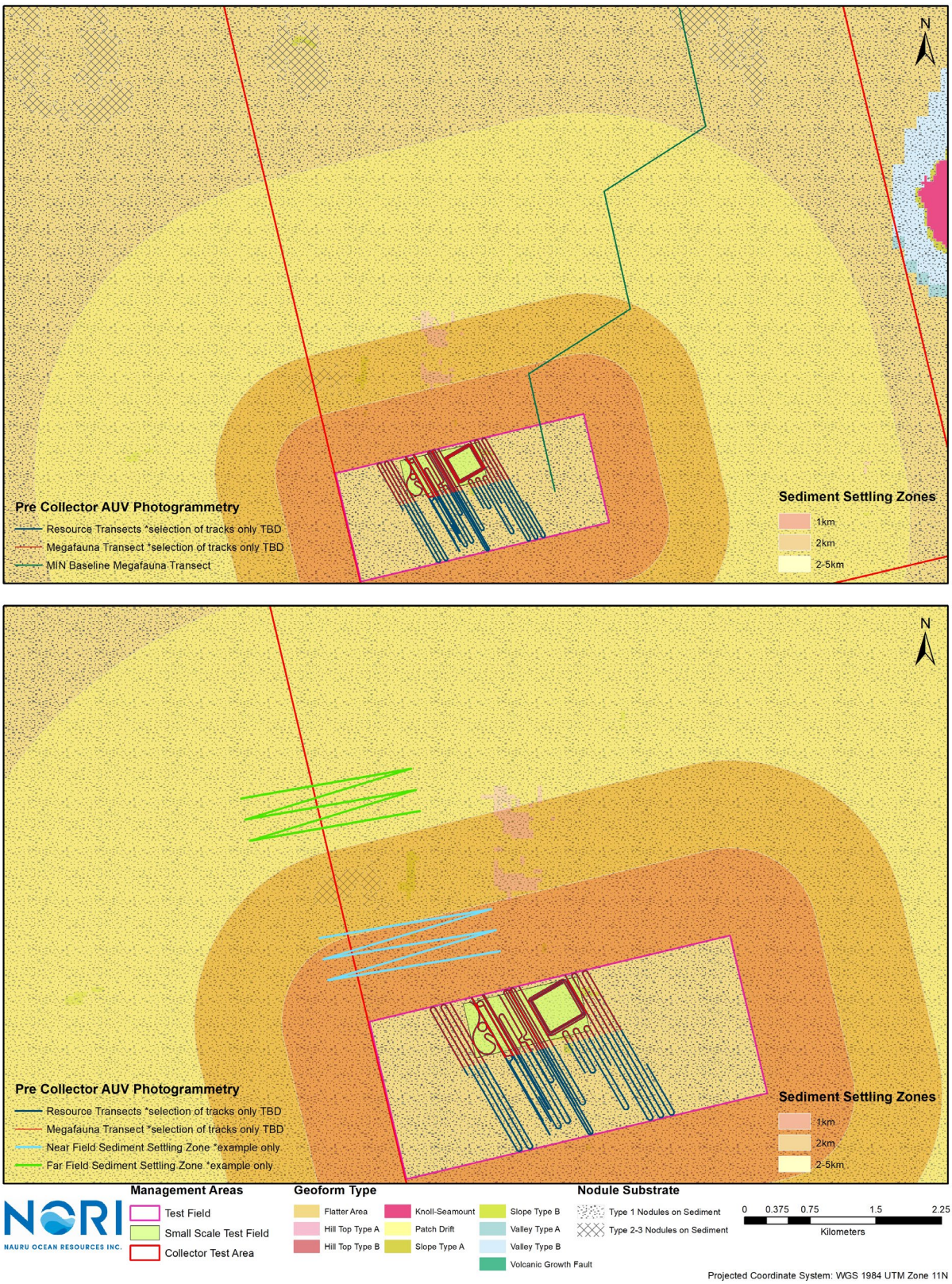
Figure 3-12. Proposed megafauna transects in CTA and TF conducted pre-collector test (A) and post-collector test (B)



3.2.19 Macrofauna

Lead: Natural History Museum and University of Gothenburg

This study will characterize changes in macrofaunal abundance, community structure and diversity as a result of disturbance by the PCV and coverage by sedimentation from the benthic plume.

Sample Collection: The contractor will conduct box core sampling on 2 campaigns using the USNEL BX-750* box core as follows:

- Maximum 15 randomized stations localized to the TF and wider CTA pre-collector test
- Minimum 23 randomized stations taken in the same area, targeting different levels of impact (including control sites) taken on a post-collector test

The contractor will collect and analyse of a total max. 40 USNEL BX-750 box core samples which will be fully processed and analysed using standardized cold-chain methods for DNA-taxonomy protocols (Glover *et al*. 2016) for the collection of box core samples and data at-sea. For the NORI-D baseline studies, a BX-650 boxcore was utilized. To allow temporal comparability, a 50 x 50 cm frame will be manufactured and used to sample the same footprint within the BX-750.

Once the box core is secured on deck, the temperature of top water will be measured, a downward-facing image of the box core will be taken with top water intact, the top water will be siphoned off, and a further image will be taken with top water removed. The box core sample quality will be assessed based on whether the top water was retained and the sediment surface undisturbed, both criteria will have to be met in order for the sample to be accepted for quantitative work.

Following recovery and quality assessment, box cores will be sampled quantitatively for megafauna, nodule fauna, and macrofauna, with a live-sort of a 15x15cm subsample (sampled at 0-2cm and 2-5cm depth layers).

All remaining sediment will be sieved on 300micron sieves sliced in 0-2cm, 2-5cm and 5-10cm layers and the residue retained on 300-micron sieves bulk fixed in non-denatured ethanol.

In the live-sorting process, most specimens will be individually preserved in 80% ethanol in barcoded sample jars or tubes linked to a database. Sediment residues from the 15x15cm live-sort will be returned to respective bulk-fixed depth layers following the removal and processing of animals.

Sample Analysis: The workflow will be divided up amongst both taxonomic groups and sediment/nodule fauna amongst the scientific contractors and different phases of work. For the nodule fauna (40 box cores), the contractors will use highly tested methods to pick, image, sort and provide preliminary identification at-sea of all macrofaunal and megafaunal sessile nodule metazoan fauna (this excludes protists such as xenophyophores, foraminifera) within 50x50cm quantitative box core sections. These data will be provided in the form of preliminary abundance/community composition data at the end of the cruise, with species-level refinements provided later during the analysis phase.

For the sediment fauna (40 box cores), samples will be preserved at sea in 80% ethanol, before being maintained in a chilled <10C environment until shipment. On receipt contractors will undertake initial sorting of the samples initially to class/phylum, with annelid polychaetas sorted initially to family level and putative morphospecies, with only intact individuals or heads counted.

The samples will then be split, with polychaetas, molluscs, crustacea being sent to respective taxonomic experts and the remainder of the groups (e.g., echinoderms, bryozoans, and misc. phyla, the remaining approx. 25%) being identified using DNA barcoding and database comparison. It will not be required to barcode every specimen as we will have a good working species voucher collection from the baseline phase.

Typical meiofaunal taxa such as ostracods, copepods and nematodes will be included in counts but not identified as is normal practice for macrofaunal studies. Samples will then be identified to putative species-level based on a combination of morphology with inferences from molecular phylogenetic analyses and barcoding. All data will be combined into a master database and ecological/disturbance analyses.

The data from the 40 samples will be combined with the data already collected (30 box cores) from the Baseline campaigns 5a, 5d, thus bringing the total sample number to min. 70 (more than any previous CCZ box core campaign in a single area).

Results will describe in detail the impact of the collector test on the macrofauna alongside the existing local control sites. The report will make hypotheses of the likely impact of a larger nodule collection operation, if parameters of such an operation are defined by NORI. The contractor will continue to observe and validate any potential indicator species and provide recommendations for longer-term monitoring of a larger collection operation. Methods throughout will follow tested protocols already implemented and published in the peer-reviewed literature (Glover *et al* 2016a; Glover *et al* 2016b; Glover *et al* 2016c; Dahlgren *et al* 2016; Wiklund *et al* 2017; Lim *et al* 2017; Taboada *et al* 2017; Taboada *et al* 2018).

3.2.20 Meiofauna

Lead Contractor: Florida State University

This study will characterize changes in meiofaunal abundance, community structure and diversity as a result of disturbance by the PCV and coverage by sedimentation from the benthic plume.

Sample Collection: The contractor will conduct box core sampling on 2 campaigns using the OKTOPUS MC20 multicore as follows:

- Maximum 15 randomized stations localized to the TF and wider CTA pre-collector test
- Minimum 26 randomized stations taken in the same area, targeting different levels of impact (including control sites) taken on a post-collector test

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).

The use of a multicorer will allow for retrieval of sediment cores with an undisturbed sediment-water interface. Each individual core will be extruded, and the 0-5 cm of sediment will be removed and preserved in 4% buffered formalin and stained with Rose Bengal (1%), the latter to facilitate identification in the laboratory. To avoid loss of material, supernatant water (10 cm overlying the sediment) within the core tubes will be collected on a 32 µm mesh sieve and added to the 0-5 cm sediment sample. Sediment slices will then be placed in an airtight wide-mouth sample bottle. Sediment will be stored in 4% buffered formaldehyde (Giere, 2009; Somerfield and Warwick, 1996; Somerfield and Warwick, 2013). At this stage we do not propose to preserve sediment in DESS (dimethyl sulphoxide, disodium ethylenediamine tetra-acetic acid and saturated NaCl (Abebe *et al*., 2011; Yoder *et al*., 2006) since dual use of samples for morphological and molecular purposes is unlikely given the limited size of the sediment cores and hence limited numbers of organisms available.

Sample Analysis: Sediment meiofauna samples will be carefully washed over 300 and 32µm sieves to retain the meiofauna-sized fraction as per ISA recommendations. The samples are then centrifuged via density separation using Ludox HS40 (spec. gravity 1.16 to 1.18, calibrated with hydrometer) to extract the organisms (spec. gravity 1.13) (Giere, 2009). Density separation is repeated three times so that all organisms are extracted (95 to 100%; Giere, 2009) and then stored in an alcohol solution (industrial methylated spirits [>70%]). Meiofauna individuals will be identified and counted at higher taxon level (Higgins and Thiel, 1988; Schmidt-Rhaesa, 2020) under stereomicroscope (250× magnification). From

each sample, 120 (or all if less individuals are present) nematodes will be picked out and placed in cavity blocks with a glycerol solution (5% glycerol, 45% ethanol, 50% purified water) and left semi-covered overnight in a 60°C oven to enable evaporation to pure glycerol. Nematode specimens are then mounted on glass slides for genus/species identification under a compound microscope (400 to 1,000× magnification) following latest taxonomic literature (Schmidt-Rhaesa *et al*., 2014) and Nemys, the online world database on nematode taxonomy linked to the World Register of Marine Species (Bezerra *et al*., 2019). Where specimens cannot be assigned to genus, family level information will be recorded, and putative genera will be established.

Further to taxonomic identification, biomass measurements will be made, and the feeding type of each nematode will be assigned based on their buccal cavity morphology (Wieser, 1953): selective deposit feeder (1A), non-selective deposit feeder (1B), epistratum feeder (2A), and predators/scavengers or omnivores (2B). Each nematode will also be assigned a life-history strategy based on c-p scores (following the K-r, opportunist vs. persister model) on a scale ranging from 1 (colonizer with short generation times and rapid reproductive rates) to 5 (persister with long generation times and slow reproductive rates) (Bongers, 1990; Bongers *et al*., 1991; Bongers *et al*., 1995).

Further to morphological identification, life stage (adult/juvenile) and adult sex (male/female) will be recorded. Morpho-taxonomic data will be cross-referenced with the World Register of Marine Species and APHIA IDs will be assigned. Data will be presented in list format (MS Access, to enable queries from the data) and standardized Excel matrices (ready for uploading in statistical software).

The statistical analysis procedures for meiofaunal and nematode morphological data will yield a comprehensive set of univariate and multivariate parameters and indices, including those routinely used in meiofauna baseline surveys and impact monitoring research programs. We will use alpha diversity methods (diversity, evenness, dominance), (Magurran and McGill, 2011; Rex and Etter, 2010; Warwick and Clarke, 1998). Traditional diversity measures such as taxon richness, Hill's diversity indices, Shannon Diversity, Simpson Diversity, Rarefaction, Taxonomic Diversity Index (empirically related to Shannon Diversity but with an added component of taxonomic separation), and Taxonomic Distinctness will be generated. Functional diversity metrics, as well an assessment of metrics that indicate ecological quality status will be provided. These have been reliable indicators of disturbance which as demonstrated in numerous publications and different types of habitats, as well as for different types of disturbances including physical disturbance and sedimentation. These metrics include trophic diversity index (Heip *et al*., 1998) (TDI), Maturity Index (MI)(Bongers, 1990; Bongers *et al*., 1991; Bongers *et al*., 1995), and Ecological Quality Status (EcoQS)(Moreno *et al*., 2011; Semprucci *et al*., 2013). Calculating these metrics will provide information on the level of impact of the collector test in the direct impact area, the plume settlement zone and the control or reference areas.

Patterns and differences will be investigated using univariate and multivariate methods to assess changes in taxa composition, diversity, and abundance of benthic communities, because of the collector test impacts.

Available environmental data will be statistically analyzed in conjunction with the meiofaunal and nematode data to discern the most important environmental drivers of these communities.

3.2.21 Foraminifera

Lead Contractor: Eckerd College

The ISA recommends that data on foraminiferal abundance, biomass, and species structure should be obtained through a quantitative analysis of samples from corers (Recommendations III.A.13; III.B.14; III.B.15.(d).(i)-(ii); IV.B.22). The ISA also recommends the documentation of "changes in species composition, diversity and abundance of benthic communities, including microbes and protozoa, including recolonization and changes in foundation species," (ISBA/25/LTC/6/Rev.1).

This will use the foraminiferal monitoring tool developed from baseline campaigns to determine pre-collector test baselines and post-collector test impact in a sampling array that captures impact from the mining track and accounts for plume generation and settling.

Sample Collection: The contractor will conduct multicore sampling on 2 campaigns using the OKTOPUS MC20 multicore as follows:

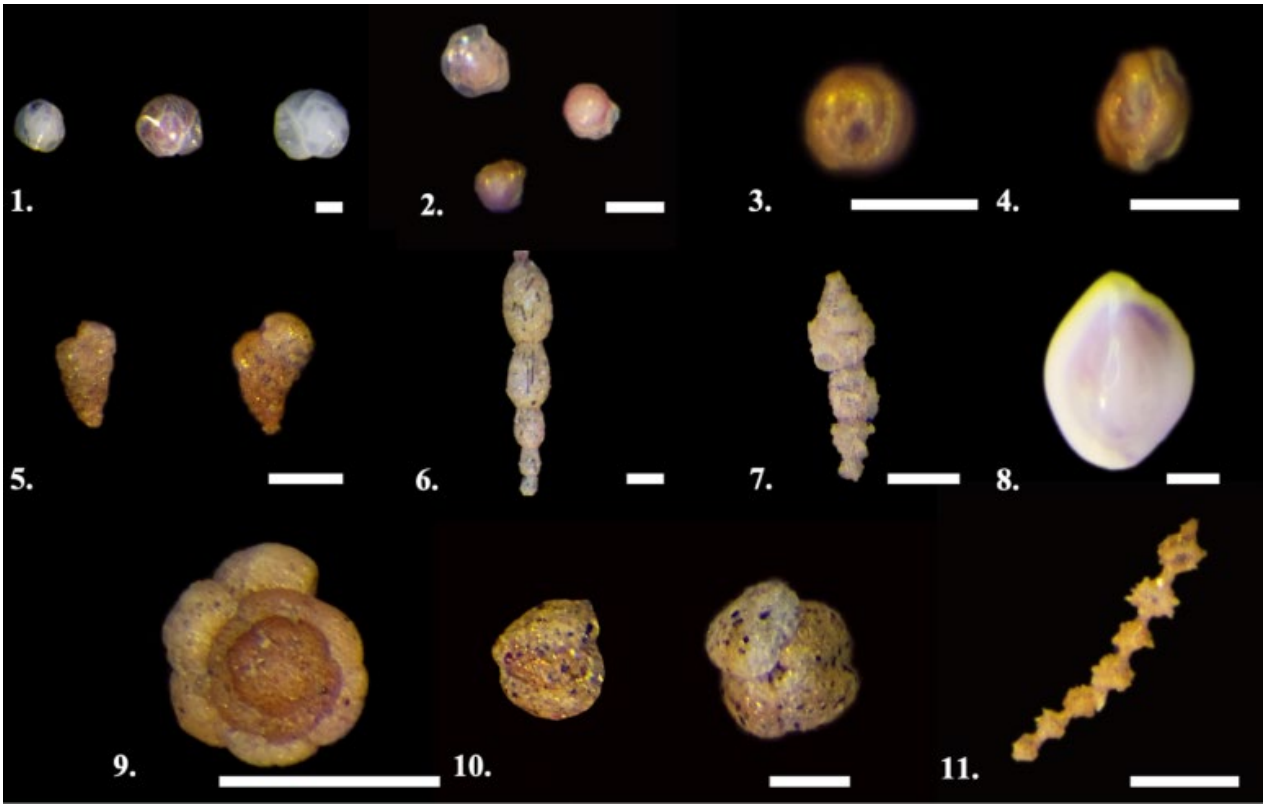
- Maximum 15 randomized stations localized to the TF and wider CTA pre-collector test
- Minimum 26 randomized stations taken in the same area, targeting different levels of impact (including control sites) taken on a post-collector test

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).
Foraminifera cores will be extruded and sampled in 1cm increments to 5cm in accordance with ISA recommendations. Supernatant water will be filtered through a 63-µm mesh sieve to avoid the loss of foraminifera living in the fluffy interstitial sediment layer. Each sediment layer will be placed in a 500 mL polypropylene sample bottle and preserved with 70% undenatured ethanol. Nodules will be picked from the surface and photographed at sea for later identification. At-sea photography is preferred because nodule dwelling forms are very fragile and are often heavily weathered in transport. Nodules will then be preserved in a polypropylene sample bottle with 70% ethanol, ready to be shipped for taxonomic analysis.

Sample Analysis: In the lab, samples will be stained with Rose Bengal solution (2g Rose Bengal: 1L DI water) for 24 hours and then washed over a 63-µm sieve. Due to the dominance of soft-bodied forms (monothalamids), sieving must be done with delicate care. Soft-bodied foraminifera and fragile monothalamids do not survive a traditional drying process in an oven. To preserve cellular integrity, a freeze-drying method was developed for baseline surveys 5a and 5d and tested to dessicate specimens without any structural distortion. After washing, Dimethyl Sulfoxide is then added to the washed sample to prevent ice crystallization. The samples are then frozen in an ultra-low freezer. Once frozen, the sample are lyophilized with a Thermo-Fisher Scientific freeze-dryer set to a gentle cycle. The processed samples are then dry-sieved to the >150-µm fraction and the <150-µm fraction and poured onto a gridded picking tray. All foraminifera are picked onto a separate storage slide and identified using the following taxonomic references: Brady (1884); Cushman (1922); Cushman (1927); Loeblich and Tappan (1964); Saidova (1975); Lukina (1980).

Tube species are counted as complete specimens when the proloculus is intact and visible, while fragmented tubes are recounted separately and not included in the diversity indices. Because of the high occurrence of undescribed species and the need for standardization, undescribed species are grouped into higher “morphological groupings “as described in Goineau and Gooday (2019). In order to maintain taxonomical consistency of undescribed species, a photograph is taken of every species and paired with its higher morphological grouping combined with a serialized number (e.g., Saccamminid sp. 1). Baseline abundance, density, diversity indices (Shannon, Fisher’s alpha, Evenness) will be calculated using statistical software. Reported data will include diversity, density and abundance, relative abundance of bioindicator species (Figure 3-13), the Epifaunal/Infaunal ratio and the Live/Dead ratio.

Figure 3-13. Noted bioindicator species identified from Campaign 5A in NORI-D



Note: These are species that have been documented as opportunistic (#1-4), as first-order recolonizers (#5-8), and as second-order recolonizers (#9-11) from sediment plume events such as the Mt. Pinatubo ashfall and the Deepwater Horizon Oil Spill (Kaminski *et al*., 1988; Hess *et al*., 2001; O'Malley *et al*., 2021).

3.2.22 Benthic ecotoxicology

Lead Contractor: Heriot-Watt University

This study will characterize changes in demersal predator and scavenger ecotoxicology as a result of exposure to the effects of disturbance by the PCV and coverage by sedimentation from the benthic plume.

Sample Collection: See section 3.2.1.1(g) for WET sample program. The contractors will conduct baited trap landers (TL) as follows:

- Minimum 5 TL randomized stations taken within the TF

Sample Analysis:

i) *Trace Element Analysis*: Baseline samples have been collected from the previous campaigns and sent for analysis to the ICP-MS Laboratory at the University of Edinburgh for quantitative trace elements analysis (including Fe, Mn, Ni, Zn, Cd, Pb, and Cu). This will establish baseline metal concentrations in organism tissues from NORI-D. Comparisons will be made with samples collected post-collector test to identify any changes in tissue concentrations. The accuracy of metal analysis will be confirmed using certified reference materials. Metal concentrations in individual tissues will eventually be expressed as micrograms per gram of dry tissue weight.

Analysis of variance (ANOVA) will be performed to test for statistical differences in tissue metal concentrations between species and sampling station, and comparisons will be made between CCZ data and historical data from different deep-sea regions. Principal components analysis (PCA) will be used to reveal the co-variance of variables (e.g., species, site, depth, metals

concentrations). Significant differences will be set at $p < 0.05$. All statistical analysis will be done using R software (version 4.1.1, R Core Team, 2021).

ii) *Total RNA extraction for gene-expression analysis:* Lander deployments with copper-spiked bait will be deployed, total RNA will be been isolated from pooled samples of an amphipod species identified to species (e.g., *Paralicella caperesca* and/or *Paralicella tenuipes*) and known to be commonly distributed in the region, as well as fish liver samples from identified species (e.g., *Coryphaenoides* spp.), with the RNeasy Mini Kit using previously described methods and DNase treated to eliminate any genomic DNA contamination, as well as the phenol/chloroform method of RNA extraction for samples thought to yield smaller concentrations of RNA. Total RNA will then be eluted into 30µL of sterile RNase/DNase free water, and confirmation of RNA quantity and the presence of impurities will be assessed using a NanoDrop spectrophotometer. Samples with 260/230 and 260/280 ratios between 1.8- 2.2 will be diluted to produce a final concentration of 100 ng/µL to be used for downstream analyses. RNA samples will be used for transcriptomic sequencing and cDNA will generated for qPCR analyses with reverse transcriptase from 2µg of total extracted RNA using the Precision nanoScript ™2 Reverse Transcription kit following the manufacturer's instructions. RNA samples of both fish and amphipod samples from the control and dietary exposure landers will then be sent to Edinburgh Genomics for sample preparation using TruSeq stranded RNA-seq cDNA library preparation with a round of rRNA depletion, and subsequent short-read sequencing on their Illumina NovaSeq 6000 platform.

iii) Raw sequence data will be provided in FastQ format and will be error corrected using the k-spectrum based correction software, Rcorrector with the -k 31 setting (Song and Florea, 2015) and uploaded to the Galaxy web-platform (Afgan *et al*., 2016) where read quality will be visualised using FastQC (Andrews, 2010). Corrected reads will be filtered to remove adapter sequences and the lowest quality bases (Phred ≤ 5) (Freedman, 2020; MacManes, 2014) using Trimmomatic (Bolger,Lohse and Usadel, 2014). Trimmed reads will be quality assessed a second time with FastQC to confirm adapter removal before de novo transcriptome using Trinity (Grabherr *et al*., 2011). The assembled transcriptomes will be analysed for completeness (BUSCO, Simão *et al*., 2015), annotated using Diamond BLAST (Buchfink,Xie and Huson, 2014) and functionally annotated with Gene Ontology terms, followed by GO enrichment analysis using the R package TopGO (Alexa, Rahnenfuhrer and Lengauer, 2006). Differentially expressed genes between exposed samples and controls will be identified through analysis and visualisation using the R package edgeR (Robinson, McCarthy and Smyth, 2010).

iv) *Targeted gene expression analysis through quantitative PCR:* To support the results of the transcriptomic analysis and to further develop gene biomarkers of metal exposure in deep-sea samples, transcripts identified through differential expression analysis and functionally annotated with appropriate GO terms to identify genes likely induced by exposure to metals will guide targeted qPCR analysis. Sequenced and annotated transcripts will be used to design and produce appropriate primers and applied to cDNA samples (prepared as mentioned above) from in situ experiments. Quantitative PCR analysis will be carried out using Primer Design PrecisionPLUS mastermix kits following manufacturer's protocols.

v) The ΔΔCt method will be used to determine the relative fold change in expression of each target gene, the transcription levels of the control group are normalized to 1 and data is presented as fold changes relative to the controls. All data will be tested for normal distribution (Shapiro-Wilk test) and homogeneity of variances (Bartlett test). Analysis of variance (ANOVA) will be performed to test for statistical differences in gene expression. Significant differences will be set at $p < 0.05$. All statistical analysis will be done using R software (version 4.1.1, R Core Team, 2021).

3.2.23 Ecosystem function

Lead Contractor: Heriot-Watt University

Benthic chamber landers are used to quantify a variety of important ecosystem and biological properties and functions, such as biodiversity, nutrient fluxes, metabolic activities of microbes, meio- and macrofauna and biological respiration (Sweetman and Witte 2008a, Sweetman *et al*. 2019). This study will characterize changes in key ecosystem and biological properties and functions as a result of the effects of disturbance by the PCV and coverage by sedimentation from the benthic plume.

Sample Collection: Contractors will conduct ecosystem lander operations on 2 campaigns using Benthic respirometer lander and micro profiling system (RL); Benthic camera lander (CL) and Baited trap lander (TL) as follows:

- Maximum 5 RL; 5 CL randomized stations localized to the TF and wider CTA pre-collector test
- Minimum 5 RL; 5 CL randomized stations taken in the same area, targeting different levels of impact taken on post-collector test.

Sample Analysis:

Benthic respiration and metabolic activity studies: Seafloor respiration and CO_2 production will be quantified at 5 sites during each campaign using a benthic chamber lander system equipped with 3 seafloor respirometers. Each lander deployment will last 36-hrs. During each lander deployment, benthic O_2 consumption (seafloor respiration) will be assessed in 3 chambers (i.e. replicates) by continuously logging O_2 concentrations in the overlying water with Contros Hydroflash® O_2 sensors. In addition, water samples will be extracted from each chamber at pre-programmed times by an onboard syringe sampler allowing CO_2 concentrations and fluxes to be quantified from the change in CO_2 concentration (in the seawater within each chamber) over time. CO_2 concentrations will be analyzed by coulometry at Heriot-Watt University.

Baited camera and trap studies to document the diversity of demersal predators and scavengers: The scavenger studies will be repeated over pre and post collector test to quantify the magnitude of temporal variation in scavenger biodiversity and processes. Comparisons of scavenger biodiversity and processes within NORI-D will be made to existing data sets to address the question of whether unique populations/ processes and/or endemic species exist in the NORI-D, and to develop a biogeography of the scavenging fauna.

3.2.24 eDNA for microbial

To comprehensively characterize microbial communities, metabarcoding represents a convenient and efficient tool for deep-sea surveys due to its high sensitivity, high throughput, and low volumes of material needed. A few recent studies have taken advantage of eDNA metabarcoding to unveil the prokaryote assemblages associated with sediments and nodules within the CCZ (e.g., Wang *et al*. 2010; Lindh *et al*. 2017; 2018; Shulse *et al*. 2017).

Sample Collection: The contractor will conduct multicore sampling on 2 campaigns using the OKTOPUS MC20 multicore as follows:

- Maximum 15 randomized stations localized to the TF and wider CTA pre-collector test
- Minimum 26 randomized stations taken in the same area, targeting different levels of impact (including control sites) taken on a post-collector test

It is anticipated that future deep-sea routine monitoring surveys will focus primarily on eDNA from the top sediment layer as it is more economic and applicable to routine, large scale monitoring. However, it is important to also consider that eDNA only protocols work quite differently, using different chemicals and

volumes of material. As such, the following approach to minimize the number of samples to be processed with the eDNA and eRNA extractions, library preparation and sequencing. Sediment (~ 1 g) per replicate core, per sediment layer and station will be pooled together and homogenized. A subset of 10 sediment samples (stations) will be processed with the RNeasy PowerSoil DNA and total RNA kits for eDNA/eRNA co-extraction. Once extracted, single stranded RNA will be converted to complementary DNA (cDNA) using random hexamer primers and the SuperScript® III reverse transcriptase enzyme (Thermo Fisher Scientific Inc.), as in Laroche *et al*. (2017). In addition, pooled samples from each station will be processed for eDNA only using the DNeasy PowerSoil pro kit and the QIAcube robotic workstation. This will allow us to characterize the microbial community from all stations and determine whether eDNA retrieved from a semi-automatized protocol that uses 1/8 of the sediment volume (0.25 g for DNeasy PowerSoil instead of 2 g for RNeasy PowerSoil DNA) and which do not involve phenol and chloroform (2 extremely toxic chemicals used for RNA extraction) provide comparable results.

3.3 Compliance Monitoring & Management

Compliance monitoring is implemented throughout the collector test operations to ensure that the prescribed mitigation measures are effective in reducing the residual impacts to acceptable levels. Although the Collector Test EIS has demonstrated that no significant impacts will arise from the proposed activities, NORI has committed to 28 mitigation measures that will be implemented throughout the collector test to ensure that environmental impacts are minimized. These mitigation measures will be monitored throughout the test and the performance of the measures will be reported in the post-campaign report.

The 28 mitigation measures that NORI committed to in the Collector Test EIS are described in Table 3-4, together with descriptions of KPIs, corrective actions, documentation of compliance and responsibilities for implementation and reporting.

Table 3-5 describes the VECs and impact pathways that the various mitigation measures have been designed to protect and mitigate. The numbers in the first column of Table 3-4 correspond with those in the last column of Table 3-5, to show which mitigation measures will be applied to minimize the impacts on specific VECs.

Table 3-4. Compliance monitoring metrics

No	MITIGATION MEASURE	KPI	CORRECTIVE ACTION	DOCUMENTATION OF COMPLIANCE	RESPONSIBILITY
1	The Collector Test System is 20% the scale of a full-size commercial system and is considered sufficient to meet the testing objectives while minimizing the environmental disturbance footprint	As built engineering drawings show that prototype collector system has been constructed at 20% scale of full commercial system	Collector Test is not allowed to proceed if collector system has not been constructed as specified	As built collector system drawings	Allseas Chief Engineer
2	The Prototype Collector Vehicle is 50% scale of a full-size commercial vehicle and is considered sufficient to meet the testing objectives while minimizing the environmental disturbance footprint.	As built engineering drawings show that prototype collector vehicle has been constructed at 50% scale of full commercial vehicle	Collector Test is not allowed to proceed if prototype collector vehicle has not been constructed as specified	As built prototype collector vehicle drawings	Allseas Chief Engineer
3	The nozzles of the PCV have been designed to exploit the Coandă effect, (the tendency of a fluid jet to stay attached to a convex surface) to minimize sediment disturbance during nodule pickup	As built engineering drawings show that the nozzles of the PCV have been designed to exploit the Coandă effect	Design modification if nozzles of the PCV do not operate as designed	As built prototype collector vehicle drawings. Post-campaign performance evaluation of the PCV	Allseas Chief Engineer
4	Nozzle head height adjustment allows for fine tuning of the Coandă effect by changing the relative force of the water jet and suction combination on the seabed. The ability to fine tune in this manner will optimize the efficiency of nodule pick-up whilst minimizing sediment disturbance.	As built engineering drawings show that the nozzle heads have been designed to allow height adjustment for fine tuning of the Coandă effect by changing the relative force of the water jet and suction combination on the seabed.	Design modification if nozzles of the PCV do not operate as designed	As built prototype collector vehicle drawings. Post-campaign performance evaluation of the PCV	Allseas Chief Engineer
5	The first stage of the nodule processing system is designed to separate nodules from sediment inside the PCV. Special pump equipment is used for separating fines from the nodule flow stream, keeping as much sediment as possible at the seafloor.	As built engineering drawings show that the nodule processing system is designed to separate nodules from sediment inside the PCV.	Design modification if nodule processing system does not operate as designed	As built prototype collector vehicle drawings. Post-campaign performance evaluation of the PCV	Allseas Chief Engineer

No	MITIGATION MEASURE	KPI	CORRECTIVE ACTION	DOCUMENTATION OF COMPLIANCE	RESPONSIBILITY
6	The PCV tracks will be fitted with water jets, powered by a dedicated pump which will clean sediment from the outer track surface and inner sprocket path prior to ascending to the surface, reducing the amount of benthic sediment transported to the surface.	As built engineering drawings show that PCV tracks are fitted with water jets.	Design modification if water jets do not operate as designed	As built prototype collector vehicle drawings. Post-campaign performance evaluation of the PCV	Allseas Chief Engineer
7	Where possible, all chemicals used in submersible equipment (i.e., ROV and PCV), will be biodegradable and compliant with OSPAR (2009) standards for the protection of the marine environment.	During the campaign ROV operator uses chemicals which are biodegradable and compliant with OSPAR (2009) standards for the protection of the marine environment.	ROV operator reconsiders use of any chemicals which are not OSPAR compliant and looks for viable alternatives	ROV maintenance logbook, a copy of which will be included in the post-campaign report.	Ocean Infinity ROV Supervisor Allseas Chief Engineer
8	The nodule surface separator and storage system has been fitted with a 2-way diverter valve that can send the slurry stream directly to the buffer tank. This provides a protection from sudden unexpected over-load and spill.	As built engineering drawings show that the nodule surface separator and storage system has been fitted with a 2-way diverter valve	Design modification if the 2-way diverter valve does not operate as designed	As built *SSV Hidden Gem* refit drawings. Post-campaign performance evaluation of the collector system	Allseas Chief Engineer
9	The depth of the return water outlet has been set at 1,200 m, 200 m below the measured oxygen minimum zone. Due to the particle momentum of the outfall the effective discharge depth may be as deep as 1,280 m.	As built engineering drawings show that the return water outlet has been set at 1,200 m	Design modification if 1,200m does not prove to be the optimal depth for return water discharge	As built riser and return water system drawings. Post-campaign performance evaluation of the riser and return water system	Allseas Chief Engineer
10	All vessels used during the Collector Test will adhere to MARPOL regulations aimed at preventing both accidental pollution and pollution from routine vessel operations.	Zero pollution events from vessels	Ship captain implements procedures to address any violation of MARPOL regulations.	Pollution event incident report is developed by the ship captain and included in the post-campaign report. Any MARPOL required reporting is completed.	Captains of the *SSV Hidden Gem* and *OSV Island Pride*

No	MITIGATION MEASURE	KPI	CORRECTIVE ACTION	DOCUMENTATION OF COMPLIANCE	RESPONSIBILITY
11	Use of modern ships and offshore supply vessels that comply with IMO (2014) guidelines, will minimize noise generation.	Vessels selected for campaign comply with IMO guidelines (2014)	Non-conforming vessels are not selected	Vessel specifications will be included in post-campaign report	NORI offshore manager
12	Use of modern and efficient thruster systems and dynamic positioning systems (e.g., DP II in preference to DP I, or DP III in preference to DP II). will minimize noise generation	Vessels selected for campaign use modern and efficient thruster systems and dynamic positioning systems	Non-conforming vessels are not selected	Vessel specifications will be included in post-campaign report	NORI offshore manager
13	The of Vertical Transport System (VTS) using airlift riser technology rather than noisier technologies such as risers with multiple slurry pumps or risers fitted with a Subsea Slurry Lift Pump (SSLP) fitted with individual positive displacement pump module displacement pump at its base	As built engineering drawings show that Vertical Transport System (VTS) has been used in the design.	Design modification if VTS does not prove to be the optimal solution in terms of technical or environmental performance.	As built Vertical Transport System (VTS) drawings. Post-campaign performance evaluation of the Vertical Transport System (VTS)	Allseas Chief Engineer
14	The outlet of the return process wastewater pipe will be located at 1,200 m depth, which is below the biologically productive epipelagic zone 90–200 m depth and upper mesopelagic zone (200– 1,000 m depth), as well as minimizing activities in the sound-fixing-and-ranging (SOFAR) channel (typically at a depth of ~1000 m in the CCZ) within which low-frequency sound is transmitted over very long distances (hundreds to thousands of kilometres).	As built engineering drawings show that the return water outlet has been set at 1,200 m	Design modification if 1,200m does not prove to be the optimal depth for return water discharge	As built riser and return water system drawings. Post-campaign performance evaluation of the riser and return water system	Allseas Chief Engineer
15	The GHG emissions for the Collector Test have been calculated and will be offset.	GHG emissions from the Collector Test campaign are accurately calculated	Suitable mechanisms are identified to offset the GHG emissions arising from the campaign	GHG emissions calculation and offset certification	NORI Chief Sustainability Officer

No	MITIGATION MEASURE	KPI	CORRECTIVE ACTION	DOCUMENTATION OF COMPLIANCE	RESPONSIBILITY
16	All Collector Test operation will be confined to an 8 km2 TF.	All direct disturbance from the PCV tracks are confined to the Test Field	Real-time adjustment of PCV course if it leaves the boundary of the TF	Post-campaign report, including campaign survey report.	NORI Offshore Client Representative / Allseas project manager
17	The duration of the entire Collector Test is limited to 860 hours, and the duration of system testing (period of maximum plume generation) is limited to 259 hours. Most impacting activities associated with the Collector Test will be temporary, short in duration, and spatially contained.	Duration of Collector Test is limited to 860 hours	Monitoring of program duration. Notification of ISA if total duration exceeds 860 hours.	Post-campaign report, including campaign survey report.	NORI Offshore Client Representative / Allseas project manager
18	The ROV and all associated equipment will be maintained and inspected for leaks prior to deployment.	During the campaign ROV Supervisor and technicians perform regular pre-dive checks on the ROV.	ROV Supervisor and technicians identify any leaks from the ROV and rectifies the issue prior to each dive	ROV maintenance logbook, a copy of which will be included in the post-campaign report.	Ocean Infinity ROV Supervisor
19	A specially designed Launch and Recovery System (LARS) for the PCV has been fitted to the side of the Hidden Gem. The LARS affords a very high degree of control for raising and lowering the PCV through the splash zone, allowing the operation to be paused or slowed at any time and minimizing the likelihood of any significant interactions with marine fauna.	As built engineering drawings show that the specially designed Launch and Recovery System (LARS) has been built to specifications	Design modification if the Launch and Recovery System (LARS) does not function as expected.	As built Launch and Recovery System (LARS). Post-campaign performance evaluation of the Launch and Recovery System (LARS)	Allseas Chief Engineer

No	MITIGATION MEASURE	KPI	CORRECTIVE ACTION	DOCUMENTATION OF COMPLIANCE	RESPONSIBILITY
20	The area of seabed that will be directly disturbed by the PCV has been contained to just 0.5km2; considered to be the minimum level of disturbance required to credibly assess the functionality of the system and potential environmental impacts. This represents just under a quarter (23%) of the 2.2 km2 that was disturbed within the 10.8 km2 DISCOL experiment in the Peru basin.	The trial runs are completed as specified in the Collector Test EIS and the overall area of direct disturbance by the PCV tracks does not exceed 0.5km^2.	Monitoring of area of direct disturbance during trial runs. Notification of ISA if are of directly disturbed seabed exceeds 0.5km^2.	Post-campaign report, including campaign survey report.	NORI Offshore Client Representative / Allseas project manager
21	CTA has been located in the 'Flatter area' which is the largest geoform by area (8,553.70 km2) in NORI-D, rather than in the 'Abyssal hills' geoform. This placement is intentional as Abyssal hills and seamounts have been shown to be higher in species richness and standing stock biomass compared to adjacent areas devoid of topographic	No deviation from the Test Field specified in the Collector Test EIS.	Notification of ISA for prior permission to utilize a different Test Field if necessary.	Post-campaign report, including campaign survey report.	NORI Offshore Client Representative / Allseas project manager
22	The area of seabed experiencing sedimentation rates above the demonstrated natural range of variation (i.e., ≥0.1 mm) is limited to 25km2. This is considered the minimum level of disturbance required to credibly assess the functionality of the system and potential environmental impacts.	Area of seabed experiencing sedimentation rates above the demonstrated natural range of variation (i.e., ≥0.1 mm) does not exceed 25km^2.	Monitoring of plume dispersal and sediment deposition. If the total area of deposition exceeds 25km^2, refinements will be made to the plume model for commercial EIS.	Post-campaign report, including campaign survey report.	NORI Offshore Client Representative / Allseas project manager
23	Measures recommended by the International Maritime Organisation for minimising the risk of collisions between ships and whales will be implemented during the Collector Test campaign, including good route planning for transit to the site, keeping watch, continued	Zero collisions between ships and whales	Ship captain implements procedures to address any violation of IMO recommendations.	Vessel strike incident report is developed by the ship captain and included in the post-campaign report.	Captains of the *SSV Hidden Gem* and *OSV Island Pride*

No	MITIGATION MEASURE	KPI	CORRECTIVE ACTION	DOCUMENTATION OF COMPLIANCE	RESPONSIBILITY
	scientific research into the migratory species that utilize NORI-D.				
24	The wet weight of nodules collected during the Collector Test will be restricted to 3,600 – 4,600 tonnes, limiting the impacts of the test due to loss of nodule habitat and direct impacts to benthic biota.	The wet weight of nodules collected during the Collector Test does not exceed 3,600 – 4,600 tonnes	Monitoring nodule recovery tonnage. If the total tonnage exceeds expectations, modifications will be made to the recovery rates for the commercial EIS	Post-campaign report, including campaign survey report.	NORI Offshore Client Representative / Allseas project manager
25	Nodules >80 mm in diameter will not be collected. Larger nodules will be left in the TF where they may continue to provide habitat value for nodule obligate biota, if not buried by sediment.	Evidence that nodules >80 mm in diameter are being left on the sea floor	Design modification of the commercial system if the PCV does entrain nodules >80mm.	Pre- and post-disturbance photography of the PCV tracks which show evidence of nodules being left at the seafloor	NORI Offshore Client Representative / Allseas project manager
26	Modelling predicts that mid-water exceedances of ≥0.1 mg/l will be laterally contained to 200 - 250 m from the point of discharge and an overall plume dispersal footprint will be just 16km^2; this is 8% of the 200 km2 plume footprint generated by the Muñoz Royo *et al*. (2021) study	Mid-water exceedances of ≥0.1 mg/l are laterally contained to 200 - 250 m from the point of discharge and overall plume dispersal footprint is ≤16km2	If sediment levels of ≥0.1 mg/l are not laterally contained to 200 - 250 m from the point of discharge and/or overall plume dispersal footprint >16km^2, design modifications will be made to alter the flow rate and sediment load of the discharge to the commercial system	Post-campaign report, including campaign survey report.	NORI Offshore Client Representative / Allseas project manager

No	MITIGATION MEASURE	KPI	CORRECTIVE ACTION	DOCUMENTATION OF COMPLIANCE	RESPONSIBILITY
27	A marine mammal observer (MMO) will be present during all offshore operations and to act immediately to protect species of concern should they enter the vessel's exclusion zone prior to and sometimes during operations. The MMO will advise personnel onboard to delay or shutdown operations until the animals are at a safe distance and also to record behaviour and sightings at other times	No impact from the operations to marine mammals	Potentially disturbing operations are suspended until the animals are at a safe distance.	Post-campaign report, including MMO report.	NORI Offshore Client Representative / MMO
28	The air lift will be in operation during the Riser installation and commissioning, System Integration Test and System Test Runs. This is considered to be the minimum operating time required to meet the objectives of the Collector Test and limits exposure to potentially impactful underwater noise to approximately 529 hours.	Duration of System Integration Test and System Test Runs is limited to 529 hours	Monitoring of System Integration Test and System Test Runs. Notification of ISA if test runs exceed 529 hours.	Post-campaign report, including campaign survey report.	NORI Offshore Client Representative / Allseas project manager

Table 3-5. VECs and Impact Pathways that will be protected/mitigated by mitigation measures in Table 3-4

ACTIVITY	VULNERABLE VECS	IMPACT PATHWAYS	MITIGATION MEASURES
Transit of the vessel from San Diego to the CCZ	Air quality/GHG	Vessel's diesel engines will emit fumes into the atmosphere reducing local air quality and contributing to GHG emissions.	15,
	Noise/vibration/light	Vessel's diesel engines will generate noise and vibrations which could disturb birds, cetaceans, and turtles. Vessel will emit light.	11, 12, 13
	Cetaceans/turtles	Vessel strike on cetaceans or turtles	23, 27
	Water quality	Intentional or accidental release of pollutants from the vessels could negatively impact water quality	7, 10
Offshore Inspection and Preparation	Water quality	Leakage of hydraulic fluids, oil, or other substances from the ROV could negatively impact water quality throughout the water column during its descent to the seabed.	7, 18

ACTIVITY	VULNERABLE VECS	IMPACT PATHWAYS	MITIGATION MEASURES
	Noise/vibration/light	Deployment of ROV to the seabed has potential to generate noise, vibration, and light.	27,
	Benthic Biota (sediment, nodule, free swimming)	Deployment of the ROV and other equipment (inc. LBL network) to the seabed has the potential to physically disturb sediment and nodule dwelling animals.	1, 2,
	Benthic Habitat Quality	Deployment of other equipment (inc. LBL network) to the seabed will physically disturb benthic habitat by creating contours in the sediment.	1, 2,
PCV Deployment	Cetaceans/Turtles	Lowering the PCV through the splash zone could disturb or physically strike cetaceans or turtles that are in close proximity to the vessel.	19, 27
	Water Quality	Leakage of hydraulic fluids, oil, or other substances from the PCV could negatively impact water quality throughout the water column during subsea lowering.	7, 18
	Benthic Biota (sediment, nodule, free swimming)	Touchdown of the PCV on the seabed will physically disturb, displace or kill sediment and nodule dwelling animals.	1, 2, 16, 20, 21
	Benthic Habitat Quality	Touchdown of the PCV on the seabed will physically disturb the benthic habitat by creating contours in the sediment and/or moving or crushing nodules.	1, 2, 16, 20, 21
Jumper and Riser Deployment	Cetaceans/Turtles	Lowering the jumper and riser tubes through the splash zone has the potential to disturb or physically strike cetaceans or turtles that are in close proximity to the vessel.	27
	Water Quality	Leakage of hydraulic fluids, oil, or other substances from the ROV during manipulation of the jumper or riser could negatively impact water quality throughout the water column.	7, 18
Riser Commissioning	Noise/Vibration	Surface and/or subsea noise or vibrations caused by pressure testing of the riser pipe could disturb birds, cetaceans, and turtles.	13, 27, 28
	Cetaceans/Turtles	Surface and/or subsea noise or vibrations caused by pressure testing of the riser pipe could disturb birds, cetaceans, and turtles	13, 27, 29
Subsea Connection of Jumper on PCV	Water Quality	Leakage of hydraulic fluids, oil, or other substances from the ROV during connection of the jumper on the PCV could negatively impact water quality throughout the water column.	7, 18

ACTIVITY	VULNERABLE VECS	IMPACT PATHWAYS	MITIGATION MEASURES
System Testing	Cetaceans/Turtles	Riser installation and commissioning tests, system integration testing, and system test runs all have the potential to create noise and vibration disturbances at the surface and throughout the water column from use of the air lift and through pressure testing of the system which could disturb diving and foraging behaviour.	28
	Microbes	Manoeuvring the PCV on the seabed, pick-up test runs, and system test runs will physically disturb the sediments and nodules potentially disrupting the microbial community structure in the surface layers of the sediment, and seafloor metabolic activity	1, 2, 3, 4, 5, 6, 16, 20, 21, 24, 25
	Water Quality	Manoeuvring the PCV on the seabed, pick-up test runs, and system test runs will physically disturb the sediments and nodules creating a sediment plume and potentially mobilizing particle-bound nutrients and trace metals.	1, 2, 3, 4, 5, 6, 16, 20, 21
	Noise/Vibration/Light	Manoeuvring the PCV on the seabed and pick-up test runs will create noise and vibration which could disturb or displace motile large macrofauna. Riser installation and commissioning tests, system integration testing, and system test runs all have the potential to create noise and vibration disturbances at the surface and throughout the water column from use of the air lift and through pressure testing of the system. PCV will emit light.	28,
	Benthic Biota (sediment, nodule, free swimming)	Manoeuvring the PCV on the seabed and pick-up test runs will create noise and vibration which could disturb or displace motile large macrofauna.	1, 2,16, 20, 21, 24, 25
		Riser installation and commissioning tests, system integration testing, and system test runs all have the potential to create noise and vibration disturbances at the surface and throughout the water column from use of the air lift and through pressure testing of the system. PCV will emit light.	28,
		Manoeuvring the PCV on the seabed and pick-up test runs will physically disturb or remove sediment and nodule dwelling animals.	1, 2, 3, 4, 20, 21, 24, 25

ACTIVITY	VULNERABLE VECS	IMPACT PATHWAYS	MITIGATION MEASURES
		System test runs will create a benthic plume, as entrained sediment is ejected from the rear of the PCV; this plume will be denser than that formed during the manoeuvrability and pick-up test runs and will blanket and smother surrounding sessile biota.	1, 2, 3, 4
	Sediment Geochemistry	Manoeuvring the PCV on the seabed, pick-up test runs, and system test runs will mix the surface layers of the sediment, disrupting oxygen concentration gradients in the surface layers and potentially mobilizing particle-bound nutrients and trace metals.	1, 2, 3, 4
	Benthic Habitat Quality	Manoeuvring the PCV on the seabed and pick-up test runs will physically disturb the benthic habitat by creating contours in the sediment, disrupting surface layers of sediment, and/or moving or crushing nodules.	1, 2, 3, 4
		System test runs will create a benthic plume, as entrained sediment is ejected from the rear of the PCV; this plume will be denser than that formed during the manoeuvrability and pick-up test runs and will blanket and smother surrounding sessile biota.	1, 2, 3, 4, 20, 21, 22
	Nekton	Nekton in the mesopelagic and bathypelagic zones could be impacted by noise and vibration from the air lift system and by suspended sediment and mobilized chemicals released from the return water pipe outlet at 1,200 m.	9, 13, 14
	Zooplankton	Zooplankton in the euphotic, pelagic and bathypelagic zones could be impacted by noise and vibration from the air lift system and by suspended sediment and mobilized chemicals released from the return water pipe outlet at 1,200 m.	9, 13, 14, 26
	Water Quality	Water quality in the bathypelagic zone and below could be impacted by increased turbidity caused by suspended sediments and mobilized chemicals released from the return water pipe outlet at 1,200 m.	8, 9, 13, 14
	Climate Regulation	Emissions of GHGs to the atmosphere through travel, operation of equipment or mobilization of sequestered C in benthic sediments.	16
Emergency Shutdown Testing	N/A	There are no environmental aspects anticipated to be associated with the emergency shutdown testing of the system.	N/A

ACTIVITY	VULNERABLE VECS	IMPACT PATHWAYS	MITIGATION MEASURES
Riser and PCV Recovery	Cetaceans / Turtles	Rising the jumper hose, riser pipe, and PCV through the splash zone could disturb or physically strike cetaceans or turtles that are in close proximity to the vessel.	27
	Water Quality	A ROV will be used for recovery, leakage of hydraulic fluids, oil, or other substances from the ROV could negatively impact water quality throughout the water column.	7, 18
Transit of the vessel from the CCZ to San Diego	As for previous transit	As for previous transit	N/A
Cumulative Impacts	Ecosystem Function	Disruption of key ecosystem functions as a result of additive or synergistic impacts from project related activities.	16, 17, 22, 26
	Ecosystem Services	Disruption of climate regulation capacity	16, 17, 22, 26

3.4 Long-Term Monitoring

ISBA/25/LTC/6/Rev.1 identifies Impact Reference Zones (IRZs) and Preservation Reference Zones (PRZs) as being important in identifying natural variations in environmental conditions against which the impacts of mining can be assessed.

The ISA recommends that a PRZ should be representative of the pre-mining condition so that impacts in mined areas can be benchmarked against it. Therefore, it is important that the composition and condition of the biotic and abiotic components of the PRZ are representative of those of the pre-mined IRZ, including comparable geochemistry and species composition. It has also been recommended that multiple control sites are desirable to detect disturbances that do not affect long-term mean abundances of a population, but, instead, alter the temporal pattern of variance of abundance (Jones *et.al.*, 2020).

To satisfy these recommendations both a PRZ and a maximum two control sites have been established within the NORI-D contract area. The PRZ is in the NE corner of NORI-D covers an area of 750 km^2. The primary role of the PRZ is the long-term preservation of examples of the geoforms and associated habitats that may be directly or indirectly impacted by nodule collection activities. The baseline condition of habitats in the PRZ is being established and they will be monitored for change as part of the long-term monitoring program developed for NORI-D.

Specific to the Before-After-Control-Impact (BACI) studies to monitor recovery in the IRZ, two additional control sites have been established. These sites have been chosen specifically to be representative of the conditions at the IRZ only, rather than multiple habitats that will be impacted during commercial operations. The control sites are in the same geoform and nodule type as the IRZ (i.e., flat area with Type 1 nodules) and as close as possible to it without being impacted by collector test activities. Baseline studies demonstrate that the geochemistry and benthic species composition of the control sites are comparable to that of the TF (see NORI Collector Test EIS, Section 5.13.3 and Section 6.3).

The sites identified in Figure 3-14 have been designated as potential BACI control sites. Recently acquired data suggests that they are far enough away from the TF that impacts from collector test activities are unlikely for the following reasons:

- Modelled data from DHI (2021), Aleynik *et.al*., (2017) and from unpublished data from the JPI-Oceans project (Haeckel, 2021) suggest that the majority of the plume will settle out within a few hundred metres of the tracked regions.
- Sedimentation modelling indicates that sedimentation depths >0.1mm will be restricted to an approximate 5 km radius from the point of mobilization (see NORI Collector Test EIS, Section 7.2.2.5)

These assumptions will be tested during the collector test.

Figure 3-14. Location of PRZ relative to the TF (A) and example Before-After-Control-Impact sites for the IRZ (B).