Draft Guidelines for the establishment of baseline environmental data
Developed by the Legal and Technical Commission

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Background

1. During the continuation of the twenty-sixth session, the Legal and Technical Commission (the Commission) considered draft guidelines for the establishment of baseline environmental data pursuant to annex IV of the draft regulations on exploitation of mineral resources in the Area (ISBA/25/C/WP.1). The draft guidelines were prepared by a technical working group of the Commission with the assistance of a consultant.

2. The purpose of the acquisition and establishment of baseline data is to enable an assessment of the possible impacts of exploitation activities on the marine environment prior to those activities taking place. Baseline data also forms the basis for long-term monitoring of environmental impacts to make sure that impacts are in line with the environmental impact assessments and environmental monitoring and management plan once exploitation commences.

3. Annex IV of the draft regulations recommends an environmental impact statement submitted by an applicant or contractor describes environmental reference baseline data established as part of a contract for exploration pursuant to the relevant exploration regulations and the terms and conditions of an exploration contract.

4. To give effect to the recommendations contained in annex IV, the Commission considered that it was necessary to prepare: (i) Guidelines (Appendix I) for the establishment of baseline environmental data.

Appendix I

Draft Guidelines for the establishment of baseline environmental data

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I. INTRODUCTION

1. The environmental impact statement to be prepared and submitted by an applicant for a Plan of Work under the Regulations on exploitation of mineral resources in the Area (Exploitation Regulations) should be based on the environmental reference baseline data established as part of a contract for exploration pursuant to the relevant exploration regulations and the terms and conditions of an exploration contract.

2. These Guidelines focus primarily on deep-sea polymetallic nodules found in the central and NW Pacific and Indian Oceans. Some elements may not apply to all mineral types. Further iterations will be issued in the future to cover polymetallic seafloor massive sulphides and cobalt-rich ferromanganese crusts.

3. These Guidelines provide guidance on how an applicant or contractor may fulfil the requirements concerning the acquisition of oceanographic and environmental baseline data and build on the recommendations for the guidance of contractors for the assessment of the possible environmental impacts arising from exploration for marine minerals in the Area (ISBA/25/LTC/6/Rev.1 and Corr.1).

4. These Guidelines should be read in conjunction with the Exploitation Regulations, the relevant Exploration Regulations, other relevant International Seabed Authority rules, regulations and procedures, as well as other relevant Standards and Guidelines, including but not limited to those related to:

- Environmental Impact Assessment and Environmental Impact Statement;
- Environmental Management and Monitoring Plan; and
- Environmental Management Systems.

II. PURPOSE AND SCOPE

5. The primary goal of the acquisition of baseline data is to enable an assessment of the possible impacts of exploration and exploitation activities on the marine environment prior to those activities taking place. It also forms the basis for long-term monitoring of environmental impacts to make sure that those are in line with the environmental impact assessments and environmental monitoring and management plan once exploitation commences.

6. Sampling is the cornerstone of environmental surveys and monitoring. If samples are not taken with the correct equipment and follow the Best Available Techniques and Good Industrial Practice then all the subsequent data and analyses are flawed or compromised.

7. These Guidelines provide guidance on the following aspects:

- Scope, coverage and standard of baseline data needed to characterize the physical, chemical, geological as well as sediment properties and biological communities in the Area;
- Review and evaluation procedures to assess the quality of environmental baseline data and the statistical rigour needed to be able to detect and differentiate change from baseline/background levels; and
- Data management, particularly relating to metadata needed to support data deposition and reporting of environmental baselines.

99 8. The baseline data that should be collected are grouped in these Guidelines under the
100 following headings:

101 - Physical Oceanography
102 - Chemical Oceanography and Biogeochemistry
103 - Geological Properties
104 - Biological Communities

105 **III. SAMPLING AND DATA ACQUISITION**

106 9. Baseline data should be multidisciplinary to allow for a holistic assessment of
107 oceanographic and environmental conditions and processes. Appropriate representation is
108 necessary to identify if any identified changes are associated with mining operations or
109 represent spatial and temporal variability and trends that occur naturally. Without this
110 knowledge, deviations from pre-mining conditions observed during mining operations could
111 only be assigned to exploitation activities. As such a comprehensive understanding of the
112 natural variability in baseline conditions should be determined during the exploration phase.

113 **A. Spatial and Temporal Variability**

114 10. The magnitude and spatiotemporal scales of variability will be different for different
115 variables and will differ between ecosystem components. Consequently, the replication and
116 frequency needed to address variability will differ between components. To achieve a robust
117 coverage of temporal and spatial variability, and to reduce the uncertainty of data, replicate
118 observations should be obtained to detect changes as a result of time (seasons, interannual
119 variability) and space (horizontal and vertical), and differentiate between different regions.

120 11. Care should be taken that the baseline sampling sites are aligned with requirements for
121 monitoring during later mining operations and should, therefore, be at appropriate locations to
122 later serve as Impact Reference Zones (IRZs) and Preservation Reference Zones (PRZs) and in
123 sufficient numbers to address effects connected to both direct and indirect impacts with the
124 necessary statistic rigor. The arrangement should consider typical ocean current directions and
125 substantial topographic features as these will have an influence on the direction and distance
126 sediment plumes generated by the mining collector may disperse and resettle.

127 12. The use of standard references for global ocean biogeography should be used to
128 determine the relevant large-scale biomes, e.g. Longhurst (1998) for the epipelagic and Sutton
129 *et al.* (2017) for the mesopelagic. The main currents should be mapped and relevant mesoscale
130 and sub-mesoscale features (dimensions 1-100km) within the sampling area such as meanders,
131 eddies, fronts should be identified as well as features influenced by sea bed topography such
132 as seamount wakes and Taylor columns. Archived remote sensing satellite altimetry and sea
133 surface temperature data should be accessed and analysed to identify currents and surface
134 oceanographic features and the area considered should extend beyond the contract area. A time-
135 series extending back at least 20 years, covering microwave and infrared temperature data to
136 detect seasonal and interannual changes, enhanced by ocean colour data, should be reviewed
137 for the areas considered to validate the biomes and determine interannual variability. Major
138 seasons should be identified.

139 13. In homogeneous stable areas such as within a gyre province over an abyssal plain there
140 may be only one identifiable vertical layer. Latitudinal or longitudinal gradients may indicate

141 more than one stratum. In the vicinity of fronts and mid-ocean ridges there may be considerable
142 spatial heterogeneity leading to multiple strata. In eddy fields sampling should be flexible to
143 include anticyclonic and cyclonic eddies.

144 14. For sediment, pore water and benthic biological sampling, a nested stratified sampling
145 scheme should be used to ensure that the collection of samples and data encompasses the range
146 of environmental settings at the scale of an exploration contract area. Based on the data
147 collected from other variables (primarily physical oceanography, section IV), regions should
148 be divided into different biogeochemical and bathymetric entities and within each of these
149 entities, a nested set of physiographic zones and units with different topographies and different
150 nodule coverage (abundance and size) should be established to fully cover conditions that are
151 expected to represent important drivers of changes in community and biogeochemical
152 functions. Such physiographic units typically include seamounts, abyssal plains, hills, valleys,
153 with low to high abundances of nodules of different sizes. Other additional units should be
154 defined as needed to cover the specific conditions and their variability in the respective contract
155 areas. This is visually demonstrated in Figure 1 below. The location and extent of these units
156 should be defined based on a ship-based bathymetry and seafloor acoustic and optical imagery
157 with AUVs or cable-deployed gear at a high resolution.

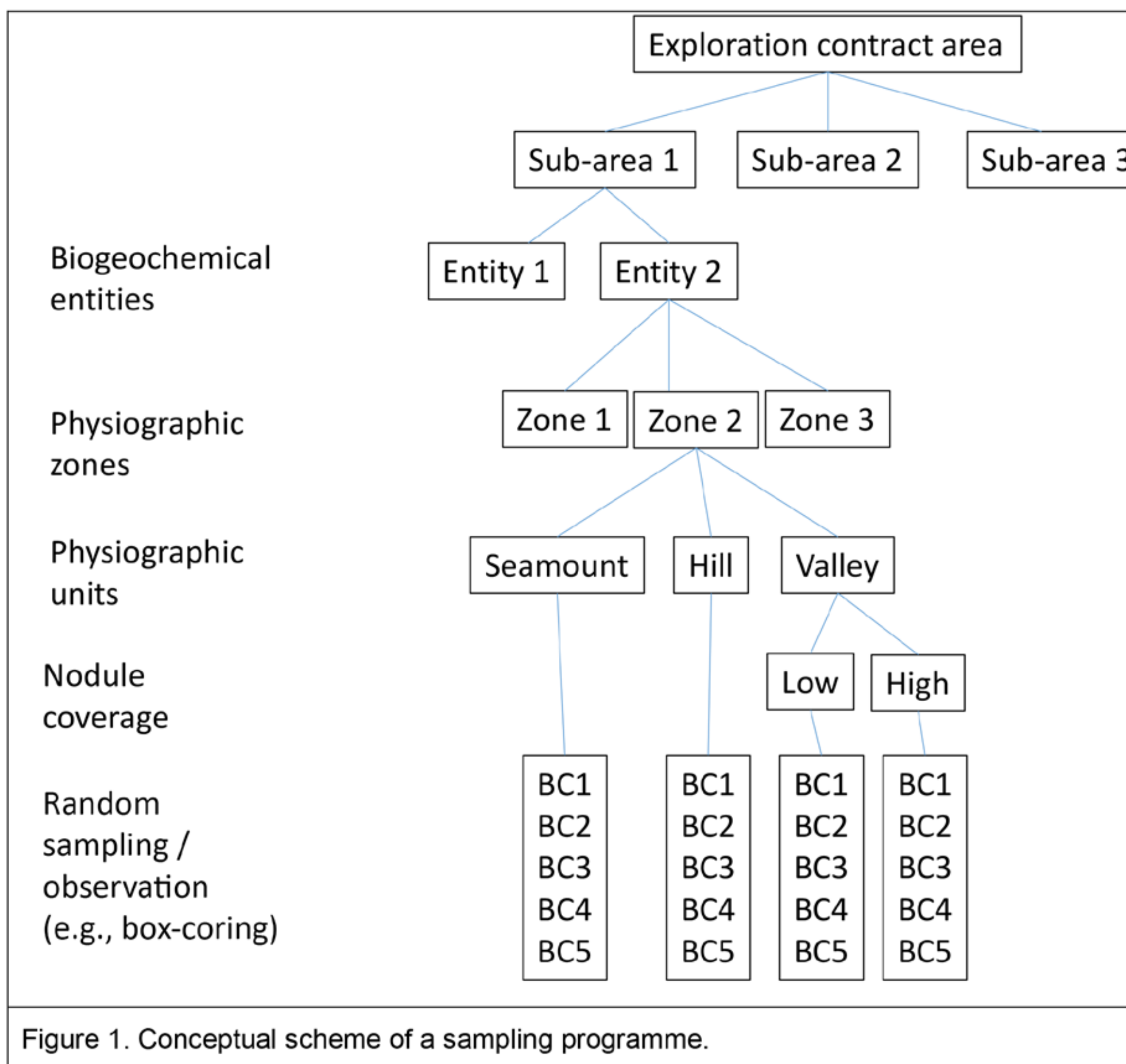

Figure 1. Conceptual scheme of a sampling programme.

158

159 15. Observations should be carried out at different times of the year to cover seasonal
160 changes in productivity and hydrodynamic conditions specifically covering periods of
161 contrasting bottom water flow regimes and at different seasons in terms of organic matter
162 availability. In addition, diel changes throughout the 24-hour cycle should be quantified where
163 they are relevant (e.g. pelagic systems).

164 16. For variables that are not expected to show significant seasonal variability (e.g.,
165 sediment infauna and biogeochemistry of deeper sediment layers) this should be validated at
166 least once by a comparison of observations at contrasting seasons.

167 17. Observations in similar seasons or environmental conditions should be carried over at
168 least in three different years to assess interannual variability and increase the chance to capture
169 periodic events. In addition, the temporal sampling strategy should also cover interannual
170 changes including possible periodic variations, e.g., connected to the El Niño–Southern
171 Oscillation (ENSO). Other natural stressors such as global warming and rising atmospheric
172 CO_2 levels, their impact on the environment where baseline data is being collected and their
173 temporal variability, should be considered when developing an environmental baseline.

174 18. When temporal or spatial comparisons are being made, the other component should be
175 kept constant. For example, to compare between seasons, samples from the same
176 physiographic unit and depths should be compared.

177 19. Unless stated differently within the sections on specific variables, the vertical sampling
178 resolution should be as follows:

179 • For the water column sampling (including physical measurements unless stated
180 differently in section IV.B) - Higher resolution of sampling should be used in the
181 surface 200m (3-4 samples with the depths determined by local variability) and
182 bottom 500m (e.g., at 5m, 10m, 25m, 50m, 75m, 100m, 150m, 200m and 500m
183 above the seabed), noting that surface weather conditions and localised
184 topography may impact the resolution possible very close to the seabed.
185 • For the seabed sampling, unless a higher resolution is stated under the specific
186 variables below, vertical resolution should focus on 0-0.5cm, 0.5-1cm, every
187 centimetre down to 10cm, every 2cm from 10 to 20cm depth or down to the
188 sediment depth that is expected to be impacted by the mining equipment
189 (whatever is deeper). Where deeper measurements are required, every 5cm
190 between 20cm and 50 cm and every 20 cm in deeper layers over a sediment
191 column of up to 5m. This resolution should be considered a guide and increased
192 where initial studies carried out at high resolution (e.g. for determining redox
193 zonation) indicate that the above layers are insufficient to suitably characterize
194 vertical profiles.

195 20. Random replicates should be obtained from each sample site and sufficient replication
196 should be obtained to cover the variability and discriminate between units. Determination of
197 the number of replicates required to characterize baseline conditions in a specific zone depends
198 on a number of factors, including the variable being addressed and is likely to differ among
199 contract areas therefore the number of replicates used should be justified using appropriate
200 statistics. Lower temporal and spatial variability are expected in deeper sediment layers. Hence,
201 to assess conditions in deeper sediment layers, measurements in single long core from each site
202 that are repeated during a few campaigns may be sufficient unless significant temporal or small-
203 scale spatial variabilities are observed.

204 21. Samples or data collected from the same deployment of a single platform (e.g., cores
205 form a single multicore deployment or multiple sensors on a single lander) should be
206 considered one sampling point (i.e., one 'biological replicate'). Where samples are sub-divided
207 it should be to obtain different variables from the same sample and not create pseudo-samples.

208 22. Where specific detail is not provided under the detail for specific variable regarding the
209 sampling required to determine spatial and temporal variability, the protocol should follow
210 those outlined in document ISBA/25/LTC/6/Rev.1 and Corr.1.

211 **B. Adaptability of Sampling Strategies**

212 23. Initial sampling and observation strategies should build on the existing knowledge and
213 should be regularly revised as more information becomes available to ensure it is fit for purpose
214 and capturing the relevant spatial and temporal variability. It should be demonstrated that
215 observations in areas or on spatial scales that have been considered homogeneous indeed show
216 less variability as compared to data from areas where differences were expected. Also, it should
217 be established if observations at similar seasons are less variable than those from different times
218 of the year. However, changes in sampling strategy, especially where they involve
219 discontinuation of observations at specific sites or seasons, should be done with caution so as
220 not to miss episodic events or fail to resolve interannual variability or lead to inconsistencies
221 that prevent temporal analysis.

222 24. Expert input has been obtained to ensure the methodologies in these Guidelines
223 correspond to best practice. However, techniques and processes are subject to development
224 over time and as such, to adequately characterise the environment, the Best Available
225 Technology should be used, and when it is not, a justification provided. Independent feedback
226 should be sought to enable suitable adjustment as required. Where data collection has already
227 commenced, care should be taken to make sure that data obtained with different approaches
228 are consistent to allow for integrated assessments of all data obtained.

229 25. As the details of the technology that will be used for resource exploitation become
230 available, and exploration activities progress, the sampling programme should be adjusted if
231 required to ensure the baseline data is focused on areas where mining is expected to take place
232 and any impacts are likely to be seen. This is particularly relevant where the potential depth of
233 mining exceeds the suggested sampling depths in the individual variables or where significant
234 variability in the parameters are identified.

235 **C. Coordination and Cooperation**

236 26. Where possible, measurement of different variables should be aligned, both temporally
237 and spatially, to facilitate integrated data analyses and to strengthen explanatory power. This
238 is particularly important for variables that target interconnected or similar processes, fall within
239 the same or closely connected disciplines (e.g., geology and sediment biogeochemistry,
240 oceanography with ocean chemistry and pelagic biology), or need to be combined to create
241 derived products. Where the methodology is compatible, samples from single sediment cores
242 should be used for the analyses of multiple parameters (e.g. the same cores for pore water and
243 sediment characteristics). Box cores for macrofaunal should not be subsamples (see section
244 VII.C.2).

245 27. Collaboration and exchange of data and information with the scientific community and
246 between contractors should occur wherever possible to enable analyses that extend beyond the

247 contract area of individual contractors. This will provide context on larger scale patterns to
248 facilitate the interpretation and use of baseline observations to enable a larger scale analysis to
249 support regional environmental management plans whilst also reducing the burden on
250 individual contractors.

251 28. Sharing data between contractors and the scientific community is recommended to
252 assure that high quality data have been acquired following state-of-the-art methodology.

253 29. Many of the variables in these guidelines are also addressed by the Global Ocean
254 Observing System (GOOS, https://www.goosocean.org). GOOS has created a framework
255 around Essential Ocean Variables (EOV) to enable a cost-effective plan to provide an optimal
256 global view for each EOV. Many of the variables in these guidelines have an associated EOV
257 factsheet created and disseminated by the Expert Panels, including identifying what
258 measurements are to be made, various observing options, and data management practices and
259 refer to best practices, guides and background information. These factsheets provide
260 supplementary information to these guidelines. The current set of EOVs corresponds to
261 physical and biogeochemical oceanography observations but are lacking important information
262 on biology and benthic biogeochemistry.

263 **D. Data Quality**

264 30. As a minimum quality control, all measured data should be compared to observations
265 available in the scientific literature as well as in other sources from the same region or similar
266 depth and oligotrophic areas. A good agreement between state-of-the-art models and
267 observations of the different variables is considered a strong indication of a baseline data set
268 of good quality, consistency, and completeness. A comparison of observations to model results
269 should therefore be a core component of reporting and should include reference to all
270 information needed to run the model and reproduce results. Where discrepancy between
271 measurements and the model occur, these should be investigated to resolve the error. This may
272 require adaption of the model or collection or more samples.

273 31. If large deviations are observed that cannot be assigned to differences in environmental
274 settings, methods should be checked or cross-validated with other laboratories.

275 32. The full workflow, including detailed information on the measurement methodology
276 and quality control (e.g., standards and blanks measured), should be fully documented,
277 especially in cases where no standards were available or where applied methods deviated from
278 agreed standards. Where non-standard methods are used these should be openly shared by
279 publication in relevant journals or in established method databases. (e.g., in IOC's Ocean Best
280 Practices System or at protocols.io).

281 33. The number of replicates required within each physiographic unit will depend on the
282 existing natural variability (see above) and statistical methods, such as power analysis (Jumars,
283 1981), should be used to decide on the sampling effort required to detect relative changes at an
284 appropriate resolution.

285 34. Uncertainty and limits of detection of methodology should be presented along with any
286 measurements.

287 35. Where data are corrected for depth, temperature, sample size or any other variable,
288 details of the correction should be provided and the exact procedure explained. This should
289 also be accompanied by the raw data.

290 36. Where different methodologies are used as a result of adaptability of sampling
291 strategies or through cooperation across studies, any details about standardisation methodology
292 to make results comparable should be provided.

293 37. Where sampling devices need calibration, this should be done as near to use as possible
294 to their use (e.g. for in-situ microprofiling of pH, the electrodes should be calibrated on board
295 the sampling vessel prior to deployment).

296 38. The information contained in these Guidelines concerns the minimum requirements.
297 Any extra sampling or analysis beyond what is outlined in here will increase the quality and is
298 therefore encouraged.

299 **E. Data and Sample Management**

300 39. Data, samples and specimens should be archived using the appropriate long-term
301 preservation standards to enable revisiting of the raw information should further analysis or
302 quality control be required.

303 40. Raw data should be archived by the contractor in a way that allows to trace them back
304 to their origin including space, time, and methodology used.

305 41. Raw and derived data should be submitted to established and long-term sustained
306 Global Data Assembly Centres that provide open access.

307 42. Digital data, including relevant metadata, should be safely stored locally and in cloud-
308 storage to guarantee its long-term availability and be provided to the secretariat of the ISA as
309 laid out in the International Seabed Authority Recommendations for the guidance of
310 contractors on the content, format and structure of annual reports (ISBA/21/LTC/15).

311 43. Data and findings should be published in international, peer-reviewed and open access
312 scientific journals and presented at international scientific conferences to facilitate the
313 dissemination of new information. Publication also enables feedback and approval from
314 multiple independent experts.

315 44. Latitude and longitude should be collected in decimal degrees, WGS84 and time and
316 date recorded in Coordinated Universal Time (UTC).

317 45. Standard metadata (including position, water depth, expedition and station ID, principal
318 investigator) should be recorded following established metadata standards.

319 46. Detailed information on the sensors and sampling device being used (type,
320 manufacturer, ID, date and method of last calibration) and a detailed description of the
321 measurement and sample analysis methods, including deployment details for sampling
322 equipment, reference to adopted standards, best practices, or method descriptions in scientific
323 publications, should also be provided.

47. Where meta information refers to publications (e.g., cruise reports, method descriptions) persistent identifiers, or duplicates, should be provided to ensure long-term availability.

48. For derived data, relevant metadata and all information that is necessary to reproduce the analyses and/or conversions applied has to be supplied. Reference should be provided to the raw data including core measurements as well as all supporting variables that have been used for calculations. Specific protocols, software, and code that were used should be provided via open access sustained online resources that allow for version control and provide persistent identifiers (e.g., GitHub, Protocols.io).

49. These principles apply to all variables with additional information provided in each section below.

IV. PHYSICAL OCEANOGRAPHY

A. Introduction

50. The main objectives for establishing a baseline of the physical oceanography of a contract area are:

- To define the hydro physical and hydrodynamic conditions and structure of water column and its variability in order to:
  - understand the habitats of marine organisms and
  - define the detailed sampling strategy for other sampling measures
- To assess the potential dispersion and size of any operational and discharge plume

51. The following variables should be determined in order to define the physical oceanography baseline:

- Temperature, pressure and salinity – The sea-water parameters that discrete water masses within which other variables should be measured and determine water column stratification. These variables will also be required when deriving information from other data.
- Currents - The knowledge of currents is crucial to understand the connectivity of populations and to assess the dispersion of any operational and discharge plumes.
- Tides and Waves - Tides and waves interact with current flow to influence mixing processes. Tides also have effects on some marine organisms (tides cycles).
- Turbulence - Vertical turbulent mixing is a dominant factor in controlling vertical material flux in the water column and bottom-enhanced turbulent mixing has an important role on water mass transformation.
- Optical properties - Light penetration and its availability are crucial for many processes in the upper part of the water column including the formation of biomass by oceanic phytoplankton through photosynthesis, biogeochemical cycling through photochemical reactions and the heating of the upper ocean.
- Noise - Noise is created by numerous sources located both inside the ocean and on its surface and can affect communication in marine mammals and other marine organisms.

B. Sampling Resolution

52. Many of the physical sampling methods should be taken from collocated devices, greatly increasing the resolution and this should be achieved wherever possible.

53. Variability in physical parameters should be determined using different sampling methodology as follows:

- Spatial variability (vertical) – Stations (CTD and water samplers), LADCP, floats/drifter, AUVs/Gliders, ship mounted ADCP
- Spatial variability (horizontal) – Sections (CTD and water samplers), floats/drifters, AUVs/Gliders, ship mounted ADCP, satellite remote sensing
- Temporal variability – Moorings/buoys with ADCP or other current meters, repeat stations/sections, floats/drifters, bottom landers, satellite remote sensing

54. Oceanographic and hydrochemical measurements and sampling should be undertaken at the same stations where biological sampling is performed. In cases when distances between physiographic zones are more than 50km, it is recommended that additional stations of at least one station every 50km both in latitudinal and longitudinal directions are included, with higher resolution of every 10-30km in areas of significant horizontal gradients or large-scale topographic features.

55. Use of CTD with additional sensors (e.g., turbidity, dissolved oxygen, pH, fluorescence, PAR, etc.) combined with a rosette water sampler should be used to study vertical variability both of physical and chemical properties of the water column. The resolution of sampling for physical parameters will be higher than for other parameters and as such in addition to the depths noted in section III.A, sampling should be performed at of 0m, 10m, 25m, 30m, 50m, 75m, 100m, 125m, 150m, 200m, 250m, 300m, then every 100m down to 1600m, 1750m, 2000m, and then every 500m to 200m above the seabed.

56. This sampling scheme should be modified as required to ensure all-important features of the water column are captured.

57. To study diurnal variability of the water column properties, a diurnal station should be established for each physiographic unit with samples taken from surface to the depth of 200 meters. As noted in section III.A, sampling should also be repeated every season for at least three years to determine annual and inter-annual variability.

58. The use of a combination of a limited number of ADCP mounted on different carriers should be used to obtain and sample data on the spatial (vertical and horizontal) and temporal variability of currents. Lowered ADCP (LADCP) (combined with CTD or in isolation) should be used to obtain high quality absolute depth velocity profiles. Use in combination with a ship-mounted ADCP and/or a secondary ADCP pointing upward improves quality of the data obtained (Thurnherr *et al.*, 2010). Current measurements should be determined throughout the water column and in addition to the depths noted in section III.A at the following depths, surface, 10m, 25m, 50m, 100m, 200m, 300m, 500m, 750m, 1000m, 1200m, 1500m, and then every 500m to 200m above the seabed. This scheme should be modified if the vertical structure of water masses indicates it is required.

59. Ship-mounted ADCP provides data on spatial distribution of currents at the upper layer of the water column up to 600/800 – 1000/1600m (depending on the model). However, there

407 is large measurement error for measurements for long ranges (800m and 1600m respectively).
408 To provide better resolution in the upper 100-200m, a combination of two ship mounted ADCP
409 (e.g., OS75 or OS38 with OS150 or WH300) should be used (Firing and Hummon, 2010).

410 60. Moorings with ADCP (or other current-meters) should be used to study temporal
411 variability of currents and other water column characteristics. At least one mooring per
412 physiographic zone, and ideally one per physiographic unit, should be used (depending on the
413 presence of large-scale topographic features). In cases where distances between physiographic
414 zones are far more than 50km, additional moorings should be deployed with one mooring in
415 each 50km x 50km area (with higher resolution in case of significant horizontal gradients or
416 large-scale topographic features). Moorings should be deployed for a minimum of 12-13
417 months (to cover one annual cycle) with longer deployments providing better information. The
418 number of ADCP (or other current-meters) should ensure detailed coverage of the near-bottom
419 200 metres. The use of additional ADCP (or other current-meters) in surface, intermediate and
420 abyssal layers is strongly recommended.

421 61. Recommendations are available in the published literature for lowered ADCP
422 (LADCP) (Thurnherr *et al.*, 2010), ship-mounted ADCP (Firing and Hummon, 2010) and
423 towed ADCP and ADP (Sgih *et al.*, 2001).

424 62. Sediment traps and other relevant equipment should be deployed at moorings in order
425 to obtain data on the temporal variability of other water characteristics and sedimentation
426 processes.

427 63. Floats and drifters should also be deployed to study temporal variability of currents at
428 appropriate depths.

429 **C. Measured variable – Temperature and Salinity**

430 64. CTD-profiling of water-column or remote sensing by ROV, AUV or glider should be
431 used to characterise the physical conditions of the water column. Seawater should be described
432 following the Thermodynamic Equation of Seawater - 2010 (TEOS-10) standard. Besides a
433 standard configuration that measures pressure (converted into depth), conductivity (converted
434 into salinity) and temperature, any CTD sampling should be complimented by additional
435 sensors for other parameters where possible (e.g., turbidity, dissolved oxygen, pH,
436 fluorescence, photosynthetically active radiation, nitrates, altimeter). Key considerations for
437 the collection of quality CTD data and data standards are given in ICES Data and Information
438 Group (DIG), 2006.

439 65. CTDs or appropriate sensors can be mounted on drifters/floats, moorings/buoys or
440 bottom landers or used as underway CTD (UCTD). Underway CTD is when the probe is
441 launched from portable or fixed launchers and then is recovered by reeling the line back.

442 66. Satellite remote sensor measurements should be used for getting information about
443 oceanographic parameters on a synoptic time-scale. In addition to surface temperature and
444 surface salinity, sea ice distribution, wave height, surface height, radar backscatter, ocean
445 colour can also be measured by satellites. A large amount of information about satellites and
446 data sets can be found on the websites of NASA (in particular, NASA Jet Propulsion
447 Laboratory's Physical Oceanography DAAC (PO.DAAC)), NOAA, the European Space
448 Agency (ESA), and the Japan Aerospace Exploration Agency (JAXA).

449 67. Drifters and floats can have sensors for measurements of sea surface temperature/
450 seawater temperature, sea surface pressure/ seawater pressure, sea surface salinity/ seawater
451 salinity, wind velocity, dissolved oxygen concentration, fluorescence and ocean colour, mixed
452 layer temperature, partial pressure of dissolved carbon dioxide (pCO_2). They also can be used
453 to collect biological information (e.g., about dispersion of fish larvae, etc.) and to study currents
454 and ocean waves. Key considerations for the collection of quality buoy data, data standards and
455 processing are given in documents of Data Buoy Cooperation Panel, Drifter Data Management
456 Team (IFREMER) and the Argo program community.

457 **D. Measured variable – Currents**

458 68. Currents should be determined using both Eulerian methods (time-series measurement
459 of current speed and direction at fixed location) and Lagrangian methods (the path followed by
460 each fluid particle is observed as a function of time) to enable a holistic view. For Eulerian
461 methods, either mechanical current meters or non-mechanical current meters could be used.
462 Protocols and methodologies of the FixO3 (Fixed point Open Ocean Observatory Network)
463 project should be used for moorings and other types of Eulerian systems (Coppola *et al.*, 2016).
464 For Langrangian methods, surface drifters, subsurface floats or "pop-up" floats drifters or floats
465 could be used. Satellite images of sea surface temperature and colour could be used as "pseudo-
466 drifters" to study surface currents with the assumption that the entire displacements of surface
467 features seen in the imagery are caused by surface current advection. A brief review and
468 references to all the different methodologies, including the advantages and disadvantages of
469 each, can be found in Thomson and Emery (2014).

470 69. The data obtained should be used to develop and validate a numerical circulation model.
471 Coupled with an adequate sediment transport model this model will integrate the effects of
472 particle aggregation and disaggregation that can be used to understand the potential dispersion
473 of operational and discharge plumes.

474 70. An important step of current data analyses is their graphical representation. More details
475 can be found in Joseph (2014) both for both measured and modelled data.

476 71. The parameters that should be measured depend on the equipment used but should
477 include magnitude and direction of current velocity, zonal and meridional velocity components
478 and vertical velocity.

479 72. From these measurements, the current regime of the water column and especially of the
480 layer from the bottom boundary layer up to 200 metres above the seafloor should be
481 characterised. This should include analyses of field structure; spatial variations of current
482 velocity and direction (with particular attention to areas of complex geomorphology); and
483 temporal variations of current velocity and direction. Temporal variability should be
484 characterised diurnally, seasonally and inter-annually.

485 **E. Measured variable – Tides and Waves**

486 73. Tides should be measured either using pressure sensors on fixed mooring or satellite
487 altimetry. Modern oceanographic instruments on fixed moorings can resolve pressure
488 variations to a fraction of millimetre at full ocean depth, but for accurate depth measurements
489 they require temperature correction and information on the pressure sensor drift (roughly 1
490 cm/year). The use of dual pressure sensors helps to correct such drift. Consideration on sea
491 level measurement and interpretation can be found in IOC manuals. Satellite altimetry can be

492 used to determine tides by estimating variability of the sea surface from repeated passes of the
493 satellite radar. Altimetry data (including data from TOPEX/Poseidon, Jason-1, ERS-1 and
494 ERS-2, EnviSat, DORIS) and the necessary software and handbooks, are available through the
495 Web site AVISO (Archiving, Validation and Interpretation of Satellite Oceanographic data:
496 https://www.aviso.altimetry.fr/)

497 74. Any of the commonly accepted methods for determining surface gravity wave
498 measurements should be used. These include satellite altimetry, wave buoys with
499 accelerometers, wave gauges (including resistance-type, capacitance-type, and wave pressure
500 gauges) or Synthetic Aperture Radars on satellites.

501 75. The parameters that should be measured are pressure or sea level data depending on
502 whether fixed mooring or satellite altimetry is used.

503 76. From these measurements, tidal amplitude and period, main tidal constituents and
504 inequality, wave height and direction should be determined.

505 **F. Measured variable – Turbulence**

506 77. Either of the two accepted methods for the estimation of turbulent intensity should be
507 used. They are the direct method using the data from the velocity shear probe and indirect
508 methods using the data from CTD or Acoustic Doppler Current Profiler (ADCP), Acoustic
509 Doppler Velocimeter (ADV) or DopplerCurrent Profiler (DCP) (Thorpe, 2007).

510 78. Observations to determine turbulence intensity should be made as close to the sea floor
511 as possible. Because the bottom-enhanced turbulence generally propagates upward across the
512 bottom boundary layer, field measurements should be made up to the ocean interior including
513 entire bottom boundary layer. Turbidity near the bottom is closely related to turbulence
514 intensity so turbulence measurements should be combined with turbidity investigation (section
515 G). When using the direct method, a horizontally profiling microstructure probe attached to an
516 Autonomous Underwater Vehicle (AUV) is recommended to infer the spatial distribution of
517 turbulent intensity. If the Thorpe scale method is used, CTD casting should be made very
518 accurately as close to the bottom as possible. If the Acoustic Doppler method is used, the
519 current profiler mooring should be placed on the seafloor floor.

520 79. The parameters that should be measured depend on the methodology used:

521 • For direct measurement - Microscale velocity shear, lowering speed of
522 instrument, lateral acceleration of instrument, high-resolution temperature
523 • For indirect measurement – Temperature, conductivity, pressure and velocity

524 80. From these measurements, turbulent kinetic energy dissipation rate, density, buoyancy
525 frequency, vertical velocity profile, microstructure temperature fluctuations, vertical eddy
526 diffusivity, Thorpe Scales and temperature dissipation rates should be determined.

527 **G. Measured variable – Optical Properties**

528 81. Optical properties of seawater can be divided into Apparent Optical Properties (AOPs)
529 and Inherent Optical Property (IOP).

- Apparent Optical Properties depend on the nature of seawater and its dissolved material and particulates along with the angular distribution (geometry) of solar radiation and should be measured using spectroradiometers that use a variable monochromator to separate the light into specific wave bands.
- Inherent Optical Property depends upon light's wavelength and the aquatic medium but is independent of the ambient light field and its angular distribution and should be measured using a monochromatic beam attenuation meters (transmissometer), spectral absorption-attenuation meters, scattering (or backscattering sensors), liquid waveguide capillary cells, laser diffraction instruments or flow cytometry.

82. Optical properties should be obtained using one of the following:

- Physical shipboard sampling at stations (vertical profiling and sampling, tethered or hand-held radiometric measurements) or when underway (ship-mounted, tethered or hand-held radiometric measurements; sampling using flow-through systems or towed undulating or fixed depth devices or chains with appropriate sensors).
- Measurements from AUVs, gliders, fixed Eulerian platforms (moorings, bottom tripods and other bottom landers) and/or Lagrangian devices (drifters and floats).
- Remote sensing from shipboard, aircraft, or satellite platforms. These measurements can be passive (the Sun is the source of illumination) or active (a signal from the sensor platform is used as such source, generally laser illumination is applied)

83. Optical properties should also be determined using inverse (See details in Werdell *et al.,* 2018) or bio-optical models (see details in Ogashawara, 2015)

84. Different types of fluorometers can be used to measure fluorescence, or photo-emission and bioluminescence (which can also be an addition to modern acoustic methods for biomass estimation). More details on each can be found in Moore *et al.* (2009), and references therein. The sensors for measuring turbidity can be in a variety of configurations and there are numerous methods and configuration standards for them (e.g., ISO 7027. See also Petihakis *et al.*, 2014; Tamburri, 2006). Remote sensing of fluorescence and bioluminescence can also be used to measure plankton fluorescence from satellites (e.g., Erickson *et al.*, 2019).

85. The sensors for measuring turbidity (nephelometers and backscatter sensors) can be in a variety of configurations and there are numerous methods and configuration standards for them (e.g., ISO 7027. See also Petihakis *et al.*, 2014; Tamburri, 2006).

86. The parameters that should be measured (depending on the methodology) are radiance, irradiance, scalar irradiance, light diffuse attenuation coefficient, attenuation coefficient for scalar irradiance, Photosynthetic Available Radiation, irradiance reflectance, radiance reflectance, absorption coefficient, scattering coefficient, beam attenuation coefficient, volume scattering function (VSF), ocean colour, fluorescence, bioluminescence, transparency, turbidity.

87. From these measurements, chlorophyll a and other pigments, visibility, suspended sediment volume, phytoplankton biomass, concentration of particulate and dissolved organic carbon (POC and DOC), productivity in the form of POC, species composition (for the

573 presence of harmful algal blooms and nitrate analysis) should be determined (also see sections
574 V.H and VII.D).

575 88. Optical measurements can also be used for validation and calibration of remote sensing
576 measurements.

577 **H. Measured variable – Noise**

578 89. Two noise characteristics should be determined, ambient noise and the patterns of
579 sound propagation. The fundamental mechanisms, measurements and numerical modelling of
580 oceanic ambient noise can be found in Carey and Evans (2011). Noise measurements can be
581 made from ships (at stations or underway), AUVs, gliders, floats, drifters, moorings, buoys,
582 bottom landers and tripods. It should be taken into consideration that some other sensors create
583 noise so single hydrophone tripods or hydrophone arrays should be connected some distance
584 from the instrument platform for noise reduction. Sound velocity should be measured directly
585 (with help of a sound velocity profiler or sensor) or derived from values of temperature, salinity
586 (conductivity) and pressure measured by a CTD (see section C). The method for deriving sound
587 velocity is described in Wong and Zhu (1995).

588 90. The parameters that should be measured are noise levels and, potentially, sound
589 velocity.

590 91. From these measurements ambient noise levels in vertical profiles through the water
591 column from the sea surface to the seabed, temporal variability in ambient noise levels, the
592 depths of the sound fixing and ranging (SOFAR) channel and sound velocity (if not measured
593 directly) should be determined.

594 **I. Data quality**

595 92. The different analysis techniques, including statistical methods, that should be used for
596 data acquisition, processing and presentation, error handling, analysis of spatial data fields and
597 time series of such techniques and methods can be found in Thomson and Emery (2014).

598 93. To obtain the highest quality data, corrections should be applied to the CTD sensors.
599 Calibration procedures will vary from one laboratory to another, but it is generally accepted
600 that whilst the pressure and temperature sensors can be subject to pre- and post-cruise
601 calibrations in the laboratory, the conductivity sensor is best calibrated by comparison with
602 samples collected for salinity analysis (ICES Data and Information Group (DIG), 2006;
603 Petihakis *et al.*, 2014 and information and manuals from manufacturers).

604 94. For data quality control of CTD data, then information from EuroGOOS DATA-MEQ
605 Working Group (2010), IOC (2010) or U.S. Integrated Ocean Observing System (2020a,
606 2020b) should be used

607 95. For data quality control and correction associated with AUVs and gliders, Allen *et al.*
608 (2018, 2020); U.S. Integrated Ocean Observing System (2016) and Woo (2011) should be
609 consulted and the EGO Gliders Data Management Team (2020) for data management

610 96. For measurements of sea-surface temperature and salinity Le Menn *et al.* (2019) and
611 Data Buoy Cooperation Panel (2011) should be consulted.

612 97. For more information on the different types of drifters and floats, opportunities and
613 advantages of their use, constraints and innovations, see Lumpkin *et al.* (2017).

614 98. Values of temperature should be converted to potential temperature considering the
615 effect of hydrostatic pressure. Density (potential density) should be calculated indirectly from
616 salinity, temperature (potential temperature) and pressure using the equation of state (TEOS-
617 10).

618 99. Guidance on ADCP data quality control can be found in U.S. Integrated Ocean
619 Observing System (2019a) and EuroGOOS DATA-MEQ Working Group (2010). Information
620 on mooring data correction and processing (ADCP, RCM, Microcat) can be found in
621 Karstensen (2005).

622 100. Calibration is crucial for accurate measurements of the noise and the following
623 guidelines and publications should be referenced for calibration deltails, Biber *et al. (*2018);
624 Robinson *et al.* (2014) and for quality control U.S. Integrated Ocean Observing System (2017).

625 101. Any models should be validated and accepted by the ocean modelling community. Van
626 Sebille *et al.* (2018) provides a review of Lagrangian codes for online and offline particle
627 tracking with references to relevant literature (see also Numerical Models, 2000).

628 102. Spatial resolution of modern radiometers is 1 km (Advanced Very High-Resolution
629 Radiometer (AVHRR)), but they can work only in cloudless weather. Passive microwave
630 sensors can observe even in cloudy conditions because they use longer wavelengths (6-12
631 GHz), but they have much poorer spatial resolution (25-50km) (Talley *et al.*, 2011). Microwave
632 radiometers can be used to measure sea surface salinity with spatial resolution of 50-100km at
633 temporal scales of week to month respectively (Talley *et al.*, 2011, Thomson and Emery, 2014).
634 In addition to surface temperature and surface salinity, sea ice distribution, wave height, surface
635 height, radar backscatter, ocean colour can also be measured by satellites. More information
636 about satellite remote sensing can be found in the literature (e.g., Stewart, 1985; Robinson,
637 2004; IOC, 1992), documents of the International Ocean-Colour Coordinating Group (IOOGP)
638 and Ocean Optics Protocols for Satellite Ocean Colour Sensor Validation).

639 103. Over the past decades, large datasets have been accumulated under different
640 international scientific programmes. These data are in open access and should be used for
641 comparison with baseline data collected for quality assurance. Examples are:

642 • World Ocean Circulation Experiment (WOCE) 1990 – 2002:
643 https://www.nodc.noaa.gov/woce/wdiu/;
644 • WOCE Subsurface Float Data:
645 https://www.aoml.noaa.gov/phod/float_traj/index.php;
646 • World Ocean Database (WOD)
647 https://www.nodc.noaa.gov/OC5/WOD/pr_wod.html;
648 • The Global Temperature and Salinity Profile Programme (GTSPP):
649 https://www.nodc.noaa.gov/GTSPP/;
650 • SeaDataNet: https://www.seadatanet.org/;
651 • CORA: Coriolis Ocean database for ReAnalysis:
652 http://www.coriolis.eu.org/Data-Products/Products/CORA;
653 • PANGEA data repository: https://www.pangaea.de/?t=Oceans

- the Global Drifter Program (GDP) (formerly the Surface Velocity Program (SVP): https://www.aoml.noaa.gov/phod/gdp/index.php;
- Global Ocean Currents Database (GOCD): https://www.ncei.noaa.gov/products/global-ocean-currents-database-gocd;
- the ARGO floats: http://www.argo.ucsd.edu (ARGO home page) and http://www.argo.net (the International Argo Project Home Page); https://biogeochemical-argo.org/ (Data of biogeochemical ARGO float);
- Archived Drifter Data, Integrated Science Data Management, Fisheries and Oceans Canada: http://www.dfo-mpo.gc.ca/science/data-donnees/drib-bder/index-eng.html
- Electronic atlases can also be useful:
    - World Ocean Atlas 2018 (WOA18): https://www.nodc.noaa.gov/OC5/woa18/;
    - eWOCE: https://www.ewoce.org/.

J. Data Management

104. Data and metadata should be provided to the ISA as outlined in section III.E. Additional guidance for specific variables can obtained from references noted above.

V. CHEMICAL OCEANOGRAPHY AND BIOGEOCHEMISTRY

A. Introduction

105. An understanding of the chemical environment of the water column and sediments (that is porewaters and solid fraction) is required to characterize baseline oceanographic and biogeochemical conditions in order to later assess both direct impacts of mining activities on the seafloor as well as indirect impacts from suspended sediment plumes that may be produced, including potential blanketing of the seafloor and impacting processes in the water column.

106. The development of suspended sediment plumes largely depends on the future mining techniques. They could potentially transfer over larger distances (1-10s km), may have a different chemical composition to the surrounding water, and will resettle away from the source and as such have potential to impact pelagic and benthic ecosystems, their functions and marine biogeochemical cycles in larger areas.

107. Marine biogeochemistry focuses on seafloor processes and functions and combines studies of chemical conversions with the observations of the biological and geological processes involved. Observations focus on benthic processes that are involved in the remineralization of the organic material exported from surface waters in a cascade of redox reactions. Measurements are mostly based on sediment sampling and subsequent, layer-wise extraction of pore waters and solid-phase subsamples for analyses. In some cases, such as oxygen uptake rates and pH distribution, measurements need to be obtained directly at the seafloor (i.e. in situ). For all porewater variables that will later be used to quantify pore water release and plume dispersion additional sampling should target the bottom water to construct a baseline that allows the identification of the release of solids or pore waters as a consequence of seafloor disturbances or discharge of material and their effect (i.e., the distribution, transport, and conversion of the reactants and products of these reactions).

108. The chemical variables that should be measured in the water column, sediments and pore water are:

- Nutrients - The availability of inorganic macronutrients (nitrate (NO_3), nitrite (NO_2), ammonium (NH_4) phosphate (PO_4), silicic acid ($Si(OH)_4$)) in the upper ocean frequently limits and regulates the amount of organic carbon fixed by phytoplankton, thereby constituting a key control mechanism of organic matter availability at the seafloor. Nutrient concentrations in porewaters (nitrate (NO_3), nitrite (NO_2), ammonium, phosphate) provide information on biogeochemical cycling of organic matter and redox conditions in different sediment layers.
- Oxygen - Oxygen concentrations in the water column provide information on organic matter production in the surface layer and its remineralization during export towards the seafloor and the distribution of oxygen in the sediment and the flux across the sediment-water interface provides a measure of benthic organic matter remineralization and the activity of the benthic community.
- Carbonate system – This system constrains primary production, organic carbon remineralization, metal oxidation in sediment plumes, ocean acidification and deoxygenation in the water column as well as organic matter remineralization and secondary redox reactions and induced porewater-mineral reactions in the sediment which all affect ecosystem functions.
- Trace metals - Many trace metals are essential elements for the maintenance of cellular functions in microorganisms, however, under elevated concentrations, those elements may result in potential toxicity that is metal, and organism, dependent.
- Organic and Inorganic Matter - The provision of organic matter to the seafloor is the key driver of biogeochemical processes and provides food to sustain biomass and biodiversity of benthic organisms as the interaction in the benthic food web. Observations in the water column address productivity and export while measurements at the seafloor quantify the amount and quality of the organic material that is available to seafloor and the dynamics of benthic organic matter cycling.
- Radioactive isotope tracers (Radiotracers) – The analysis of radioisotopes associated with the solid sediment phase is required for the quantitative characterization of bioturbation activity in the sediments and the determination of sedimentation rates. The distribution of naturally occurring radioisotopes serves as a baseline to determine direct impacts of mining activities on the sediments and water column (including the release of porewaters) as well as enabling the assessment of radioisotopes in the nodules once mining commences.

B. General Methodology

109. For most of the chemical and biogeochemical variables, community-wide accepted methods exist and these should be used to ensure high-quality accurate and precise data that are comparable across licence areas and contractors.

110. Water column chemical parameters should be sampled using the most relevant of the 4 techniques:

- water bottle sampling with CTD casts: for nutrients, oxygen, carbonate system, trace metals (using trace metal-clean CTD/Go-Flo bottles), dissolved organic

740 matter (DOM), suspended particulate material (SPM) including particulate
741 organic matter (POM)

742 • in situ pumps for radioisotope activity, trace metals, and SPM concentrations
743 • moored and tethered sediment traps for particle concentrations and particle fluxes
744 • BGC-Argo (Biogeochemical Global Array of Profiling Floats) for pH, nitrate,
745 oxygen, etc

746 111. While CTD stations, in situ pump deployments, and tethered sediment traps require
747 stationary work limiting the flexibility of data acquisition, moored sediment traps should be
748 deployed in the water column for up to 2 years for time-resolved observations. In addition,
749 autonomous floats, drifters etc., equipped with (bio)chemical and optical sensors, should be
750 used to provide spatial and temporal data for chemical variables.

751 112. Samples for sediment and pore water analysis should be obtained using a multicorer or
752 ROV-pushcores for the top decimetres of sediments and a gravity corer for deeper samples.
753 The method publications of the Integrated Ocean Drilling Program (IODP), the Global Ocean
754 Ship-based Hydrographic Investigations Program (GO-SHIP), the GEOTRACES initiative,
755 and in the Ocean Best Practices repository hosted by the International Oceanographic Data and
756 Information Exchange (IODE) of the Intergovernmental Oceanographic Commission (IOC)
757 should be consulted for commonly accepted and agreed methods for chemical oceanographical
758 and biogeochemical sampling.

759 113. Pore water should be extracted using appropriate methods for each variable directly
760 after the recovery of cores and, where possible, as many biogeochemical variables as possible
761 should be determined from the same porewater samples. The process of porewater extraction
762 should be undertaken within a couple of hours of collection. For some dissolved components
763 that are expected to change rather slowly (e.g., phosphate and silicic acid) the porewater
764 samples can be stored at -20°C or -80°C until they are returned to shore for analysis. Sediment
765 cores not investigated for porewater can be stored at 4°C or colder (before subsampling into
766 sediment horizons). For some sensitive constituents (e.g., nutrients), pore water analysis should
767 be undertaken on board as soon as possible after pore waters are extracted from the sediment
768 while other analyses may be performed in the onshore lab on samples transported frozen or
769 cooled and appropriately preserved.

770 114. As biogeochemical processes and solute fluxes across the sediment-water interface are
771 affected by conditions in the overlying water, the water overlying the sediment in the core liner
772 should always be sampled as the seawater "endmember" for the pore water and, as this may be
773 altered during recovery or sample handling, it should be compared to the deepest water column
774 samples from the CTD.

775 115. Sampling of suboxic sediment and pore water should be conducted in a glove bag under
776 an oxygen-free atmosphere (filled with an inert gas e.g. Nitrogen or Argon) to preserve metal
777 speciation and other redox sensitive variables.

778 116. References to existing up-to-date best practices are provided under each variable,
779 noting where modifications are required to be relevant to deep-sea mining purposes. If no
780 common best practice exists yet (e.g., colloidal/nanoparticle size fractionation for trace metals)
781 a methodology is recommended and reference to state-of-the-art scientific publications are
782 provided. The Global Ocean Observing System (GOOS - www.goosocean.org/) is a sustained
783 collaborative system of ocean observations, encompassing in situ networks, satellite systems,

784 governments, UN agencies and individual scientists and the majority of the variables belong to
785 the Essential Ocean Variables (EOVs) as defined by the Global Ocean Observing System
786 (GOOS).

787 117. As methods may be subject to change (e.g. new technology developments) best practice
788 online repositories should be used to capture methodology updates. The Ocean Best Practices
789 Repository (https://repository.oceanbestpractices.org) is recommended as a hub to search and
790 find existing best practices in ocean research, observation and data/information management.
791 The Ocean Best Practices System Repository (OBPS-R) is an open access, permanent, digital
792 repository of community best practices in ocean-related sciences and applications maintained
793 by the International Oceanographic Data and Information Exchange (IODE) of the UNESCO-
794 IOC as an IOC (IODE, GOOS) coordinated activity.

795 **C. Sampling Resolution**

796 118. Archived remote sensing satellite altimetry and sea surface temperature data, ocean
797 colour data, and hydrography data from data repositories should be used to approximate the
798 expected spatial and temporal variations of surface oceanographic features controlling primary
799 productivity within a licence area. This information should be combined with information
800 about oceanic and atmospheric processes in order to identify the appropriate temporal and
801 spatial sampling strategy for chemical parameters in the water column within a specific region
802 to cover zones of different primary productivity and changing oceanographic features. At least
803 one CTD station and two sediment traps shoudl be established in the water column above each
804 impact reference zone (IRZ), preservation reference zone (PRZ), and intended mining area
805 within the contract area. In addition, transects with regularly spaced CTD stations at distances
806 of about 100 km should be conducted throughout the licence area.

807 119. For water column measurements, samples should be taken throughout the water
808 column, ensuring all zones identified by the physical oceanographic data (section IV) are
809 characterised (e.g. mixed surface layer, the pycnocline, the extent of the oxygen minimum
810 zone, and the individual oceanographic water masses in the thermocline, intermediate and
811 deep-water regions).

812 120. As noted in paragraph 19, a higher vertical sampling resolution is recommended near
813 the seabed as this covers the expected vertical space for the dispersal of the operational plume
814 and is also the most likely depth for the dispersal of the discharge plume. If the depth of the
815 discharge plume is still to be determined at the time of the baseline studies, all potential release
816 depths should be characterized.

817 121. Integrated data acquisition with CTD water sampling, in situ pumping and sediment
818 trap deployment should be undertaken and for the assessment of natural benthic (metal) fluxes
819 from the sediment into the overlying bottom water sampling should be performed as close to
820 the seafloor as possible. Besides point sampling with CTD, this should include long-term
821 deployments of passive samplers along a vertical gradient from the seabed up to 10 m above
822 the seafloor.

823 122. Sampling should be collocated wherever possible (section III.C) and follow the nested
824 stratified sampling scheme and the general considerations to cover spatial and temporal
825 variability (section III.A) with further details provided for specific variables in the sections
826 below.

827 **D. Measured variable - Nutrients**

828 123. The recommended best practices approach that should be used for the determination of
829 dissolved inorganic macronutrients, (Nitrate (NO_3^-), Nitrite (NO_2^-), (Phosphate (PO_4^{3-}) and
830 Silicic acid ($Si(OH)_4$)) in both the water column and-porewater is documented in the (revised)
831 GO-SHIP manual by Becker *et al.* (2019) and in the standard protocols of Gieskes *et al.* (1991)
832 and Grasshoff *et al.* (1999). Measurements should be performed using continuous (segmented)
833 flow analysis (CFA/SFA) methods with certified reference material (CRM) and/or reference
834 material for nutrients in seawater (RMNS) to ensure quality control during analysis.

835 124. Even with high-precision equipment, quantification of ammonium in deep-sea
836 porewaters is difficult because of very low concentrations and, therefore, in cases where
837 concentrations prove to be close to the detection limit, the determination of porewater
838 ammonium can be excluded until better analytical methods become available. Silicic acid in
839 deep-sea porewaters does not have high diagnostic potential for the determination of the
840 benthic geochemical system, and thus, can also excluded from baseline observations.

841 125. Nutrient concentrations, particularly nitrate and nitrite, should be determined
842 immediately after sampling or analysed within 1-2 weeks if, upon collection, the water and
843 pore water samples are immediately frozen at – 80°C.

844 126. The methodology for the determination of seawater and porewater nitrate and nitrite
845 (and concomitantly phosphate and silicic acid using SFA) concentrations that should be used
846 is:

847 - A few mL of freshly extracted (or freshly thawed) untreated (pore)water should
848 be analysed usually upon 3-fold dilution (porewater) or 2-fold dilution (water),
849 while the SFA-system is constantly flushed with nitrogen.
850 - Total NOx (nitrate + nitrite) concentrations should determined colorimetrically at
851 520-540 nm after the reduction of nitrate to nitrite at pH 8 using a copperized
852 cadmium coil.
853 - Nitrite is measured separately colorimetrically at 520-540 nm after its
854 reaction with sulphanilamide under acidic conditions.
855 - Nitrate concentrations are determined by the subtraction of measured
856 nitrite concentration from total NOx values.
857 - Phosphate should be determined colorimetrically at 820 nm (dihydrazine sulfate)
858 or 880 nm (ascorbic acid) using the molybdenum blue method.
859 - Silicic acid should be determined colorimetrically at 660 nm (stannous chloride)
860 or 820 nm (ascorbic acid) as silica molybdate complex.

861 127. Data should be reported in mol/L (nmol, µmol, mmol - depending on the specific
862 concentration range of the constituent) and solid-phase data in mg/kg or wt.%. Data should
863 always be reported with blank information (if applicable), limits of quantification (LOQ) as
864 well as CRM/RMNS results. Each sample should be analysed in duplicate or triplicate
865 measurements. Analytical precision for each sample should not exceed > 5 %RSD. Calibrations
866 for each pore-water nutrient constituent should be performed using IAPSO (International
867 Association for the Physical Sciences of the Ocean) standard seawater with at least six
868 standards. The coefficient of determination (r2) for each calibration curve should be > 0.98.
869 Average nutrient concentrations should be calculated from duplicate or triplicate measurements

870 and displayed as depth plots. Information on the analytical quality (i.e., accuracy, precision)
871 during measurement should be indicated.

872 128. The parameters that should be measured in both the water column and porewater are
873 Nitrate (NO_3^-), Nitrite (NO_2^-), Ammonium (NH_4^+), Phosphate (PO_4^{3-}), Silicic acid ($Si(OH)_4$)

874 129. From these measurements primary production (water column only), respiration rate,
875 remineralization, deoxygenation and benthic fluxes should be determined along with the redox
876 zonation within the sediment.

877 **E. Measured variable – Oxygen**

878 130. The methodology that should be used for measuring oxygen distribution in the water
879 column is described in Langdon (2010), McTaggart *et al.* (2010), and Uchida *et al.* (2010).
880 Bittig *et al.* (2018) should be consulted for review on optodes. An automated laboratory method
881 that could be used, with software support, is presented by the Oceanographic Data Facility at
882 SCRIPPS (https://scripps.ucsd.edu/ships/shipboard-technical-support/odf/chemistry-
883 services/dissolved-oxygen).

884 131. Observations of oxygen at the seafloor should cover both measurements of oxygen
885 consumption as well as the depth of penetration of oxygen into the sediments. Consumption
886 measurements focus on the upper sediment layer and need to be carried out *in situ* (i.e., directly
887 at the seafloor). Measurements of oxygen distribution along the sediment column should be
888 obtained in the laboratory from retrieved cores obtained with Multicorers (for the top
889 decimeters) and with gravity cores to determine the penetration depth, (i.e., the depth where
890 oxygen concentration drops to zero) (e.g., Mewes *et al.,* 2014). Oxygen should be measured
891 with sensors, either optical oxygen sensors (optodes) or Clark-type electrodes to allow for
892 measurements at the required spatial resolution and avoid the risk of contamination with
893 atmospheric oxygen associated with sampling-based methods. Microsensors (microelectrodes
894 and fiberoptical optodes) should be used to record vertical profiles of oxygen concentration in
895 pore waters. Larger and temporally more stable optical sensors (macrooptodes) should be used
896 for time series measurements of oxygen in benthic chambers or bottom waters.

897 132. Strong spatial and seasonal dynamics are expected in case of seafloor oxygen uptake so
898 *in situ* measurements carried out during expeditions with microprofilers and / or chambers
899 should cover different time intervals relative to major productivity and export events (e.g., algal
900 blooms, peaks in vertical fluxes, and phytodetritus deposition incidents). To fully address
901 seasonal variability, those measurements should be supplemented by time series of oxygen
902 uptake measurements performed autonomously by repeated profiling and / or chamber
903 incubations (see below) with mobile platforms (benthic crawlers) over longer periods of several
904 months or year-long.

905 133. Oxygen uptake measurements should be determined *in situ* using benthic chambers and
906 microprofilers (Boetius and Wenzhöfer, 2013). Chamber incubations determine total oxygen
907 uptake (TOU) and microprofiler measure diffusive oxygen uptake (DOU). Total oxygen uptake
908 (TOU) is also referred to as sediment community oxygen consumption (SCOC). For DOU
909 measurements, oxygen microsensors are lowered in small vertical steps into the sediments by
910 means of microprofilers. To fully address oxygen uptake, in situ oxygen measurements should
911 generally include both total (TOU) and diffusive oxygen uptake (DOU). If methodology and
912 the quantity being addressed (i.e., TOU or DOU) are consistent throughout the baseline
913 observations, one of the two quantities are considered sufficient. If only one approach is

914 selected, TOU measurements are preferred as they cover the entire sediment community and
915 include oxygen uptake taking place in the nodules and respiration of nodule epifauna. However,
916 DOU measurements, that mostly address microbial respiration, represent an acceptable
917 alternative as the contribution of fauna is typically low in deep-sea sediments and most of the
918 respiration is expected to take place in the sediments rather than the nodules.

919 134. The deployment time for TOU analyses should be long enough for a robust
920 determination of the rate of decrease from the oxygen recordings based on the at the given
921 sensor performance. Diffusive oxygen uptake should be calculated from the oxygen depth
922 profile by matching the measurements with a 1D diffusive transport and respiration model. As
923 *in situ* profiles generally do not reach the oxygen penetration depth in deep-sea environments
924 with low respiration rates, measurements should cover the sediment layer where significant
925 oxygen uptake takes place.

926 135. For vertical profiles, both *in situ* measurements targeting fluxes as well as
927 measurements in cores focusing on oxygen penetration depth, the sensor tip diameter and
928 vertical intervals between consecutive measurements should inversely scale with the slope of
929 the oxygen gradient and, hence, should be smaller in the top decimetres than below. Generally,
930 tip diameters should be <100 µm for the top 0.5m and <1mm in deeper layers. Vertical intervals
931 may start with 250 µm while they can increase to the small cm- to decimetre-range below 0.5m.
932 Changes in concentration in consecutive depth intervals should be well below 2% of the bottom
933 water concentration. *In situ* profiles used for DOU calculations should cover the layer that
934 significantly contributes to the overall oxygen uptake. They should cover at least the top 20cm
935 or reach the depth at which volumetric respiration rates (as determined by 1D transport-reaction
936 modelling) drop to <10 % of the maximum rate observed in the upper part of the profile. In the
937 case of TOU measurements with chambers, frequency of observations is not critical as decrease
938 in oxygen is slow and one reading every couple of minutes suffices. Higher frequencies may
939 be used in case sensor readings depict a large scatter.

940 136. To address oxygen penetration depth and redox zonation throughout the oxic sediment
941 layer, oxygen measurements should be obtained from bottom waters overlying the sediments
942 and continued in pore waters on long cores down to the depth where oxygen drops to zero or
943 reaches a minimum.

944 137. The parameter that should be measured is dissolved oxygen (O_2) with raw data provided
945 as concentrations (mol m^{-3})

946 138. From oxygen observations in the water column, apparent oxygen utilization, Net
947 Community Production (NCP), Net carbon export flux, ocean oxygen inventories,
948 deoxygenation and oxidation consumption due to oxidation of reduced metals should be
949 determined. For the sediments, oxygen penetration depth, volumetric respiration of the
950 different sediment layers, rates of sediment community oxygen consumption / oxygen uptake,
951 carbon remineralization rates, net rates of organic matter flux to the seafloor should be
952 determined. The redox zonation in the sediment should also be characterised

953 **F. Measured variable – Carbonate system**

954 139. Instead of carbonate alkalinity (as described in ISBA/25/LTC/6/Rev.1 and Corr.1) total
955 alkalinity (TA) should be used to characterise the carbonate system as molecules other than
956 HCO_3^- and CO_3^{2-}, such as borate, hydrogen sulphide, and dissolved organic carbon (DOC)
957 typically contribute to this variable.

140. Detailed information on data acquisition of the different variables of the carbonate system, including data quality, should be obtained from the chemical oceanography and biogeochemistry literature such as Dickson *et al.* (2007) and European Commission (2011).

141. Any two of dissolved inorganic carbon (DIC), carbonate alkalinity (CA), pCO_2, and pH, along with pressure, temperature and salinity should be used to constrain the full suite of the seawater carbonic acid system (i.e., $[CO_2]$, $[H_2CO_3]$, $[HCO3^-]$, $[CO3^{2-}]$, $[H^+]$) (Millero, 2013). While total alkalinity is a robust variable of the carbonate system that can be measured ex situ without inducing artefacts, DIC, pH, and pCO_2 are sensitive to changes in pressure and temperature as well as induced degassing upon retrieval of samples from the deep seafloor to the sea surface and therefore, pH and pCO_2 should be measured in situ, using ROV-deployed profiling devices to avoid ex situ sampling artefacts that cannot be corrected during data processing.

142. To account for contributions to total alkalinity from other chemical such as borate and hydrogen sulphide, additional measurement should be taken for sediment porewater. Since it is difficult to measure the individual species, these additional variables are usually total boron concentration (i.e. the sum of borate and boric acid) and total sulphide concentration (i.e. the sum of $[S^{2-}]$, $[HS^-]$, and $[H_2S]$). These are robust variables that can be measured ex situ.

143. The GOOS EOV specification sheet should be consulted for further information on current global observing networks including available sensor techniques (mainly CO_2 and pH, e.g., Biogeochemical ARGO), and future observing capacity.

144. The carbonate system should be determined using total alkalinity and at least one of dissolved inorganic carbon, pH or pCO_2 (Dickson *et al.*, 2007; European Commission, 2011). Other variables, such as total boron concentration, total sulphide concentration, and dissolved organic carbon (DOC), should to be considered as well if contributing to TA (Luff *et al.*, 2001; Zeebe and Wolf-Gladrow, 2001).

145. The methodology for each of these is as follows:

- In porewater samples, Total alkalinity (TA) should be determined in aliquots of extracted pore water by titration with diluted HCl solution observing the pH change spectroscopically, potentiometrically, or optically (e.g. using a suitable pH indicator) and bubbling of solution in the titration vessel with nitrogen or argon gas to strip the produced Carbon dioxide (CO_2) and Hydrogen Sulphide (H_2S) from the solution (e.g., Wallmann *et al.*, 2006; Haffert *et al.*, 2013).
- In water column samples, the methodology outlined in the guidelines of the ocean acidification community, i.e. Dickson *et al.* (2007) and European Commission (2011) should be followed.
- Total Dissolved Inorganic Carbon (DIC) should be determined coulometrically in aliquots of extracted pore water. Samples should be preserved against further microbial degradation by adding $HgCl_2$ solution and stored in tightly closed vials that have been flushed with nitrogen gas to avoid gas exchange with the atmosphere. The DIC should be converted to Carbon dioxide (CO_2) by treating the sample with phosphoric acid and the gas transferred to the coulometer with a purified Helium carrier gas for the measurement. Dissolved sulphides in the sample should be precipitated as Copper monosulphide (CuS) by adding Copper Sulphate ($CuSO_4$) to the sample. In an equivalent procedure the δ13C isotopic

1002 signature of DIC should be determined by isotope ratio mass spectrometry
1003 (IRMS). The DIC stable carbon isotope signature provides additional information
1004 that helps to discriminate organoclastic DIC production from methane oxidation
1005 pathways.

1006 • pH profiles should be determined in situ using glass micro-electrodes (e.g.,
1007 Wenzhöfer *et al.*, 2001; Revsbech and Jorgensen, 1986).
1008 • pCO_2 or dissolved CO_2 concentration should be determined in situ using micro-
1009 optodes (e.g., Wenzhöfer *et al.*, 2001).
1010 • Total boron concentration (TB) should be determined by inductively coupled
1011 plasma optical emission spectrometry (ICP-OES) or inductively coupled plasma
1012 mass spectrometry (ICP-MS) as described in section 6.7.
1013 • Total sulphide concentration (TS) should be determined spectrophotometrically
1014 as methylene blue (Grasshoff *et al.*, 1999; Haffert *et al.*, 2013).
1015 • Total dissolved carbon (DOC) should be determined on the same sample as DIC
1016 as described in section H.

1017 146. As the marine carbonate system is constrained by measuring some of its variables to
1018 calculate the other species (e.g., Luff *et al.*, 2001; Zeebe and Wolf-Gladrow, 2001), the
1019 propagated uncertainties of the calculated variables should be reported. The most important
1020 factor for uncertainty propagation of the marine Carbon dioxide (CO_2) system is the choice of
1021 input uncertainties themselves (Orr *et al.*, 2018). As samples can be preserved easily and the
1022 measurements made with low uncertainty, the measurement of the sum variables TA, DIC, TB,
1023 TS should be used, but other combinations, such as pH and DIC to calculate carbonate
1024 alkalinity and the carbonate species can be used if contributions from borate and hydrogen
1025 sulphide to total alkalinity can be neglected.

1026 147. The use of certified reference material (CRM) samples for both DIC and TA analyses
1027 is a critically important approach for assessing seawater chemistry over time and accurate
1028 calculation of pCO_2 and pH for seawater samples and as such seawater reference material
1029 should be obtained from the International Association for the Physical Sciences of the Oceans
1030 (IAPSO) or the A.G. Dickson Laboratory, Scripps Institution of Oceanography. Dickson *et*
1031 *al.* (2007) should be used as a guide for the calculation of standard deviation of measurements
1032 and for consideration of uncertainty and its propagation the documentation in Orr *et al.* (2018)
1033 should be consulted. These refreremnces should also be used for links to, and documentation
1034 of, the respective add-on routines for the different software packages to calculate carbonate
1035 chemistry variables (seacarb, CO2SYS-Excel, CO2SYS-MATLAB, mocsy). Other open-
1036 access software packages also consider other acid-base systems, such as borate and sulphide,
1037 contributing to pH and TA (AquaENV; Hofmann *et al.* 2010) as well as pressure effects
1038 (SUGARToolbox; Kossel *et al.*, 2013).

1039 148. From these measurements saturation states for carbonate minerals, such as aragonite
1040 and calcite, and silicate minerals, carbonate compensation depth (CCD), lysocline and reaction
1041 rates for carbonate/silicate mineral dissolution, organic matter remineralization, and oxidation
1042 of reduced metals should be calculated and the redox zonation should be constrained.

1043 **G. Measured variable - Trace metals**

1044 149. The GEOTRACES Cookbook should be consulted for specific recommendations on
1045 appropriate sampling, cleaning procedures and sample handling for trace elements (particulates

and total dissolved) and their isotopes in seawater, including procedures to obtain accuracy and precision measures.

150. For the assessment of trace element cycling and toxicity assessments, physical and chemical speciation of dissolved trace metals should be determined, rather than total dissolved concentrations. Methods for physical size speciation of trace metals in the total dissolved pool (which includes colloids and nanoparticles as well as truly dissolved species) are not covered in the GEOTRACES cookbook and there is no best practice guide yet published on this topic so the most up to date literature should be consulted at the time of sampling.

151. For physical size speciation of seawater and pore water, potential methods include:

- Sequential filtration resulting in different size fractions: >0.2 µm (particulates), < 0.02 µm (total dissolved), 0.02 µm to 0.2 µm (inorganic colloids such as Fe oxyhydroxides, clays, Mn oxides), <0.02 µm (soluble: small organic colloids, truly dissolved), on-board
- Ultrafiltration 1KDa MWCO (size pool between 1 KDa to 0.2 µm contains all colloidal and nanoparticulate matter, size pool < 1KDa defined as truly dissolved pool), on-board depending on sample volume availability, which is the main problem when aiming to do ultrafiltration for pore waters

152. Other methods are available to assess chemical speciation including:

- Voltammetric methods, home lab analysis
- Diffusive gradients in thin film (DGT) passive samplers for labile metal concentrations, on-board sampling, home lab analysis

153. Samples should be adequately preserved (e.g. acidification with ultrapure HCl to a pH ~1.8 for trace metal concentration analyses, also see GEOTRACES cookbook for details) or frozen (e.g. chemical speciation analyses, ligand analyses).

154. Planquette and Sherrell (2012) should be consulted for details on sampling and sample treatment for particulate trace metals in the water column using in situ filtration, bottle filtration and sediment traps.

155. The best analytical methods for trace metals in seawater and pore water are subject to change due to technological developments and instrument availability and so various analytical methods are possible. Appropriate analyses and data processing should be proven with the requested metadata. Generally, metal concentration data should be determined using Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES) or Inductively Coupled Plasma-Mass Spectrometry (ICP-MS). Sediment samples should be acid pressure or microwave digested prior to ICP analysis with suitable acid combinations, e.g. HF+$HClO_4$ or HF+HCl+HNO_3 (Paul *et al.*, 2018, Nöthen and Kasten, 2011). The ICP-MS can be coupled with a seaFAST for seawater and pore water trace metal analysis. Certified reference materials (CRM) for trace metals and inorganic contaminants in solid phase (MESS-4, NIST-2702) and seawater (e.g. NASS-7, CASS-6, SLEW-3) or, if they do not exist, in-house standards (e.g. for pore water) should be processed and measured together with the samples to document analytical accuracy and precision.

156. The parameters that should be measured are concentrations of Iron, Manganese, Cobalt, Copper, Nickel, Zinc, Cadmium, Arsenic, Lead and Vanadium. The results should be presented

1088 in fractions of a mole per unit mass or volume (e.g., nmol kg^{-1} or nmol l^{-1}). These should be
1089 determined in each of the operationally defined size fractions (particulate, total dissolved <0.2
1090 µm and nanoparticulate/colloidal >0.02 µm to 0.2 µm) noting chemical speciation (total
1091 concentrations, labile, redox speciation, complexation with organic ligands).

1092 157. From these measurements, trace metal fluxes, distribution between different physical
1093 and chemical species, labile concentrations and types and concentrations of nanoparticles and
1094 colloids (NPC) and the redox zonation (including its spatial and temporal variability) in the
1095 sediment should be determined.

1096 **H. Measured variable – Organic and inorganic matter**

1097 158. Baseline observations should address quantity, quality and lability of dissolved and
1098 particulate organic matter as well as particulate inorganic carbon in the water column and at
1099 the seafloor including their temporal and spatial variability using measurements of appropriate
1100 proxies. Observations of particulate matter in the water column should include organic and
1101 inorganic particles.

1102 159. The main emphasis of baseline observations should be on a well-replicated
1103 characterization of Particulate Inorganic Carbon (PIC), Particulate Organic Matter (POM) and
1104 Dissolved Organic Matter (DOM) in the water column and uppermost decimetres of the
1105 sediment where biogeochemical conversion rates are highest, and where current knowledge
1106 suggests the impacts are most likely to be expected. For sediment analyses, in addition to the
1107 resolution identified in section III.A, PIC and POM should also be measured in deeper and
1108 older sediment at some sites to help characterize the different settings found in the area
1109 including past productivity and deposition regimes.

1110 160. For seabed analysis, the distribution of PIC and POM should be determined in
1111 subsamples taken from distinct depth layers of retrieved cores while DOM should be analysed
1112 in pore-waters extracted from distinct depth layers. Samples for analyses in the top decimetres
1113 of the sediment should be taken with state-of-the-art samplers that are able to recover the fluffy
1114 semi-liquid surface layer (e.g., multiple corer, ROV-manipulated push corers). Deeper strata
1115 should be cored with a gravity corer or a piston corer.

1116 **1. Dissolved Organic Matter (DOM)**

1117 161. The amount of DOM should be quantified in terms of DOC alongside with
1118 measurements of Total Dissolved Nitrogen (TDN), typically by catalytic oxidation at high
1119 temperature and after removal of inorganic carbon and volatile organic matter by means of
1120 acidification and purging with inert gas. The ratio of DOC to dissolved organic nitrogen (DON,
1121 calculated by subtracting the sum of NH_4^+, NO_3^-, and NO_2^- from TDN) provides a first
1122 indication of the quality of DOM so the DOM's chemical composition should be characterised.
1123 A general molecular characterization of DOM should be determined based on optical analyses
1124 of the coloured (C-DOM) and fluorescent (F-DOM) pool. This can be performed with off the
1125 shelf instruments that readily collect excitation emission spectra using fluorescence
1126 spectroscopy and combine this with absorption spectroscopy-based measurements.

1127 162. Dickson *et al.* (20007) should be consulted for best practice in measuring DOC in the
1128 water column.

163. The parameters that should be measured in the water column are dissolved organic carbon (DOC) and dissolved nitrogen (DN).

164. The parameters that should be measured for the pore water are dissolved organic carbon (DOC), total dissolved nitrogen (TDN), dissolved amino acids and carbohydrates, and DOM optical characteristics (C-DOM, F-DOM)

165. For the water column, the observations should be used to determine the contribution of DOC to net community production and carbon export fluxes.

166. For the sediments, observations should be used to determine the quantity and quality of organic matter and its spatiotemporal variability to quantify and explain organic matter remineralization rates, and provides contextual information on metal complexation and bioavailability.

2. Particulate Matter

167. Particulate matter includes a number of variables that describe the suspended particulates (total suspended matter; TSM) and particulate matter transport in the ocean, both organic and inorganic fractions. Particles can be collected in the water column using different sampling techniques:

- by filtration of water from Niskin or GoFlo bottles,
- with *in situ* pumps
- with sediment traps

168. Each of these sampling techniques has advantages and disadvantages and therefore a combination of all of them should be used. While sampling techniques based on filtration of water samples collected with water sampling devices such as NISKIN or GO-FLO bottles are limited to relatively small volumes <12l, in situ pumps specifically aimed at collecting larger masses of particles are capable of filtering large volumes (hundreds of litres per hour) required for some investigations (such as activities of specific radioisotopes). A depth profile should be collected by attaching individual in-situ pumps in sequence onto a wire (e.g., CTD cable) and programming them to pump at target depths for 2–4 h. Filtered seawater from bottles and pumps should be used for particle concentrations, type and quantity, and is suitable for trace metal investigations. The size spectrum of these particles reflects a mixture of sinking and non-sinking particles. Export fluxes should be indirectly deduced by measuring the activity of radiotracers (see section I). The most direct method of measuring particle flux uses sediment traps, which collect sinking particles at a certain depth over a period of several days to months. Quantity, type, and quality of sinking particulate matter should be directly assessed.

169. The GEOTRACES cookbook (Sampling and Sample-handling Protocols for GEOTRACES Cruises), Bishop *et al.* (2012) and Planquette and Sherrell (2012) should be consulted for guidance on best practice sampling and sample processing methods for particulate matter investigations using *in-situ* filtration and on-deck filtration from GO-Flo bottles, with special focus on trace metals. The Cookbook should also be referenced for recommended modifications on the determination for particulate organic carbon (POC) and particulate nitrogen (PN) being originally published in the JGOFS Report 19 (Knap *et al.*, 1996), which encompasses the recommendations for the Joint Global Ocean Flux Study and represents a widely employed and cited POC and PN method for small-volume samples (i.e., < 10 L)).

170. McDonnell *et al.*, (2015) should be used for a review on collection methods for particulate matter >0.2 µm and their application in studies of biogeochemical cycling derived from bottles, *in situ* pumps and sediment traps, with details on recommended filter types, sediment trap sampling protocols including cleaning, sample preservation and processing, and sediment trap collection biases. Details on particle sampling, sample treatment/processing and the determination of particle types, composition and concentration, as well as mass of suspended particles and particle fluxes should be obtained from Lam *et al.* (2018), Boxhammer *et al.* (2018), and Huffard *et al.* (2020), and can additionally be deduced from the Guidelines of ocean observation published by the Oceanographic Society of Japan and in the IOCCG protocol on POC sampling and measurements.

171. A review on optical techniques for remote and in-situ characterization of marine particles without collection and retrieval is should be found in Boss *et al.* (2015) which covers techniques to assess bulk properties including particle mass, particle size distribution, particle shape information, and also single particle optical properties, such as individual particle type and size. The authors also review advances in imaging technology and its use to study marine particles in situ. More details can be found in Giering *et al.* (2020) and Huffard *et al.* (2020).

172. See also GOOS EOV specification sheet for further information on current global observing networks and links to literature on autonomous data observation innovations.

173. The parameters that should be measured for the water column are POM (POC, PON, POP), BSi, PIC, total organic carbon (TOC), total nitrogen (TN), Total Suspended Matter (TSM), POC flux, Calcium Carbonate ($CaCO_3$) flux, Biogenic Silica (BSi) flux, lithogenic particles, iron and manganese (oxyhydr)oxides, concentration of particulate matter, carbon supply/carbon demand and POM Redfield (C:N:P) stoichiometry.

174. The quantity and quality of sinking material varies seasonally and interannually so aprticualor emphaisis should be placed on weekly to monthly sampling of primary production and monthly to annual resolution for export fluxes.

175. Organic matter observations in the sediments should address the quantity of particulate matter, as well as the amount of bioavailable organic matter and its quality (i.e., freshness / lability). Different approaches can be used (e.g., Pusceddu *et al.*, 2009; Meckler *et al.*, 2004, and references therein) but a core set of proxies should be consistent throughout the baseline studies. Information on the amount of bioavailable organic matter should be obtained by measurements of total organic carbon (TOC) and total nitrogen (TN), typically by means of an elemental analyser after removal of inorganic carbon by acidification. The Ratio of TOC/TN (C:N ration) provides a first indication of POM quality. More specific information on organic matter quality should be obtained by chloroplastic pigments equivalents ('CPE', the sum of chlorophyll a and its degradation products, i.e., 'phaeopigments'), by simple fluorometric analysis or HPLC or by wet-chemical analyses of 'biopolymeric carbon' (the sum of hydrolysable carbohydrates, proteins, and lipids). Information on POM freshness should be obtained based on the Chlorophyll *a*:CPE-ratio (or the similar 'Chlorine Index') or based on analyses of the specific composition of biomolecule classes (e.g., ratio of hydrolysable to total carbohydrates, proteins, and lipids; 'degradation index' based on amino acid composition; rates of fatty acid with different levels of saturation).

176. In conjunction with measurements of TOC and TN also PIC should be measured with a CNS element analyzer. PIC is often reported as calcium carbonate ($CaCO_3$) content in weight-percent of the dry sediment sample.

1217 177. POM distribution is expected to be heterogeneous, especially near the sediment surface.
1218 Because of low density of organic matter particles, their deposition at the seafloor typically
1219 depends on small scale patterns of currents and seafloor morphology, leading to patchy
1220 distributions with local accumulations, e.g. in small depressions. Appropriate statistical
1221 methods should be used to decide on the number of replications required and the appropriate
1222 resolution. This information should be provided along with the raw data. The number of
1223 replicates should never be lower than three cores per site and sampling campaign. Seafloor
1224 imaging surveys (cable-based imaging systems, AUVs) or time series (lander-based systems,
1225 benthic crawlers) should be used where possible to obtain semi-quantitative information on
1226 spatial and temporal variability in the supply, standing stock, and processing of fresh POM at
1227 the seafloor (semi-quantitative observations of greenish phytodetritus distribution in colour
1228 imagery, quantitative observations of chloroplastic pigment with fluorescence imaging or
1229 hyperspectral techniques).

1230 178. From measurements of particulate matter in the water column, products such as primary
1231 production, ocean acidification, export fluxes and carbon supply, and attenuation of organic
1232 matter in the water column should be derived. Based on the inorganic particle fraction (PIC,
1233 Bsi) the main origin of biomass, (i.e., calcifying or silicifying organisms) should be determined
1234 as well as the amount of POC ballast which is a main driver for POC export from the euphotic
1235 zone (Klaas and Archer, 2002). For the sediments, observations should be used to quantify
1236 benthic carbon standing stock and turnover-and assess its availability for remineralization by
1237 benthic communities. This information should be combined with observations of organic and
1238 inorganic particulate matter export fluxes, oxygen uptake, the carbonate system, nutrients, and
1239 trace metals, by means of transport-reaction models to quantitatively assess benthic
1240 biogeochemical cycling in organic matter, nutrients, and trace elements.

1241 **I. Measured variable - Radioactive isotope tracers (Radiotracers)**

1242 179. Sampling, sample processing and analysis for long-lived radionuclides (e.g., ^{230}Th) and
1243 short-lived radionuclides (e.g., ^{210}Pb) in seawater, suspended sediment plume particles and
1244 sediments should follow the detailed recommendations provided in the GEOTRACES
1245 cookbook. The parameters that should be measured are dissolved, colloidal and particulate
1246 ^{230}Th, ^{234}Th, ^{210}Po, ^{210}Pb, ^{231}Pa, ^{224}Ra, ^{226}Ra, ^{228}Ra, ^{227}Ac and gross alpha radiation.

1247 180. For the determination of short-lived radionuclide (e.g., ^{210}Pb) activities in sediments:

1248 • A few grams of dried, homogenized sediment samples should be sealed gas-tight
1249 and left for at least several weeks to ensure that the radioisotopes are in secular
1250 equilibrium (i.e., constant radioisotopic activity because production rate is equal
1251 to decay rate).
1252 • Total ^{210}Pb and ^{226}Ra activities should be determined directly by gamma
1253 spectrometry (High Purity Germanium (BEGe) detector).
1254 • Total ^{210}Pb can also be measured indirectly by alpha spectrometry (Passivated
1255 Implanted Planar Silicon (PIPS) detector) via its granddaughter isotope ^{210}Po.
1256 • External calibration should be performed using certified reference material
1257 (CRM) such as IAEA-RGU-1 (Uranium Ore).

1258 181. The determination of long-lived radionuclide (^{230}Th and ^{231}Pa) activities in sediments,
1259 particles and in the water column (Ra series) should be performed by:

1260 - gamma spectrometry (Yokoyama and Nguyen, 1980)
1261 - alpha spectrometry (Lao *et al.*, 1992)
1262 - mass spectrometry (Geibert *et al.*, 2019)
1263 - For sediments and particle analyses IAEA-385 (Irish Sea sediment; Pham *et al.*,
1264 2008) should be used as certified reference material
1265 - For water column analyses IAEA-443 (Irish Sea water, Pham *et al.* 2011) could
1266 be used as certified reference material

1267 182. The determination of gross alpha radiation can be replaced by measuring ^{230}Th, ^{226}Ra
1268 and ^{231}Pa individually, then calculating expected gross alpha radiation based on equilibria with
1269 their respective daughter isotopes.

1270 183. Activities should be presented as total, dissolved and particulate activities, in dpm/g or
1271 Bq kg-1. All radioisotope activities (except for their ratios) should be corrected for the
1272 interference of pore-water salt during analysis (Kuhn, 2013; Geibert *et al.*, 2019) and the exact
1273 procedure and corrections recorded.

1274 184. From these measurements, concentrations and activities, ^{230}Th deficit, radionuclide
1275 fluxes, sinking elemental fluxes, sedimentation rates should be determined. In addition,
1276 bioturbation depth, bioturbation activity, bioturbation mode (i.e. diffusive or non-local mixing),
1277 radiation level and porewater-mineral reactions (e.g. carbonate dissolution/precipitation)
1278 should be determined within the sediment.

1279 185. Numerical transport-reaction models or analytical solutions are available for analyzing
1280 the data. For example, the Constant Initial Concentration (CIC) model provides a simple
1281 approach to calculate sedimentation rates for deep-sea sediments: Using the average activity of
1282 either ^{230}Th or ^{231}Pa within the bioturbated layer of an undisturbed sediment (in which no
1283 significant depth trend for ^{230}Th excess or ^{231}Pa excess is seen) the depth at which the activity
1284 has decayed to one half of this level is determined. The difference between this depth and the
1285 bottom of the bioturbated layer, divided by the respective half-life, is a rough approximation
1286 of the sedimentation rate at this location.

1287 **J. Data Quality**

1288 **1. Chemical oceanography**

1289 186. Five programs working with oceanographic data, namely the Alliance for Coastal
1290 Technology (ACT), the AtlantOS project, the Integrated Marine Observing System (IMOS),
1291 the Joint Technical Commission for Oceanographic and Marine Meteorology (JCOMM), and
1292 the U.S. IOOS Quality Assurance/Quality Control of Real-Time Oceanographic Data project
1293 jointly published a review on existing quality assurance best practices (Bushnell *et al.*, 2019)
1294 and this should be consulted for details on QA record-keeping, check lists, maintenance
1295 recommendations, how to improve measurement uncertainty and general QA
1296 recommendations regarding oceanographic data. The document further identified the recently
1297 created Ocean Best Practice System as one means of developing, sharing, documenting, and
1298 curating more specific QA processes and should be the standard adhered to.

1299 187. In chemical oceanography, uncertainties for data values obtained from water samples
1300 are associated with the sampling process, sample treatment and analytical measurements and
1301 can be reduced by the number of observations. This needs to be distinguished from the
1302 uncertainty/variability on a data value for similar environmental conditions in space and time

that arises from repeated sampling or data recording e.g., same location sampled in three different years at same time, or three samples at similar but not same location within a radius of around 10km. High analytical rigor (i.e., accuracy and precision) helps to distinguish between sources of uncertainty.

188. For trace metals GEOTRACES states that two categories of replicates should be measured: field and analytical replicates. Analytical replication is the repeated analysis of a single sample and is a measure of the greatest precision possible for a particular analysis. Field replication is the analysis of two or more samples taken from a single sampling bottle and has an added component of variance due to sub-sampling, storage, and natural within sample variability. The variance of field and analytical replicates should be equal when sampling and storage have no effect on the analysis (assuming the analyte is homogeneously distributed within the sampling bottle).

2. Biogeochemistry

189. The number of replicate samples or observations that are required to properly describe biogeochemical baseline conditions in the respective physiographic units (see section III.A) will depend on the existing natural variability but also on the relative changes in response to mining activities that need to be identified. Appropriate statistical tools, such as power analysis (Sweetman *et al.*, 2019). should be used to assess the sampling effort that is required to detect a change at a specific level and with a specific statistical power. The target level of change to be resolved for specific variables will mainly depend on the magnitude of change typically associated with mining-related impacts, and the relevance of the variable to serve as indicator of ecosystem status, deterioration, and recovery. As a guide the chosen replication should allow the detection of deviations of <30% compared to baseline conditions at a statistical power of at least 0.8. Statistics on the level of change that can be detected for the individual variables should be reported together with the baseline data.

190. To decide on an initial sampling effort, available information on natural variability should be collected but three replicates should always be considered a minimum. As more information on natural variability and relevance of the respective variables becomes available from baseline observations, impact studies and integrated modelling of baseline conditions and changes, the replication required for the different variables should be regularly revised.

K. Data Management

191. The technical notes of the International Ocean Discovery Program (IODP) and its predecessor the Ocean Drilling Program (ODP) provide details on data and sample management and curation (as well as biogeochemical and geological sampling and analyses) that should be followed.

192. Metadata are required to document appropriate sampling and analyses and to trace provided data back to their origin and need to be provided for all chemical variables. Metadata related to sampling, sample logging, and resulting data should follow the guidelines defined by the International GEOTRACES Data Assembly Centre (http://www.bodc.ac.uk/geotraces/), the International Council for the Exploration of the Seas (ICES) and the Working Group on Marine Data Management (WGMDM)). More information and metadata protocols are provided in the Data Management Best Practices Guide compiled by the Biological and Chemical Oceanography Data Management Office (BCO-DMO) based on experience from GLOBEC and JGOFS ocean research programs, and comprises a collection of better practice

1347 recommendations for the management of data from research cruises. This guide is available as
1348 download from: http://bco-dmo.org/resources. Guidelines of (meta)data management can also
1349 be found in the Ocean Best Practices System, and within the Argo program community.

1350 **VI. GEOLOGICAL PROPERTIES**

1351 **A. Introduction**

1352 193. In combination with biogeochemical parameters (section V), geological properties are
1353 targeted to characterize the habitat and to determine the heterogeneity of the seafloor and
1354 subsoil environment (bathymetry, geological evolution, sediment and sedimentation records,
1355 diagenesis and remobilization, resource and substrate geochemistry and mineralogy) and assist
1356 in the placement of suitable sampling locations to characterize the distribution and composition
1357 of faunal communities.

1358 194. The following variables form the basis of a geology baseline:

1359 • Bathymetry - used to map large and small-scale morphologic features of the
1360 seabed and can be used to plan other types of sampling.
1361 • Sediment properties and habitat classification –important to characterise the
1362 benthic habitat. Additionally, the properties should be used to quantify
1363 deformation and changes of seafloor sediment physical properties during mining
1364 gear operations, and for the design of the mining system.

1365 195. Resource properties are important for habitat characterization and they constitute the
1366 main target of any exploration activity in the Area. Some of the resource characteristics may
1367 constitute information of commercial interest and may be subject to confidentiality under the
1368 contracts with the ISA. However, an assessment of the relevant information needed to establish
1369 the environmental baseline should be presented.

1370 **B. General Methodology**

1371 196. Data and information on the geology and deep seafloor morphology can be collected
1372 using

1373 • Multibeam echo sounding (hull-mounted and/or from Remotely Operated
1374 Vehicle, (ROV) or AUV);
1375 • Side-scan sonar profiling (towed from the vessel, from ROV, AUV or other);
1376 • Sub-bottom profiling;
1377 • Photography and video recording obtained by TV grab, sledge, ROV, AUVs or
1378 submersibles.

1379 197. There are diverse methodological approaches to carry out geological surveys and
1380 acquire high-quality accurate data of the geological variables and any of the commonly
1381 accepted practises should be used.

1382 198. Sediment samples, for sediment analysis, should be obtained using a multicorer or
1383 ROV-pushcores for the top decimetres of sediments and gravity corers for deeper samples.

199. Specific methodologies for sediment sampling and bathymetry can be found in publications of the Integrated Ocean Drilling Program (IODP), and in the Ocean Best Practices Repository (https://repository.oceanbestpractices.org).

200. Standards for hydrographic surveys are published by the International Hydrographic Organization (2020) and these should be consulted.

C. Sampling Resolution

201. The resolution of sampling will be dependent on whether the information is to be used for large-scale resource assessment or local habitat mapping and should be adjusted to be appropriate to the use. For large scale surveys of the entire exploration area bathymetric and backscattered maps with resolutions greater than 80-100m should be produced. In areas where other discrete sampling is being undertaken, where conditions indicate higher variability, or in areas predicted to be indirectly impacted by mining (sediment and discharge plumes) higher resolution sampling should be obtained.

D. Measured variable – Bathymetry

202. Multibeam bathymetry, backscattered mapping, side-scan sonar (SSS), or synthetic aperture sonar (SAS) methods from ship-based, deep towed, ROV or AUVs should be used for seafloor mapping to provide high spatial resolution data on the physical status of seafloor habitats.

203. Suitable calibration is required to obtain reliable and consistent seafloor bathymetric and backscatter data (Lemarche and Lurton, 2018). Constancy of acquisition settings and specific design of backscatter-dedicated surveys, are recommended and should be comparable across licence areas and contractors. Standards for hydrographic surveys are found in publications of the International Hydrographic Organization (e.g. International Hydrographic Organization, 2020). References on standardization of undersea feature names are also found in: https://iho.int/en/bathymetric-publications and in https://www.gebco.net

E. Measured variable – Sediment Properties

204. To describe the sediment properties, the lithology and stratigraphy, particle size distribution and porosity should all be measured. Lithology refers to the physical characteristics of the sediment or rock, and the stratigraphy refers to the classification, nomenclature and description of the layered deposits. Core samples should be taken using a suite of different tools to sample the uppermost 30cm of sediment (push core and multicore), the uppermost 50cm (box core) and several metres deep (gravity core).

205. Physical oceanographic phenomena can generate sedimentary structures on the deep seafloor as well as mining processes. Therefore, seafloor sedimentary structures should be identified and mapped using optical imaging. Optical imaging acquired by deploying a variety of platforms including ROVs, AUVs, towed or drop-down cameras allow quantitative or qualitative characterization of geological, sedimentological (ripples, marks and casts related to seabed bottom currents) and biological elements or patterns, and their interrelationships. Rates and depths of bioturbation and type of structures should be described. GIS-based mosaicking approaches should be used to image complex or larger areas of seafloor (Garcia *et al.*, 2015), indicating the used overlap percentage.

206. Core samples should be handled and stored in such a way as to maximize their utilization for scientific studies, following best practices for transportation, sampling and storage (Basu *et al.*, 2020).

207. Grain size is a fundamental physical property of sediment. It is correlated with the dynamic conditions of the marine environment and it is important for interpreting its stability under load. The introduction of automated grain size measuring techniques can add efficiency and precision to grain size determination (Jaijel *et al.*, 2021). According to the authors, a typical modern Laser Diffraction Spectrometer will have a size scale range that measures up to 2000µm, which covers the great majority of soft bottom sediments of the world's oceans. The grain size distribution of the bulk sediments (GSD_{bulk}) should be determined using a standard methodology with appropriate handling (see Jaijel *et al.*, 2021 and references therein).

208. Lithological characterization of the sediment should be described by the examination under the petrographic microscope of smear slides of unconsolidated sediment or thin sections of hard rock and carry out (e.g. Marsaglia *et al.*, 2013, 2015a and b). The mineralogical composition should be determined qualitatively and quantitatively. Both are usually analyzed by X-ray diffraction and/or automated quantitative mineralogy (AQM) using mineral liberation workflows and quantitative scanning electron microscopy techniques. These methods should be used to provide a quantitative modal analysis and virtual petrography. Quantitative measurements should also be performed using the Rietveld analysis, especially to fully characterize the seafloor surface of the future mining areas and to define the clay fraction (particles < 2 microns in size) for modelling of potential environmental harm by plumes.

209. Sediment chemical composition should be performed using laboratory analysis operating with quality systems based on international standards, including X-Ray Fluorescence (XRF), Inductively Coupled Plasma Mass Spectrometry (ICP-MS) and Inductively Coupled Plasma Optical Spectrometry (ICP-OS) measurements (see section V).

210. Details on visual core description procedures and analytical equipment and sediment sampling, sample preparation and general analysis and techniques can be found in Przeslawski *et al.* (2018), Simpson and Batley (2016), Marsaglia *et al.* (2013, 2015a and b), Rothwell and Rack (2006), Mazzullo *et al.* (1988) and other resources at https://repository.oceanbestpractices.org/, and http://publications.iodp.org/index.html.

211. The parameters that should be measured are bedding thickness and attitude (orientation or angle), bedding contacts (e.g. gradational, sharp and scoured), sedimentary structures (e.g. laminated bedding, graded bedding, cross bedding, fractures or micro-faults, fluid scape structures and bioturbation), sediment colour (e.g. use a Munsell Soil Colour Chart for classification), composition, texture (sand, silt, clay), accessory components (concretions, microfossils, biogenic material), diagenesis and lithification or cementation degree (presence of silicic or calcareous cements), identification of macroscopic biogenic and non-biogenic components, specific gravity, bulk density, sediment porosity, fluid saturation, shear strength and grain size, sediment depth of change from oxic to suboxic conditions.

212. From the information collected, seabed substratum characteristics and geomorphic features for the detailed understanding of pre-mining conditions of claim areas should be determined.

1467 **F. Habitat Classification**

1468 213. Mapped qualitative descriptions of basic geomorphic features, habitat classifications
1469 and the presence of non-biogenic disturbances resulting from coring should be produced at a
1470 scale appropriate to the resource and habitat variability to support other sampling using the
1471 terminology for standardization of undersea feature names provided by the International
1472 Hydrographic Organization (2019).

1473 **G. Data quality**

1474 214. References on quality assurance of oceanographic observations including standards and
1475 guidance include Bushnell *et al.* (2019). All methodology should be checked against quality
1476 assurance plans (Simpson and Batley, 2016). Guidance on quality control for hydrographic
1477 surveys and guidelines for data processing can be accessed at https://iho.int/en/standards-and-
1478 specifications.

1479 **H. Data Management**

1480 215. A suite of representative pre-mining cores of the sea floor sediment, with appropriate
1481 metadata, should be stored in a suitable repository for later comparison and additional testing
1482 should it be required.

1483 216. All observations should be recorded in a worksheet following conventional data formats
1484 and accompanied by high quality close-up photographs with the reference scale.

1485 217. A best practices document template on data management is provided by the Ocean Best
1486 Practices System (2020). This template is available as download from:
1487 https://repository.oceanbestpractices.org/handle/11329/1245

1488 **VII. BIOLOGICAL COMMUNITIES**

1489 **A. Introduction**

1490 218. The environmental baseline for biological communities should include spatial and
1491 temporal data on both the pelagic and benthic communities and their ecosystem functions as
1492 well as information on sea mammals, birds and large gatherings of surface nekton. The data
1493 collected will be diverse and should be extensive enough to allow the assessment of the
1494 potential impact of mining activities on the seafloor and in the water column.

1495 219. The following variables should be determined in order to define the biological
1496 communities:

1497 • Pelagic communities - The pelagic system comprises the entire water column
1498 from the sea surface down to the sea floor. The water and the organisms within
1499 this vast volume move across potential mining site(s), so sampling should extend
1500 beyond the immediate zone of direct mining impact to include all the water and
1501 organisms entering, potentially interacting with, and exiting, the zone of mining
1502 impact.
1503 • Benthic Communities– The benthos is the biota living in or near the seafloor as
1504 adult. Benthic organisms, from bacteria and protists to metazoans, are mostly
1505 sedentary or with limited ability to move and escape disturbances so will be

directly impacted by mining activities through habitat removal or habitat disaggregation, as well as indirectly impacted through increased turbidity and sediment redisposition.
- Connectivity - Understanding the genetic diversity, molecular connectivity patterns and turnover is essential to determine the potential recovery to a disturbance.
- Ecosystem Functioning – a knowledge of ecosystem functioning enables and understanding of how small-scale disturbances can lead to shifts in food-web structure and organic-matter cycling activity by the resident benthic community.
- Ecotoxicology - Metals released during mining operations may impact organism physiology and therefore it is important to understand the potential toxicity of these.
- Whales, sharks, turtles and surface nekton– it is important to record the presence of sensitive or protected species that occur in the general contract area as they may have seasonal migration routes through the area and be impacted by noise and light as well future mining operations.
- Seabirds – Seabirds are one of the most threatened bird groups worldwide, have their behaviour affected by marine installations, and are good indicators of the overall health of the ecosystem as they bioaccumulate heavy metals and other toxic substances. They are also easier to study than any other marine vertebrates.

B. General methodology

220. Temporal sampling is necessary to capture seasonal variability in tissue metal and other contaminant concentrations for ecotoxicology studies, but also to include consideration of life-history traits, such as migration patterns of pelagic species that might travel through the contract or reference area.

221. To document regional diversity and connectivity patterns, it is likely that comparisons with distant sites will be required. Such comparisons may require sampling of distant sites as part of the baseline or may rely on comparisons with third-party data sources.

222. All taxonomic identifications should be to the best resolution possible. Molecular samples of the different taxonomic units should be used to support identification.

C. Sampling Resolution

1. Pelagic sampling

223. In the pelagic realm, biological communities are partitioned with respect to depth, in the photic zone where there is sufficient light for photosynthesis by phytoplankton (0-200m), in the mesopelagic or twilight zone dominated by animals of the deep-sea scattering layers (DSL, 200-1000m) and the bathypelagic, or ocean interior, inhabited by specialised organisms of the dark ocean depths (>1000m). Finer layers occur within these depth zones. By contrast, the horizontal distributions may be quite homogeneous over hundreds of kilometres but punctuated by transitions at oceanic fronts or eddy systems.

224. Samples should be taken within vertical strata within each biome. Rather than specific point samples indicated in section III.A, depth profiles should extend from the surface to 50m; 50–100m; 100–200m; 200–500m; 500–1,000m; 1,000m to 10m above the seafloor.

1548 225. Particularly below 1000m, beyond the maximum range of ships' sonars, net sampling
1549 can be augmented by imaging systems. These include the Underwater Video Profiler that is
1550 lowered on a wire to take a vertical profile, oblique profiling by submersibles as demonstrated
1551 by Robison *et al.* (2013) and bioluminescence profiling as described by Heger *et al.* (2008)
1552 AUVs are likely to become important for such deep surveys.

1553 **2. Benthic Sampling**

1554 226. Benthic sampling should span the range of size classes, different substrates (sediments
1555 and nodules), biogeochemistry (section V), ecosystem functioning and genetics. Details for
1556 specific variables are provided in separate sections below.

1557 227. Best practice should be used for the operation of the sampling devices and on-board
1558 handling of samples such as:

1559 • Sediment sampling equipment should be gently landed on the seafloor to
1560 minimize the bow wave effect (deployment from the side of the ship, low wire
1561 speed, use of telemetry);
1562 • Box cores for macrofauna should not be subsampled. Subsamples from a single
1563 box core, and separate cores from the same multiple corer deployment, are
1564 'pseudo-replicates' and should not be regarding as true replicates. (see section
1565 III.A);
1566 • Samples and specimens should be kept as cold as possible to improve DNA
1567 quality (sieve in a cold room, sorting on ice and preferably onboard, preserve
1568 specimens and sieve residues in cold ethanol, maintain the cold chain during
1569 transport and storage of samples).

1570 228. The number of samples required should be determined using power analysis (Jumars,
1571 1981) based on exploratory sampling of 5-10 cores per physiographic unit. Previous studies
1572 have indicated that to have an adequate baseline to statistically compare pre- versus post-
1573 mining macrofaunal abundance in a physiographic unit, at least 20, but preferably more than
1574 30, full box cores would be necessary, but this should be determined following power analysis
1575 specific to the area of investigation.

1576 229. The sampling strategies should focus on physiographic units that are going to be
1577 directly impacted by mining (e.g. plains with dense nodule coverage) and those indicted by
1578 other variables to be potentially affected by secondary impacts (e.g. areas where plumes may
1579 settle).

1580 **D. Measured variable - Pelagic communities.**

1581 230. To describe the vertical structure of the water column an acoustic echosounding using
1582 a ship-borne system (Simrad EK6.0, or equivalent) operating at multiple frequencies, 18, 38,
1583 70, 120 and 200 kHz, calibrated before the commencement of each voyage should be
1584 undertaken. Transects should be undertaken during daytime to estimate total biovolume or
1585 biomass, e.g. at each location 10 line transects, each 8 nautical miles long with the ship moving
1586 at 8 knots (Cox *et al.*, 2013). The data should be processed to estimate biomass as a function
1587 of depth and total integrated biomass from the surface to 1000m depth (Irigoien *et al.*, 2014).
1588 The sound scattering layers should be identified and classified according to multifrequency
1589 analysis to discriminate fish, squid and crustacea (Benoit-Bird *et al.* 2017). Acoustic echo

1590 sounder surveys using a Simrad EK60 (or equivalent) should be continued through at least
1591 three 24 h cycles to quantify diel vertical migration as described by Klevjer *et al.* (2016).

1592 231. Where possible reference should be made to historic reference points accessible by
1593 examining global sound scattering data available in various archives such as World
1594 Oceanographic Data Centres as well as national data centres; global databases such as
1595 Mesopelagic Biogeography (Proud *et al.*, 2017)

1596 232. The components of the pelagic communities, and the appropriate sampling
1597 methodology, are:

1598
- To understand the phytoplankton, primary production (Chlorophyll-a) should be
1599 mapped across the sampling area from appropriate satellite multispectral imagery,
1600 (AVHRR, SeaWiFS, MERIS and MODIS). Sampling is necessary to calibrate
1601 and verify satellite estimates of primary production. Replication is required to
1602 determine natural variation both spatially and temporally. Water samples using
1603 Niskin bottles within a CTD provide phytoplankton data at different depths.

1604
- Zooplankton (Mero and Holo-) should be sampled using nets to retrieve voucher
1605 specimens for identification and DNA sequencing with different sampling for
1606 each size class:

1607
  - Zooplankton – should be sampled with a mesh size less than 1 mm, using
1608 Bongo nets or plankton pumps in deeper waters and/or with a multiple
1609 open and closing net enabling discrete depth samples to be taken on a
1610 single tow (ISBA/25/LTC/6/Rev.1 and Corr.1). The nets should be
1611 equipped with flow meters to measure volume sampled, depth and
1612 temperature sensors. Sampling should be from 100m above the sea floor
1613 to the surface with a minimum of two tows at each sampling station.

1614
  - Mesopelagic nekton. A larger net should be used such as the macro-
1615 zooplankton or "krill" net described by Wenneck et al. (2008) which is a
1616 pelagic trawl suitable for catching representative samples of scattering
1617 layer fishes, crustacea and other organisms in discrete depth layers with
1618 five cod-ends each equipped with a 7 litres bucket. Larger versions of the
1619 MOCNESS can also be used. Sampling should be from 100m above the
1620 sea floor to the surface with horizontal tows at the depth of each scattering
1621 layer which should be simultaneously observed on the echo-sounder to
1622 ensure correct targeting, sample processing is described by Cook et al.
1623 (2013).

1624
- Nekton covers a large size range from small micronekton ranging from 2-20cm
1625 through to large fish with different sampling for each size class:

1626
  - Small nekton should be collected using net samplers, i.e. MOCNESS

1627
  - Larger elements should be sampled using mid-water trawls to collect
1628 specimens as well as acoustic methods to estimate biomass and categorise
1629 the deep scattering layer.

1630 233. The various elements of the zooplankton should be characterised into the lowest
1631 taxonomic level possible. Holoplankton should be identified to species level. With
1632 meroplankton it may be necessary to identify to a more general grouping, e.g. echinoderm
1633 larvae, polychaete trochophore, egg, etc. Molecular analyses can assist in the identification of
1634 both holo- and meroplanktonic taxa.

234. For all faunal groups imaging and taxonomic information should be obtained, with molecular techniques used to provide genetic characters for taxonomic comparison between contract areas.

235. The parameters that should be measured are Chlorophyll-a (Chl-a) concentration (µg L^{-1}), phytoplankton composition and biomass, diel migration of zooplankton, abundance and composition of other faunal groups

236. From these measurements, primary productivity, density and diversity (univariate and multivariate) of the different faunal groups, size classes and functional groups should be determined.

E. Measured variable - Benthic communities

237. Benthic communities can be divided into a number of size-class and functional groups. Whilst sampling should be aligned wherever possible, each group is subject to different considerations. The groups are:

- Megafauna – organisms visible in images; usually >1cm in size
- Macrofauna – usually annelids, amphipod, tanaid and isopod crustaceans, molluscs, smaller echinoderms, usually retained on a mesh size of 250-300 µm. Abyssal samples also contain numerous macrofauna-sized foraminifera (Bernstein *et al.*, 1978) as well as large meiofaunal organisms such as nematodes, although these are rarely studied. Hessler and Jumars (1974) suggested excluding from the macrofauna *sensu stricto* the smaller taxa that are best represented in samples of the meiofauna and that is the approach for these guidelines. Populations of the larger species among meiofaunal taxa may still be more accurately sampled in the larger sampling unit typically used for the macrofauna and considered as part of the macrofauna *sensu lato*. In the CCFZ, the macrofauna *sensu stricto* is dominated by two taxonomic groups, the Polychaeta and the Tanaidacea.
- Meiofauna – usually nematodes, harpacticoid copepods, ostracods, kinorhynchs and other small animals (the metazoan meiofauna) retained on a 32 µm sieve. This size class also includes abundant smaller-sized foraminifera (the foraminiferal meiofauna). For practical reasons these are typically limited to those retained on a 150, 125 or 63 µm sieve.
- Fauna associated with Polymetallic Nodules - Nodules are an important source of benthic habitat structure in areas where they are abundant. The nodule epifauna is dominated by octocorals, sponges, actiniarians, and foraminiferans. The nodule infauna, found in sediments within nodules crevices is dominated by meiofaunal organisms.
- Microbiota - Organisms invisible to the naked eye, smaller than meiofauna. Operationally defined as <32 µm.
- Demersal Fishes and Scavengers - mobile animals that are active predators in the benthic boundary layer but also species that exploit dead carcasses (e.g. fish and whales) that fall to the sea floor.

1676 **1. Megafauna**

1677 238. The megafauna should be assessed over broad scales of relevance to mining operations
1678 using imaging assessments along straight line transects, replicated within specified strata or
1679 physiographic units. Image assessment based on photographs (still images) rather than videos
1680 (moving images) should be used wherever possible as analysis and quality control are greatly
1681 facilitated. Stills can be extracted from very good quality video, but the quality of photographs
1682 is almost always higher than that obtained from video footage.

1683 239. Still cameras should be >10 Megapixels resolution and capable of manual control of
1684 exposure settings. Video can also be used if sufficient resolution (high-definition i.e. 1080
1685 pixels minimum frame dimension; or >1M pixels per image or greater) for reliable
1686 characterisation of megafauna >10mm in size. Images should ideally be obtained in RAW
1687 format, i.e. minimally processed data from the image sensor.

1688 240. Seabed images should be obtained using a platform capable of acquiring well-lit, high-
1689 resolution images or a consistent scale and quality that allow the reliable identification of
1690 megafaunal individuals of the determined size (usually 10mm). This platform may be an AUV,
1691 ROV or towed camera platform. The survey altitude should be kept constant so that images are
1692 obtained at a constant altitude above the seabed. Navigation information for the platform
1693 obtained using an acoustic transponder system should be collected automatically at a regular
1694 interval (e.g. 1 Hz).

1695 241. The start positions and transect heading should be randomised. Transects should be
1696 replicated. The number of replicates should be determined and justified using statistical power
1697 analysis. At least 5 replicates should be obtained for each target stratum. Transects should be
1698 independent of each other (i.e. not splitting up a long line transect into adjacent segments).
1699 Efficient strategies for obtaining independent transects are available, for example collecting
1700 multiple straight-line transects in a zig-zag pattern.

1701 242. Transect length should be determined using existing data for the region to ensure
1702 sufficient megafaunal organisms are encountered in each transect for effective and robust
1703 evaluation of the metrics of interest. For biodiversity assessment, transects should be designed
1704 with the aim of encountering > 500 individual organisms in each transect.

1705 243. Transect width should be determined by effective imaging altitude (typically around
1706 2m). If sufficient positioning information and spatially accurate sampling approaches are
1707 available, adjacent overlapping transects should be obtained to create mosaicked images and
1708 cover a wider area, as long as the mosaicked image still has sufficient resolution to reliably
1709 identify organisms >10mm in size.

1710 244. Taxa that cannot be determined to be alive, e.g. invertebrates living in a shell or tube
1711 (most polychaete and gastropod taxa) should be listed. It may be necessary to exclude them
1712 from quantitative analyses. Xenophyophores (protistan megafauna; Gooday *et al.*, 2017,
1713 2020b) should be analysed separately. Their numbers are typically several times higher than
1714 those of metazoan megafauna.

1715 245. Transects of images should be analysed as sample units (i.e. all organisms recorded in
1716 each transect should be summed to form a single sample unit) for the majority of analyses.

1717 246. All images should be scaled using photogrammetric approaches using known optical
1718 properties of the camera, the camera position on the collecting device, altimeter records and
1719 vehicle pitch and roll data. The area of seafloor covered should be stated in reporting.

1720 247. Images should be annotated using specialist annotation software, such as BIIGLE
1721 (Langenkämper *et al.*, 2017). Any of the range of different image-annotation tools available
1722 that are highly suitable for seabed image analysis cam be used (Gomes-Pereira *et al.*, 2016;
1723 Schoening *et al.*, 2016).

1724 248. Images should be analysed in random order (to minimise any sequence- or time-related
1725 bias). All megafaunal individuals greater than 10mm should be detected and annotated. They
1726 should be identified to the lowest taxonomic resolution possible (i.e. morphotype: typically
1727 Genus or Family level). The physical dimensions of each individual should be measured based
1728 on known image pixel sizes.

1729 249. Results should be presented in a way that facilitates future use and comparison with
1730 other studies, thus allowing integration of data into regional and other assessments. Typically,
1731 this includes providing morphospecies abundance matrices and presenting density values
1732 (numbers per m^2), Hill's diversity numbers of order 0, 1, and 2 (0: morphospecies richness [S],
1733 1: the exponential form of the Shannon index [exp H´], and 2: the inverse form of Simpson's
1734 index [1/D]) and multivariate assessment (ideally including past data for comparison).

1735 250. The parameters that should be measured are numerical abundances of specimens per
1736 area sampled (ind. m^{-2}) for appropriate taxonomic/functional groups and for the whole
1737 metazoan/xenophyophore community. The size of each individual encountered and any
1738 observations of its location (such as whether it was attached to a nodule) should also be
1739 recorded.

1740 251. From these measurements, density, statistics to describe community structure
1741 (univariate and multivariate diversity measures) and distribution patterns should be determined.

1742 **2. Macrofauna**

1743 252. Macrofauna should be sampled using the methodology outlined in ISA Technical Study
1744 No. 13: Deep Sea Macrofauna of the Clarion-Clipperton Zone with additional information
1745 provided in ISBA/25/LTC/6/Rev.1 and Corr.1.

1746 253. Both macrofauna living on nodules and those in the sediment should be collected.

1747 254. For nodule living fauna, when box cores are recovered, nodules with obvious epifauna
1748 should be identified. Nodule fauna still attached to the nodules should be imaged live in special
1749 small aquaria with cold filtered seawater, fauna removed, snippet sample for DNA taken in a
1750 2 ml tube with cold 96% Ethanol and the animal fixed in a separate tube. The nodule should be
1751 returned to the original container. All water that was in contact with the nodules should be
1752 sieved over 32μm sieve and residue is added to the original container. The size and weight of
1753 the nodules should be recorded and preserved in formalin or cold ethanol.

1754 255. For sediment fauna, all processing should be performed in a wet laboratory. The
1755 sediment should be divided into 0-3cm, 3-5cm and 5-10cm depths and each sieved with cold
1756 filtered seawater. The uppermost sample should be sorted immediately, the residue from the
1757 deeper slices should be kept in a cold lab in cold filtered seawater until they are

1758 processed. Increasingly samples are needed for both morphological and molecular analyses and
1759 so the use of formaldehyde as a fixative should be carefully considered. For morphology and
1760 ecological analyses, in the refrigerated laboratory, the 0-3 cm layer of sediment should be
1761 sieved with CFS, the residues preserved in 10% buffered formaldehyde. In the wet lab, the 3-
1762 5 cm and 5-10 cm layers of sediments should be sieved with cold filtered seawater, and the
1763 residues fixed in 10% buffered formaldehyde or 96% Ethanol. If there are large volumes of
1764 residues, stronger concentrations of formaldehyde may be needed to ensure fixation of
1765 specimens. Formaldehyde should not be used for fixing crustacean groups such as isopods; for
1766 such taxa preservation in 96% Ethanol is advised. Samples should be fixed in formaldehyde
1767 solution for at least 24 hours, then as soon as is practicable, all samples should be transferred
1768 from formaldehyde solutions into 70-80% EtOH solution.

1769 256. For molecular, morphology and biodiversity studies, the residues of the upper 0-3 cm
1770 layer should be sieved and retained, the sample kept as cold as possible sorting all metazoans
1771 into easily identifiable taxonomic groups over an “ice bed”. Live images of specimens should
1772 be taken before preserving them in Ethanol. DESS can be used to preserve nematodes. Other
1773 layers should be sieved and the residues examined as above or preserved in 96%
1774 Ethanol. Polychaete should be preserved in cold 80% Ethanol, nematodes in DESS (and stored
1775 at 4°C), and all other groups in cold 96% Ethanol. The ethanol should be changed after 24-48
1776 hours and the samples stored at -20°C.

1777 257. The parameters that should be recorded are taxonomic classification for each
1778 morphospecies, species by station matrices showing abundance (ind/per sampler) and gene
1779 sequences.

1780 258. From these measurements, density, species richness, statistics to describe community
1781 structure (univariate and multivariate diversity measures) and distribution patterns should be
1782 determined.

1783 **3. Meiofauna (including foraminiferal meiofauna)**

1784 259. Metazoan meiofauna should be sampled using the methodology outlined in ISA
1785 Technical Study No. 7: Marine Benthic Nematode Molecular Protocol Handbook (Nematode
1786 Barcoding).

1787 260. For biodiversity analyses, meiofauna should be restricted to those elements of the
1788 sediment fauna commonly recognised as meiofauna, e.g. nematodes, harpacticoid copepods,
1789 kinorhyncha, etc. Macrofaunal elements captured in meiofaunal samples can be noted but
1790 should not be included in the meiofaunal abundance estimates.

1791 261. At least one core per multiple-corer deployment should be dedicated to the
1792 morphological characterisation of metazoan meiofauna, and one core for the morphological
1793 characterisation of Foraminifera. Additional cores should be allocated for molecular
1794 characterization of these groups and other small-sized eukaryotes (e.g., small naked protists;
1795 Gooday *et al.*, 2020a) via barcoding and/or metabarcoding.

1796 262. If nodules are abundant, they may disrupt the sediment as a result of movement during
1797 coring, causing varying degrees of disturbance. Therefore, different analyses should be
1798 prioritised in advance of each deployment with the least disturbed cores assigned to those with
1799 the highest priority, with the priority rankings being rotated between deployments.

263. Once onboard, all cores should first be photographed. Overlying water of the core for metazoan meiofaunal analyses should be siphoned off over a 32 µm sieve with the use of a plastic hose, and processed together with the surface sediments. The slicing of the core should be determined based on visual inspection. Typically, the presence of nodules prevents slicing, in which case the entire unsliced 0-5cm section of the core should be preserved. Alternatively, nodules can be removed and cores sliced using a cutting plate into the following layers: 0–1cm, 1–2cm, 2–3cm, 3–4cm, 4–5cm (the depths identified in section III.A but only going down to 5cm into the sediment).

264. The core used for foraminiferal analysis should be sliced as described above and each sediment slice preserved separately in in borax-buffered 4% formaldehyde solution (= 10% formalin).

265. Preservation of meiofaunal samples should be explicitly mentioned. For example, samples for morpho-molecular study (i.e. barcoding) should be preserved with a solution containing dimethyl sulphoxide, disodium ethylenediamine tetra-acetic acid and saturated salt (DESS) (Yoder *et al.*, 2006) at 4°C. Samples preserved in this way can be used for the study of morphological characteristics (i.e. vouchering), while also maintaining the possibility of extracting genetic material (i.e. DNA barcode) from the same specimen, thus establishing a link between morphology and molecular identification (Bhadury *et al.*, 2006). Samples for metabarcoding analyses should be frozen to at least -20°C immediately after sampling (Macheriotou *et al.*, 2020). Samples should also be preserved with borax-buffered formaldehyde-seawater solution 4-8% but these specimens can only be used for morphological analysis.

266. At least one core should be subsampled for metabarcoding of small-sized eukaryotes (protists and metazoans). From each, three sediment subsamples (approximately 2 ml volume) should be taken using a sterile spoon, placed directly in plastic vials with 5 ml of a suitable soil preservation solution and stored at -20°C.

267. Where nodules are encountered, these should be preserved separately for further analyses of crevice fauna.

268. Once in the laboratory, samples should be processed using any standard meiofauna extraction procedure. For metazoan meiofauna, a flotation and centrifugation (4000 rpm) method should be used as it is known to yield up to >80% of the fauna (Mclntyre and Warwick, 1984). Because floatation methods yield inconsistent results, foraminiferal samples should be sorted by hand. Efforts should be made to include the single-chambered (monothalamous), 'soft-shelled' component in biodiversity assessments, since they are abundant and dominate foraminiferal diversity in CCFZ samples. However, for monitoring purposes, analyses can focus on the multi-chambered, 'hard-shelled' taxa, which are less abundant and diverse, but better known and less time-consuming to study than monothalamids (the 'micropalaeontological approach').

269. Sieves with mesh sizes of 150, 125 and 63 µm are commonly used in foraminiferal studies. The choice of mesh size is a trade-off between the increasing effort required to analyses finer-sized residues, the larger number of species and data that finer fractions yield (Gooday and Goineau, 2019). A 125-µm-mesh sieve is recommended for general use in biomonitoring studies (Schönfeld *et al.*, 2012), but the 63-µm fraction can yield additional information about environmentally sensitive species (Lo Giudice Capelli and Austin, 2019), while the 150-µm fraction retains diverse larger monothalamids poorly represented in finer fractions (Goineau

1845 and Gooday, 2017, 2019). Ideally, all three fractions (>150, 125-150, 63-125 µm) should be
1846 analysed, but if this is impractical, one fraction (>150, >125, or >63 µm) should be used
1847 consistently.

1848 270. Sieve residues should be stained in Rose Bengal solution (1 g in 1 litre tap water), for
1849 example, by placing the sieve containing residue in a dish of stain solution overnight and then
1850 washing the residue on the sieve to remove excess stain. Foraminiferal sorting should be carried
1851 out in water, e.g., in a Petri dish. Delicate monothalamids should be removed from the dish
1852 using a pipette and stored in glycerol on glass cavity slides, the slides being left uncovered so
1853 that specimens remain accessible. The more robust hard-shelled species should be stored on
1854 dry micropaleontological slides. For additional details about processing foraminiferal samples,
1855 including wet splitting and sediment sieving, as well as distinguishing 'live' and dead
1856 specimens and the problem of fragmentation, Goineau and Gooday (2017, 2019) and Gooday
1857 and Goineau (2019) should be consulted. These papers and their supplementary materials also
1858 include numerous photographs of common and mainly undescribed monothalamids. Schönfeld
1859 *et al.* (2012) and Alve *et al.* (2016) should be used for recommendations regarding the
1860 'micropalaeontological approach' to using multichambered foraminifera in monitoring studies.

1861 271. The parameters that should be recorded are species/genus lists, species/genus by
1862 stations matrices providing abundance density per 10cm^2, and gene sequences.

1863 272. From these measurements, density, statistics to describe community structure
1864 (univariate and multivariate diversity measures) and environmental drivers for distribution
1865 patterns should be determined.

1866 **4. Fauna associated with Polymetallic Nodules**

1867 273. The extremely slow growth rates of nodules mean that it will be millions of years before
1868 this hard-substrate is re-established once removed, therefore it is also important to determine
1869 the extent to which species are shared between soft sediments and nodules in abyssal nodule
1870 fields.

1871 274. Samples should be collected using a box corer (sampling area of minimum 0.25m^2), by
1872 ROV or using any other similar benthic device that can collect undisturbed sediment and nodule
1873 samples.

1874 275. All polymetallic nodules in the sediment should be carefully removed, photographed
1875 and examined for the presence of epifauna. Further processing depends on which fauna are
1876 being investigated.

1877 276. For metazoan meiofauna, all the epifaunal organisms should be photographed
1878 immediately, carefully removed from the nodule and stored in 90% ethanol for further
1879 microscopic and other laboratory analysis. Each nodule should be then washed separately on a
1880 25µm mesh sieve and stored in 90% ethanol along with the sieved material for further analysis.
1881 The soft sediment on the nodule should also be washed separately, preferably on a fine-mesh
1882 sieve (20-25 µm) and sieved material should be considered as part of the fauna containing
1883 sediment of their respective layers. In the laboratory, nodules should be examined for crevice
1884 fauna, washed, measured and weighed. The clean nodules should be broken down mechanically
1885 to sand-sized grains and fixed in 90% ethanol. This will yield the sample to be considered as
1886 nodule crevices fauna. This sample then can be processed using any standard meiofauna
1887 extraction procedure. However, it is recommended to use a flotation and centrifugation (4000

1888 rpm) method which is known to yield up to >80% of the fauna (Mclntyre and Warwick, 1984).
1889 The supernatant has to be then washed onto 20-32 µm mesh size sieve. The sieve residue should
1890 be carefully examined under a stereo-microscope (40× magnification). All the faunal
1891 organisms should be identified to the species level, counted, sorted and stored separately in
1892 90% ethanol so they can later be used for molecular identification.

1893 277. For foraminiferal studies, nodules should be taken from the surfaces of box cores or
1894 multicores, placed in separate containers, and preserved in borax-buffered 4% formaldehyde
1895 solution (10% formalin). Wide-mouthed jars should be used so the nodules can be easily
1896 removed without damaging delicate encrusting foraminifera. In the laboratory, nodules should
1897 be carefully washed, if necessary, by squirting water onto the surface with a pipette to remove
1898 any adhering sediment. However, washing should be kept to a minimum and nodules should
1899 be handled as carefully and as little as possible. When clean, the nodules should be placed in a
1900 bowl of water, sufficiently deep to cover them completely, and examined under a stereo
1901 microscope fitted with a digital camera. Foraminifera are typically more common on the upper
1902 surfaces, and may concentrate on higher points, but can also be found on the undersides.
1903 Different morphotypes should be photographed in order to build up a catalogue documenting
1904 their diversity. Where possible, the number of specimens of each type should be recorded.
1905 However, this is difficult to do in the case of some forms, e.g. large reticulated formations and
1906 tubular systems with poorly-defined limits.

1907 278. The parameters that should be recorded are taxonomic identification lists at lowest level
1908 possible (ideally species level), abundance per nodule (nodule volume/weight) and gene
1909 sequences

1910 279. From these measurements, density, statistics to describe community structure
1911 (univariate and multivariate diversity measures) and distribution patterns should be determined.

1912 **5. Microbiota**

1913 280. Sediment samples should be collected with ROV push corer, manned submersible push
1914 corer, (TV) box corer, (TV) multiple corer, or (TV) grab with the sampler sealed as close to
1915 collection point as possible to prevent contamination during recovery.

1916 281. Water samples should be collected with a CTD Rosette with water sampler or an *in-situ*
1917 filtration/extraction for particles, such as McLane Water Transfer System with the sampler
1918 sealed as close to collection point as possible to prevent contamination during recovery.
1919 Samples should be collected at important water layers as defined by the water column sampling
1920 (see section V). The layers to sample include, but are not limited to, the surface layer,
1921 subsurface chlorophyll maximum layer, anoxic layer and near-bottom layer.

1922 282. Samples for cultivation approaches should be stored at 4 °C. Samples for culture-
1923 independent approaches should be stored at - 80 °C or in liquid nitrogen (after being filtrated
1924 using microbe filtration device with micro-filtration film in the case of water samples).

1925 283. Microbial count should be obtained using fluorescent staining method with DNA-
1926 specific dyes (e.g. DAPI) or real-time PCR method with groups-specific oligonucleotide
1927 primers (Labrenz *et al.*, 2004). Where cultivation techniques are used, this should be performed
1928 on board the sampling vessel.

1929 284. Microbial DNA should be obtained by the Phenol Chloroform DNA Extraction method
1930 or DNA extraction kits, and spectrophotometry and DNA agarose gel electrophoresis used to
1931 detect the DNA purity and integrity, respectively. Qualified microbial DNAs should be
1932 sequenced in high-throughput sequencing platform (e.g. Illumina Hiseq X platform, PacBio
1933 RSII platform). Additional amplicon sequencing should be performed for important marker
1934 genes (e.g. 16S rRNA gene).

1935 285. Microbial RNAs should be obtained by RNA extraction kits or similar reagents, and
1936 spectrophotometry and RNA agarose gel electrophoresis used to detect the RNA purity and
1937 integrity, respectively. Qualified microbial RNAs should be sequenced in a high-throughput
1938 sequencing platform. Additionally, specific RNAs should be analysed with real-time PCR
1939 method with specific oligonucleotide primers.

1940 286. There is currently no standard method for splice of high-throughput sequencing. The
1941 commonly accepted methods mentioned are FastQC for quality control; SPAdes for assembly
1942 of sequencing reads; MetaBAT for contig binning; BLAST+ for sequence alignment and gene
1943 annotation; CheckM for assembly and binning quality assessment (Breitwieser *et al.*, 2017).

1944 287. Results of genome sequencing analysis or metagenomic binning of microbial
1945 population should be provided.

1946 288. The parameters that should be recorded are identification, abundance and gene
1947 sequences.

1948 289. From these measurements, the microbial diversity, community composition,
1949 abundance, functional differences of different groups should be determined

1950 **6. Demersal Fishes and Scavengers**

1951 290. One or more of the three main categories of sampling should be used: image transects,
1952 bottom trawls or baited systems. Image transects should follow the approach outlined in section
1953 1. Bottom trawls can be towed independently or behind a camera sledge, the catch provides
1954 voucher specimens for taxonomy and DNA sequencing. Traps and long lines have the
1955 disadvantage that they are species selective and so should not be used for biodiversity studies.
1956 Baited cameras mounted on landers provide unbiassed sampling of the bait-attending fauna in
1957 any given area. For amphipods, small minnow-type traps can be attached to the legs of the
1958 camera lander to catch voucher specimens (Jamieson, 2015).

1959 291. A disadvantage of camera systems is that species are often difficult to discriminate in
1960 images but if utilised, a minimum of ten replicate baited camera drops should be used.

1961 292. The parameters that should be recorded are taxonomic identification lists at lowest level
1962 possible (ideally species level), abundance, gene sequences (when samples are collected), size
1963 measurements of individuals, arrival time after bait touchdown and the maximum observed
1964 number of individuals for each species (for baited landers).

1965 293. From these measurements, density, species richness statistics to describe community
1966 structure (univariate and multivariate diversity measures) and distribution patterns should be
1967 determined.

1968 **F. Measured variable - Connectivity**

1969 294. Population connectivity studies of key species based on sampling from different
1970 geographic locations and/or habitats should be undertaken. For each species, the number of
1971 individuals in each population should ideally be relatively large (>10-20 individuals/site), so
1972 only relatively abundant species might be assessed and used as proxies for the wider
1973 assemblage. However, given the relatively low density for some species found in the CCFZ,
1974 even lower numbers (3–5 individuals/site) should still be enough to conduct connectivity
1975 studies (Taboada *et al.*, 2018).

1976 295. Depending on the setting, collecting enough individuals to undertake connectivity
1977 studies may require employing additional samplers to those identified above. For example,
1978 collecting methods such as epibenthic sledges in benthic habitats may be necessary to ensure
1979 enough macrofaunal individuals are collected. Samples for connectivity studies should be
1980 collected and stored in order to preserve DNA in its best condition, as detailed by Glover *et al.*
1981 (2016). When preserving large specimens or parts of larger specimens, 96% ethanol instead of
1982 80% ethanol should be used.

1983 296. For analysis, the Reverse Taxonomy approach should be used (Janssen *et al.*, 2015).
1984 Vouchers of the specimens under study should be maintained as further detailed examination
1985 of morphological characters (for example using scanning electron microscopy techniques) is
1986 needed to distinguish cryptic species identified molecularly

1987 297. The selection of appropriate molecular markers will depend on the taxon selected. In
1988 some cases, standard approaches, such as the use of most common molecular markers (e.g.
1989 *COI*, *16S rRNA gene*) may not provide sufficient genetic variability to enable further analyses.
1990 A combined approach, using common molecular markers and microsatellite markers, including
1991 highly polymorphic microsatellites should be applied (Taboada *et al.* 2018), which can be used
1992 for small-scale studies.

1993 298. In addition to microsatellites for population genetic studies, other molecular techniques
1994 should be explored, including using single nucleotide polymorphisms (SNPs) generated from
1995 reduced representation genome studies that can be easily applied to non-model organisms at
1996 relatively low costs. For instance, the double-digest Restriction site-Associated DNA
1997 Sequencing (ddRADseq), is able to generate 100s to 1000s of SNPs providing not only the
1998 power to perform fine-scale population genomics studies, but also to investigate
1999 phylogenomics, adaptation strategies, or introgression, among other population-level processes
2000 (Andrews *et al.,* 2016).

2001 299. Modelling approaches using a range of available tools should be used. Gene flow and
2002 migration patterns inferred from the genetic data should be compared with environmental
2003 factors such as oceanographic currents. The use of oceanographic models (section IV.D) to
2004 estimate larval transport may explain some of the patterns in the large-scale population
2005 differentiation and connectivity of the species (Taboada *et al.*, 2018; Kenchington *et al.*, 2019).

2006 300. A variety of programs and software are being developed continuously, so the results of
2007 baseline studies should clearly indicate the tools used in the analyses, together with their
2008 underlying assumptions.

2009 301. From these studies the connectivity and biogeography of key species for each functional
2010 grouping should be determined and inferred for the wider assemblages.

302. Specific metrics to be determined include:

- Minimum genetic distances, using haplotype networks, based on uncorrected *p*-distance and Kimura two-parameter (K2P) models between and within species to establish which are the within and between species genetic distances.
- For genetic diversity, expected (*H*e) and observed (*H*o) heterozygosities, and inbreeding coefficients (F_{IS}) should be calculated for each species, sampling station and region, using R packages or for example the program *GENODIVE* (Meirmans and Van Tienderen, 2004).
- For population structure, one of the following two methods should be used:
  - Clustering Methods such as determined by the programs *STRUCTURE* (Pritchard *et al.,* 2000) and *DAPC* –Discriminant Analysis of Principal Components–, the later included with the *adegenet* R package (Jombart *et al.,* 2010), which provides graphic information on the genetic affinities between samples.
  - Distance Methods such as F_{ST} statistic (Fixation Index) to measure the extent of genetic differentiation among populations. Using pairwise F_{ST} values comparing sampling sites and regions and Analysis of Molecular Variance (*AMOVA*) to determine the hierarchical distribution of genetic variation.
- For migration patterns the *divMigrate* function of the *diveRsity* R package (Keenan *et al.* 2013) should be used to estimate the relative contemporary migration between sampling stations. Alternatively, the programs *LAMARC* (Kuhner, 2006) or *MIGRATE* (Beerli and Palczewski, 2010) can also be used to calculate migration patterns.
- Isolation-by-distance and Genetic breaks a Mantel Test correlating geographic distances with log-transformed and correlated to Slatkin's linearized pairwise F_{ST} estimates ($F_{ST}/1-F_{ST}$) should be calculated using different R packages or using programs such as *GENODIVE.* Occurrence of possible barriers determining the genetic structure of populations should also be evaluated using programs such as BARRIER (Manni *et al.*, 2004).

G. Measured variable - Ecosystem functioning

303. Infauna samples (a minimum of 10-12 randomly selected sites) for natural isotope abundance for food-web structure analysis should be sampled at 0-1, 1-2cm for meiofauna and 0-1, 1-5 and 5-10cm for macrofauna. Megafauna should be sampled for natural abundance isotopes wherever possible such that at least 10 individuals of a particular taxon (e.g., Ophiuroidea) are sampled. Isotope labelling experiments should be undertaken at a minimum of 10 randomly selected sites with replicate benthic chamber measurements made at each site (Sweetman *et al.* 2019).

304. Meiofauna for stable isotope analysis should be sampled using megacores or multicorers and sampled from the 0-1cm and 1-2cm layers. They should be sieved over a 32 or a 63 µm sieve using cold (0-2°C) filtered seawater. Macrofauna should be collected using a 0.25m^2 box-corer and sampled at 0-1, 1-5 and 5-10cm sediment depth and the sediment slices sieved on a 300 µm sieve using cold-filtered seawater.

305. Samples for basic food-web structure of infauna (e.g., number of trophic levels etc) should be collected at the same locations that samples are collected for meiofauna and

2056 macrofauna community structure and include samples from a minimum of 10-12 randomly
2057 selected sites. Where possible, megafauna (e.g., holothurians) should be collected by ROV
2058 during ROV transects or via trawling and efforts should be made to collect at least 10 animals
2059 of each major megafaunal taxon. Isotope labelling studies to quantify microbial and faunal
2060 activities and food-web linkages should be undertaken *in situ* using benthic chamber platforms
2061 (ROV or landers) at a minimum of 10 randomly selected sites with replicate benthic chamber
2062 measurements made at each site (Sweetman *et al.* 2019).

2063 306. Meiofauna and macrofauna sieve residues should be placed in a plastic bag and flash-
2064 frozen in liquid Nitrogen, and subsequently stored at -20ºC. Alcohol-based fixatives should
2065 never be used when fixing samples for stable isotopes. Megafauna collected by ROV or trawl
2066 should be immediately transferred to a cold room and up to 10 individuals of each taxon
2067 individually sealed in plastic bags, and flash-frozen in liquid Nitrogen and subsequently stored
2068 at -20ºC.

2069 307. Meiofauna and macrofauna should be sorted once back in the laboratory with care being
2070 taken to minimize sample warming. Fauna should be washed of attached organic debris in cold,
2071 filtered seawater, and placed in pre-weighed tin or silver (if calcareous) isotope analysis cups.
2072 Target tissues (e.g., body wall, muscle, ophiuroid arm) from megafauna should be removed in
2073 the laboratory, taking care to minimize tissue warming, and placed on foil. All samples should
2074 be dried for 2-3 days at 45ºC and megafauna tissues ground by hand with a mortar and pestle.
2075 Calcareous megafauna tissues should be placed in silver isotope analysis cups. Calcareous
2076 animals and tissues (e.g., ophiuroid arms) should then be acidified with 10% HCl to remove
2077 carbonates and dried again at 45ºC for 3 days, followed by an additional acidification step if
2078 not all the carbonates are removed. Isotope samples should then be prepared for isotope
2079 analysis (as specified by the laboratory that is analysing the samples) and sent away to be
2080 analysed as described in the literature (e.g., Hardy *et al*,. 2008; Levin *et al.*, 2009; Sweetman
2081 *et al.*, 2013).

2082 308. To quantify the dominant food-types for the fauna, sediment trap POM samples and
2083 sediment samples (section V.H) should also be prepared for stable isotope analyses and their
2084 isotope signatures corrected if samples have been preserved in formaldehyde solution.

2085 309. Isotope labelling studies to document food-web activities and linkages should be
2086 undertaken *in situ* using ROV operated benthic chambers or benthic chamber landers. To
2087 document heterotrophic microbial and faunal metabolic activity labelling studies should use
2088 ^{13}C-labelled phytoplankton cultures (Sweetman *et al.* 2019), while autotrophic microbial
2089 activity can be determined using ^{13}C labelled bicarbonate as a tracer. Labelling studies using
2090 ^{13}C-labelled bicarbonate or ^{13}C-labelled glucose will also allow for further detection of food-
2091 web linkages, such as identifying which fauna feed on microorganisms (Sweetman *et al.* 2019).
2092 In situ labelling studies should follow the methods of Stratmann *et al.* (2018) or Sweetman *et*
2093 *al.* (2019) and run for between 36 and 48 hours. Metabolism of organic C (from ^{13}C labelled
2094 phytoplankton) into CO_2 can also be quantified in these experiments if the chambers being used
2095 have syringe sampler capabilities. If so, samples should be collected at set times (e.g., every 6-
2096 8 hrs) during the experiment by the syringe sampler. In the lab, samples should be filtered
2097 (0.45µm cellulose acetate filter) and fixed in exetainers with 5-10 µl of 6% mercury chloride
2098 for total DIC and ^{13}C isotope-ratio mass-spectrometry analysis (Sweetman *et al.*, 2010). The
2099 depth of the water in the chamber and the area of the camber should always be noted to
2100 determine the volume of water in the chamber at the end of each experiment. At the end of the
2101 experiment, push / blade cores should be used to sample sediments for microbes and fauna

2102 from ROV operated chambers, while benthic chamber landers, for the most part, will
2103 automatically collect the sediment that has been exposed to the labelled substrate. Once
2104 onboard, sediments should be transferred to a cold room and sampled for microbe samples at
2105 0-1, 1-5, and 5-10cm depth, homogenised and flash frozen in glass bottles (previously washed
2106 with methanol and dichloromethane in a 1:1 ratio and dried) using liquid Nitrogen and
2107 transferred to -20ºC. Separate samples should be collected at the same depth horizons for
2108 sediment water content. Meiofauna should be sampled from a push core (ROV operated
2109 chambers) or syringe corer (benthic chamber lander) at 0-1 and 1-2cm depth, sieved on a 32
2110 µm or 63 µm sieve and transferred to 4% buffered formaldehyde-seawater solution (i.e., 10%
2111 formalin). Macrofauna should be sampled from blade corers (ROV operated chambers) or the
2112 rest of the chamber in the case of a benthic chamber lander sample, sieved on a 300 µm sieve
2113 and preserved in formalin. Samples for "background" microbial and fauna isotope signatures
2114 should be collected using ROV push-, box- or mega-cores and prepared and preserved in the
2115 same way. Although formalin preservation can affect delta ^{13}C signatures by 0.5-1 parts per
2116 thousand, the labelling of the fauna is likely to be significantly higher than this (500-1000 parts
2117 per thousand) negating the need to freeze the samples. Moreover, the preservation of
2118 background samples in formalin will cancel out the formalin preservation effect on the isotope
2119 signatures when calculating the faunal feeding rates. Once back in the laboratory, the amount
2120 of label uptake into microbial fatty acids and fauna biomass (i.e., metabolic / feeding activity)
2121 should be determined using the approaches described in Stratmann *et al.* (2018) and Sweetman
2122 *et al.* (2019).

2123 310. Natural abundance isotope (^{13}C, ^{15}N) data from fauna, sediment trap samples and
2124 sediments should be generated using an isotope ratio mass spectrometer that are available at
2125 academic institutions and commercial laboratories. The data from samples preserved in
2126 formalin should be corrected for formalin preservation. The corrected values plus the food web
2127 sources should be used determine the basal food-sources that the sampled fauna is feeding on
2128 using an isotope mixing model (e.g., MixSIAR, Harbour *et al.*, 2020), plus the number of
2129 trophic levels present within the benthic food web.

2130 311. The parameters that should be recorded for natural isotope analysis are species lists,
2131 delta 13C signatures, delta 15N signatures, and means together with number of samples and
2132 appropriate error estimates.

2133 312. The parameters that should be recorded for isotope labelling studies are species lists,
2134 rates of uptake of carbon by microbes, meiofauna and macrofauna from different organic and
2135 inorganic sources (in mmol C m^{-2} d^{-1}), identification of key fauna feeding on microbes and
2136 depth of mixing of organic matter into sediments over short term time scales if sediment
2137 samples are collected for $TO^{13}C$. Means together with number of samples and appropriate error
2138 estimates should be provided.

2139 313. Isotope signatures (^{13}C, ^{15}N) in tissues of benthic fauna, Production of ^{13}C-labeled
2140 dissolved inorganic C, ^{13}C-signatures of microbial fatty acids and faunal biomass, depth
2141 distribution of ^{13}C-labelled detritus through sediments should also be recorded.

2142 314. From these measurements, the amount of Carbon taken into the biomass of seafloor
2143 microbes and fauna per unit area per unit time (i.e., the metabolic or feeding activity), the
2144 number of trophic levels present within the food web, the dominant food sources being
2145 consumed, and contribution of different foods to the diets of different fauna, the trophic
2146 structure of meiofauna and macrofauna, microbial and faunal Carbon cycling rates, rates of
2147 short-term sediment mixing and respiration rates should be determined.

2148 **H. Measured variable - Ecotoxicology**

2149 315. Establishing the potential ecotoxicological risk for ore mining should involve multiple
2150 sources of data (or 'Lines of Evidence' LoE) that should be collected prior to mining. These
2151 sources of data can be compartmentalized into discrete components to build a Weight of
2152 Evidence (WoE) to establish a relative toxic risk (Regoli *et al.*, 2019) for a particular resource
2153 and a particular mining operation.

2154 316. WoE should integrate data from 4 LoEs which are:

2155 - sediment physico-chemical properties
2156 - laboratory ecotoxicological bioassays
2157 - bioaccumulation of metals in indicator species
2158 - sublethal effects/biomarkers in indicator species

2159 317. Each LoE should be analysed using the most suitable quantitative methods and all
2160 should be determined during the baseline data collection.

2161 318. Resource mineralogical characterisation to determine the relative proportion of mineral
2162 metal species, should be used to identify the metal and metal mixtures that will contribute to
2163 the overall potential toxic risk to biological species.

2164 319. In addition, biological specimens of key biomass or food web dominant species (from
2165 a minimum of three taxonomic groups, but see discussion in part R.10.3.2 of ECHA, 2008) for
2166 both benthic and pelagic (full water depth) compartments should be recovered on multiple
2167 occasions (> 4 occasions) through a *minimum* of a 12 months seasonal cycle in order to
2168 determine baseline concentrations of metals, other organic contaminants and the levels of
2169 biochemical and cellular biomarkers in key benthic, abyssopelagic and bathypelagic species.
2170 These biomarkers are the early warning signals of distress to ecosystem health (Andersen,
2171 1997; Mestre *et al.,* 2017).

2172 320. Established biomarkers assays, such as of tissue superoxide dismutase (SOD) activity,
2173 using the spectrophotometric determination of the reduction of cytochrome c by the xanthine
2174 oxidase/hypoxanthine system at 550 nm (e.g. McCord and Fridovich, 1969), should be used to
2175 assess the activation of antioxidant detoxification pathways. Other antioxidant assays could
2176 include the quantification of metallothionein protein concentration (e.g. Bebianno and
2177 Langstone, 1989; Mourgaud *et al.*, 2002) using differential pulse polarography, as well as
2178 enzymatic assays of catalase (CAT), glutathione peroxidase (GPx) and glutathione-S-
2179 transferase (GST) activities (Auguste *et al.*, 2016).

2180 321. Thereafter, the relative ecotoxicity of different bulk mineral phases (e.g. particulate and
2181 aqueous) to biological organisms should be established using proxy biological species in
2182 controlled, standardized laboratory experiments. Bulk toxicity of a resource can be established
2183 without *a priori* knowledge of the precise mineral composition. Using established laboratory
2184 protocols, the relative toxicity (relative to known pure mineral standards – e.g. chalcopyrite
2185 $CuFeS_2$) of different phases of the bulk resource should be quantified. Aqueous (e.g. metal
2186 minerals leached from a freshly-exposed mineral surface) and solid phase experiments should
2187 be conducted to mimic the intended mining operation, replicating fragment/particle size and
2188 duration/temperature of leaching (e.g. Brown and Hauton, 2018; Knight *et al.*, 2018).
2189 Internationally recognised standard protocols should be employed to establish bulk resource
2190 toxicity (e.g. ECHA 2008, ECHA 2016).

2191 322. The potential toxicity of dewatering plumes should be determined, based on the
2192 operator plan for 'at sea' processing, surface transfer and shipment, and dewatering including
2193 any additives such as chelating agents or lubricants, to proxy biological species relevant to the
2194 intended depth of discharge. Model biological species may include cultures of cyanobacteria
2195 (e.g. *Prochlorococcus*, *Synechococcus,* or *Cyanobium*) in the epipelagic zone, zooplankton
2196 (e.g. calanoid or cyclopoid copepods) or cnidarians (e.g. *Aurelia* or *Nematostella*) for discharge
2197 plumes in the meso- and bathypelagic zone, as well as fish (e.g. marine medaka *Oryzias*
2198 *melastigma* (Bo *et al.*, 2011; Kong *et al.*, 2008)).

2199 323. Lethal concentration LC_{50} or Lethal Dose LD_{50} toxicity of potential dewatering plumes
2200 to appropriate macrofaunal proxy species, chronic, or sub-lethal toxic effects of exposure to
2201 solid or aqueous phases of the bulk mineral or the dewatering plume, and the most relevant
2202 biomarkers activity should be determined.

2203 **I. Measured variable - Whales, sharks, turtles and surface nekton**

2204 324. To obtain an understanding of whales, sharks, turtles and surface nekton a combination
2205 of ship-borne visual line transects using standard methods as described in Buckland *et al.*
2206 (2001), Barlow and Forney (2007), Verfuss *et al.* (2018) and SCANS II project
2207 (http://biology.st-andrews.ac.uk/scans2/inner-finalReport.html) should be used. This should be
2208 undertaken during daylight hours with the ship moving at constant speed of 9-10 knots on a
2209 grid pattern at each station, supplemented by towed hydrophones for detection of marine
2210 mammal vocalisation. This information should be supplemented by Passive Acoustic
2211 Monitoring (PAM) stations deployed on oceanographic moorings to continuously monitor the
2212 vocalisations of marine mammals over several complete annual cycles.

2213 325. The parameters that should be recorded are species encountered (for sea mammals it
2214 may be possible to identify specific individuals) and the abundance of those species.
2215 Photographs should be obtained where possible.

2216 **J. Measured variable - Seabirds**

2217 326. To obtain a thorough understanding of seabird distribution, abundance and impacts of
2218 any human activity at sea several sources of information should be used. Monitoring seabird
2219 attraction and collisions to infrastructures and stationary ships, systematic seabird censuses and
2220 the compilation and analysis of previous collected seabird tracking data should all be
2221 undertaken along with analysis of monitoring programs (breeding numbers, demographic
2222 parameters, breeding success, etc.) on relevant breeding sites. In addition, where possible
2223 tracking of relevant species and populations should be undertaken.

2224 327. Seabird abundance and attraction should be studied from stationary platforms or ships
2225 using visual surveys, imaging or radars. Visual surveys from stationary ships should be
2226 conducted using instantaneous counts, or snapshots, of birds within a semi-circle radius
2227 (usually up to 300-500m) for 10-15 minutes at regular time intervals (e.g. from 20 to 60
2228 minutes) (Gjerdrum *et al.*, 2012; Bolduc and Fifield, 2017). Marine radars should be used to
2229 estimate seabird abundance and collision risk (Gauthreaux and Belser, 2003; Desholm and
2230 Kahlert, 2005; Bertram *et al.*, 2015; Assali *et al.*, 2017). In addition, seabird abundance and
2231 attraction should be assessed by censusing seabirds using line-transects from ships or
2232 aeroplanes (Camphuysen *et al.*, 2004; Ronconi and Burger, 2009; Gjerdrum *et al.*, 2012).

2233 328. Whenever possible, seabird carcasses killed by collisions should be collected by
2234 systematic searches, preserved frozen in a permanent infrastructure for future reference of
2235 emerging contaminants, and analysed for contaminants in different tissues (Gochfeld, 1973;
2236 Barbieri *et al.*, 2010; Amélineau *et al.*, 2016) to create a baseline against which to compare
2237 tissue content in carcasses collected during operations. A wide range of contaminants,
2238 particularly those that may be released during mining activities, should be analysed.

2239 329. Relevant data sets should be requested and used for assessing the importance of a
2240 specific area for seabirds (among other marine predators). At-sea tracking data exist for many
2241 marine top predators, and there are currently compilations of information on marine migratory
2242 species regularly collated by a number of global initiatives, such as the Seabird Tracking Data
2243 Base (http://www.seabirdtracking.org/), the Migratory Connectivity in the Ocean
2244 (https://mico.eco/) or the Movebank for Animal Tracking Data
2245 (https://www.movebank.org/cms/movebank-main).

2246 330. Tracking data offer the opportunity to identify the source of the seabirds occurring in a
2247 specific area, allowing for a further identification and monitoring of their population of origin.
2248 It also allows for obtaining precise estimation of the population and species using a specific
2249 area (some of them difficult to identify at sea from a ship or a platform), breeding status,
2250 seasonal variation, specific populations visiting a certain area and even the age and sex
2251 structure of the visiting animals. This information should be used to identify the source
2252 breeding colonies. Monitoring programmes in those breeding colonies act as additional
2253 baseline data that should be reviewed. If there is any evidence or reasonable suspicion that
2254 contractors' activity is producing seabird mortality in significant numbers on specific seabird
2255 populations, monitoring programmes should established to study the demography of the
2256 affected populations.

2257 331. The parameters should be recorded year-round as follows:

2258 • From visual surveys, censuses, imaging or radars counts - relative and absolute
2259 abundances of seabirds identified to the lowest taxonomic level possible, usually
2260 at species level, and whenever possible by sex, age, seasonal and morph plumage
2261 variations; diversity indices and the use of the area over time
2262 • From tracking data - proportion of birds from each colony using the area over
2263 time by species, population, breeding colony, breeding status, sex and age;
2264 • From monitoring programmes - population size, breeding success,
2265 juvenile/immature/adult survival, recruitment age, population trends and
2266 estimations on population viability and time to extinction;
2267 • From collisions and collected carcasses - numbers of deaths/day over time by
2268 species, sex, sexual maturity, moulting and body condition; tissue collection
2269 (liver, muscle, fat and feathers) and concentration of contaminants (Stockholm
2270 Convention list) on these tissues; microplastics and microfibres in the stomach.

2271 **K. Data Quality**

2272 332. Temporal sampling should revisit the same sampling locations as previous surveys
2273 where possible. Samples for temporal analysis should have sufficient size for robust
2274 determination of the parameters of interest. To improve comparability, sample size should be
2275 kept constant between surveys.

2276 333. When comparing datasets collected or analysed by different researchers,
2277 standardisation across datasets should be carried out. This is particularly important in time-
2278 series investigations or those using multiple operators. Where inconsistencies are found, further
2279 quality control will be necessary.

2280 334. Comparisons between megafaunal surveys can be made even if the acquisition
2281 methodology is not identical. However, robust comparison relies on having accurately
2282 quantified (scaled) images and as much consistency in image quality (resolution, lighting,
2283 colour balance etc.) as possible. The possibility of methodological bias between surveys should
2284 be carefully evaluated in any subsequent comparisons, for example key taxa controlling
2285 patterns should be evaluated to ensure they are clearly distinct in different datasets. It should
2286 be the assumption that there is methodological bias until proven otherwise.

2287 335. To ensure suitable quality of images, lighting should be sufficient to maintain near
2288 uniform lighting of the entire seafloor image at the target altitude, consistent imager settings
2289 (zoom, exposure etc.) should be maintained throughout the survey and the camera should not
2290 be moved relative to the camera platform (e.g. using a pan-and-tilt unit on a ROV) for any
2291 transect.

2292 336. All images should be accurately scaled using a photogrammetric approach, which
2293 requires accurate information on image altitude, pitch and roll. Altimeter data should be
2294 accurate to ± 10mm. Test images of known scale should be obtained on the seafloor to verify
2295 calculations. Use of lasers projected onto the seafloor is an alternative approach.

2296 337. Many organisms can only be identified to species level by examination of features not
2297 visible on photographs (for example those hidden, internal or microscopic). Furthermore, other
2298 tools, such as molecular approaches (genomics, transcriptomics, population genetics etc.)
2299 require specimen material. As such, precise samples of individual specimens should be
2300 obtained that are linked in situ images, ex situ images, tissue samples and a sample for
2301 morphological analysis from the same individual. Such samples are best obtained by remotely
2302 operated or human occupied vehicle. This is particularly important for the many taxa,
2303 particularly soft-bodied forms (e.g. anemones), that look very different alive on the seabed than
2304 they do after recovery to the surface.

2305 338. All identifications should be the lowest taxonomic level possible. Taxonomic keys and
2306 references used to determine these designations should also be provided in order to ensure
2307 equivalence between identifiers.

2308 339. Molecular identification through barcoding (Sanger sequences) and metabarcoding
2309 (Amplicon Sequence Variants, ASVs) should provide a species or genus list resulting from
2310 matching the acquired genetic data to that available in public reference databases such as
2311 GenBank. This can be achieved through Basic Local Alignment Tool (BLAST) or the RDP
2312 classifier.

2313 340. Biomass is appropriate when it is evaluated by an ecological material cycle model, and
2314 in that case, classification based on size is better than classification based on taxonomy.

2315 341. When larger samples are needed than can be collected using precise approaches, trawl
2316 or epibenthic sledge sampling may be appropriate in these cases. Care should be taken as these
2317 techniques have the potential to disturb relatively large areas of the seafloor, which may require
2318 an EIA (see ISBA/25/LTC/6Rev.1 and Cor.1) and may affect other sampling efforts.

342. To determine whether sufficient individuals have been collected to characterise the communities, a collector's curve or Chao analysis should be undertaken. The latter is likely to be required given the low numbers of individuals and the high diversity.

343. To ensure statistical robustness, a sufficient number of replicates should be sampled. The number of replicate samples will depend on the density or richness of the taxon of interest and its variance. In order to demonstrate statistical robustness, the power of a BACI analysis of variance should be reported based on actual data provided by the baseline. The power analysis should be presented considering Cohen's d scale of effect size (low d=0.2, medium d=0.5, high d=0.8) (Cohen, 1988). The number of replicate samples required to achieve a power of 80% should be provided.

344. The number of nodules required for studying the faunal association depends on the abundance of the nodule in the study area, and the number of nodules actually collected in a box corer or sampler. A minimum of ~25 nodules should be collected randomly for the benthic biodiversity study. To get better spatial sample coverage, samples from at least three-box cores per physiographic area should be collected during the baseline data generation and monitoring study.

345. Where sampling design is unbalanced, diversity indices should be rarefied to the lowest number of replicates.

346. Numbers of seabirds will be specific for that site and it will not be possible to understand the origin, breeding status, age or sex of the observed seabirds. Seabird identification at sea is not an easy task and should be carried out by trained ornithologist using one of the global seabird identification guides (Harrison, 2000; Howell and Zuflet, 2019). Most seabird tracking data are biased or limited to a number of species (some small but mostly medium to large size species) and to specific periods of the annual cycle and to specific life stages (usually adult breeders).

L. Data Management

347. Metadata for all the specimens collected should be generated, including depth, latitude, longitude and substrate where they occur (e.g. nodule, infauna, associated to other organisms). This should be used to create catalogues of species using the Darwin Core layout.

348. Vouchers for all the specimens should be deposited in museums or national collection facilities in order to make them available for the scientific community using storage appropriate to the analysis (e.g. formalin or ethanol for morphologic identification, ethanol or freezing for molecular analysis). For some analyses (e.g. ecotoxicology), where the entire specimen cannot be stored, several tissue samples (at least muscle, feathers, intestinal fat and liver) should be taken and individually stored.

349. DNA extractions should be preserved in cryofacilities of museums. Genetic sequences should be deposited in free repositories such as GenBank (https://www.ncbi.nlm.nih.gov/genbank/). Genotypes should be deposited in free repositories such as Dryad (https://datadryad.org/stash) or Pangaea (https://www.pangaea.de/). RADseq data should be deposited in free repositories such as the NCBI SRA database (https://www.ncbi.nlm.nih.gov/sra). Sanger and HTS data should be archived in publicly available databases along with all relevant metadata, especially georeferencing information. GenBank should be used for Sanger data, the Sequence Read Archive (SRA) should be used

for HTS data; note that the latter should be uploaded demultiplexed, i.e. two read files per sample.

350. Wherever possible, identifications should be documented using photographic evidence should the information need to be revisited.

351. Image data should be ideally stored as raw files (as obtained by the camera) and the files used for analysis (with processing applied) and these should be linked to the survey metadata through the unique image name, so the datasets can be easily combined.

352. Raw data, and information about where and how specimens are stored should be submitted to the ISA as part of the Annual Reports and as metadata in the contractor's data submissions to the ISA DeepData database.

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