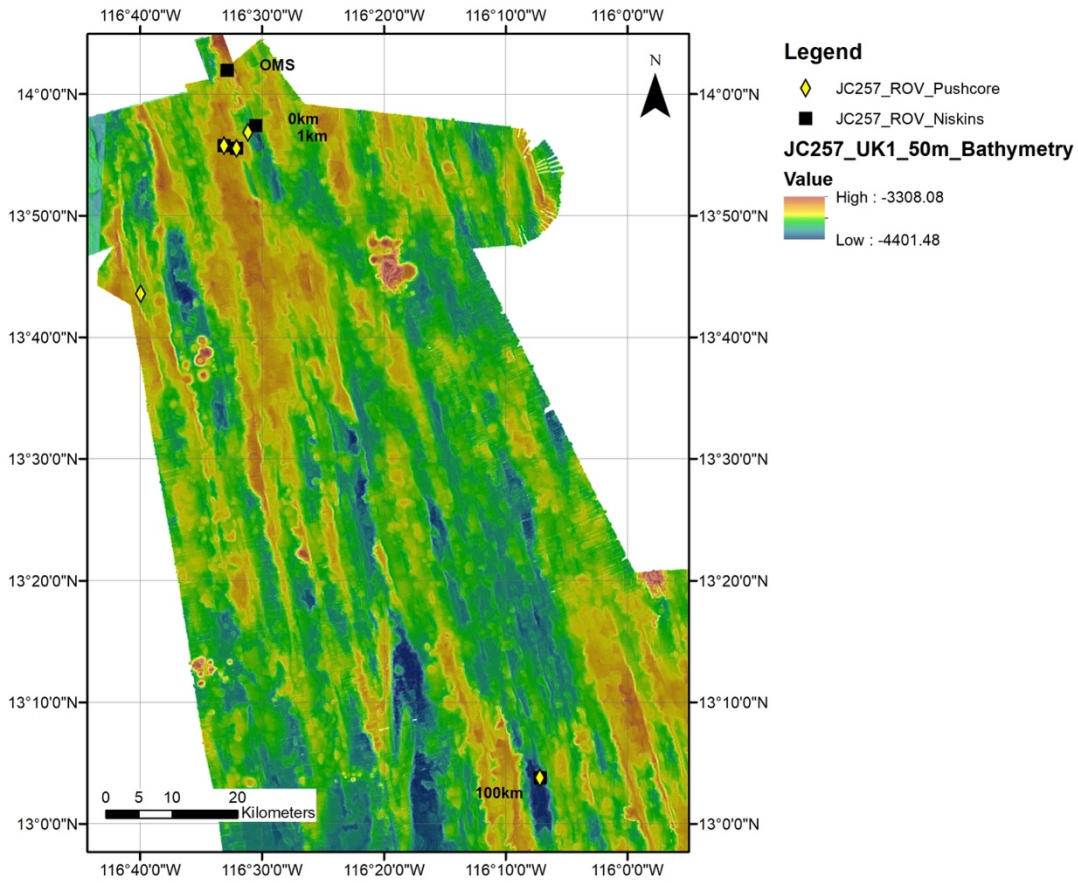


Figure 3.7.5. Location of ROV pushcore and niskin samples taken for microbial ecology and eDNA analysis in the UK-1 area.

Prior to sectioning, all equipment (core extruder, rings) were cleaned with 5% bleach and rinsed thoroughly with Milli-Q water. The SWI was sampled using a sterile syringe and nodules removed using sterile tweezers. Each core was then sectioned into 2 cm intervals, down to 12 cm and the sectioning equipment was rinsed, then dipped in 5% bleach followed by a rinse in Milli-Q in between each section. The remaining section was transferred into a sterile bag for metagenomics and then stored at -80°C.

Table 3.7.4. Summary of ROV push core samples taken during JC257.

Station	ROV dive	Location
15	431	UK-1 1km
19	432	UK-1 1km
49	435	UK-1 0km
105	443	UK-1 100km
115	445	Bone and wood colonisation 0m
115	445	Bone and wood colonisation 1m
115	445	Bone and wood colonisation 25m

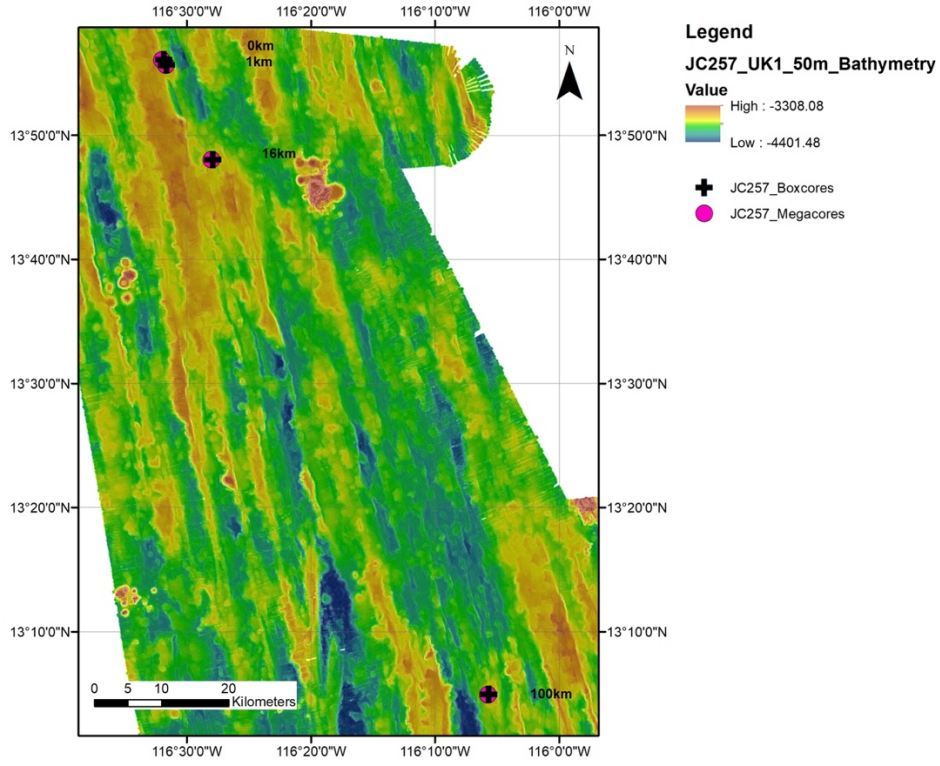


Figure 3.7.6, Location of box core and megacore sediment sampling for microbial ecology/eDNA at the UK-1 site

Megacore

Sediment for microbial and eDNA were collected from 3 megacores at each of the sites at UK-1 (Table 3.7.5) following the method described in Section 2.4. The cores were 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 and nodules were removed using sterile tweezers. Each core was then sectioned into 2 cm intervals, down to 12 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 3.7.5. Summary of megacore samples taken during JC257 including nodule presence in each core. MetaT = metatranscriptomics, MetaG = metagenomics.

Station	Location	Samples collected	Nodule
11	UK-1 1km	MetaT, MetaG	Y
46	UK-1 0km	MetaT, MetaG	Y
73	UK-1 16km	MetaT, MetaG	Y
99	UK-1 100km	MetaT, MetaG	Y

Box core

Sediment for eDNA and microbial ecology was collected in triplicate from four of the UK-1 sites (Table 3.7.6).

Table 3.7.6. Summary of box cores sub-sampled for molecular analysis during JC257, including whether nodules were collected or not.

Station	Location	Nodules collected
43	UK-1 1km	3
80	UK-1 0km	3
89	UK-1 16km	3
97	UK-1 100km	3

Following the draining of the overlying water from the box core, 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.

3.7.6. 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 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 three gene regions (cytochrome c oxidase I, 18S 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. 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.

3.7.7. 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)

3.8. Scottish Association of Marine Science - Ecosystem Function

3.8.1. Benthic cube studies

To measure benthic ecosystem function, specifically the rate of C-processing by the benthos and what happens when the benthos is exposed to varying levels of sediment burial, stable-isotope pulse chase experiments were undertaken during JC257 (Table 3.8.1) with benthic cubes. During the first three sets of cube experiments, four benthic incubation CUBES were deployed, while on the last ROV Cube deployment dive only two were undertaken. At the seafloor, the cubes were removed from the ROV tool tray and placed into the sediment where the isotopically labelled algae and sediment was deployed by syringe. Cubes were deployed so they were spaced 15 – 20 m apart.

Table 3.8.1 Description of depths and position of CUBE deployments during JC241.

ROV dive	AKS #	Cube #	Sediment load	Depth (m)	Latitude	Longitude	Date deployed (UTC)	Time (UTC)	Date deployed (UTC)	Time (UTC)
JC257_015	353	1	High sediment	4138	13º 55.44N	116º 31.97W	16.2.24	21:16	20.2.24	22:58
JC257_015	353	2	High sediment	4099	13º 55.45N	116º 31.96W	16.2.24	20:25	20.2.24	22:04
JC257_015	353	3	Control	4138	13º 55.45N	116º 31.97W	16.2.24	21:35	20.2.24	23:20
JC257_015	353	4	Control	4139	13º 55.44N	116º 31.96W	16.2.24	20:53	20.2.24	22:39
JC257_052	356	1	High sediment	4124	13º 56.21N	116º 32.56W	24.2.24	20:11	28.2.24	20:48
JC257_052	356	2	High sediment	4124	13º 56.22N	116º 32.54W	24.2.24	19:39	28.2.24	20:19
JC257_052	356	3	Control	4123	13º 56.22N	116º 32.55W	24.2.24	19:54	28.2.24	20:35
JC257_052	356	4	Control	4124	13º 56.21N	116º 32.53W	24.2.24	19:24	28.2.24	20:10
JC257_078	358	1	Medium sediment	4137	13º 49.60N	116º 29.48W	1.3.24	07:16	4.3.24	13:06
JC257_078	358	2	Medium sediment	4137	13º 49.60N	116º 29.50W	1.3.24	07:38	4.3.24	13:47
JC257_078	358	3	Medium sediment	4138	13º 49.60N	116º 29.48W	1.3.24	07:06	4.3.24	12:48
JC257_078	358	4	Medium sediment	4137	13º 49.60N	116º 29.50W	1.3.24	07:38	4.3.24	13:26
JC257_094	359	1	Low sediment	4160	13º 6.38N	116º 5.14W	5.3.24	06:56	8.3.24	14:48
JC257_094	359	3	Low sediment	4159	13º 6.38N	116º 5.13W	5.3.24	07:26	8.3.24	15:14

All CUBES contained one syringe previously filled with filtered seawater and 0.2g of isotope-labelled *Phaeodactylum tricornutum* (grown in media with 25% ^{13}C and ^{15}N) and either two (low sediment, n=2), three (medium sediment, n=4) or five (high sediment, n=4) syringes filled with sediment (box-cored abyssal mud) to simulate different degrees of sediment burial (Figure 3.8.1). Control cubes (n=4) with just algae were also deployed. Immediately after the CUBES were placed and pushed into the sediment, a safety pin was removed, and the syringe depressed by the ROV manipulator to start the experiment (Figure 3.8.2).

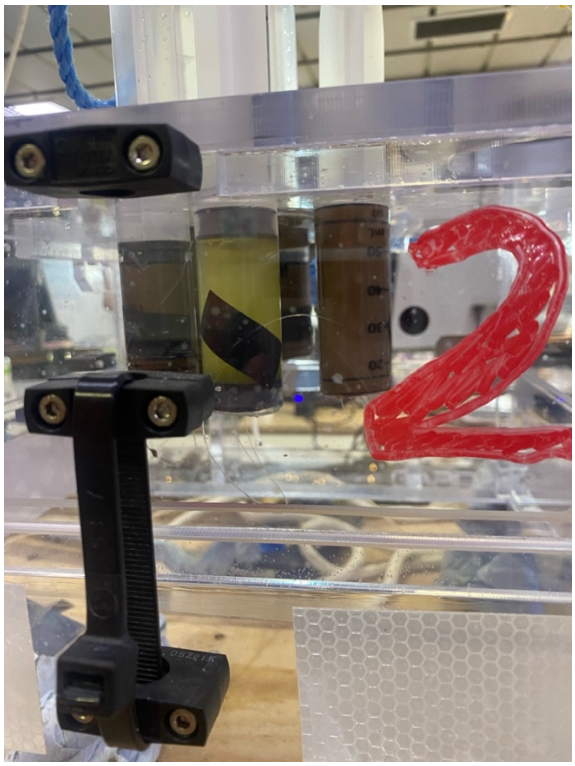


Figure 3.8.1. A benthic incubation CUBE with hydrated stable isotope labelled P. tricornutum (green colour at the bottom of the syringe) and other syringes filled with hydrated sediment.

The CUBES were sampled between 72 to 96 hrs after the experiment began (Table 3.8.1, Figure 3.8.2). To sample the CUBES, the ROV manipulator was used to carefully rock the cube back and forth to free it from the sediment before it was lifted out of the sediment and placed back on the ROV. Three push-cores were then collected from inside the imprint of each CUBE (Figure 3.8.3) and placed in the quivers.

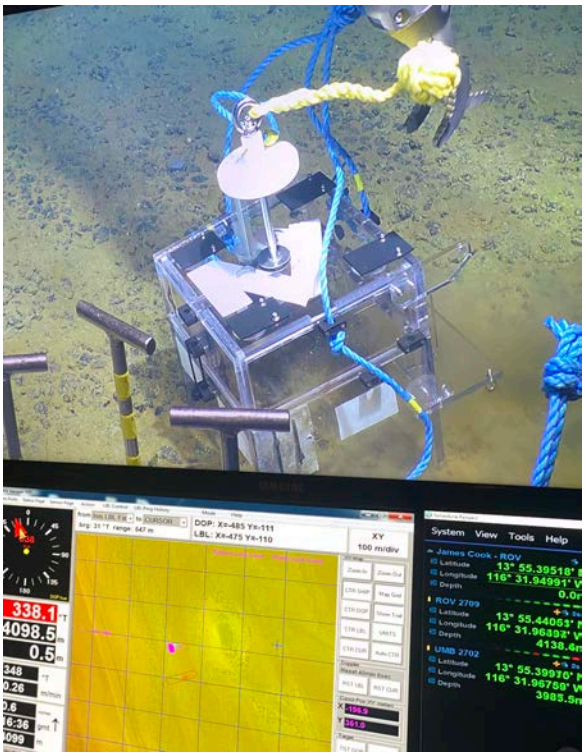


Figure 3.8.2 Approaching one of the CUBES to depress the syringes and start the experiment.

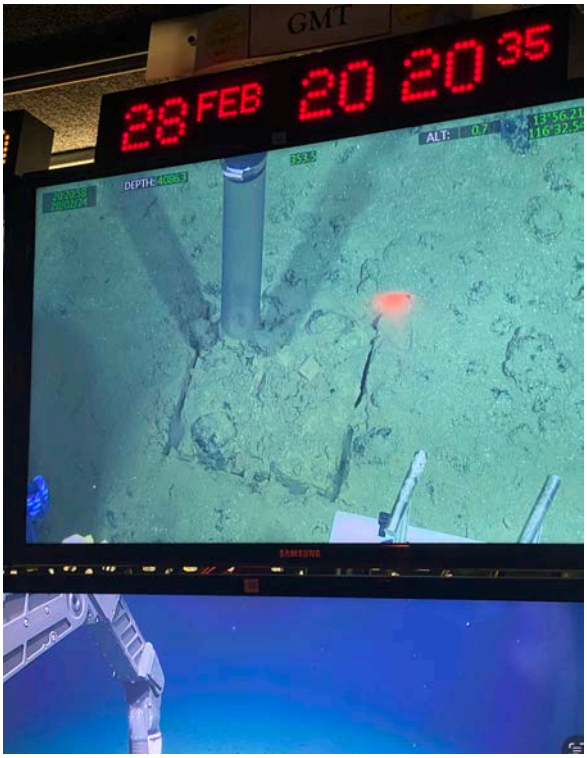


Figure 3.8.3 Sampling the CUBE footprint with a push core.

Following the ROV dive, the push cores were placed in a bucket and transferred to the cold room. Here, a push core for microbial PLFA analysis was extruded at 0-2, 2-5 and 5-10cm depth using a spackle knife and core extruding fence. The other two push cores were sectioned at 0-10cm and placed in two separate bottles for meiofauna and macrofauna. The microbial PLFA samples were then placed in the freezer at -80^{o}C, while the fauna samples were preserved in 4% buffered formaldehyde for later processing.

3.9. Herriot-Watt University - Fish Ecotoxicology

3.9.1. Samples collected

Six specimens, 4x *Coryphaenoides sp*, one *Barathrites sp* and one *Pachycara sp* were caught. Fish length was measured, and photos taken for later identification (Figures 3.9.1 to 3.9.6). Specimens were immediately transferred to the CT room and processed.

Figure 3.9.1. AKS354-10 Coryphaenoides ssp 63 cm.

Figure 3.9.2. AKS354-11 Coryphaenoides ssp 87 cm.

Figure 3.9.3. AKS357-12 Coryphaenoides ssp 95 cm.

Figure 3.9.4. AKS357-13 Coryphaenoides ssp 72 cm.

Figure 3.9.5. AKS357-14 Pachycara sp 30 cm.

Figure 3.9.6. AKS357-15 Barathrites sp 59 cm.

Blood samples were taken and processed for Comet assay on board.

Figure 3.9.7 Lab setup in the chemistry lab of RRS James Cook.

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 3.9.8).

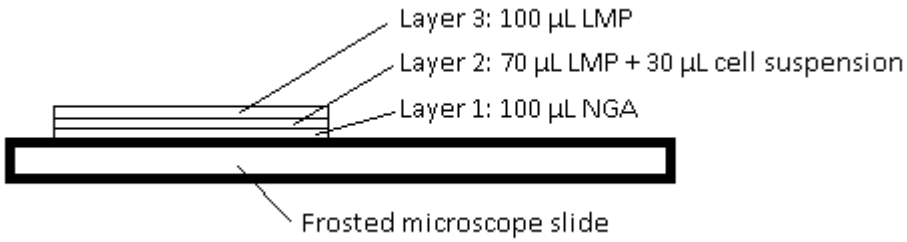


Figure 3.9.8 Microscope slide with gel sandwich.

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 3.9.9) 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.

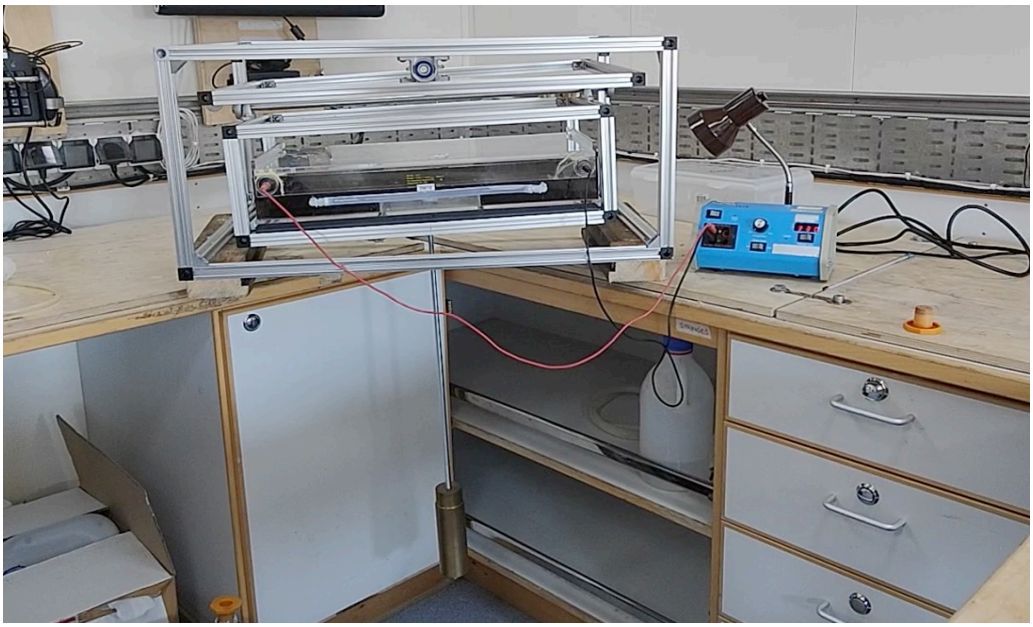


Figure 3.9.9 Electrophoresis tank mounted on a gimbal table which was screwed to the sacrificial benchtop in the CT room of RRS James Cook.

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: gill samples preserved in 4% formalin for histological assessment; muscle samples were frozen for stable isotope assessment; muscle samples were preserved in RNA-later for DNA barcoding; the stomach was frozen for later microplastic content assessment.

References

Coughlan, B. M., Hartl, M. G. J., O'Reilly, S. J., Sheehan, D., Mothersill, C., van Pelt, F. N. A. M., O'Halloran, J. & O'Brien, N. M. (2002). Detecting genotoxicity using the Comet assay following chronic exposure of the Manila clam Tapes semidecussatus to polluted estuarine sediments. Mar. Pollut. Bull.(44/12): 1359-1365.

Woods, J. A., O'Leary, K. A., MCCarthy, R. P., O'Brien, N. O. B. (1999) Preservation of Comet assay slides: comparison with fresh slides. Mut. Res. 249: 181-187.

3.10. University of Liverpool - Stable isotopes, amino acids & lipids

3.10.1. Goals

Sediment samples were collected at all UK1 sites during JC257 to characterise the sedimentary organic matter by way of changes in total carbon and nitrogen. In addition, tissue samples from the ROV megafauna collection dives were taken in collaboration with the team from NHM. As the sediments act as a food source for a variety of fauna, stable isotopes of C and N and lipid biochemistry within the sediments and faunal tissue, together with additional δ15N and δ13C amino acids in the faunal tissue will be used to quantify characteristics of the food webs including, trophic position, food chain length, and trophic niche width from this undisturbed area.

3.10.2. Sediment samples – Megacores

Sampling strategy and protocol

To obtain adequate representation of the natural variability of sediments, four megacore deployments were undertaken at each site (UK1-0 km, UK1-1 km, UK1-16 km, UK1-100 km). In order to recover enough material for all planned analysis, 3 cores were obtained from each deployment, except for stations JC257-046 and 073 where an insufficient number of suitable cores were collected to cover all requirements. At these stations 2 cores were obtained. A total of 46 cores were collected from 16 deployments during the cruise.

All core processing took place in the CET lab with 2 h of recovery. The overlying water was removed from the core tubes using Nalgene tubing 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 100 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 100 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 station number, megacore number 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 core length are provided in Table 3.10.1.

Table 3.10.1 Details of megacores taken for stable isotope analysis and lipid biochemistry including station number, latitude, longitude, location, megacore and tube number, core length and slicing depth.

Station Number	Lat N	Lon W	Location	Core No (Tube No)	Core details
JC257_011	13˚55.65	116˚31.67	UK1-1 km	MC1 (3)	Core length: 440 mm; sliced to 400 mm
				MC2 (4)	Core length: 430 mm; sliced to 100 mm
				MC3 (2)	Core length: 440 mm; sliced to 100 mm
JC257_017	13˚55.593	116˚31.683	UK1-1 km	MC4 (5)	Core length: 400 mm; sliced full depth
				MC5 (1)	Core length: 400 mm; sliced to 100 mm
				MC6 (2)	Core length: 360 mm; sliced to 100 mm
JC257_025	13˚55.626	116˚31.730	UK1-1 km	MC7 (2)	Core length: 365 mm; sliced to 360 mm
				MC8 (1)	Core length: 410 mm; sliced to 100 mm
				MC9 (3)	Core length: 410 mm; sliced to 100 mm
JC257_029	13˚55.634	116˚31.616	UK1-1 km	MC10 (2)	Core length: 410 mm; sliced to 400 mm
				MC11 (1)	Core length: 410 mm; sliced to 100 mm
				MC12 (8)	Core length: 410 mm; sliced to 100 mm
JC257_031	13˚56.052	116˚31.808	UK1-0 km	MC13 (4)	Core length: 400 mm; sliced full depth
				MC14 (3)	Core length: 410 mm; sliced to 100 mm
				MC15 (2)	Core length: 410 mm; sliced to 100 mm
JC257_034	13˚56.113	116˚31.864	UK1-0 km	MC16 (7)	Core length: 390 mm; sliced full depth
				MC17 (8)	Core length: 400 mm; sliced to 100 mm
				MC18 (5)	Core length: 410 mm; sliced to 100 mm
JC257_046	13˚56.077	116˚31.972	UK1-0 km	MC19 (7)	Core length: 400 mm; sliced full depth
				MC20 (3)	Core length: 390 mm; sliced to 100 mm
JC257_050	13˚56.109	116˚31.786	UK1-0 km	MC21 (3)	Core length: 410 mm; sliced to 400 mm
				MC22 (4)	Core length: 410 mm; sliced to 100 mm
				MC23 (1)	Core length: 400 mm; sliced to 100 mm
JC257_068	13˚47.994	116˚27.875	UK1-16 km	MC24 (4)	Core length: 450 mm; sliced to 440 mm
				MC25 (3)	Core length: 440 mm; sliced to 100 mm
				MC26 (1)	Core length: 420 mm; sliced to 100 mm
JC257_073	13˚48.027	116˚27.982	UK1-16 km	MC27 (8)	Core length: 410 mm; sliced to 400 mm
				MC28 (5)	Core length: 400 mm; sliced to 100 mm
JC257_088	13˚47.927	116˚27.941	UK1-16 km	MC29 (5)	Core length: 410 mm; sliced to 400 mm
				MC30 (6)	Core length: 430 mm; sliced to 100 mm

				MC31 (3)	Core length: 440 mm; sliced to 100 mm
JC257_092	13˚47.969	116˚27.885	UK1-16 km	MC32 (1)	Core length: 430 mm; sliced to 420 mm
				MC33 (2)	Core length: 420 mm; sliced to 100 mm
				MC34 (8)	Core length: 410 mm; sliced to 100 mm
JC257_099	13˚4.914	116˚5.722	UK1-100 km	MC35 (6)	Core length: 400 mm; sliced full depth
				MC36 (4)	Core length: 400 mm; sliced to 100 mm
				MC37 (2)	Core length: 400 mm; sliced to 100 mm
JC257_101	13˚4.785	116˚5.63	UK1-100 km	MC38 (3)	Core length: 340 mm; sliced full depth
				MC39 (3)	Core length: 330 mm; sliced to 100 mm
				MC40 (6)	Core length: 400 mm; sliced to 100 mm
JC257_104	13˚4.919	116˚5.650	UK1-100 km	MC41 (7)	Core length: 250 mm; sliced full depth
				MC42 (5)	Core length: 170 mm; sliced to 100 mm
				MC43 (3)	Core length: 180 mm; sliced to 100 mm
JC257_110	13˚4.799	116˚5.717	UK1-100 km	MC44 (6)	Core length: 400 mm; sliced full depth
				MC45 (5)	Core length: 450 mm; sliced to 100 mm
				MC46 (7)	Core length: 360 mm; sliced to 100 mm

3.10.3. Megafauna samples

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).

Sample details

Megafauna were collected during 7 ROV dives and collection of specimens was lead by the NHM team. Details of the collections and dissection of the faunal samples can be found in section 3.3. Samples for the food web studies were taken where sufficient fauna and tissue was available. In summary, 61 tissue samples were taken from 42 specimens including; 29 sponges (Holascus sp., Hyalonema, Sympagella, tulip sponge, cauliflower sponge), 2 urchins (Plesiodiadema), 1 (possibly 2) anemone, 2 sea stars, 2 crinoids, 1 brisingid and 5 holothurians (Synallactus, Amperima). Details of samples, including station number, location, Liverpool ID, NHM voucher number, specimen and tissue taken are provided in Table 3.10.2. Tissue samples were placed in cryovials, snap frozen in liquid nitrogen and then stored at - 80˚ C. In addition to the ROV collection specimens, 2 muscle samples from fish collected in the fish trap at UK1-1 km (JC257_016, AKS-10 & 11) and 4 muscle samples from the fish trap at UK1-16 km (JC257_066, AKS-12, 13, 14 &15) were provided by Andrew Sweetman and Mark Hartl. Amphipods were also collected from the 16 km fish trap (Liverpool ID JC257_0035 & 36). A tuna muscle sample was also provided by Andrew Sweetman from near the UK1 - 0 and 1 km sites. Furthermore, opportunistic squid specimens were collected from individuals that had been blown on deck at UK1-0 km during station JC257_082 and at UK1_16 km during the ROV 440 dive (JC257_078). These additional samples were bagged and placed in a - 80˚ C freezer as soon as possible.

Table 3.10.2 Details of megafauna samples collected for stable isotope, amino acid and lipid analysis [during JC257 including, station number, location, Liverpool ID, NHM voucher number, specimen and tissue collected.

Station number (ROV dive)	Location	Liverpool sample ID	NHM number	Specimen	Sample collected
JC257_015 (ROV 431)	UK1 – 1 km	JC257-0001	12627	Hyalonema (sponge)	Body
		JC257-0002	12628	Hyalonema (sponge)	Body
		JC257-0003	12631	Holascus euonyx (sponge)	Body
		JC257-0004	12633	Holascus	Body
		JC257-0005	12630	Sympagella	Body
		JC257-0006	12629	Sea star	Arm

JC257_019 (ROV 432)	UK1 – 1 km	JC257-0007	12695	Tulip sponge	Body
		JC257-0008	12696	Big Hyalonema	Body
		JC257-0009	12697	Big Hyalonema	Body
		JC257-0011	13077	Cauliflower sponge	Body
		JC257-0012	13081	Holascus	Body
		JC257-0013	13247	Large tulip sponge	Body
JC257_057 (ROV 437)	OMS	JC257-0014	13453	Tulip sponge	Body
		JC257-0015	13455	Cauliflower sponge	Body
		JC257-0016	13456	Cladorhizon - carnivorous sponge (probably associated anemone)	Probably anemone tissue
		JC257-0017	13456	Cladorhizon stalk	Stalk
		JC257-0018	13457	Anemone	Body
JC257_063 (ROV 438)	UK1 – 0 to 1 km	JC257-0019	13597	Ring sponge	Body
		JC257-0020	13599	Bowl sponge	Body
		JC257-0021	13600	Holascus euonyx	Body
		JC257-0022	13605	Plesiodiadema globulosum	Tissue
		JC257-0023	13606	Plesiodiadema globulosum	Tissue
		JC257-0024	13696	Plesiodiadema globulosum	Gonads
		JC257-0025	13607	Brisingid	Arm
		JC257-0026	13608	Ophuroid	Arm
JC257_078 (ROV 440)	UK1 – 16 km	JC257-0027	13863	Holascus	Body
		JC257-0028	13864	Holascus	Body
		JC257-0029	13864	Holascus	Body
		JC257-0030	13866	Hyalonema	Body
		JC257-0031	13867	Holascus	Body
		JC257-0032	13868	Synallactus (cucumber)	Resp tree/gonads
		JC257-0033	13868	Synallactus (cucumber)	Mid gut
		JC257-0034	13868	Synallactus (cucumber)	Fore gut
JC257_066	Fish trap 16 km	JC257-0035	_____	Amphipods	Fish trap 16k
	Fish trap 16 km	JC257-0036	_____	Amphipods	Fish trap 16k
JC257_091 (ROV 441)	UK1-16 km	JC257-0037	14154	Sympagella	Body
		JC257-0038	14155	Sympagella	Body
		JC257-0039	14156	Hyalonema	Body
		JC257-0040	14159	Synallactus	Mid gut
JC257_094 (ROV 442)	UK1-100 km	JC257-0041	14221	Holascus	Body
		JC257-0042	14223	Big vase sponge	Body
		JC257-0043	14224	Sympagella	Body
		JC257-0044	14225	Sympagella	Body
		JC257-0045	14228	Amperima (1)	Gonad

JC257_105 (ROV 443)	UK1-100 km	JC257-0046	14394	Amperima (2)	Body (E)
		JC257-0047	14390	Hyalonema	Body
		JC257-0048	14394	Amperima (2)	Gonads
		JC257-0049	14394	Amperima (2)	Body wall
		JC257-0050	14394	Amperima (2)	Body wall
		JC257-0051	14394	Amperima (2)	Muscle
		JC257-0052	14394	Amperima (2)	Muscle
		JC257-0053	14394	Amperima (2)	Cloaca
		JC257-0054	14394	Amperima (2)	Cloaca
		JC257-0055	14398	Synallactus (pink)	Gonads
		JC257-0056	14398	Synallactus (pink)	Fore gut
		JC257-0057	14398	Synallactus (pink)	Mid gut
		JC257-0058	14398	Synallactus (pink)	Cloaca
		JC257-0059	14398	Synallactus (pink)	Mid-hind gut
JC257_107 (ROV 444)	UK1-100 km	JC257-0060	SG-03	Golf ball sponge	Body
		JC257-0061	SG-05	Crinoid	Arm

3.10.4. Additional sampling – CTD seawater collection

Seawater samples were taken for the determination of inorganic macronutrients (nitrate+nitrite, silicate, phosphate) to provide additional and complimentary data to the sediment cores detailed above. Between 7 and 10 depths were sampled, including depths where seawater was collected for eDNA (Susan Evans, NOC, section 19, Microbial and eDNA) as the nutrient data may be useful for that project.

Sampling strategy and sample processing.

Seawater was collected during physics CTD deployments at all UK1 sites (0 km, 1 km, 16 km, 100 km), at 1 km south of the long mooring location and on the last upcast of the physics 'Yo Yo' deployment south of UK1-0 km (JC257_116). Between 7 and 10 depths were sampled, depending on niskin bottle availability, which included; as close to the bottom as possible (generally 5 to 10 m), 10 m above this, the oxygen 'bulge', salinity minimum, oxygen minimum, pycnocline, chlorophyll maximum (Cmax) and 5 m below the surface. Features such as, oxygen 'bulge', salinity minimum, oxygen minimum, pycnocline and chlorophyll maximum were located on the downcast using the fluorescence, conductivity and oxygen sensors fitted to the CTD. Where a deep chlorophyll maximum (DCM) was present, this was also targeted. The number of Niskin bottles fired at each depth was dependant on the parameters required. eDNA had two or three dedicated bottles, whereas nutrients and salinity shared a bottle. Upon recovery of the CTD, nutrient samples were collected, unfiltered, directly from the Niskin bottles using acid-clean tubing. Starting with the deepest sample, a 125 mL acid cleaned HDPE bottle was rinsed three times with the sample and then filled to just below the shoulder. This process was repeated for all samples. Bottles were labelled with station number and Niskin number and then frozen at - 20˚C for storage. Analysis for nutrients will be conducted at the home laboratory at the University of Liverpool. Details of station number, latitude, longitude, location, samples taken, depths and analyses to be conducted are provided in Table 3.10.3.

Table 3.10.3. Details of inorganic macronutrient samples (nitrate+nitrite, phosphate, silicate) taken during JC257 including, station number, latitude, longitude, location, water depth and depths sampled.

Station number	Latitude (N)	Longitude (W)	Location	Sounding depth	Depths sampled
JC257_030	13˚54.619	-116˚29.293	UK1–0 km	4227 m	4227 m, 4217 m, 3000 m 600 m, 200 m, 120 m 70 m, 5 m
JC257_044	13˚52.8018	-116˚28.8717	UK1-1 km south of long mooring	4252 m	4247 m, 4237 m, 2996 m, 300 m, 200 m, 115 m, 58 m, 5 m

JC257_062	13˚56.0763	-116˚31.973	UK1-1 km	4090 m	4083 m, 4074 m, 602 m, 302 m, 125 m, 70 m, 5 m
JC257_065	13˚48.021	-116˚27.939	UK1-16 km	4115 m	4105 m, 4095 m, 2597 m, 600 m, 351 m, 115 m, 60 m, 5 m
JC257_102	13˚4.227	-116˚7.7465	UK1-100 km	4339 m	4341 m, 4331 m, 2495 m, 600 m, 450 m, 120 m, 70 m, 6 m
*JC257_116	13˚52.801	-116˚28.87	*South of UK1-0 km	4259 m	4241 m, 4231 m, 4000 m, 3600 m, 2400 m, 1200 m, 600 m, 8350 m, 70 m, 5 m

**YoYo deployment*

3.11. Other Reports and Observations

3.11.1. Geological collections of polymetallic nodules

Loïc Van Audenhaege, Bethany Fleming, Daniel Jones (NOC)

Nodules were photographed in a tray after epibenthic fauna removal. Nodules were not measured due to their high number (i.e., measurement of nodules of JC257-012 took 2 hours). Nodules were bagged and labelled by station.

Although, the overall density varied greatly, there was a great number of small nodules not exceeding 3x3x3 cm (Table 1). Based on their angular morphology, small nodules may be fragment of larger ellipsoid nodules, and originate from the splitting of the later. This is supported by their crumbly texture and easiness of hand breaking.

JC257-012

JC257-021 (part 1)

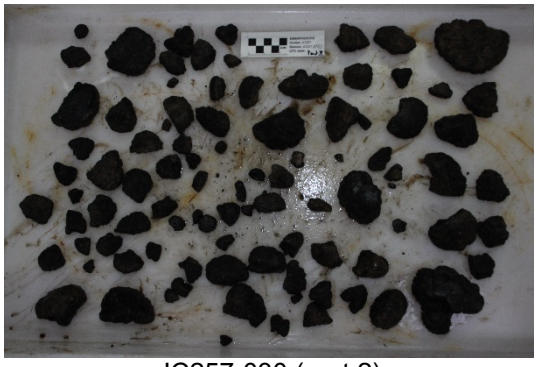
JC257-021 (part 2)

JC257-024 (part 1)

JC257-024 (part 2)

JC257-028

JC257-030 (part 1)



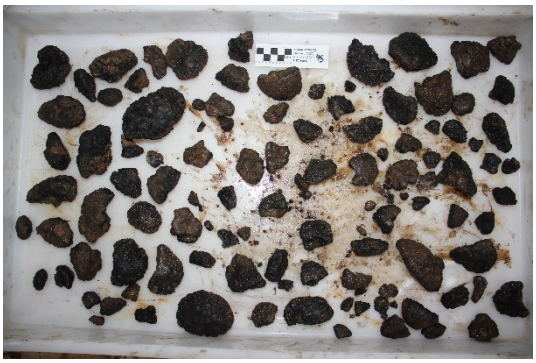
JC257-030 (part 2)

JC257-032

JC257-038

JC257-040

JC257-043



JC257-045

JC257-048

JC257-051

JC257-055

JC257-058

JC257-059

JC257-060

JC257-061

JC257-067

Photo not available

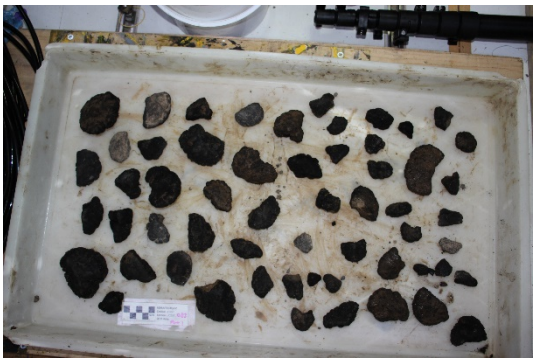
JC_069

JC257-072

JC257-074

JC257-080

JC257-082



JC257-083 (part 1)

JC257-083 (part 2)

JC257-085 (part 1)

JC257-085 (part 2)

JC257-087 (part 1)

JC257-087 (part 2)

JC257-089

JC257-097

JC257-098

JC257-100

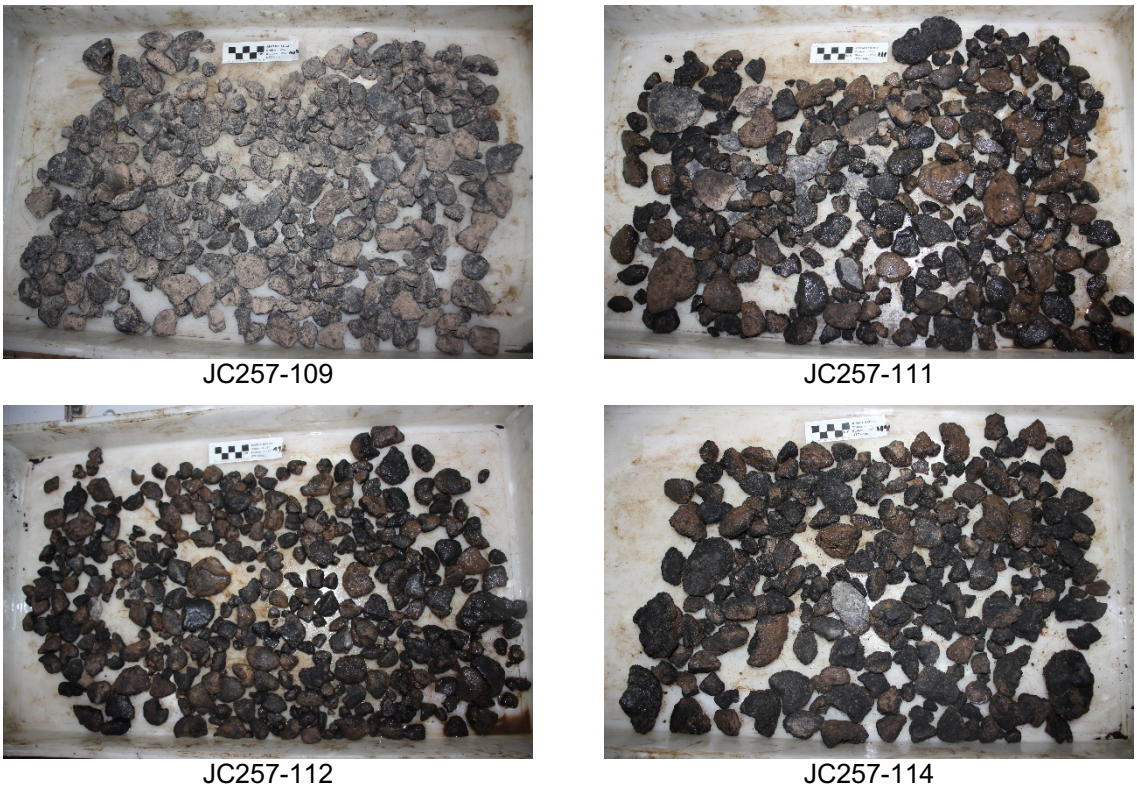


Figure 3.11.1 Nodules retrieved from box core sampling. Top shots taken after removal of epibenthic fauna. All top shots include station number (JC257_###), also written on the scale bar. Note that top shot of JC257-069 is missing.

3.11.2. Samples for DEFRA DEEPEND (biodiscovery) project

Marine organisms are a promising resource for useful natural products such as medicines. The potential use of biodiversity, or marine genetic resources (MGR), has yet to be thoroughly explored in the deep sea. These organisms offer the exciting potential discovery of new gene clusters that direct the formation of enzymes and small molecules (Saide *et al*., 2021). These could have useful biotechnological and pharmaceutical applications, including the discovery of novel antibiotics, coming at a time when society faces an antimicrobial resistance crisis (Murray *et al*., 2022). The DEFRA funded DEEPEND project aims to explore deep-sea MGR, begin to understand the value of deep-sea biodiversity and assess the consequences of resource extraction.

SMARTEX JC257 has collected pushcore and ethanol samples that will be analysed under the remit of the DEFRA funded DEEPEND project from the UK1 site using the ROV.

DEEPEND Pushcore Sampling

The ROV collected 25 pushcore samples for DEEPEND over 9 deployments and 6 sites. See section 2.1 for more information on pushcore equipment. Upon arrival on deck, the cores are taken to the cold temperature lab where they are removed from the quiver, but kept in the plastic tubes which are capped at the end of where the bung is removed, tin foiled at the other, bagged and sealed with a cable tie, labelled and stored in the -80 °C freezer (Figure 3.11.2). The cores will arrive in the UK laboratory frozen and stored at -80°C until processed. On processing, they will be removed from the plastic tubes and the top portion (approx. 10cm) removed. A sterile saw and spatula will be used to access the inner sediment of the core which has not touched the sides of the corer. This ‘uncontaminated’ sediment is dried in a laminar flow hood in a sterile Petri dish, then plated using a dry/stamping method with a sterile foam bung in a clockwise direction to create a serial dilution effect on minimal media with antifungal and anti-Gram-negative bacterial antibiotics. Once bacterial colonies appear resembling actinomycete morphology, they will be subcultured until pure using the isolation media and preserved as glycerol stocks at -80 °C. These strains will be cultured, tested for antibacterial activity against a range of clinically relevant bacterial pathogens and their metabolites profiled using liquid-chromatography high resolution tandem mass spectrometry. The goal of this work is to isolate actinomycete bacteria (unsurpassed for producing chemistry with antibacterial properties) and profile their biomedical potential.

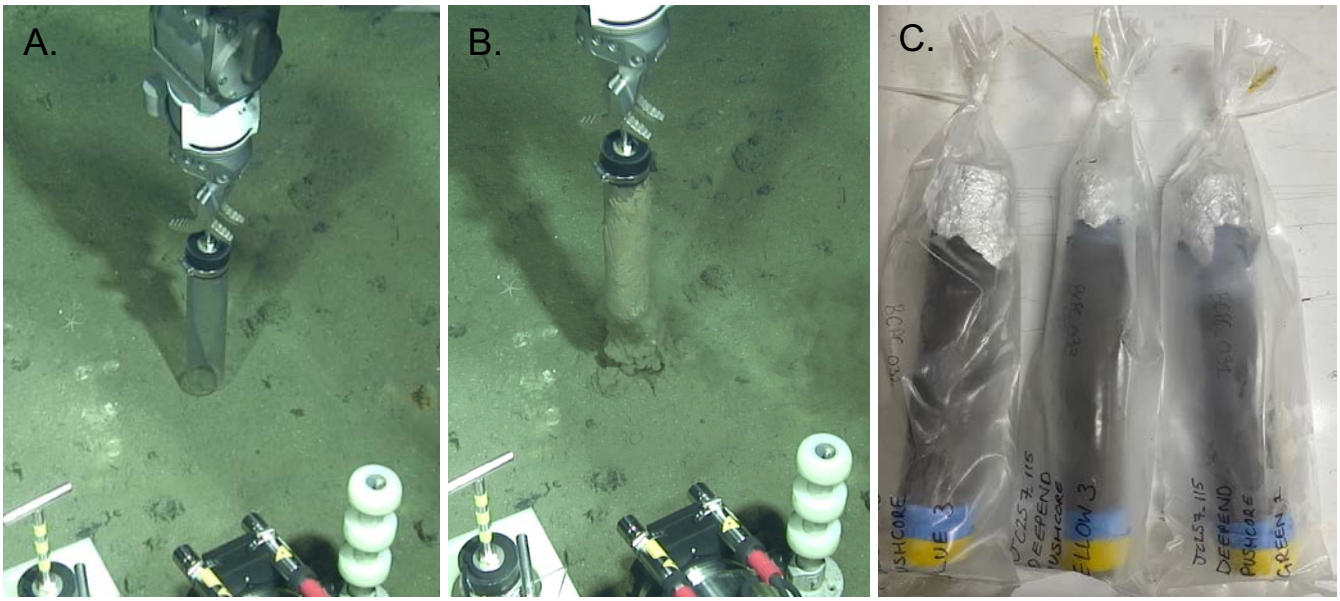


Figure 3.11.2 Examples of the ROV pushcoring process (A, B) and the frozen processed pushcores (C).

Table 3.11.1 DEEPEND pushcore samples. Time and coordinates are taken from sample logs at the seafloor.

Deployment #	Dive #	Site	Date	Time	Latitude		Longitude		Depth (m)	Pushcore Number	NHM#	Notes
JC257_015	431	UK1_1km	16/02/24	23:05	13	55.454	-116	31.997	4096	White 1	12677	
JC257_015	431	UK1_1km	16/02/24	23:08	13	55.453	-116	31.997	4096	White 2	12678	
JC257_015	431	UK1_1km	16/02/24	23:10	13	55.454	-116	31.999	4096	White 3	12679	
JC257_019	432	UK1_1km	17/02/24	13:26	13	57.409	-116	30.449	4143	Yellow 2	12765	
JC257_019	432	UK1_1km	17/02/24	13:38	13	57.408	-116	30.448	4143	Blue 2	12766	
JC257_019	432	UK1_1km	22/02/24	13:40	13	57.408	-116	30.449	4143	Blue 3	12767	
JC257_042	434	UK1_1km	22/02/24	15:06	13	55.309	-116	33.151	4077	Blue 1	13083	
JC257_042	434	UK1_1km	22/02/24	15:06	13	55.309	-116	33.151	4077	Blue 2	13084	
JC257_042	434	UK1_1km	22/02/24	15:08	13	55.309	-116	33.151	4077	Blue 3	13085	
JC257_049	435	UK1_0km	24/02/24	01:59	13	55.759	-116	33.059	4079	Green 1	13255	
JC257_049	435	UK1_0km	24/02/24	02:02	13	55.759	-116	33.059	4079	Green 2	13256	
JC257_049	435	UK1_0km	24/02/24	02:03	13	55.759	-116	33.058	4079	Green 3	13257	
JC257_057	437	UK1_0km (OMS)	24/02/24	23:14	14	02.102	-116	32.754	4078	White 1	13465	1m from colonisation experiment
JC257_057	437	UK1_0km (OMS)	24/02/24	23:06	14	02.101	-116	32.755	4078	Yellow 1	13497	0 m from colonisation experiment
JC257_063	438	UK1_0km	25/02/24	08:02	13	55.955	-116	30.658	4145	Green 1	13621	0 m from colonisation experiment
JC257_063	438	UK1_0km	25/02/24	08:20	13	55.956	-116	30.660	4144	Blue 1	13622	1m from colonisation experiment
JC257_094	442	UK1_16km	04/03/24	14:21	13	05.472	-116	04.881	4138	Yellow 1	14280	
JC257_094	442	UK1_16km	04/03/24	14:19	13	05.472	-116	04.881	4138	Yellow 2	14278	
JC257_094	442	UK1_16km	04/03/24	14:27	13	05.472	-116	04.881	4138	Green 1	14281	
JC257_094	442	UK1_16km	04/03/24	14:25	13	05.472	-116	04.881	4138	Green 2	14279	
JC257_094	442	UK1_16km	04/03/24	14:23	13	05.472	-116	04.881	4138	Green 3	14284	

JC257_105	443	UK1_100km	05/03/24	13:02	13	03.792	-116	07.136	4261	Blue 2	14488	
JC257_115	445	Bone colonisation	08/03/24	23:14	13	43.546	-116	39.940	4131	Green 1	BCPC_031	0m from bones
JC257_115	445	Bone colonisation	08/03/24	00:47	13	43.543	-116	39.939	4131	Blue 3	BCPC_032	1m from bones
JC257_115	445	Bone colonisation	08/03/24	02:43	13	43.575	-116	39.948	4132	Yellow 3	BCPC_033	10m from bones

DEEPEND Ethanol Sampling

The ROV collected 34 specimens where ethanol was set aside for DEEPEND chemical analysis over 13 deployments and 6 sites, made up of 3 phyla: Chordata (1), Porifera (9) and Echinodermata (24). These samples have been double sealed and kept in refrigerated conditions. Upon arrival in the UK, they will be sent to partners at Aberdeen University for chemical analysis with the aim of creating a library of extracts, fractions and pure compounds for antimicrobial testing against medically important diseases including ESKAPE pathogens and diseases of livestock including bovine mastitis (Schabauer *et al*., 2018).

Table 3.11.2 DEEPEND ethanol samples

Deployment Number	Site	Sampler	NHM#	Phylum
JC257_015	UK1_1km	ROV	12941	Echinodermata
JC257_015	UK1_1km	ROV	12942	Echinodermata
JC257_019	UK1_1km	ROV	12943	Echinodermata
JC257_015	UK1_1km	ROV	12944	Porifera
JC257_015	UK1_1km	ROV	12945	Porifera
JC257_015	UK1_1km	ROV	12946	Porifera
JC257_015	UK1_1km	ROV	12947	Echinodermata
JC257_019	UK1_1km	ROV	13017	Echinodermata
JC257_020	UK1_1km	ROV	13018	Echinodermata
JC257_021	UK1_1km	ROV	13019	Echinodermata
JC257_022	UK1_1km	ROV	13020	Echinodermata
JC257_023	UK1_1km	ROV	13021	Echinodermata
JC257_042	UK1_0km	ROV	13236	Porifera
JC257_042	UK1_0km	ROV	13237	Echinodermata
JC257_042	UK1_0km	ROV	13238	Echinodermata
JC257_049	UK1_0km	ROV	13371	Echinodermata
JC257_049	UK1_0km	ROV	13372	Echinodermata
JC257_057	OMS	ROV	13594	Porifera
JC257_057	OMS	ROV	13595	Porifera
JC257_057	OMS	ROV	13596	Echinodermata
JC257_063	UK1_0km	ROV	13797	Echinodermata
JC257_063	UK1_0km	ROV	13798	Echinodermata
JC257_063	UK1_0km	ROV	13799	Porifera
JC257_063	UK1_0km	ROV	13800	Echinodermata
JC257_078	UK1_16km	ROV	14074	Echinodermata
JC257_078	UK1_16km	ROV	14075	Echinodermata
JC257_105	UK1_100km	ROV	14503	Echinodermata
JC257_105	UK1_100km	ROV	14504	Chordata
JC257_105	UK1_100km	ROV	14505	Echinodermata
JC257_105	UK1_100km	ROV	14506	Echinodermata
JC257_105	UK1_100km	ROV	14507	Echinodermata
JC257_105	UK1_100km	ROV	14508	Porifera
JC257_117	Sponge Garden	ROV	SG_038	Echinodermata
JC257_117	Sponge Garden	ROV	SG_039	Porifera

Figure 3.11.3 Examples of ethanol colour after 24 hours preservation of sea cucumber, this ethanol was kept at cool temperature for posterior chemical analysis.

References

Murray, C. J. L., *et al*. (2022) Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. *The Lancet*, 399(10325): P629-655. DOI: https://doi.org/10.1016/S0140-6736(21)02724-0

Panda, S. K., *et al*., (2022). Recent advances to combat ESKAPE pathogens with special reference to essential oils. *Frontiers in Microbiology*, *13*. DOI: https://doi.org/10.3389/fmicb.2022.1029098

Saide, A., Lauritano, C., & Ianora, A. (2021). A treasure of bioactive compounds from the deep sea. *Biomedicines*, *9*(11), 1556. DOI: https://doi.org/10.3390/biomedicines9111556

3.11.3. National Oceanography Centre - sediment traps

The sediment traps from both short moorings (JC257_079 and JC257_084) were on their last bottles, so the last bottle sample should be considered an incomplete record.

Both sediment traps on the long moorings had completed their rotations and were on the open hole. The last bottle on these two traps should be considered a complete cycle.

The upper sediment trap on the long mooring (1000m) came up with some tangling of the line in the floatation above and below the trap. This probably was caused on recovery, but the data should be checked to ensure that instruments were where they should be.

Sediment trap bottles

Sediment trap bottles were recovered with evidence of surprising amounts of sedimentation.

Figure 3.11.4 Sediment trap bottles from JC257_120. Long mooring 1 - 100 m above seabed.

Figure 3.11.5 Sediment trap bottles from JC257_120. Long mooring 1 - 1000 m above seabed.

Figure 3.11.6 Sediment trap bottles from JC257_084. Short mooring 2 - 100 m above seabed.

Figure 3.11.7 Sediment trap bottles from JC257_079. Short mooring 3 - 100 m above seabed.

3.11.4. Natural History Museum - basalt block colonisation experiment

Background to the experiment: a simple colonisation experiment was conceived during project planning for the RC01 cruise in November 2019 to test if nodule-fauna are able to colonise similarly-sized basaltic blocks to nodules within a 2-3 year time frame with a recovery as part of SMARTEX (at that point scheduled for 2022). Basalt blocks from the East Pacific Rise, collected by DSV Alvin by Dan Fornani (Woods Hole Oceanographic Institution) were provided by Greg Kurras (SFI) and used to construct an ROV-deployable experimental rig during project mobilization in Panama. 50cm x 50cm frames were constructed from PVC plastic pipe each with 10 rocks held on a grid by cable ties. Above the frames, floating polypro line lead to a piece of syntactic foam and bucket lids, equipped with ROV-light reflecting tape. Together, the bucket-lids and foam make a reasonable sonar-targeton the seabed for subsequent experiment location (Figure 3.11.8). The UKBio team constructed and oversaw deployment of these experiments in three localities during RC01, two sites in AOI-2 (both next to UTB positioning beacons) and 1 in AOI-3 (also next to one of the AUV UTB positioning beacons).The design was created to enable the basalt blocks to sit as near to the surface of the seafloor as possible, with a minimum amount of corrosive materials and to allow easy ROV deployment and recovery. The experiments were visible on the KD scanning sonar from 10-20m away.

Recovery: Two of the three original experiments were recovered, station JC257_057 in OMS, on ROV dive 437; and JC257_063 in UK1 (stratum A), ROV dive 438. The experiment in UK1 stratum B was not recovered for logistical reasons (time of travel to site). In both cases the experiment was retrieved and placed in an aluminium box (bio box) attached to the ROV. Basalt blocks were processed following the same pipeline as for box core nodule fauna (Section 3.5)

Nodule fauna were present on the basalt rocks, including multiple serpulid worms, a *Plenaster craigi* sponge, an encrusting bryozoan an a Nausithoe. The experiment successfully illustrates that colonisation is possible by some nodule fauna within a four-year window. Serpulid worms (albeit small) were particularly common.

Fauna were also found in the box wash (sediment from the bio box that the experiment was recovered in), including several monoplacophorans, and the on the experiment ropes, including a large pycnogonid and several ophiuroids (visible, along with potential chaetognaths during the dives). These additional fauna were processed following the box core cold chain pipeline outlined in Section 3.5. A summary of the collected fauna from this experiment is presented in (Table 3.11.3).

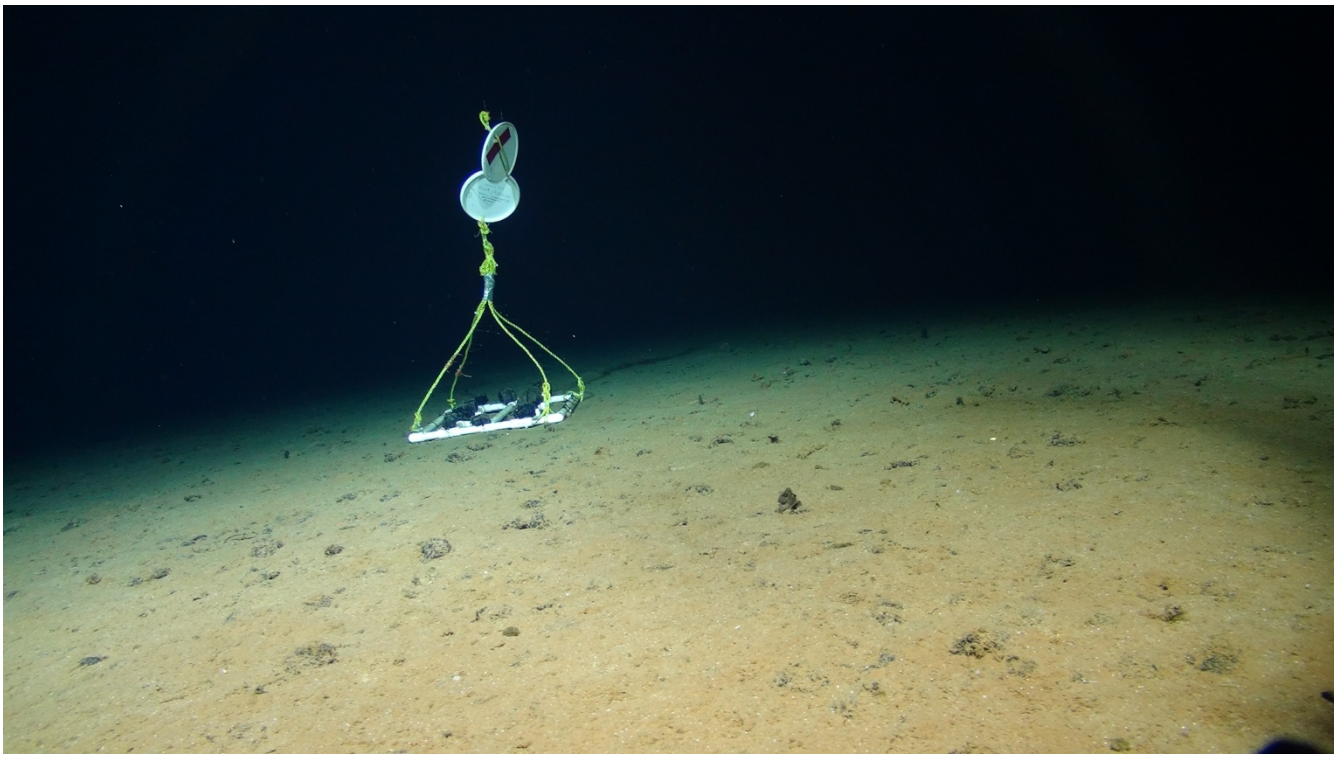


Figure 3.11.8 Colonisation experiment in situ prior to pick up.

Table 3.11.3 Number of individuals per phylum collected during colonisation experiment, split by fauna on the blocks, associated fauna in wash of blocks, and experiment ropes.

Phylum	Basalt blocks	Box wash	Ropes	Grand Total
Annelida	18	5	-	23
Arthropoda	-	7	1	8
Bryozoa	3	-	-	3
Cnidaria	2	-	-	2
Echinodermata	-	4	4	8
Mollusca	-	26	-	26
Nemertea	-	1	-	1
Porifera	2	-	-	2
Grand Total	**25**	**43**	**5**	**73**

3.11.5. Natural History Museum - whale and wood bone colonisation experiment

Large organic falls on the deep-sea floor provide extreme levels of nutrients in an otherwise nutrient poor environment. The science around this type of ephemeral habitats has a long history with the first whale fall specialists described a century ago from bones getting caught in fishing trawls (Marshal 1900). Many of the species found here rely on energy from symbiotic chemoautotrophic bacteria using sulphides released from the organic falls (Smith & Baco 2003).

More recently experimentally deployed bone and wood have been studied in various areas of the ocean, from shelfs to deep-ocean sites (Dahlgren et al. 2006, Glover et al. 2005, Glover et al. 2012). While wood can drift long distances, it is hypothesized that whale carcasses sink more readily and are concentrated along areas that whales occupy most, e.g. along migration routes largely following continental shelf breaks (Young et al. 2022). Here is also where most experiments have been conducted resulting in a growing understanding of the taxonomy and ecology of the fauna specialized in this type of habitat. The most known of these species may be the bone eating worms *Osedax*, with more than thirty species discovered since they were first described in 2004 (Rouse et al. 2004). Population genetic and biogeography data suggest that they have a remarkable dispersal capacity with bone being colonized within a short time after deployment and at most sites where experiments have been conducted (Berman et al. 2023). There is only one published record of whale-fall ecosystems at

abyssal depth from off Brazil (Sumida et al. 2016) but no record from the Pacific and none from abyssal basins at a distance from continental slopes or other areas where whales are frequently observed (Berman et al. 2023).

To address questions regarding dispersal and distribution of whale and wood fall specialist in the abyssal plains of the Central East Pacific three simple moorings were deployed during the first Abyssline cruise to the UK1 exploration contract area onboard the research vessel *Melville* on October 22, 2013. Each of the moorings consisted of two whale bones from a whale carcass stranded in Washington State and a piece of wood. They were fitted with an 8 kg weight as anchor, and a small piece of syntactic foam and a bucket lid aiding the discovery at the seafloor with an ROV (Figure 3.11.9).

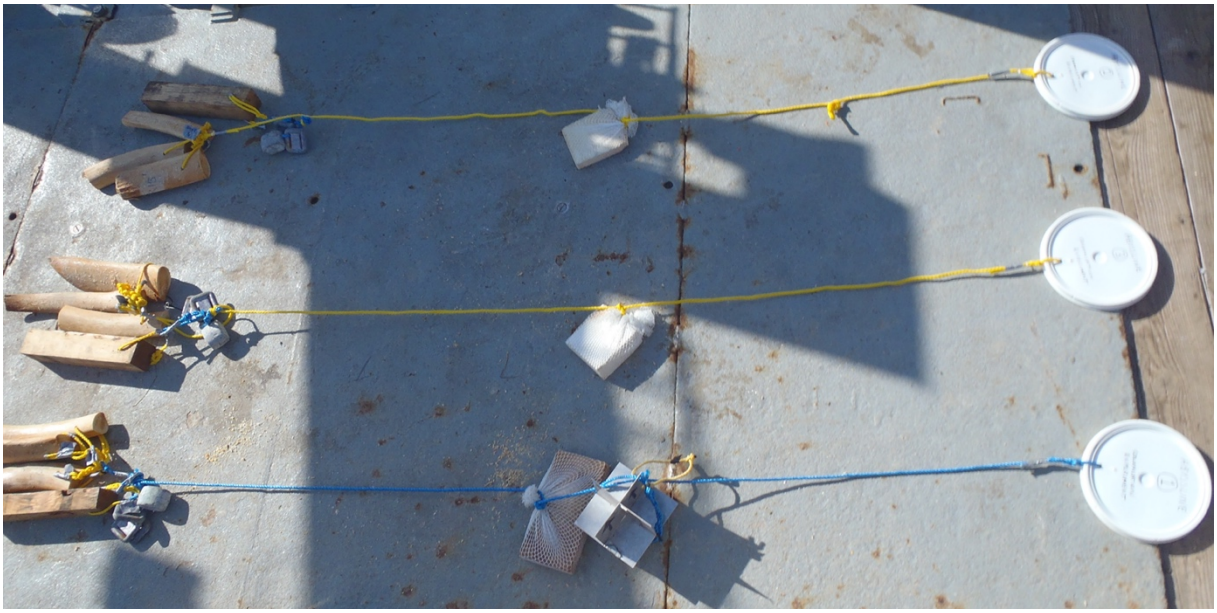


Figure 3.11.9 The three wood and bone mooring on the deck of the research vessel Melville prior to deployment in on October 22, 2013. Note than an additional 6 kg iron plate was added as weight to each of the mooring before deployment (not in image).

During ISIS Dive 445 (JC257_115) of the SMARTEX (JC257) cruise 6 hours of bottom time was dedicated to the experiments and two of the three moorings originally deployed where sampled. The moorings were found within a radius of around 100 m from the location they were deployed (13°43.563 N; 116°39.895 W, 4160m depth). One mooring (#1) was sampled using manipulators and push cores, the other (#3) was recovered entirely with manipulators, with pushcores taken from the surrounding mud afterwards. The final mooring (#2) was not collected due to time constraints on the dive. Pushcores for background data were taken at one site in the vicinity (≈ 50 m) of the moorings (Table 3.11.1).

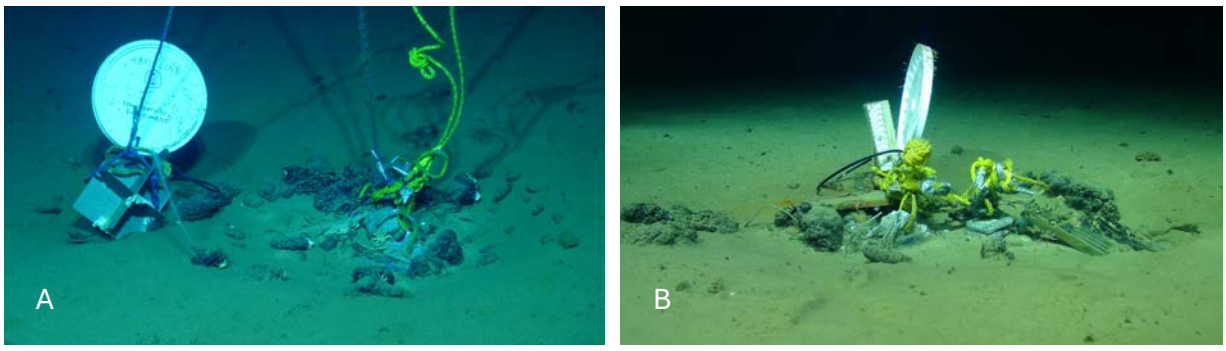


Figure 3.11.10 Bone and wood moorings experimentally deployed in October 2013 and retrieved 11.5 years later on the SMARTEX II cruise. Two of the three original moorings were sampled, mooring #1 (A) and mooring #3 (B).

In the laboratory, specimens were picked directly from the bone or wood for imagery and fixation. In addition, ROV bioboxes that contained experiments during retrieval were sieved for associated fauna (box wash). Due to high abundances of animals on the experiments and from the box wash, only a small portion was live sorted and imaged at sea. While final estimates of abundance and diversity of experiment-associated fauna await further analyses, a preliminary summary table of processed fauna is presented in Table 3.11.4. Bone and wood

experiments were fixed in ethanol for further processing. Unsorted sediment and sieve samples were also bulk fixed in ethanol for further analysis.

Table 3.11.4 Number of individuals per phylum collected during bone and wood experiment, including individuals from box wash etc. – does not include remaining bulk fix sediment, bulk fixed but not counted animals, and remaining animals to pick from bones etc. – qualitative quick glance.

Phylum	bone picking	wood picking	box wash	Grand Total
Annelida	26	6	115	147
Arthropoda	3	30	103	136
Cnidaria	-	2	1	3
Echinodermata	-	-	9	9
Metazoa	-	1	-	1
Mollusca	-	14	38	52
Nematoda	9	-	-	9
Nemertea	-	-	6	6
Grand Total	**38**	**53**	**272**	**363**

Notably, after 11.5 years at the seafloor there was still a considerable number of animals attracted to the experiments suggesting that they continued to provide energy as well as acting as reefs. However, the presence of a large number of empty tubes from sulphophilic worms in the family Siboglinidae resembling tubes of *Lamellibrachia* provide some evidence of that a significant proportion of this food were spent at an earlier time (Feldman et al. 1998). The wood was also heavily devoured by shipworms of the group Xylophagaidae with only some wood remaining, most of which parts that were buried in mud. Only two individuals of Xylophagaidae were retrieved also suggesting that most of the wood was spent at the time of retrieval. The retrieved fauna included a large number of annelid worms belonging to the families Phyllodocidae, Hesionidae and Polynoidae along with a few cirratulids and nereids. Most of the bone material at the experiments was consumed after 11.5 years. Only three smaller pieces was retrieved with associated fauna including at least three different *Osedax* species. These species included one belonging to the nude palp clade and one with long pinnulae on the palps suggesting a placement in one of the two identified pinnulate clades (Rouse et al. 2018). The third species were of a very small size requiring more detailed analyses. The wood pickings included a number of bivalves similar to members of Lucinidae with dark gray colored gills and a large green/gray colored organ suggesting symbiotic bacteria. There were also a number of Munidopsid crabs on or in the close vicinity of the experiments of which a sample were collected.

References

Berman, G., Johnson, S., Seid, C., Vrijenhoek, R., Rouse, G., 2023. Range extensions of Pacific bone-eating worms (Annelida, Siboglinidae, Osedax). BDJ 11, e102803. https://doi.org/10.3897/BDJ.11.e102803

Dahlgren, T.G., Wiklund, H., Källström, B., Lundälv, T., Smith, C.R., Glover, A.G., n.d. Ashallow-waterwhale-fall experiment in the north Atlantic.

Feldman, R.A., Shank, T.M., Black, M.B., Baco, A.R., Smith, C.R., Vrijenhoek, R.C., 1998. Vestimentiferan on a Whale Fall. The Biological Bulletin 194, 116–119. https://doi.org/10.2307/1543041

Glover, A.G., Källström, B., Smith, C.R., Dahlgren, T.G., 2005. World-wide whale worms? A new species of *Osedax* from the shallow north Atlantic. Proc. R. Soc. B. 272, 2587–2592. https://doi.org/10.1098/rspb.2005.3275

Glover, A.G., Wiklund, H., Taboada, S., Avila, C., Cristobo, J., Smith, C.R., Kemp, K.M., Jamieson, A.J., Dahlgren, T.G., 2013. Bone-eating worms from the Antarctic: the contrasting fate of whale and wood remains on the Southern Ocean seafloor. Proc. R. Soc. B. 280, 20131390. https://doi.org/10.1098/rspb.2013.1390

Marshall, J. T. (1900). On a British species of *Myrina*, with a note on the genus *Idas*. *J. Malacol*, *7*(7), 167-170.

Rouse, G.W., Goffredi, S.K., Vrijenhoek, R.C., 2004. Osedax: Bone-Eating Marine Worms with Dwarf Males 305, 5.

Rouse, G.W., Goffredi, S.K., Johnson, S.B., Vrijenhoek, R.C., 2018. An inordinate fondness for Osedax (Siboglinidae: Annelida): Fourteen new species of bone worms from California. Zootaxa 4377. https://doi.org/10.11646/zootaxa.4377.4.1

Smith, C. R., & Baco, A. R. (2003). Ecology of whale falls at the deep-sea floor. In *Oceanography and marine biology* (pp. 319-333). CRC Press.

Sumida, P.Y.G., Alfaro-Lucas, J.M., Shimabukuro, M., Kitazato, H., Perez, J.A.A., Soares-Gomes, A., Toyofuku, T., Lima, A.O.S., Ara, K., Fujiwara, Y., 2016. Deep-sea whale fall fauna from the Atlantic resembles that of the Pacific Ocean. Sci Rep 6, 22139. https://doi.org/10.1038/srep22139

Young, E., Halanych, K., Amon, D., Altamira, I., Voight, J., Higgs, N., Smith, C., 2022. Depth and substrate type influence community structure and diversity of wood and whale-bone habitats on the deep NE Pacific margin. Mar. Ecol. Prog. Ser. 687, 23–42. https://doi.org/10.3354/meps14005

3.11.6. National Oceanography Centre - marine mammal observations

We carried out one hour of marine mammal observation for each start of the ship's multibeam echosounder. A soft start was started after 40 minutes, if no animal entered the mitigation zone. If an animal entered the mitigation zone, the soft start was delayed of 20 minutes from the sight of the animal.

List of certified observers:

- Catherine Wardell
- Bethany Fleming
- Lucy Harris
- Prof. Daniel Jones
- Dr. Loïc Van Audenhaege

Full recording sheets are provided in Appendix 5.

Imagery of marine mammals observed during JC257 are provided here in Figure 3.11.11.

Figure 3.11.11 Marine mammal observations – Dolphins. Top left: bottlenose dolphins Tursiops truncatus (during transit 7 Feb 2024 14:18 UTC). Top right: short-beaked common dolphin Delphinus delphis (during transit 8 Feb 2024 12:28 UTC). Upper Middle left: possibly pantropical spotted dolphin Stenella attenuata (during transit 9 Feb 2024 13:00 UTC). Upper Middle right: Possibly bottlenose dolphin in pod following large shoal of tuna (on station at 0 km site UK-1; 10 Mar 2024 17:30 UTC). Lower middle: Minke whale (on station at UK-1 site; 9 Mar 2024 18:45 UTC). Bottom: Killer whale (on transit back 16 Mar 2024 22:47 UTC).

3.11.7. National Oceanography Centre - Gravity Core

Details are provided in the equipment section 2.6.

3.11.8. Trainee report - Tanga Morris, Seabed Mineral Authority, Cook Islands

The Cook Islands, a Pacific Small Island Developing State (PSIDS), are actively exploring their deep-ocean mineral resources. As a Knowledge Management Officer with the Cook Islands Seabed Minerals Authority (SBMA), a regulatory body under the government, we have had the opportunity to engage in both past and ongoing collaborations with DEEPEND, a research initiative focused on deep-ocean resources and biodiversity affiliated with the National History Museum (NHM). Months prior to the expedition, SBMA expressed my interest, and I was fortunate to be selected as a trainee from the Pacific to join the SMARTEX expedition. This expedition marks my longest duration at sea for scientific purposes and is a pioneering experience for a young female Cook Islander like myself.

SMARTEX has served as a platform for knowledge sharing and collaboration among scientists from diverse professional backgrounds, significantly enhancing my understanding of the deep sea. Through this experience, I have acquired a range of hard and soft skills essential for conducting deep-sea research at sea.

Table 3.11.5 Tanga Morris Trainee Report: Hard Skills.

Hard Skills	Trainer	Learning Outcomes	Summary of skills
Lab preparation	Dr Adrian Glover, Dr Belen Arias	Preparation of 2 successful labs, deck, and cold lab.	Both labs serve their specific purposes to which biological work take place. Packing, organizing and labelling equipment in cabinets/benches. Decoration to create a live and fun work environment. Ensuring all equipment is secure and ready for operation once we reach the first sampling station.
Box core	Dr Adrian Glover, Dr Thomas Dahlgren	Logged and processed box cores.	Assessment criteria, draining of topwater, nodule wash, slicing 0-2, 2-5, 5-10cm, sieving, live sort, specimen photography, sample storage, metadata logging.

Megacore	Dr Adrian Glover	Logged and processed megacore deployments.	Assessment criteria, photography and measurement of each core, core removal, slicing the cores, storage for foraminifera analysis. Fixing in ETOH 80% storage and metadata logging.
ROV Data	Ms Bethany Flemming	Logged and recorded ROV dives.	Logging of dive, media, and sample log sheets during dives. Systems used: OFOP (map calibration, ship and ROV position, event, and sample logging). Setting waypoints, video capture, copying, locking, cleaning and storage.
ROV Megafauna Collection	Dr Adrian Glover, Dr Georgina Glaser, Ms Bethany Flemming	Megafauna collections and processing	Retrieving all megafauna from ROV once back on deck. Prefilling, labelling of equipment needed (buckets with cold filtered seawater with correct labelling). Cold room prep (prefilling aquarium tanks, camera set up and ETOH 80% prep). Photography of megafauna, fixing, labelling and storage (each megafauna required a second change in ETOH 80% after 24hrs).
ROV Pushcore	Dr Muriel Rabone	Processed pushcores	Retrieving from ROV on deck and move to cold lab. Removal of from capsule, drain top water, sealing bottom of tube with cap and tape, storage in bags, labelling and storage in -80 freezers.
Photography	Dr Adrian Glover, Dr Thomas Dahlgren	Visual documentation and camera skills.	Photography of the following scientific activities: top shot box core, individual megacore shots, nodules and specimen shots (TD and 90TD), ROV megafauna collection shot and mammal observations. This improves ability to adjust and fit camera settings along with flashes.
Taxonomy	Dr Adrian Glover, Dr Thomas Dahlgren	Classification of megafauna and macrofauna	Morphological species identification via images taken on 90TD camera for nodule collection or live sort from box core.
Science Communication	Dr Adrian Glover	Blog writing	Wrote my first scientific blog sharing initial experience of life out at sea on RRS James Cook.
Sample labelling	Dr Adrian Glover, Dr Georgina Glaser, Dr Muriel Rabone	Quality control and efficient inventory system.	Labelling samples involves attention to detail, organization, and precision. This makes things easier for inventory purposes once vessel arrives back in South Hampton Port. Finding samples and easy transitioning to respected organisations.
Stock taking & cleaning	Dr Adrian Glover, Dr Georgina Glaser	Re-stock of equipment after every shift	Pre-filling 2ml tubes, ETOH 80% containers, cold filtered sea water bottles, container labelling. Cleaning and sterilizing all equipment after use, always keeping lab tidy. This creates good work environment while on shift and for the next. Leaving work stations tidy and organized.
Pack-down and Sample Storage	Dr Belen Arias, Dr Adrian Glover	Demobilisation of both labs, packing and inventory of samples	Safely packing of all equipment from benches and cabinets into metal boxes, proper storage of samples (in boxes), washing and bleaching all equipment used (get rid of sea salt), full wipe down and bleach of benches and floors.

Table 3.11.6 Tanga Morris Trainee Report: Soft Skills.

Soft Skills	Summary of Skills
Knowledge	My participation in SMARTEX has provided invaluable knowledge and experience for small island developing states with limited experience in deep sea research at sea. The Cook Islands are a large ocean state, and it only makes sense to build capacity in at sea experience, for this we are truly grateful for this opportunity. The cruise has offered insights into deep-sea ecosystems, geological features, and habitats contributing significantly to our understanding of the marine environment. Furthermore, collaborations and networking opportunities with other research institutions (such as NHM, NOC, SAMS, UoL, and others) can lead to potential future partnerships. The cruise has

	also provided a platform to share my experiences with my country, aiming to inspire our people to explore similar fields.
Teamwork	Working with passionate and precise individuals was enjoyable. Together, we shared and tackled challenges. Our teamwork was instrumental in boosting efficiency, as we assigned tasks based on each team member's strengths, thereby improving problem-solving and decision-making. Safety is a top priority while working at sea, and it was impressive to see everyone proactively stepping in to minimize risks. All groups - the science team, technical crew, and vessel crew - worked exceptionally well together to reach our target cruise goals. Despite starting the cruise as strangers, we now part ways as family. In the Pacific, we have a saying that goes, "It takes a village to raise a child," and I want to thank everyone for being that village for me during this stellar experience.
Organisation/Time Management	Time management is critical during at-sea expeditions. Every task, from deploying equipment to collecting samples and analysing data, was done promptly some things I will take home with me. My first experience in 12hr shifts was a challenge but as many say “if its easy everyone can do “and they sure did. Seeing tasks unfold and completed despite minor issues proves hard work and commitment come a long way towards success.
Adaptability/diversity	I knew from the start that participating in this cruise would introduce me to a diverse group of individuals from around the world. This experience opened opportunities to learn about and understand very diverse ethnicities, languages, and behaviours, which is something we in the Pacific don’t encounter daily.

On behalf of SBMA and the Cook Islands, I want to express my heartfelt gratitude to Chief Scientist Dr. Adrian Glover, Co-chief Scientist Dr. Dan Jones, the respective members of the science team, technical crew, and vessel crew for giving me the opportunity to make dreams a reality. As a young, female Cook Islander from the Pacific, bridging the gap between science and at-sea experiences, I feel incredibly fortunate. I will bring this knowledge back to my country and strive to inspire others. Meitaki ranunui, te Atua, te Aroa.

Figure 3.11.12 Trainee Tanga Morris participating in a wide variety of tasks on JC257.

3.11.9. Trainee report - Lucy Harris, University of Southampton

As an early career researcher currently in the position of Research Associate for the DEFRA DEEPEND project I joined the SMARTEX JC257 cruise as a trainee. It has been my first experience of a scientific cruise and conducting science at sea. Prior to the cruise, the CCZ and deep-sea ecology has been a focus of study during my current write-up of papers, “A Cost Benefit Analysis of Deep-Sea Mining” and, "Global distribution of novel bioactive compounds from deep-sea organisms: exploring the influence of phylogeny and environment". Thus, the opportunity to join SMARTEX has provided significant insight into the work I am continuing to explore academically and has substantially improved my skillset for biosampling science protocols and operations at sea.

I have largely been working with the bio-sampling NHM team to process micro- to megafauna as well as assisting with food web samples (UoL), pushcores, ethanol (DEEPEND) and gonads and eggs for Life History analysis (UoS).

Table 3.11.7 Lucy Harris Trainee Report: Hard Skills.

Hard Skills	Date of Training (2024)	Trainer	Learning Outcomes	Summary of skills
Marine Mammal Observer	8th January	Intelligent Ocean, JNCC	Completed Marine Mammal Observer mitigation training, assisted with MMO watch prior to turning on multibeam.	Conducted a visual MMO watch prior to the multibeam being switched on, mammal sightings successfully logged.
Lab preparation	5th – 8th February	Dr Adrian Glover, Dr Belen Arias	A fully prepared deck lab and cold temperature lab (CTL) before arrival at the research site. Learnt about the types of equipment required to set-up a lab at sea and the process of installation.	Bleaching all lab surfaces in the cold temperature and deck lab. Unpacked, set-up and secured required lab equipment (computers, hard drives, microscopes, PPE, containers (falcon tubes, cryo tubes, petri dishes, varied screw lid container volumes), stationery and dissection kits).
Box core	13th February	Dr Adrian Glover, Dr Belen Arias	Processed ~20 box cores.	Logging the box core's top water clarity and temperature, disturbance, megafauna presence and taking a topshot photo. Quantitatively sieving the top water and slicing and sieving the layers; 0-2, 2-5 and 5-10 cm. Live sorting the nodule wash and fixing the sieved material with 80 % ethanol (ETOH 80). Photographing nodule fauna. Assigning NHM numbers.
Megacore	17th February	Dr Daniel Jones, Dr Louisa Norman	Logged and processed ~13 megacore deployments.	Metadata logging (length, quality and photographing each core), removal of cores from the mega corer instrument. Slicing the cores using an extruder and slicing equipment for Foraminifera analysis. Fixing in ETOH 80 and assigning NHM numbers. Assisting food web slicing (UoL).
ROV Data	12th February	Emre Mutlu	Logged and recorded ~15 ROV dives.	Using the OFOP window and rough logs to record ROV movements and updates, megafauna observations, collections, and waypoints. Backing-up and replacing recording discs.
ROV Megafauna Collection	16th February	Dr Belen Arias	Processed ~8 megafauna collections.	Logging all megafauna during the ROV dive, retrieving them from the vehicle on deck, processing the samples in the CTL, including photography, subsampling and preservation in different methods, fixing voucher specimens (ETOH 80, RNA Later and Formalin) and assigning NHM numbers.
ROV Pushcore	16th February	Dr Louisa Norman	Processed ~15 pushcores	Logging all pushcores during the ROV dive, retrieving them from the vehicle on deck, processing the samples in the CTL, including removing top water, quiver, and bung. Capping, sealing, assigning NHM numbers and freezing the core at -80 °C for microbiology analysis onshore.
Photography	16th – 20th February	Dr Regan Drennan	Comfortably and effectively using photography on all deployments where required.	Utilising four cameras for box core topshots and megacores (600D), nodule and macrofauna (TD and microscope mounted 90D), megafauna (850). Improving skills by adjusting lighting, aperture, shutter speed and different focus settings.
Science Communication	16th – 20th February	Dr Adrian Glover, Dr Dan Jones	Blog successfully posted on 29th February.	Built on science communication skills by writing a blog about my experience of going to sea for the first time.
Cruise Pack-up and Sample Storage	14th March	Dr Belen Arias, Dr Adrian Glover, Dr Georgina Glaser	A fully demobbed ship with all equipment logged on packing lists and all samples stored appropriately prior to docking.	Ensuring the safe packing of equipment and samples so they can be easily moved by crew during transit if required and upon arrival in Southampton. Ensuring the preservation of samples in refrigerated conditions.

Table 3.11.8 Lucy Harris Trainee Report: Soft Skills.

Soft Skills	Summary of Skills
Knowledge	**Perception of deep-sea communities**: Over the course of the cruise, particularly during ROV dives and boxcore processing, I gradually improved my understanding of CCZ ecology by learning from the experts around me and using the CCZ catalogue. I was able to begin identifying species using their taxonomic names to the best of our knowledge and gain an insight into the communities that live in the CCZ. This strengthened my perception of deep sea not being a "desert" but a wide variety of unique ecological niches, occupied by highly specialised species. **Perception of deep-sea fieldwork**: The cruise has given me an insight into the challenges of undertaking deep-sea research. The remote nature of the CCZ requires highly technical equipment that can suffer failures of different kinds. This gave me an insight in the need to be flexible with science plans, whilst ensuring that we are capitalising on expensive time at sea. This also comes into sampling, where I gained an insight into prioritising certain species, such as Sympagella for population genomics and not spending time collecting other, better understood species.
Teamwork	Working in a team has been critical in achieving the scientific goals of the SMARTEX cruise. Largely this has been ensuring I can operate at a level where I am trusted to complete all tasks associated with my role on board thus allowing the team to work efficiently on multiple tasks at once. This also extends to proactively working in other teams e.g., UoL and NOC when required to promote the efficiency of the science during my shift. I have also learnt how important it is to support the team at sea and prioritising certain behaviours such as positivity, respect, solidarity, getting to know people, listening to others, punctuality and generally being a good team member.
Organisation/Time Management	It's been crucial to be aware of ship activities using the whiteboards and dashboard to effectively prepare. This includes prepping the sieving table, deck lab and cold room ahead of sampling, such as filling buckets with cold filter sea water, resupplying ethanol containers, ensuring all equipment is clean, ready for use and in the correct location and workstations are clear for processing. I also learnt to deal with uncertainty and adapt to changing plans when equipment failed, or we ran out of daylight etc. and still use time efficiently to prepare for plans A, B or C.
Resilience/ Confidence	The SMARTEX cruise required ~7 weeks of time away from home, on a research ship for the first time and with the majority of the team previously unknown to me. I have grown in resilience due to the level of support offered by the team. I have felt comfortable to engage with all members of senior and junior crew, technical and science team members and have felt confident to try new skills, make new connections and enjoy this unique working environment.

Figure 3.11.13 Trainee Lucy Harris processing a pushcore for DEEPEND (A), processing ROV megafauna (Psychropotes sp.) (B), removing a megacore tube (C) and recording the dissection of parasitic snails from an Amperima holothurian (D).

3.11.10. Surface water ecological observations

Daniel Jones

A number of surface water observations of marine life were made (Figure 3.11.14, 3.11.15). These are summarised in Table 3.11.9.

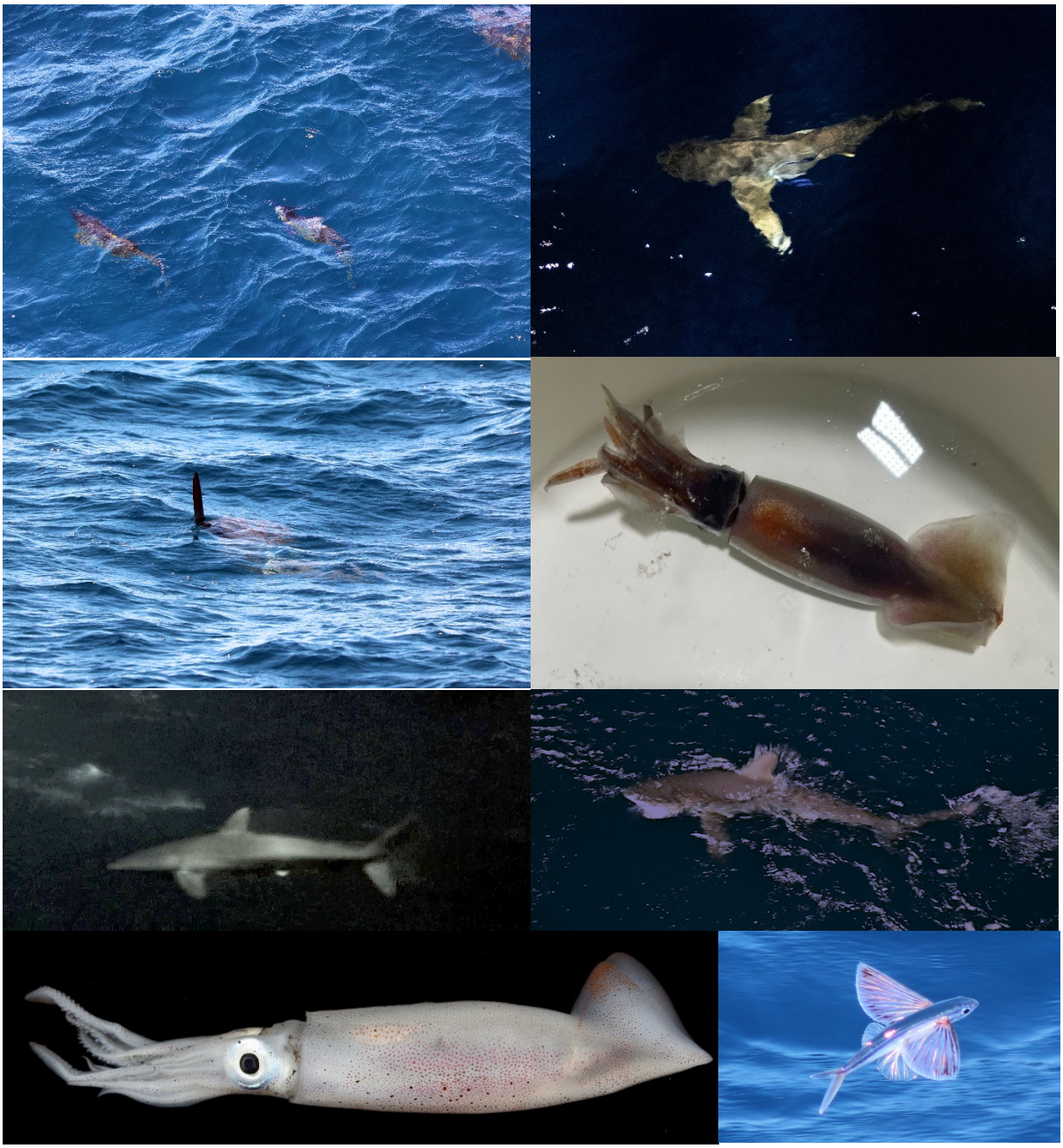


Figure 3.11.14 Surface water ecological observations. Top left: Tuna (possibly yellowfin; on station at 0 km site UK-1; 10 Mar 2024 17:30 UTC). Top right: Oceanic white tip shark Carcharhinus longimanus (on station at UK-1; 16 Feb 2024 12:17 UTC). Upper Middle left: Sunfish Mola mola (on station at UK-1; 10 Mar 2024 19:03 UTC). Upper Middle right: Squid (approx. 20cm long) captured from surface waters (at UK-1, 28 Feb 2024 06:32 UTC). Lower middle: Silky shark Carcharhinus falciformis (at UK-1 on 20 Feb 2024). Bottom: Small (8cm long) squid recovered from surface waters. Flying fish observed on transit.

Figure 3.11.15 Bird observations. Top left: Peregrine falcon with prey bird, likely petrel (observed close to Punta Arenas). Top right: Booby bird observed throughout voyage. Middle left: Booby bird observed throughout voyage. Middle right: magnificent frigatebird observed near Punta Arenas. Bottom left: Brown pelican observed near Punta Arenas. Bottom right: nest of Booby birds on bow structure of James Cook Ship.

Table 3.11.9 Observations of surface fauna made during transit and on station.

Group	Common name	Species name	Notes
Cetaceans	Common bottlenose dolphin	*Tursiops truncatus*	
	Short-beaked common dolphin	*Delphinus delphis*	Observed during transit only
	Pantropical spotted dolphin	*Stenella attenuata*	Observed during transit only
	Minke whale	*Balaenoptera acutorostrata*	At least two separate signtings were made of different individuals at UK-1
	Killer whale	*Orcinus orca*	Observed during transit back
Fish	Yellowfin tuna	*Thunnus albacares*	Confirmed with captured specimens at UK-1
	Mahi mahi	*Coryphaena hippurus*	Confirmed with captured specimens at UK-1
	Sunfish	*Mola mola*	Observed once at UK-1
	Flying fish	Family: Exocoetidae	Observed very regularly in large shoals at UK-1
	Oceanic white tip shark	*Carcharhinus longimanus*	Observed on several nights at the start of operations at UK-1. Rarely observed in the day.
	Silky shark	*Carcharhinus falciformis*	Observed regularly, on most nights, at UK-1. Rarely observed in the day. Confirmed with close up observations.
Turtles	Indet. Turtle		Possibly Olive Ridley turtle. Observed rarely at UK-1.
Siphonophores	Bluebottle	*Physalia physalis*	Confirmed with several close-up observations of small (3cm float length) individuals at UK-1.
Birds	Indet. storm petrel		Observed regularly at night at UK-1. Attracted to ship's lights. Often found on ship deck.
	Booby bird	*Sula* spp.	Likely several species observed, including the Brown Booby. Quickly colonised bow section of ship and used it as fishing platform for entire journey.
	Peregrine falcon	*Falco peregrinus*	Observed close to Punta Arenas on transit. Confirmed with photographs.
	Magnificent frigatebird	*Fregata magnificens*	Observed in first few days of transit. Confirmed with photographs.
	Brown pelican	*Pelecanus occidentalis*	Observed close to Punta Arenas on transit. Confirmed with photographs.

3.11.11. Drone observations

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.

Ideal flying conditions are <15 knots. The drone was flown from the rear deck. 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). Holding 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.

Figure 3.11.16 Examples of drone observations made during JC257. Images by Daniel Jones.

4. Public communication

A twitter (X) feed was maintained at #smartexccz. Total mentions were 550, the total reach 843,000 and the total impressions 2.8m. A cruise blog was maintained at www.smartexccz.org.

5. List of Appendices

5.1. Appendix 1: Summary Deployment Log of JC257

Data are provided at the end of this document in the form of a summary table

5.2. Appendix 2: Complete Science Log of JC257

Document provided in a separate electronic file JC257StationLog.xlsx

5.3. Appendix 3: ROV Technical Report

Document provided in a separate electronic file JC257ROVTechnicalReport

5.4. Appendix 4: AUV Technical Report

Document provided in a separate electronic file JC257AUVTechnicalReport

5.5. Appendix 5: CTD Technical Report

Document provided in a separate electronic file JC257CTDTechnicalReport

5.6. Appendix 6: OEG Technical Report

Document provided in a separate electronic file JC257OEGTechnicalReport

5.7. Appendix 7: Bathysnap Technical Report

Document provided in a separate electronic file JC257BathysnapTechnicalReport

5.8. Appendix 8: Scientific Ship Systems Technical Report

Document provided in a separate electronic file JC257ShipSystemsTechnicalReport

5.9. Appendix 9: Marine Mammal Observation Sheets

Document provided in a separate electronic file JC257MMOTechnicalReport

Appendix 1: Full Station Deployment List for JC257. Information for JC241 bathysnap and mooring recoveries (JC257_009, JC257_010, JC257_079, JC257_084, JC257_120) are based on deployment positions of gear from JC241 logs.

Station	Gear	Site	Date (UTC)	Deployment Start (UTC)	Latitude (N)		Longitude (W)		Depth (m)
					Deg	Min	Deg	Min	
JC257_001	Underway ADCP	Transit	08/02/2024	12:35:00	9	35.6300	-89	12.2400	3500
JC257_002	Multibeam	Transit	08/02/2024	13:30:00	9	40.4290	-89	23.5740	3472
JC257_003	Autosub AUV MBES	Transit	10/02/2024	12:40:00	11	3.2050	-98	14.7690	4005
JC257_004	Sound Velocity Profile	Transit	10/02/2024	14:08:00	11	3.2530	-98	14.6870	3980
JC257_005	Glider	Transit	13/02/2024	20:54:00	13	23.9900	-110	0.0000	4475
JC257_006	CTD	Transit	13/02/2024	21:27:00	13	24.0900	-110	0.0900	4471
JC257_007	CTD	Transit	14/02/2024	00:20:00	13	23.2000	-110	0.0000	4355
JC257_008	CTD	Transit	14/02/2024	14:56:00	12	40.1800	-112	20.1500	3962
JC257_009	Bathysnap	1km	18/03/2023	22:33:00	13	53.2500	-116	30.6100	4147
JC257_010	Bathysnap	1km	18/03/2023	21:50:00	13	54.8600	-116	31.2600	4103
JC257_011	Megacore	1km	16/02/2024	01:14:00	13	55.6500	-116	31.6700	4097
JC257_012	Box core	1km	16/02/2024	05:34:00	13	55.6500	-116	31.6700	4098
JC257_013	Autosub AUV MBES	1km	16/02/2024	12:34:00	13	54.9440	-116	31.4770	4102
JC257_014	CTD	1km	16/02/2024	13:24:00	13	55.1071	-116	31.4774	4102
JC257_015	ISIS ROV	1km	16/02/2024	16:39:00	13	55.5048	-116	31.7322	4100
JC257_016	Fish / Amphipod trap lander	1km	17/02/2024	06:40:00	13	56.4480	-116	33.3072	4056
JC257_017	Megacore	1km	17/02/2024	07:26:00	13	55.5950	-116	31.6840	4101
JC257_018	CTD	South of UK1	17/02/2024	12:08:00	13	54.6200	-116	29.3410	4228
JC257_019	ISIS ROV	1km	17/02/2024	18:33:00	13	56.8656	-116	31.0698	4153
JC257_020	Autosub AUV MBES	1km	18/02/2024	18:03:00	13	55.7170	-116	31.6210	4091
JC257_021	Box core	1km	18/02/2024	18:58:00	13	55.6680	-116	31.6170	4080
JC257_022	Bathysnap	1km	19/02/2024	03:11:00	13	54.8730	-116	31.2240	4106
JC257_023	Bathysnap	1km	19/02/2024	03:51:00	13	53.2500	-116	30.6200	4178
JC257_024	Box core	1km	19/02/2024	04:48:00	13	55.7140	-116	31.6920	4096
JC257_025	Megacore	1km	19/02/2024	08:47:00	13	55.6260	-116	31.7310	4101
JC257_026	CTD	1km	19/02/2024	13:13:00	13	54.6200	-116	29.3400	4220
JC257_027	Box core	1km	19/02/2024	14:26:00	13	55.6210	-116	31.6980	4097
JC257_028	Box core	1km	19/02/2024	19:42:00	13	55.6200	-116	31.6980	4098

JC257_029	Megacore	1km	20/02/2024	00:12:00	13	55.6343	-116	31.6163	4097
JC257_030	Box core	1km	20/02/2024	04:31:00	13	55.6086	-116	31.5828	4091
JC257_031	Megacore	1km	20/02/2024	08:40:00	13	56.0520	-116	31.8040	4097
JC257_032	Box core	1km	20/02/2024	13:05:00	13	55.6880	-116	31.6421	4097
JC257_033	ISIS ROV	1km	20/02/2024	17:13:00	13	55.5024	-116	31.7310	
JC257_034	Megacore	0km	21/02/2024	02:53:00	13	56.1100	-116	31.8600	4092
JC257_035	Box core	1km	21/02/2024	07:19:00	13	55.5900	-116	31.6470	4097
JC257_036	CTD	South East in AOI2	21/02/2024	11:16:00	13	54.6212	-116	29.3411	4239
JC257_037	CTD	South East in AOI3	21/02/2024	12:32:00	13	54.6193	-116	29.3416	4239
JC257_038	Box core	1km	21/02/2024	16:16:00	13	55.5930	-116	31.6490	4100
JC257_039	Fish / Amphipod trap lander	West outside of AOI2	21/02/2024	20:44:00	13	55.9700	-116	33.0910	4080
JC257_040	Box core	1km	21/02/2024	22:28:00	13	55.5860	-116	31.6210	4098
JC257_041	Autosub AUV camera	1km	22/02/2024	02:20:00	13	55.6157	-116	31.7305	4105
JC257_042	ISIS ROV	0km	22/02/2024	04:48:00	13	55.6788	-116	33.3090	4051
JC257_043	Box core	1km	22/02/2024	19:40:00	13	55.6500	-116	31.6120	4093
JC257_044	CTD	1km south of long mooring	23/02/2024	00:16:00	13	52.8010	-116	28.8710	4252
JC257_045	Box core	0km	23/02/2024	04:18:00	13	56.1130	-116	31.8640	4099
JC257_046	Megacore	0km	23/02/2024	08:15:00	13	56.0770	-116	31.9700	4090
JC257_047	Autosub AUV camera	AOI2	23/02/2024	12:06:00	13	56.1400	-116	31.8100	4105
JC257_048	Box core	0km	23/02/2024	12:47:00	13	56.1340	-116	31.8100	4091
JC257_049	ISIS ROV	0km	23/02/2024	17:28:00	13	55.6752	-116	33.3120	
JC257_050	Megacore	1km	24/02/2024	05:42:00	13	56.1090	-116	31.7870	4101
JC257_051	Box core	1km	24/02/2024	09:40:00	13	55.6760	-116	31.6360	4092
JC257_052	ISIS ROV	0km	24/02/2024	16:50:00	13	56.2170	-116	32.3052	4092
JC257_053	Box core	0km	25/02/2024	01:02:00	13	56.0120	-116	31.9250	4099
JC257_054	CTD	1km	25/02/2024	05:07:00	13	56.0760	-116	31.9700	4101
JC257_055	Box core	0km	25/02/2024	07:47:00	13	56.1700	-116	31.8300	4105
JC257_056	Autosub AUV MBES	16km	25/02/2024	14:51:00	13	48.8140	-116	29.1160	4110
JC257_057	ISIS ROV	OMS - colonisation experiment	25/02/2024	11:11:00	14	2.1048	-116	32.7468	4083
JC257_058	Box core	0km	26/02/2024	06:55:00	13	56.1090	-116	31.7880	4093
JC257_059	Box core	0km	26/02/2024	11:22:00	13	56.1870	-116	31.8750	4090
JC257_060	Box core	0km	26/02/2024	15:50:00	13	56.0130	-116	31.9250	4102
JC257_061	Box core	0km	26/02/2024	19:47:00	13	56.1700	-116	31.8300	4104

JC257_062	CTD	1km	27/02/2024	00:36:00	13	56.0763	-116	31.9730	4090
JC257_063	ISIS ROV	West 0km - colonisation experiment	27/02/2024	04:34:00	13	55.9440	-116	30.6900	4149
JC257_064	Autosub AUV MBES	16km	27/02/2024	18:43:00	13	48.0542	-116	27.9939	4112
JC257_065	CTD	16km	27/02/2024	19:53:00	13	48.0211	-116	27.9390	4113
JC257_066	Fish / Amphipod trap lander	16km	27/02/2024	23:46:00	13	49.3900	-116	29.6000	4105
JC257_067	Box core	16km	28/02/2024	00:30:00	13	48.0300	-116	27.8600	4114
JC257_068	Megacore	16km	28/02/2024	04:18:00	13	47.9960	-116	27.8760	4117
JC257_069	Box core	16km	28/02/2024	08:21:00	13	48.0760	-116	27.9220	4115
JC257_070	Multibeam		28/02/2024	11:51:00	13	48.0820	-116	27.8820	4112
JC257_071	ISIS ROV	0km	28/02/2024	16:57:00	13	56.1696	-116	32.5608	4084
JC257_072	Box core	16km	29/02/2024	01:30:00	13	47.9590	-116	27.9659	4120
JC257_073	Megacore	16km	29/02/2024	05:25:00	13	48.0270	-116	27.9820	4120
JC257_074	Box core	16km	29/02/2024	09:25:00	13	48.0580	-116	27.9550	4118
JC257_075	Box core	16km	29/02/2024	14:10:00	13	47.9788	-116	27.9393	4117
JC257_076	Autosub AUV camera	16-30km	29/02/2024	23:54:00	13	48.0430	-116	28.0100	4112
JC257_077	CTD	16km	01/03/2024	01:32:00	13	47.9770	-116	27.9370	4113
JC257_078	ISIS ROV	16km	01/03/2024	04:17:00	13	49.5870	-116	29.4912	4110
JC257_079	Mooring	0km, short mooring 3	15/03/2023	21:39:00	13	52.4530	-116	32.9520	4051
JC257_080	Box core	0km	02/03/2024	01:13:00	13	56.1000	-116	31.8200	4107
JC257_081	Autosub AUV camera	0km	02/03/2024	05:24:00	13	56.1400	-116	31.8600	4103
JC257_082	Box core	0km	02/03/2024	07:25:00	13	56.0410	-116	31.9600	4104
JC257_083	Box core	0km	02/03/2024	11:40:00	13	56.1770	-116	31.9510	4099
JC257_084	Mooring	0km, short mooring 2	15/03/2023	15:51:00	13	56.5190	-116	30.3390	4187
JC257_085	Box core	0km	02/03/2024	18:37:00	13	56.0860	-116	32.0010	4180
JC257_086	Box core	16km	03/03/2024	01:14:00	13	47.9770	-116	27.8970	4114
JC257_087	Box core	16km	03/03/2024	04:46:00	13	47.8970	-116	27.9700	4122
JC257_088	Megacore	16km	03/03/2024	08:21:00	13	47.9280	-116	27.9420	4118
JC257_089	Box core	16km	03/03/2024	12:10:00	13	48.0450	-116	27.8890	4049
JC257_090	Autosub AUV MBES	100km	03/03/2024	22:43:00	13	4.8810	-116	5.7380	4101
JC257_091	ISIS ROV	16km	04/03/2024	06:40:00	13	49.5918	-116	29.4864	4108
JC257_092	Megacore	16km	04/03/2024	17:41:00	13	47.9680	-116	27.8590	4120
JC257_093	NO DEPLOYMENT								
JC257_094	ISIS ROV	100km	05/03/2024	04:26:00	13	6.3846	-116	5.1402	4125

JC257_095	Fish / Amphipod trap lander	100km	05/03/2024	17:50:00	13	6.6980	-116	5.0000	4114
JC257_096	Autosub AUV camera	100km	05/03/2024	19:42:00	13	5.0100	-116	5.6750	4111
JC257_097	Box core	100km	05/03/2024	20:26:00	13	4.9630	-116	5.6480	4099
JC257_098	Box core	100km	06/03/2024	00:39:00	13	4.9420	-116	5.7090	4103
JC257_099	Megacore	100km	06/03/2024	04:37:00	13	4.9140	-116	5.7290	4106
JC257_100	Box core	100km	06/03/2024	10:18:00	13	4.8580	-116	5.7200	4103
JC257_101	Megacore	100km	06/03/2024	12:12:00	13	4.7830	-116	5.6310	4102
JC257_102	CTD	100km	06/03/2024	17:20:00	13	4.2270	-116	7.7460	4343
JC257_103	Autosub AUV camera	100km	06/03/2024	22:30:00	13	4.9200	-116	5.6600	4009
JC257_104	Megacore	100km	06/03/2024	23:12:00	13	4.9170	-116	5.6510	4101
JC257_105	ISIS ROV	100km	07/03/2024	03:57:00	13	4.2000	-116	7.4496	4044
JC257_106	Autosub AUV camera	100km	08/03/2024	03:45:00	13	41.5000	-116	24.4000	4112
JC257_107	ISIS ROV	100km	08/03/2024	11:59:00	13	6.3768	-116	5.1522	4127
JC257_108	Autosub AUV MBES	100km	09/03/2024	08:25:00	13	4.9480	-116	5.7000	4098
JC257_109	Box core	100km	09/03/2024	11:02:00	13	4.8900	-116	5.6900	4110
JC257_110	Megacore	100km	09/03/2024	14:39:00	13	4.7980	-116	5.7170	4111
JC257_111	Box core	100km	09/03/2024	18:53:00	13	4.9380	-116	5.6110	4101
JC257_112	Box core	100km	10/03/2024	00:53:00	13	4.8480	-116	5.7870	4112
JC257_113	Autosub AUV camera	30km	10/03/2024	09:18:00	13	41.5150	-116	24.5280	4090
JC257_114	Box core	1km	10/03/2024	13:34:00	13	55.6870	-116	31.6927	4106
JC257_115	ISIS ROV	Whalebone experiment	10/03/2024	19:30:00	13	43.5684	-116	39.9156	4127
JC257_116	CTD	South of 0 km	11/03/2024	07:01:00	13	52.8000	-116	28.8720	4296
JC257_117	ISIS ROV	OMS - sponge garden	11/03/2024	16:46:00	14	1.9848	-116	32.8260	4080
JC257_118	Autosub AUV camera	AOI2	12/03/2024	06:53:00	13	55.6600	-116	31.5600	4093
JC257_119	Gravity Corer	1km	12/03/2024	07:35:00	13	55.6500	-116	31.5600	4092
JC257_120	Mooring	0km	15/03/2023	19:30:00	13	53.4050	-116	29.3840	4222
JC257_121	CTD	Eddy west	13/03/2024	17:31:00	13	17.2929	-115	45.4999	4076
JC257_122	CTD	Eddy 2	14/03/2024	02:31:00	13	15.5700	-114	45.0000	4012