

Figure 3-8. Proposed AUV MBES pre- and post-collector survey plan (A); pre- and post-collector photogrammetry surveys (NB: current line plans are for illustrative purposes only)



3.2.13 Seafloor Geological Studies (resource box core)

Lead Contractors: AMC consultants

Sample Collection: Contractors will conduct box core sampling on pre- and post-collector test campaigns using an USNEL BX-750 box core as follows:

- Minimum 20 box cores within the planned HTR/STR and PR's track lines pre-collector
- Minimum 20 box cores within the planned HTR/STR and PR's track lines post-collector

An overview of the box core sampling sites if provided in Figure 3-9.

Sample Analysis: The abundance of nodules (and mass) along the collector path after nodule collection will be measured using AUV photogrammetry and automated long axis estimation (LAE) – section 3.2.12. The LAE estimates will be verified by sampling the collected nodule paths with box cores. The difference between the pre- and post-collection mass estimates will be the basis of the estimate of efficiency of recovery of nodules by the collector.

3.2.14 Seafloor Geophysical Studies (cone penetration testing)

Lead Contractors: Bluefield Geoservices

The proposed equipment is the Bluefield ROV cone CPT system, a lightweight CPT system for pushes up to about 4 m from a work-class ROV. It can also conduct push sampling and other in situ testing such



as T-Bar and ball cone. The cone size is 10 cm², which is the industry reference size, and the T-bar and ball are 100 cm² in line with industry norm.

Data Collection: The contractor will conduct cone penetration testing on pre- and post-collector test campaigns using the ROV cone penetration test (CPT) as follows:

- Maximum 20 in-situ ROV CPTs within proposed PR tracks pre-collector test
- Maximum 20 in-situ ROV CPTs taken in the same location post-collector test

An overview of the CPT sampling sites if provided in Figure 3-9.

Data Analysis: The in-situ ROV cone penetration test (CPT) will collect a continuous profile through the seafloor to allow interpretation of the sediment type and shear strength. These operations will be conducted both pre – and post-collector test and will allow NORI to evaluate seafloor compression as a result of the collector operations. The contractor will also repeat the CPT on recovered resource box cores (see section 3.2.13) to allow a comparison between methodologies.



Figure 3-9. Potential locations of resource box core and cone penetration testing sites in the Test Field

3.2.15 Sediment geochemistry

Lead Contractors: University of Leeds

Sample Collection: Multicoring campaigns will be conducted at strategic sites in and around the TF preand post-Collector Test. Sediment core samples will be collected using the OKTOPUS MC20 multicore as follows:

• Maximum 6 randomized stations localized to the TF and wider CTA Pre-Test Campaign

• Minimum 12 randomized stations taken in the same area, targeting different levels of impact taken on a Post-Test Campaign

Once on board, cores will be assessed for integrity using a core quality rubric developed from previous baseline campaigns and distributed amongst the different work scopes (Meiofauna/Foraminifera/eDNA/ Geochemistry).

It is proposed that sampling for sediment biogeochemistry and physical properties occurs at a sub-set of the multicore stations that have been proposed for macrofauna, meiofauna, forams and eDNA. It is important to ensure there is equivalence between the biogeochemical and biological data sets.

Sample Analysis: At sea analysis will include the collection of oxygen microprofiles, and porewater pH and alkalinity, using protocols from Campaign 5D. Porewater and solid phase samples will be preserved and retained by the contractor for onshore analysis. Porewater analytes will include nutrients and dissolved and soluble metals. Solid-phase analytes will be metals, total organic and inorganic C and total N concentrations and isotopic compositions, gamma counting for 210Pb, lipids, amino acids, water content and grain size. In most cases 22 depth intervals per core will be analysed, which takes the depth of data collection at least to the 20 cm, as required by the ISA.

In the case of 210Pb fewer depth intervals will be analysed, again in line with protocols from baseline campaigns and following ISA recommendations. Organic geochemical analyses (lipids and amino acids) will be restricted to fewer sites and the minimum depth intervals necessary to evaluate collector test impacts. Analytical protocols will be the same as those used for samples from cruises 5A and 5D and have been provided in our first annual report.

In addition, and in line with updated ISA recommendations, analysis of porewaters for dissolved organic carbon (DOC) will be conducted. Porewater samples will be collected from a dedicated sediment core at each site (11 on each of two cruises) and preserved acidified in ultra-clean evacuated glass containers. Samples will be analysed using a Simadzu TOC V. In addition, a subset of DOC porewater samples will be analysed by synchrotron to reveal unprecedented detail of composition and structure. This will facilitate fundamental understanding of the processes involved in preservation and burial of organic carbon in marine sediment.

3.2.16 Bioturbation and mapping of sedimentary radioisotopes using Thorium234

Lead Contractor: Eckerd College

The ISA recommendations state "collection of data on bioturbation is targeted at collecting the background "natural" rates of sedimentary processes, including "natural spatial and temporal variability", to model and evaluate the effects of mining activities on such processes. Rates and depths of bioturbation (i.e., the mixing of sediments by organisms) must be measured to analyse the importance of biological activity prior to a mining disturbance and can be evaluated from profiles of excess Pb-210 activity from cores, taking into account the variability in the sediment. Excess Pb-210 activity should be evaluated on at least six levels per core (suggested depths are 0–0.5 and 0.5–1.0 cm; 1–2 and 2–3 cm; and 3–5, 5–7 and 7–9 cm), and for at least four replicate cores (e.g., tubes from separate multiple corer drops) per site. Rates and depths of bioturbation (mixed layer) are to be evaluated by standard advection or direct diffusion-reaction models but may need to include non-local exchange terms. Additional methodologies include analysis of excess Th-234 and sediment profiling imagery. [Recommendation III.B.15.(e)]" (ISA, 2020).

Sample Collection: To address the recommendations of the ISA this study will: i) establish short-lived radioisotope baselines as sediment tracers, indicators of bioturbation, chronometers, and indicators of planktic fluxes, and ii) compare post-test measurements to the established baselines to determine the spatial extent and thickness of the sediment plume deposit and physical mixing after the polymetallic collector system test.



Multi-core samples will be collected for short-lived radioisotope measurements. Cores will be sectioned to the following increments: 0-0.5 cm, 0.5-1 cm, 1-2 cm, 2-3 cm, 3-4 cm, 4-5 cm, 5-10 cm. Radioisotope subsamples will be stored at ambient temperature.

Samples will be stabilized and transported to Eckerd College for analysis.

Sample Analysis: Sedimentary short-lived radioisotope activities will be measured to provide chronological context (age models for the last ~100 years), mass accumulation rates and input fluxes for bulk sediment and constituents, bioturbation and identification of surface sediment dynamics (passive transport, inventories). The most used chronometers for recent sedimentation are short-lived radioisotopes including excess 234Th and 210Pb, and 226Ra (Swarzenski, 2014; Holmes, 1998; Appleby, 2001). Sediment core samples will be analyzed for short-lived radioisotopes by gamma spectrometry on Series HPGe (high-purity Germanium) Coaxial Planar Photon Detectors for total 210Pb (46.5 keV), 214Pb (295 keV and 351 keV), 214Bi (609 keV), 234Th (63 keV). Activities will be expressed as disintegrations per minute per gram of sediment (dpm/g) using methodology described by Brooks *et al.* (2015). The primary sediment age-models will be based on 210Pbxs and will be comprised of the constant flux, constant sedimentation (CF:CS) and/or the constant rate of supply (CRS) model depending on sedimentary setting (Appleby and Oldfield, 1983; Holmes *et al.*, 1998; Appleby, 2001). An inventory approach will also be utilized to assess sediment focusing (Brooks *et al.*, 2015; Schwing *et al.*, 2017).

Considering the half-life of 234Th (24 days), it is imperative that the samples are received as soon as possible after each cruise so that they can be analyzed before 5 half-lives occur (120 days) rendering their activity undetectable.

3.2.17 Seafloor Studies – biological

Overall, the philosophy for the seafloor sampling in impact zone 3 will directly address parts of the following regulatory recommendations detailed in ISBA/25/LTC/6/Rev1 Paragraph 38 (o) and (q) and 40 (b), (c) and (g) reproduced below. Note that the parts in bold are particular focus areas. Square brackets are our additions to clarify the scope related to this section of the EMMP.

<u>C.</u> Information and measurements to be provided by a contractor performing an activity requiring an environmental impact assessment during exploration

38 (o) Delineation of the **impact reference zone** and the preservation reference zone for the impact assessment of test-mining. The impact reference zone should be the site where the test-mining and related direct impacts are to occur. The **preservation reference zone should be carefully located and far enough away not to be affected by testing activities, including effects from seabed-disturbance and discharge plumes.** The implementation of a good monitoring programme to detect any disturbance that may occur beyond the impact reference zone as a result of testing is crucial to rank the preservation reference zone location. Detection of physico-chemical and biological disturbances [to **mega/macro/meiofauna and foraminifera] in the far field from the test-mining site (>10 km) shall be conducted.** Preservation reference zones will be important in identifying natural variations in environmental conditions against which impacts of the mining tests will be assessed. Their species composition should be comparable to that of the impacted areas. Preservation reference zones established during an exploration test-mining should be within the contractor's area if possible;

38 (q) Status of regional and local environmental baseline data [for mega/macro/meiofauna and foraminifera]

<u>D.</u> Observations and measurements to be made after undertaking an activity that requires an environmental impact assessment during exploration



40 (b) Changes in species composition, diversity and abundance of pelagic (where applicable) and benthic [mega/macro/meiofauna and foraminifera] communities, including microbes and protozoa, including recolonization, changes in foundation species, three-dimensional-habitat-forming species, ecosystem engineers, bioturbation rates, chemical effects and changes in behavior of key species (subjected to impacts such as smothering by sedimentation);

40 (c) Possible changes in [benthic mega/macro/meiofauna and foraminifera] communities, including microbes and protozoa, in adjacent areas not expected to be perturbed by the activity, including discharge and seabed-disturbance plumes and food web structure

40 (f) Levels of metals found in key and representative benthic biota subjected to sediment from the operational and discharge plumes;

40 (g) Resampling of local environmental baseline data and evaluation of environmental impacts [to mega/macro/meiofauna and foraminifera];

3.2.17.1 Survey design and sampling philosophy

Analytical pre- and post-collector monitoring methods will provide data to help answer the following questions:

- i) How does the megafauna, macrofauna, meiofauna and foraminifera abundance, diversity and community structure change in the area disturbed by nodule collection activities? This may include areas directly disturbed by mining (removal of nodules, sediment compression, mechanical disturbance) and areas disturbed by re-sedimentation of material from plumes (to be quantified)?
- ii) Are there changes in megafauna, macrofauna, meiofauna and foraminifera abundance, diversity and community structure in areas that are not expected to be impacted by nodule collection?
- iii) Depending on the length of time in between the collector test and post-test campaign, will small opportunistic species have recolonized the collection tracks?
- iv) What is the status of regional and local megafauna, macrofauna, meiofauna and foraminifera baseline data?

Cumulative sedimentation modeling results are presented to a threshold of 0.01mm (DHI, 2022); this results in the capture of 97% of the deposited material (i.e., only 3% of deposited material is found in areas with sedimentation thickness <0.01mm; Figure 3-10). Based on the NORI-D sediment trap measurements (CSA, 2022), a plotting limit of 0.01mm has been adopted (i.e., 10% of the sediment trap short-term sedimentation background or equivalent order to the longer-term consolidated sedimentation rate based upon radioisotope analysis). However, given the level of variability in the sedimentation rate above the mean a potentially biologically relevant threshold of >0.1mm (i.e., 1 standard deviation rounded up) has been adopted as the limit above which a habitat is likely to experience sedimentation rates outside the normal levels of background variation. This assumption is valid as it has been demonstrated that the benthic habitat in NORI-D is subjected to sedimentation rates several times background on an intermittent basis (see NORI Collector Test EIS, 2022, Section 5.11.1.6).





Figure 3-10. Modelled benthic sedimentation footprints at various deposition depths (DHI, 2022)

The modelled footprint shown in Figure 3-10 represents results assuming a NW benthic current as it reflects expected conditions in the month of January, for which the sedimentation model was developed. However, it is anticipated that although the velocity of benthic currents will remain temporally constant, direction may vary on short (i.e., daily) and long (i.e., seasonally) timescales. Therefore, although the area of the footprint is expected to be consistent with the model regardless of the month in which the Collector Test is conducted, the direction of plume drift and sedimentation may vary from the model dependent on the prevailing benthic current at the time of the test. Hence, the location of benthic sampling sites will be finalized at sea, using real time information on current direction, benthic plume dispersal and sedimentation.

Pre-collector test: samples will be collected from replicate sites within (1) the proposed primary impact zone; (2) from the most likely area for benthic plume sedimentation (determined by the Decision System detailed in section 3.2.1.4) and; (3) control sites (Figure 3-11A)

Post-collector test: samples will be collected from replicated sites from (1) within collector tracks, (2) between collector tracks, (3) two distances downstream representing different amounts of benthic plume sedimentation (determined by section 3.2.1.1 and section 3.2.12) finally (4) repeat samples from the control areas. To ensure and verify the impact levels, ROV observed sampling will occur. (Figure 3-11B and C)





Figure 3-11. Example sample survey design for benthic operations pre- (A) and post-collector (B) and (C)



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3.2.18 Megafauna

Lead Contractors: National Oceanography Centre

The contractor will carry out data acquisition and analysis to characterize the abundance, biomass, morphotype structure and diversity of megafauna from scaled photographic transects. This will address question (i) and (ii). Methods will align with those already published in the peer reviewed literature (Simon-Lledó *et al.*, 2019) and used during previous NORI campaigns in May 2020 and November 2021.

Data Collection: To obtain high-frequency, vertically orientated, well-lit still photographs and associated dive metadata (e.g., ±1 cm precise vehicle above-seabed altitude), NORI plan to use an autonomous underwater vehicle (AUV) to obtain high-frequency good-quality (collected at <3m above seabed) digital photographs of a known area of seafloor, following a randomized and replicated sampling design

The pattern of the photographic transects will be defined by the scientific contractor with input from NORI with regards to operational constraints. The survey array will be based on existing baseline data for the TF and wider CTA (Figure 3-12A and Figure 3-12B). Transect length will be determined based on statistical sample-size analysis based on existing work at NORI-D.

In addition, a time-lapse camera will be used to examine the physical dynamics of surface sediment and document the activity level of surface megafauna and the frequency of resuspension events. Time-lapse images will be obtained in the study area for at least one year using an automated camera lander. Time-lapse images should be scalable (e.g., using photogrammetry techniques) and sufficient resolution to identify organisms greater than 1 cm in their smallest dimension.

Data Analysis: Photographs will be analysed manually with reference to a standardised image catalogue to identify (as morphotypes) and count megafaunal organisms, including protists (xenophyophores). Automated tools (e.g., BIIGLE) will be used to facilitate image annotation. Sequence bias (Durden *et al.*,



2016b) will be minimised by randomising image order. Using platform altimeter and motion sensor (e.g., pitch, roll, heading) information, the images will be scaled and the faunal counts standardised by area. This will allow calculation of a wide range of community metrics, including density, diversity, and community structure. Biomass calculations will be made using information on length-weight relationships (Durden *et al.*, 2016), ideally reinforced by measurements of collected CCZ megafauna. Temporal analysis is possible by revisiting stations assessed during the NORI-D baseline campaign (MIN). To address question (iv), the scientific contractor will compare the data collected during this programme with existing datasets held by the contractor and others academic institutes, covering multiple contract and APEI areas in the Clarion Clipperton Zone.

The changes observed at the collector test area will be compared to pre-disturbance conditions and natural variability at that site (assessed during existing contract for baseline assessment). The collector test areas will also be compared to the sites assessed in all campaigns to NORI-D with annotated image data. Replicated sampling in each area will provide some indication of spatial variability, providing the basis for statistical testing.

The approaches for analysis will depend on the data collected, but indicative approaches are provided in previous publications by the proposed research team (e.g., Simon-Lledó *et al.*, 2019)

Species identification for both techniques rely on comparison of morphology with an image catalogue. The scientific contractor has developed such a catalogue for the Clarion-Clipperton Zone, which has been standardised between many institutes and incorporates information from many taxonomic experts.

Time-lapse images will be analysed in a comparable way to transect photographs, although analysis would focus on temporal patterns in abundance and species presence. In addition, any visible changes in the physical environment (e.g., turbidity, nodule coverage, sedimentation) will be documented. The time lapse camera will be deployed at the edge of the Collector Test Area (CTA) to quantify natural variability and document the arrival and impacts of any sediment plume events. The changes observed at the CTA will be compared to pre-disturbance conditions and natural variability at that site (assessed during existing contract for baseline assessment). The disturbed area will also be compared to the sites assessed in all cruises to NORI-D with annotated image data.