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**Draft regulations on exploitation of mineral resources in the Area**

## Draft guidelines for the establishment of baseline environmental data

### Prepared by the Legal and Technical Commission

## Contents



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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. The present Guidelines are focused primarily on deep-sea polymetallic nodules found in the central and north-western Pacific Ocean and in the Indian Ocean. Some elements may not apply to all mineral types. Further iterations will be issued in the future to cover polymetallic sea floor massive sulphides and cobalt-rich ferromanganese crusts.

3. These Guidelines contain guidance on how an applicant or Contractor may fulfil the requirements concerning the acquisition of oceanographic and environmental baseline data. They are built on and should be read with 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 in conjunction with ISBA/25/LTC/6/Rev.1/Corr.1).

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

(a) The Environmental Impact Assessment process;

(b) The preparation of an Environmental Impact Statement;

(c) The preparation of an Environmental Management and Monitoring Plan;

(d) The development and application of Environmental Management Systems.

5. In the event of any inconsistency between these Guidelines and the exploitation regulations, including their annexes, or any Standards, the regulations, including their annexes, and the Standards, prevail.

# II. Purpose and scope

6. The primary goal of acquiring baseline data is to characterize the existing environment so that the possible impacts of Exploration and Exploitation on the Marine Environment can be assessed prior to those activities taking place. Baseline data also articulate the methodologies and form the basis for the long-term monitoring of environmental impacts, thereby ensuring that baseline data can effectively support the Environmental Impact Assessments and Environmental Monitoring and Management Plan once Exploitation commences.

7. When the Environmental Impact Statement is submitted, it is the responsibility of the applicant for a Plan of Work to provide assurances that no impact will surpass relevant thresholds. These Guidelines are not intended to set those thresholds, nor to address conservation issues that need to be considered at that stage. It is the responsibility of the applicant to describe how the baseline data have been used to draw conclusions about any impacts at that time. Management measures are determined when the Plan of Work is submitted, as it is dependent on the baseline data obtained and the conclusions drawn by the applicant, which are assessed against the standards of the scientific community at that time. It is therefore the responsibility

of applicants to ensure that the data are fit for purpose. Furthermore, the baseline data provided help to inform Regional Environmental Management Plans.

8. Appropriately designed sampling is the cornerstone of environmental surveys and monitoring. If samples are not taken in sufficient numbers, with sufficient spatial coverage and with the correct equipment in line with the Best Available Techniques and Good Industry Practice, then all the subsequent data and analyses will be flawed or compromised. Following this best practice also ensures that sampling does not have an unnecessary additional impact on the environment.

9. These Guidelines contain guidance on the following aspects:

(a) Scope, coverage and standard of baseline data needed to characterize the physical, chemical and geological properties of and the biological communities living in the Area and water column that may be affected by mining activity;

(b) Review and evaluation procedures to assess the quality of environmental baseline data and the statistical rigour needed for detecting and differentiating change from baseline/background levels;

(c) Data management, particularly as it relates to metadata needed to support data deposition and the reporting of environmental baselines.

10. In these Guidelines, the baseline data that should be collected are grouped under the following headings:

(a) Physical oceanography;

(b) Chemical oceanography and biogeochemistry;

(c) Geological properties;

(d) Biological communities.

# III. Sampling and data acquisition

11. Baseline data should be multidisciplinary to allow for a holistic assessment of environmental conditions and processes. Sampling and replication are necessary to create an appropriate representation of the environment so as to identify changes and determine whether those are associated with mining operations or, instead, are the result of natural spatial and temporal variability and trends or of non -mining-related anthropogenic activity. Without such knowledge, it would not be possible to attribute deviations from pre-mining conditions observed during mining operations to anything other than Exploitation activities. Therefore, comprehensive information on the natural variability in baseline conditions should be collected before beginning th e commercial mining phase.

## A. Spatial and temporal variability

12. The magnitude and spatiotemporal scales of variability are likely to differ for different variables and for different ecosystem components. Consequently, the replication and frequency needed to address variability are also likely to differ for different components. To achieve a robust coverage of temporal and spatial variability and to reduce the uncertainty associated with the data, replicate observations should be obtained to detect changes as a result of time (seasons, interannual variability) and space (horizontal and vertical), and differentiate between regions.

13. Care should be taken to align the baseline sampling sites with requirements for monitoring during future mining operations. They should therefore be located in a

manner that they can later serve as IRZs and PRZs. They should also be established in sufficient numbers so that effects connected to both direct and indirect impacts can be addressed with the necessary statistical rigour. In choosing an arrangement, consideration should be given to conditions such as the natural variation in ocean conditions, including ocean current directions, substantial topographic features and substrate type (e.g. soft and hard substrates), as those will influence in what direction and over what distance sediment plumes generated by the mining collector may disperse and resettle.

14. Standard references for global ocean biogeography should be used to determine the relevant large-scale biomes; see, for example, Longhurst (1998) for the epipelagic environment, Sutton et al. (2017) for the mesopelagic environment and Watling et al. (2013) for the benthic environment. New biogeographies are currently the focus of research efforts, as are bioregionalizations, also called broad-scale habitat maps, which may result in a more practical tool in support of spatial management approaches (McQuaid et al., 2020). The Esri ecological marine units (www.esri.com/en-us/about/science/ecological-marine-units/overview) may be a useful reference, but not as a replacement for additional site -specific data-gathering and analysis. The main currents should be mapped throughout the water column and relevant mesoscale and submesoscale features within the sampling area (size: 1–100 km) should be identified, such as meanders, eddies and fronts, as well as features influenced by seabed topography such as seamount wakes and Taylor columns. Archived remote-sensing satellite altimetry and sea surface temperature data should be accessed and analysed to identify currents and surface oceanographic features. The area considered should extend beyond the Contract Area to cover the major current systems in the region and their variability, including the areas of origin of major mesoscale features such as eddies. This is needed to decide what area to include in oceanographic modelling and to understand the possible origin of oceanographic and biological observations within the Contract Area. To detect seasonal and interan nual changes and cover the occurrence of infrequent oceanographic phenomena such as El Niño as well as decadal trends, a time series is needed that extends at least 20 years into the past and covers microwave and infrared temperature data. The time series should be enhanced by ocean colour data and the areas considered should be reviewed to validate the biomes and determine interannual variability (Henson et al., 2010). Major seasons should be identified.

15. In quasi-homogeneous areas such as within a gyre province over an abyssal plain there may be only one identifiable vertical zone. Latitudinal or longitudinal gradients may indicate more than one stratum. In the vicinity of fronts and mid -ocean ridges there may be considerable spatial heterogeneity leading to multiple zones. In eddy fields, sampling should be flexible to include anticyclonic and cyclonic eddies.

16. To obtain sediment, pore water and biological samples (including eDNA and samples intended for molecular analysis), a nested stratified sampling scheme should be used to ensure that the collection of samples and data encompasses the range of environmental settings at the scale of an exploration contract area, as depicted in the figure below. On the basis of the data collected for other variables – primarily physical oceanography (see sect. IV); chemical oceanography (see sect. V); and geological properties (see sect. VI) – regions should be divided into biogeochemical and bathymetric entities. Within each of those entities, a nested set of physiographic zones, geomorphological elements or features and units with different topographies and different nodule coverage should be established (abundance and size) to fully cover conditions that are expected to be important drivers of changes in communit y and biogeochemical functions. Each physiographic zone is a complex of physiographic units within a defined area. Such physiographic units typically include seamounts, abyssal plains, hills, slopes, crests and valleys, with low to high

abundances of nodules of various sizes. Additional units should be defined as needed to cover the specific conditions and their variability in the respective Contract Areas. This is visually represented in the figure below. The location and extent of the units should be defined on the basis of a ship-based bathymetry and high-resolution sea floor acoustic and optical imagery, such as that obtained with ROVs, AUVs or cable- deployed gear.

**Conceptual scheme of a sampling programme**

*Abbreviation*: BC, box coring.

17. Observations should be carried out at different and predetermined times of the year to cover seasonal changes in productivity and hydrodynamic conditions. Specifically, periods of contrasting bottom water flow regimes and seasons with different organic matter availability should be covered. In addition, diel changes throughout the 24-hour cycle should be quantified where they are relevant (e.g. pelagic systems).

18. Where variables show significant temporal variability that cannot be addressed by making discrete observations and where appropriate observation technologies such as autonomous platforms, sensors, and samplers exist, observations should be conducted continuously and at high frequency. Periods of continuous observation should be timed to cover the time scale for a given variable at a given location as in

the case of, for example, tidal cycles or seasonal productivity cycles. Observations should be included of benthic systems that have been shown to display significant temporal dynamics in deep-water environments (Davies et al., 2009).

19. Variables that are not expected to show significant seasonal variability should be validated at least once by comparing observations at contrasting seasons (spring/summer and winter).

20. Observations in similar seasons or environmental conditions should be made to assess interannual variability. As interannual changes may take place over several years, making observations over several years increases the likelihood of capturing periodic events. In addition, the temporal sampling strategy should cover interannual changes and should include possible periodic variations, such as those connected to the El Niño Southern Oscillation. Other natural stressors, such as global warming and rising atmospheric $CO_2$ levels, their impact on the environment where baseline data are being collected and their temporal variability, should be considered when developing an environmental baseline. Consideration should further be given to ensuring that any variability observed is not an artefact of disturbance caused by previous sampling.

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

22. Unless indicated otherwise in the sections on specific variables, the vertical sampling resolution should be as follows:

(a) For water column sampling (including physical measurements, unless indicated otherwise in sect. IV.B), a higher resolution should be used for sampling in the 200 m below the surface (three or four samples at depths determined on the basis of local variability) and in the 500 m above the seabed (e.g. at 5 m, 10 m, 25 m, 50 m, 75 m, 100 m, 150 m, 200 m and 500 m above the seabed), keeping in mind that surface weather conditions and localized topography may affect the resolution that is possible very close to the seabed;

(b) For seabed sampling, unless a higher resolution is indicated in the sections on specific variables the vertical resolution should be 0–0.5 cm, 0.5–1 cm, every 1 cm down to a depth of 10 cm, and every 2 cm from a depth of 10 cm to a depth of 20 cm or down to the depth to which the sediment is expected to be affected by the mining equipment, whichever is deeper. Where deeper measurements are required, samples should be taken every 5 cm between a depth of 20 cm and a depth of 50 cm, and every 20 cm in deeper layers over a sediment column of up to 5 m. This resolution should be considered a guide and should be increased where initial studies carried out at a high resolution, such as for determining redox zonation, indicate that more layers are needed to suitably characterize vertical profiles. Where surface sediment conditions are more fluid and taking fine-resolution slices is not possible, a more pragmatic approach needs to be taken and 0–1 cm should be sampled.

23. Random replicates should be obtained from each sample site and the replication should be sufficient to cover the variability and discriminate between physiographic units. The number of replicates required to characterize baseline conditions in a specific zone is determined on the basis of a number of factors, including the variable being considered, and is likely to differ among Contract Areas. Therefore, the number of replicates should be justified using appropriate statistics. Lower temporal and spatial variability are expected in deeper sediment layers. Hence, to assess conditions in deeper sediment layers, measurements in a single long core from each site that are repeated over several campaigns may be sufficient, unless significant temporal or small-scale spatial variabilities are observed.

24. Samples or data collected during the same deployment of a single platform, such as cores from a single multicore deployment or multiple sensors on a single lander, should be considered one sampling point (i.e. one biological replicate). Wher e samples are subdivided, the purpose should be to obtain different variables from the same sample and not create pseudo-samples. Pseudo-samples are created when subsamples are taken from the same main sample, such as a box core or several cores from a single multicorer drop, and are then treated as replicates. Such samples are not statistically independent.

25. Where the sampling needed to determine spatial and temporal variability is not detailed in the relevant sections below, the protocols to follow are those outlined in document ISBA/25/LTC/6/Rev.1 in conjunction with ISBA/25/LTC/6/Rev.1/Corr.1.

## B. Adaptability of sampling strategies

26. Initial sampling and observation strategies should be built on the best available existing research and data. The strategies should be revised regularly as more information becomes available to ensure that they are fit for purpose and adequatel y capture spatial and temporal variability. It should be demonstrated that observations made in areas or on spatial scales that have been considered homogeneous indeed show less variability than observations made in areas where more variability was expected. Also, it should be established whether observations made in similar seasons are less variable than those made during different times of the year. However, changes in sampling strategy should be made with caution so as not to miss episodic events, leave interannual variability unresolved or give rise to inconsistencies that prevent a temporal analysis, especially if the observations are discontinued at certain sites or during certain seasons.

27. Expert input has been obtained to ensure that the methodologies in the present Guidelines correspond to best practice. However, techniques and processes develop over time. Therefore, to adequately characterize the environment, the Best Available Techniques should be used or, if they are not, a justification should be provided. Independent feedback should be sought from an organization or person with relevant expertise in the field to enable suitable adjustments as required. Where data collection has already commenced, care should be taken to ensure the consistency of data obtained with different approaches so as to allow for integrated assessments of all data obtained.

28. As the details of the technology to be used for resource Exploitation become available and Exploration progresses, the sampling programme should be adjusted as required to ensure that the baseline data are focused on areas where mining is expected to take place and any impact is likely to be seen. This is particularly relevant where the potential depth of mining exceeds the suggested sampling depth s for individual variables or where significant variability in the parameters is identified.

## C. Coordination and cooperation

29. Where possible, measurements of different variables should be aligned both temporally and spatially to facilitate integrated data analysis and to strengthen explanatory power. This is particularly important for variables that are relevant to interconnected or similar processes that fall within the same or closely connected disciplines (e.g. geology – sediment biogeochemistry etc.; oceanography – ocean chemistry, pelagic biology, etc.), or that need to be combined to create derived products. Where the methodology is compatible, samples from single sediment cores should be used to analyse multiple parameters (e.g. the same cores for pore water and

sediment characteristics). Box cores for macrofaunal sampling should not be subsampled (see sect. VII, paras. 234–237).

30. Contractors should collaborate and exchange data and information with each other and with the scientific community wherever possible to enable analyses that extend beyond the Contract Areas of individual Contractors. Doing so will provide context in the form of larger-scale patterns. That context can facilitate the interpretation and use of baseline observations and underpin a larger-scale analysis, the result of which can inform Regional Environmental Management Plans. Another advantage of this approach is that it reduces the burden on individual Contractors.

31. Sharing data between Contractors and the scientific community is highly recommended to assure that high-quality data have been acquired following state-of-the-art methodology.

32. Many of the variables discussed in these Guidelines are also addressed by GOOS (www.goosocean.org). GOOS has created a framework around essential ocean variables that can be used to draw up a cost-effective plan for compiling an optimal global overview for each essential ocean variable. Many of the variables in these Guidelines have an essential ocean variables factsheet associated with them that has been created and disseminated by the expert panels. The factsheets identify the measurements to take, the observing options available and the data management practices to follow. They refer the user to best practices and guides and contain background information. The information in them is a supplement to these Guidelines. The current set of essential ocean variables corresponds to physical and biogeochemical oceanography observations but are lacking important information on biology and benthic biogeochemistry.

## D. Data quality

33. All measured data should be compared with observations from the same region or similar depth and biogeographic areas that are available in the scientific literature and in other sources. A good agreement between state-of-the-art models and observations of variables is considered a strong indication of a baseline data set of good quality, consistency and completeness. A comparison of observations to model results should therefore be a core component of reporting and should include reference to all information needed to run the model and reproduce results. Where discrepancies occur between measurements and the model, those should be investigated to resolve the error. Doing so may require adaption of the model or collection of more samples.

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

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

36. The number of replicates required within each physiographic unit depends on the existing natural variability (see above). Statistical methods, including power analysis (Jumars, 1981), should be used to decide on the sampling effort required to detect relative changes at an appropriate resolution.

37. Uncertainty and limits of detection of methodology should be presented along with any measurements.

38. Where data are corrected for depth, temperature, sample size or any other variable, details of the correction should be provided and the exact procedure explained. That information should be accompanied by the raw data.

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

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

41. The information contained in these Guidelines concerns the minimum expectation. Any extra sampling or analysis beyond what is outlined in this document and the additional documents cited will increase the quality and is therefore encouraged.

## E. Data and sample management

42. Data (including metadata), samples and specimens should be archived using the appropriate long-term preservation standards to enable revisiting of the raw information, should further analysis or quality control be required.

43. The Contractor should archive raw data in such a way that they can be traced back to their origin. Space and time of collection and the methodology used should be included.

44. Raw and derived data should be submitted, in an agreed format, to established and long-term sustained global data assembly centres that provide open access.

45. Digital data, including relevant metadata, should safely be stored both locally and in the cloud to guarantee their long-term availability. They should also be provided to the secretariat of the Authority as set out in the recommendations for the guidance of Contractors on the content, format and structure of annual reports (ISBA/21/LTC/15).

46. Data and findings should be published in international, peer-reviewed and open-access scientific journals and should be presented at international scientific conferences to facilitate the dissemination of new information. Moreover, publication enables multiple independent experts to provide feedback and give approval.

47. Latitude and longitude should be collected in decimal degrees in accordance with the World Geodetic System 1984, and time and date recorded in Coordinated Universal Time. Reporting formats should follow accepted international standards.

48. Standard metadata should be recorded (including position, water depth, expedition identifier, station identifier and principal investigator) following established metadata standards.

49. As part of their data submission to the Authority, Contractors should provide detailed information on the sensors and sampling device used (type, manufacturer, identifier, date and method of last calibration) and give a detailed description of the measurement and sample analysis methods followed, including deployment details for sampling equipment, reference information regarding standards adopted, best practices adhered to or method descriptions in scientific publications followed, in line with the relevant guidance.

50. Where meta-information contains references to publications (such as cruise reports or method descriptions), persistent identifiers or duplicates should be provided to ensure long-term availability.

51. For derived data, relevant metadata need to be supplied, including all information that is needed to reproduce the analyses, if necessary together with the conversions applied. A reference should be provided to the raw data, which should include core measurements and all supporting variables used for calculations. The protocols, software and code used should be specified in sustained, open-access online resources that allow for version control and contain persistent identifiers (e.g. GitHub or the protocols.io platform).

52. These principles apply to all variables. Additional information is provided in the sections below.

# IV. Physical oceanography

## A. Introduction

53. The main objectives for establishing a baseline of the physical oceanography of a Contract Area are:

(a) To define the hydrophysical and hydrodynamic conditions and the structure of the water column and its variability in order to:

(i) Understand the habitats of marine organisms;

(ii) Define a detailed sampling strategy for other sampling measures;

(b) To assess the potential dispersion, the size and the characteristics of any operational and discharge plumes.

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

(a) Temperature, pressure and salinity: the seawater parameters that determine water column stratification and discrete water masses within which other variables should be measured. These variables will also be required when deriving information from other data;

(b) Currents: knowledge of currents is crucial to understanding the connectivity of populations of marine organisms and to assess the dispersion of any operational and discharge plumes;

(c) Tides and waves: tides and waves interact with current flow to influence mixing. Tides also affect some marine organisms (tidal cycles);

(d) Turbulence: vertical turbulent mixing is a dominant factor in controlling the vertical flux of material in the water column. while bottom-enhanced turbulent mixing plays an important role in water mass transformation;

(e) Optical properties: light penetration and light 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. Optical properties also include the light field pertinent to fauna species that use bioluminescence to feed, hide and reproduce. Sediment particles from a dewatering plume may prevent fauna from using bioluminescence, which will reduce mating and/or feeding success;

(f) Noise: noise is created by numerous sources located both inside the ocean and on its surface. It can affect a range of marine organisms, including invertebrat es, fish and marine mammals. Marine species may be affected in their development, anatomy, physiology, behaviour, ecosystem services and mortality rates, which in turn has a socioeconomic impact on fisheries. Furthermore, noise-generating activities can affect population health, marine species welfare and ecosystem dynamics.

## B. Sampling resolution

55. For many of the physical sampling methods, the same sampling device should be used to take samples at the same time. This approach greatly increases the resolution and should be followed wherever possible.

56. Variability in physical parameters should be determined using a different sampling methodology, as shown below:

(a) Spatial variability (vertical): stations (CTD and water samplers), LADCP, floats/drifters, AUVs/gliders and ship-mounted ADCP;

(b) Spatial variability (horizontal): sections (CTD and water samplers), floats/drifters, AUVs/gliders, ship-mounted ADCP and remote-sensing by satellite;

(c) Temporal variability: moorings/buoys with ADCP or other current meters, repeat stations/sections, floats/drifters, bottom landers and remote-sensing by satellite.

57. Oceanographic and hydrochemical measurements and sampling should be undertaken at the same stations used for biological sampling, with at least one measurement made within each physiographic zone. Where distances between physiographic zones are greater than 50 km, it is recommended that additional stations be included at least one station every 50 km in both latitudinal and longitudinal directions, with a higher resolution in areas with significant horizontal gradients or large-scale topographic features (one station every 10–30 km).

58. A CTD device should be used with additional sensors (e.g. for turbidity, dissolved oxygen, pH, fluorescence or photosynthetically active radiation), and should be combined with a rosette water sampler to study the vertical variability of both the physical and the chemical properties of the water column. The sampling resolution for physical parameters should be higher than for other parameters. Therefore, in addition to the depths noted in paragraph 22, samples should be taken at 0 m, 10 m, 25 m, 30 m, 50 m, 75 m, 100 m, 125 m, 150 m, 200 m, 250 m and 300 m, then every 100 m down to 1,600 m, 1,750 m and 2,000 m, and then every 500 m to 200 m above the seabed.

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

60. To study diurnal variability of the water column properties, a diurnal station should be established for each physiographic unit. Samples should be taken from the surface to a depth of 200 m. As noted in section III.A, sampling should be repeated every season over several years to determine annual and interannual variability.

61. In addition to the depths noted in section III.A, currents should be measured on the surface and at the following depths: 10 m, 25 m, 50 m, 100 m, 200 m, 300 m, 500 m, 750 m, 1,000 m, 1,200 m and 1,500 m, then every 500 m to 200 m above the seabed. This scheme should be modified if the vertical structure of water masses indicates that doing so is required. A combination of ADCPs mounted on different carriers may be used to obtain and sample data on the spatial (vertical and horizontal) and temporal variability of currents. High-quality absolute depth velocity profiles

may be obtained using LADCP (in isolation or combined with a CTD device). Use in combination with a ship-mounted ADCP and/or a secondary ADCP pointing upward improves the quality of the data obtained (Thurnherr et al., 2010).

62. A ship-mounted ADCP collects data on the spatial distribution of currents at depths down to 1,600 m, depending on the specifications of the ADCP used. However, there is a large measurement error for measurements over long ranges (800 m and 1,600 m respectively). To obtain a better resolution in the upper 100 –200 m, a combination of two ship-mounted ADCPs (e.g. OS75 or OS38 with OS150 or WH300) should be used (Firing and Hummon, 2010).

63. Moorings with ADCPs (or other current meters) should be used to study temporal variability of currents and other water column characteristics. Moorings should be deployed for a minimum of 12 to 13 months (to cover one annual cycle); longer deployments yield better information. The number of ADCPs (or other current meters) should be sufficient to ensure detailed coverage of the near-bottom 200 m. The use of additional ADCPs (or other current meters) in surface, intermediate and abyssal layers is strongly recommended.

64. Recommendations are available in the published literature for LADCPs (Thurnherr et al., 2010), ship-mounted ADCPs (Firing and Hummon, 2010), towed ADCPs and ADPs (Sgih et al., 2001).

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

66. In addition, floats and drifters should be deployed to study the temporal variability of currents at appropriate depths.

## C. Measured variable: temperature and salinity

67. CTD profiling of the water column or sensing by ROV, AUV or glider should be carried out to characterize the physical conditions of the water column. Seawater should be described following the TEOS-10 standard. In addition to a standard configuration for measuring pressure (converted to depth), conductivity (converted to salinity) and temperature, any CTD sampling should be complemented by using additional sensors for other parameters where possible (to determine, e.g., turbidity, dissolved oxygen, pH, fluorescence, photosynthetically active radiation, nitrates and altimetry). Key considerations for the collection of quality CTD data and data standards are given in ICES Data and Information Group (2006).

68. CTD devices or appropriate sensors can be mounted on wires, drifters/floats, moorings/buoys or bottom landers, or used as under way CTD devices. An under way CTD probe is one that is launched from portable or fixed launchers and recovered by reeling the line back while the ship is maintaining its course and speed.

69. Satellite-based remote sensor measurements should be used for getting information about oceanographic parameters on a synoptic time scale. In addition to surface temperature and surface salinity, satellites can measure sea ice distribution, wave height, surface height, radar backscatter and ocean colour. A large amount of information about satellites and data sets can be found on the websites of NASA (in particular the Physical Oceanography Distributed Active Archive Center of the Jet Propulsion Laboratory), NOAA, ESA, JAXA and the Copernicus programme.

70. Buoys, moorings, drifters and floats can be equipped with sensors for measuring sea surface temperature, seawater temperature, sea surface pressure, seawater pressure, sea surface salinity, seawater salinity, wind velocity, dissolved oxygen

concentration, fluorescence and ocean colour, mixed-layer temperature and partial pressure of dissolved carbon dioxide ($pCO_2$). They can be used to collect biological information (e.g. on dispersion of fish larvae) and to study currents and ocean waves. Key considerations for the collection of quality buoy data, data standards and data processing are given in documents of the Data Buoy Cooperation Panel, the Ifremer drifter data management team and the Argo programme community.

## D. Measured variable: currents

71. Currents should be determined using both Eulerian methods (time series measurement of current speed and direction at fixed location) and Lagrangian methods (the path followed by each fluid particle is observed as a function of time) to enable a holistic view. For Eulerian methods, mechanical or non-mechanical current meters may be used. Protocols and methodologies of the FixO3 project should be used for moorings and other types of Eulerian systems (Coppola et al., 2016). For Lagrangian methods, surface drifters, subsurface floats, pop-up drifters or pop-up floats may be used. Satellite images of sea surface temperature and colour may be used as pseudo-drifters to study surface currents on the assumption that all displacements of surface features seen in the imagery are caused by surface current advection. A brief review and references to all methodologies, including the advantages and disadvantages of each, can be found in Thomson and Emery (2014).

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

73. Any models used should be accepted by the ocean modelling community as suitable for dispersal studies near the seabed and, more broadly, throughout the water column. A review of Lagrangian codes for online and offline particle tracking that includes references to relevant literature in Van Sebille et al. (2018) and Numerical Models (2000) can be used to help to identify a suitable model.

74. An important step in current data analysis is their graphical representation. Joseph (2014) explains how this can be achieved both for both measured and modelled data.

75. What parameters should be measured depends on the equipment used. However, magnitude and direction of current velocity, zonal and meridional velocity components, and vertical velocity should be included.

76. From those measurements, the current regime of the water column and, in particular, of the layer from the bottom boundary layer up to 200 m above the sea floor should be characterized. The analyses should include field structure, spatial variations of current velocity and direction (with particular attention paid to areas of complex geomorphology), and temporal variations of current velocity and direction. Temporal variability should be characterized diurnally, seasonally and interannually; episodic events such as storms and turbidity currents should be recorded.

## E. Measured variable: tides and waves

77. Tides should be measured using either pressure sensors on fixed moorings or satellite altimetry. Although modern oceanographic instruments on fixed moorings can resolve pressure variations to a fraction of a millimetre at full ocean depth, for accurate depth measurements they require temperature correction and information on the pressure sensor drift (roughly 1 cm/year). The use of dual pressure sensors helps

to correct such drift. IOC manuals describe sea level measurements and their interpretation. Satellite altimetry can be used to determine tides by estimating the variability of the sea surface on the basis of repeated passes of the satellite radar. Altimetry data (including data from Topex/Poseidon, Jason-1, ERS-1 and ERS-2, Envisat and Doris) and relevant software and handbooks are available through the Aviso+ website (www.aviso.altimetry.fr).

78. Any commonly accepted method for determining surface gravity wave measurements should be used, such as satellite altimetry, wave buoys with accelerometers, wave gauges (including resistance-type, capacitance-type and wave pressure gauges) and satellite-based synthetic aperture radars.

79. The parameters that should be measured are pressure or sea level data, depending on whether fixed-mooring or satellite altimetry is used.

80. From those measurements, tidal amplitude and period, the main tidal constituents and inequality, and wave height and direction should be determined.

## F. Measured variable: turbulence

81. The estimation of turbulence intensity should be made by direct or indirect methods using data from a velocity shear probe, CTD device, ADCP, ADV or DCP (Thorpe, 2007).

82. Observations to determine turbulence intensity should be made as close to the sea floor as possible. Because the bottom-enhanced turbulence generally propagates upward across the bottom boundary layer, field measurements should be made down to the ocean interior and should include the entire bottom boundary layer. Turbidity near the bottom is closely related to turbulence intensity. Consequently, turbulence measurements should be combined with turbidity investigation (see paras. 85–96). When using the direct method, a horizontally profiling microstructure probe attached to an AUV is recommended to infer the spatial distribution of turbulence intensity. If the Thorpe scale method is used, CTD casting should be performed very accurately, as close to the bottom as possible. If the acoustic Doppler method is used, the current profiler mooring should be placed on the sea floor.

83. What parameters should be measured depends on the methodology used:

(a) For direct measurement: microscale velocity shear, lowering speed of the instrument, lateral acceleration of the instrument and high-resolution temperature;

(b) For indirect measurement: temperature, conductivity, pressure and velocity.

84. From those measurements, the turbulent kinetic energy dissipation rate, density, buoyancy frequency, vertical velocity profile, microstructure temperature fluctuations, vertical eddy diffusivity, Thorpe scales and temperature dissipation rates should be determined.

## G. Measured variable: optical properties

85. Optical properties of seawater can be divided into apparent optical properties and inherent optical properties as follows:

(a) Apparent optical properties depend on the nature of the seawater together with its dissolved material and particulates and the angular distribution (geometry) of solar radiation, and should be measured using spectroradiometers in which a variable monochromator is used to separate the light into certain wave bands;

(b) Inherent optical properties depend on the wavelength of the light 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.

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

(a) Physical shipboard sampling at stations ( vertical profiling and sampling, tethered or hand-held radiometric measurements) or when under way (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);

(b) Measurements from AUVs, gliders, fixed Eulerian platforms (moorings, bottom tripods and other bottom landers) and/or Lagrangian devices (drifters and floats);

(c) Remote sensing from shipboard, aircraft or satellite platforms. Measurements of this type can be passive (the sun is the source of illumination) or active (a signal from the sensor platform is used as the source, generally laser illumination is applied).

87. In addition, optical properties should be determined using inverse models (see Werdell et al., 2018, for details) or bio-optical models (see Ogashawara, 2015, for details).

88. Various types of fluorometers can be used to measure fluorescence, photoemission or bioluminescence (and can serve as an addition to modern a coustic methods for biomass estimation). More details on each can be found in Moore et al. (2009), and references therein. The sensors for measuring turbidity (nephelometers and backscatter sensors) can be set up in a variety of configurations, and there a re numerous methods and configuration standards for them (e.g. ISO standard 7027). See also Petihakis et al., 2014; and Tamburri, 2006. Remote sensing of fluorescence and bioluminescence can also be used to measure plankton fluorescence from satellites (e.g. Erickson et al., 2019).

89. To enable the assessment of any excess concentration of suspended particulate matter in operational and discharge plumes, optical or acoustic turbidity data need to be converted to suspended particulate matter concentration. To that end, optical or acoustic sensors should be calibrated to the suspended particulate matter locally present in the water column. For baseline studies, this should be done by reference to suspended particulate matter concentration determined in water samples taken simultaneously with the turbidity measurements. For the monitoring of operational and discharge plumes, the same approach can be followed if water samples can be taken directly from the plume. Otherwise, sensors should be calibrated ex situ in suspensions made with filtered local seawater and added plume source material.

90. EPA method 180.1 and ISO standard 7027 are internationally recognized for verifying turbidity meter and turbidity sensor performance and method compliance.

91. Instruments that are in compliance with EPA method 180.1 are suitable for measuring turbidity levels between 0 NTU and 40 NTU. Such turbidity meters should have a resolution of 0.02 NTU or better in water with a turbidity of less than 1 NTU.

92. ISO standard 7027 specifies two quantitative methods for using nephelometers: nephelometry (for the measurement of diffuse radiation, applicable to water of low turbidity) and turbidimetry (for the measurement of the attenuation of a radiant flux, more applicable to highly turbid waters). The resulting turbidities measured according

to the first method typically range between 0.05 NTU or less and 400 NTU. Depending on the design of the instrument, nephelometry can also be applicable to waters of higher turbidity. NTU and FNU are numerically equivalent.

93. Turbidity measured by the second method is expressed in FAU, and results typically range between 40 FAU and 4,000 FAU.

94. Depending on the methodology, the parameters that should be measured are radiance, irradiance, scalar irradiance, light diffuse attenuation coefficient and attenuation coefficient for scalar irradiance, photosynthetic available radiation, irradiance reflectance, radiance reflectance, absorption coefficient, scattering coefficient, beam attenuation coefficient, volume scattering function, ocean colour, fluorescence, bioluminescence, transparency and turbidity.

95. From those measurements, the following should be determined: chlorophyll-a and other pigments, visibility, suspended sediment volume, phytoplankton biomass, concentration of particulate and dissolved organic carbon and, productivity in the form of particulate organic carbon, and species composition (to detect harmful algal blooms and nitrate analysis) (see also sects. V.H and VII.D).

96. In addition, optical measurements can be used for the validation and calibration of remote sensing measurements.

## H. Measured variable: noise

97. Two noise characteristics should be determined over a wide range of frequencies (1 Hz–20 kHz): spectral levels of noise (differentiating impulsive and ambient noise) and sound propagation. The fundamental mechanisms, measurements and numerical modelling of oceanic ambient noise can be found in Carey and Evans (2011) and Robinson et al. (2014). Noise measurements can be made from ships (at stations or under way), AUVs, gliders, floats, drifters, moorings, buoys, bottom landers and tripods. It should be taken into consideration that some other sensors create noise. Consequently, single hydrophone tripods or hydrophone arrays should be connected at some distance from the instrument platform to reduce noise. Sound velocity should be measured directly (with the help of a sound velocity profiler or sensor) or should be derived from the temperature, salinity (conductivity) and pressure measured with a CTD device (see paras. 67–70). The method for deriving sound velocity is described in Wong and Zhu (1995).

98. The following parameters should be measured: spectral noise levels and, potentially, sound velocity.

99. From those measurements, the following should be determined: ambient noise levels in vertical profiles through the water column from the sea surface to the seabed, temporal variability in ambient noise levels, the depths of the sound fixing and ranging channel, and sound velocity (if not measured directly).

## I. Data quality

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

101. To obtain data of the highest quality, corrections should be applied to the CTD sensors. Calibration procedures will vary from one laboratory to another, but it is generally accepted that – while the pressure and temperature sensors can be subject

to pre- and post-cruise calibrations in the laboratory – the conductivity sensor is best calibrated by comparison with samples collected for salinity analysis (ICES Data and Information Group, 2006; Petihakis et al., 2014, and information and manuals provided by manufacturers) and the IAPSO seawater standard.

102. For quality control of CTD data, information from EuroGOOS DATA-MEQ Working Group (2010), IOC (2010) or United States Integrated Ocean Observing System (2020a, 2020b) should be used.

103. For quality control and correction associated with AUVs and gliders, Allen et al. (2018, 2020), United States Integrated Ocean Observing System (2016) and Woo (2011) should be consulted. For data management, EGO Gliders Data Management Team (2020) should be consulted.

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

105. For more information on various types of drifters and floats, opportunities and advantages of their use, constraints and innovations, see Lumpkin et al. (2017).

106. Temperature values should be converted to potential temperature, bearing in mind the effect of hydrostatic pressure. Density (potential density) should be calculated indirectly from salinity, temperature (potential temperature) and pressure using the equation of state (TEOS-10).

107. Guidance on ADCP data quality control can be found in United States Integrated Ocean Observing System (2019a) and EuroGOOS DATA-MEQ Working Group (2010). Information on mooring data correction and processing (ADCP, RCM, Microcat) can be found in Karstensen (2005).

108. Calibration is crucial for accurate measurements of the noise. The following guidelines and publications should be consulted: Biber et al. (2018) (for calibration details) and Robinson et al. (2014), and United States Integrated Ocean Observing System (2017) (for quality control).

109. Any models should be validated.

110. The spatial resolution of modern radiometers is 1 km (AVHRR), but they work in cloudless weather only. Passive microwave sensors can be used to observe even in cloudy conditions, because they use longer wavelengths (6 –12 GHz), but they have much poorer spatial resolution (25–50 km) (Talley et al., 2011). Microwave radiometers can be used to measure sea surface salinity with a spatial resolution of 50–100 km at temporal scales of one week or one month, respectively (Talley et al., 2011; and Thomson and Emery, 2014). In addition to surface temperature and surface salinity, sea ice distribution, wave height, surface height, radar backscatter and ocean colour can be measured by satellite. More information about satellite remote sensing can be found in the literature (e.g. Stewart, 1985; Robinson, 2004; and IOC, 1992), documents of the International Ocean-Colour Coordinating Group and the Ocean Optics Protocols for Satellite Ocean Colour Sensor Validation.

111. Over the past decades, large data sets have been accumulated under various international scientific programmes. These are open-access data that should be used for comparison with baseline data collected for quality assurance. Examples are:

(a) World Ocean Circulation Experiment 1990–2002 (www.nodc.noaa.gov/woce/wdiu);

(b) World Ocean Circulation Experiment subsurface float data (www.aoml.noaa.gov/phod/float_traj/index.php);

(c) World Ocean Database (www.nodc.noaa.gov/OC5/WOD/pr_wod.html);

(d) Global Temperature and Salinity Profile Programme: (www.nodc.noaa.gov/GTSPP);

(e) SeaDataNet (www.seadatanet.org);

(f) Coriolis Ocean Database for Reanalysis (www.coriolis.eu.org/Data-Products/Products/CORA);

(g) Pangaea data repository (www.pangaea.de/?t=Oceans);

(h) Global Drifter Program, formerly the Surface Velocity Program: (www.aoml.noaa.gov/phod/gdp/index.php);

(i) Global Ocean Currents Database (www.ncei.noaa.gov/products/global-ocean-currents-database-gocd);

(j) Argo floats: Argo home page (www.argo.ucsd.edu) and international Argo project home page (www.argo.net); Biogeochemical Argo float data (https://biogeochemical-argo.org);

(k) Archived drifter data, integrated science data management, Fisheries and Oceans Canada (www.dfo-mpo.gc.ca/science/data-donnees/drib-bder/index-eng.html);

(l) In addition, the following electronic atlases may be useful:

(i) World Ocean Atlas 2018 (www.nodc.noaa.gov/OC5/woa18);

(ii) Electronic Atlas of World Ocean Circulation Experiment Data (www.ewoce.org).

### J. Data management

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

## V. Chemical oceanography and biogeochemistry

### A. Introduction

113. The chemical environment of the water column and sediments (i.e. pore waters and solid fraction) must be understood in order to characterize baseline oceanographic and biogeochemical conditions and assess, at a later stage, both the direct impact of mining on the sea floor and the indirect impact resulting from suspended sediment plumes that may be produced, including a potential blanketing of the sea floor and their impact on processes in the water column.

114. The development of suspended sediment plumes largely depends on future mining techniques. Plumes have the potential to transfer over larger distances (from 1 km to tens of kilometres), they may differ from the surrounding water in particle size and chemical composition and will resettle away from the source and for that reason have a potential impact on pelagic and benthic ecosystems, their functions, and marine biogeochemical cycles in larger areas.

115. Marine sediment biogeochemistry is focused on sea floor processes and functions. It is a combination of studies of biochemical conversions with the observation of the biological, geochemical and geological processes involved. Observations are focused on benthic processes associated with the remineralization of the organic material exported from surface waters in a cascade of redox reactions.

Measurements are based mostly on the results of sediment sampling and the subsequent layer-wise extraction of pore waters and solid-phase subsamples for analysis. In some cases, such as oxygen uptake rates and pH distribution, measurements need to be obtained directly on the sea floor (i.e. in situ). For all pore water variables to be used later to quantify pore water release and plume dispersion, additional sampling should be targeted at the bottom water so as to construct a baseline that allows for the identification of solids or pore waters released as a consequence of sea floor disturbances or material discharged, and their effect (i.e. the distribution, transport and transformation of the reactants and products of the reactions).

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

(a) Nutrients: the availability of inorganic macronutrients ($NO_3$, $NO_2$, $NH_4$, $PO_4$, $Si(OH)_4$) in the upper ocean frequently limits and regulates the amount of organic carbon fixed by phytoplankton and forms a key mechanism that controls the availability of organic matter on the sea floor. Nutrient concentrations in pore waters ($NO_3$, $NO_2$, $NH_4$ and $PO_4$) provide information on the biogeochemical cycling of organic matter and redox conditions in various sediment layers;

(b) Oxygen: oxygen concentrations in the water column provide information on the production of organic matter in the surface layer and its remineralization during export towards the sea floor. The distribution of oxygen in the sediment, the oxygen penetration depth and the flux across the sediment-water interface are a measure of benthic organic matter remineralization and the activity of the benthic community. Furthermore, the availability of oxygen affects the mobility of most metals;

(c) Carbonate system: this system constrains primary production, organic carbon remineralization, metal oxidation in sediment plumes, ocean acidification, deoxygenation in the water column, organic matter remineralization, secondary redox reactions and induced pore water-mineral reactions in the sediment, all of which affects ecosystem functions;

(d) 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 toxicity that is metal-, chemical speciation- and organism-dependent;

(e) Organic and inorganic matter: the provision of organic matter to the sea floor is the key driver of biogeochemical processes. It ensures the presence of food to sustain the biomass and biodiversity of benthic organisms through the interaction in the benthic food web. Observations in the water column are focused on productivity and export, while measurements on the sea floor serve to quantify the amount and quality of the organic material available to benthic organisms, biogeochemical cycling on the sea floor and the dynamics of benthic organic matter cycling;

(f) Radioactive isotope tracers (radiotracers): an analysis of radioisotopes associated with the solid sediment phase is required for a quantitative characterization of bioturbation activity in the sediments and a determination of sedimentation rates. The distribution of naturally occurring radioisotopes serves as a baseline to determine the direct impacts of mining on the sediments and the water column (including the release of pore waters). In addition, it enables an assessment of radioisotopes and, hence, of the intensity of natural radioactivity in the nodules once mining commences.

## B. General methodology

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

118. Water column chemical parameters should be sampled using the most relevant of the following techniques:

(a) Water bottle sampling with CTD casts obtained with the help of ROVs: for nutrients, oxygen, carbonate system, trace metals (using trace metal-clean CTD/Go-Flo bottles), dissolved organic matter and suspended particulate matter including particulate organic matter. Electrochemical and optical chemical sensors can be used to obtain continuous data and background information on chemical properties, but should not replace the collection of discrete water samples for high- precision, high-quality chemical analysis;

(b) In situ pumps for radioisotope activity, trace metals and suspended particulate matter concentrations;

(c) Moored and tethered sediment traps for particle concentrations and particle fluxes;

(d) Biogeochemical Argo for pH, nitrate, oxygen, among others.

119. While CTD stations, in situ pump deployments and tethered sediment traps require stationary work, which limits the flexibility of the data acquisition, moore d sediment traps should be deployed in the water column for up to two years for time - resolved observations. In addition, autonomous floats, drifters and similar devices equipped with chemical, biochemical and optical sensors should be used to obtain spatial and temporal data on chemical variables.

120. Samples for sediment and pore water analysis should be obtained using a multicorer, ROV-manipulated push corer or similar reliable equipment for the top decimetres of sediments, and a gravity corer for deeper samples. For biogeochemical and chemical oceanographical sampling, the method publications of the International Ocean Discovery Program (previously the Integrated Ocean Drilling Program 2003 – 2013), Go-Ship and the Geotraces initiative (which is focused on the water column) should be consulted for commonly accepted and agreed methods of chemical oceanographical and biogeochemical sampling, together with the publications in the Ocean Best Practices System repository (hosted by IODE, which is part of IOC), as well as the essential ocean variables as defined by GOOS.

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

122. As biogeochemical processes and solute fluxes across the sediment-water interface are affected by conditions in the overlying water, the water overlying the sediment in the core liner should always be sampled as the seawater end member for

the pore water. As the sample may be altered during recovery or handling, it should be compared with the deepest water column samples from the CTD device.

123. Suboxic sediment and pore water should be sampled in a glove bag under an oxygen-free atmosphere (i.e. the glove bag is filled with an inert gas, e.g. nitrogen or argon) to preserve metal speciation and other redox-sensitive variables.

124. References to existing up-to-date best practices are provided below for each variable, with indications of where modifications are required to ensure relevance for deep-sea mining purposes. If no common best practice exists yet (e.g. colloidal/nanoparticle size fractionation for trace metals), a methodology is recommended and references to state-of-the-art scientific publications are provided. GOOS (www.goosocean.org) is a sustained collaborative system of ocean observations, encompassing in situ networks, satellite systems, governments, United Nations system agencies and individual scientists; most variables are part of the essential ocean variables as defined by GOOS.

125. As methods may be subject to change (owing to new technological developments, for example), best practice online repositories should be used to capture methodology updates. The Ocean Best Practices System repository (https://repository.oceanbestpractices.org) is recommended as a hub for searching and finding existing best practices in ocean research, observation, and data and information management. It is a permanent open-access digital repository of community best practices in ocean-related sciences and applications. It is maintained by IODE, which is part of IOC.

## C. Sampling resolution

126. Archived remote sensing satellite altimetry and sea surface temperature data, ocean colour data and hydrography data available from data repositories should be used to approximate the expected spatial and temporal variations of surface oceanographic features that control primary productivity within a licence area. The information should be combined with information about oceanic and atmospheric processes in order to identify the appropriate temporal and spatial sampling strat egy for chemical parameters in the water column within a given region to cover zones of different primary productivity and changing oceanographic features. At least one CTD station and two sediment traps (one installed close to the sea floor and one about 500 m above the sea floor) should be established in the water column above the intended Mining Area within the Contract Area (including IRZ) and PRZ. CTD sampling and sampling with automated traps should be carried out repeatedly at these stations to resolve temporal variability. In addition, transects should be obtained throughout the licence area with CTD stations spaced regularly at distances of about 100 km.

127. For water column measurements, samples should be taken throughout the water column, ensuring that all zones identified by the physical oceanographic data (see sect. IV) are characterized (e.g. surface mixed layer, the pycnocline, the extent of the oxygen minimum zone, and the individual oceanographic water masses in the thermocline and the intermediate and deep-water regions).

128. As noted in paragraph 22, a higher vertical sampling resolution is recommended near the seabed, as that covers the expected vertical space for the dispersal of the operational plume and is moreover the most likely depth for the dispersal of the discharge plume. If the depth of the discharge plume is still to be determined at the time of the baseline studies, all potential release depths should be characterized.

129. Integrated data acquisition with CTD water sampling, in situ pumping and sediment trap deployment should be undertaken as close to the sea floor as possible. For the assessment of natural benthic (metal) fluxes from the sediment into the overlying bottom water, sampling should be performed as close to the sea floor as possible. In addition to point sampling with a CTD device, there should be long-term deployments of passive samplers along a vertical gradient from the seabed up to 10 m above the sea floor.

130. Sampling should be conducted with the same sampling device and at the same time wherever possible (see sect. III.C) and should follow the nested stratified sampling scheme. The general considerations to cover spatial and temporal variability apply (see sect. III.A). Further details on specific variables are provided below.

## D. Measured variable: nutrients

131. The recommended best practices approach for determining dissolved inorganic macronutrients ($NO_3^-$, $NO_2^-$, $PO_4^{3-}$ and $Si(OH)_4$) in both the water column and pore water is documented in the revised Go-Ship manual by Becker et al. (2019) and in the standard protocols of Gieskes et al. (1991), and Grasshoff et al. (1999). Measurements should be performed using continuous or segmented flow analysis methods with certified reference material and/or reference material for nutrients in seawater to ensure quality control during analysis.

132. Even with high-precision equipment, quantification of ammonium in deep-sea pore waters is difficult because of very low concentrations. Therefore, where concentrations prove to be close to the detection limit, the determination of pore water ammonium can be omitted until better analytical methods become available. Silicic acid in deep-sea pore waters does not have high diagnostic potential for the determination of the benthic geochemical system and can therefore also be omitted from baseline observations.

133. Nutrient concentrations, particularly nitrate and nitrite, should be determined immediately after sampling or analysed within one or two weeks if, upon collection, the water and pore water samples are immediately frozen at -80°C.

134. The methodology that should be used for determining seawater and pore water nitrate and nitrite content (and, concomitantly, phosphate and silicic acid concentrations using segmented flow analysis) is as follows:

(a) A few millilitres of freshly extracted (or freshly thawed) untreated water or pore water should be analysed, usually upon twofold dilution (water) or threefold dilution (pore water), while the segmented flow analysis system is constantly flushed with nitrogen;

(b) Total $NO_x$ (nitrate + nitrite) concentrations should be determined colorimetrically at 520–540 nm after the reduction of nitrate to nitrite at pH 8 using a copperized cadmium coil;

(i) Nitrite content is measured separately colorimetrically at 520 –540 nm after its reaction with sulphanilamide under acidic conditions;

(ii) Nitrate concentrations are determined by the subtraction of measured nitrite concentration from total $NO_x$ values.

(c) Phosphate content should be determined colorimetrically at 820 nm (dihydrazine sulphate) or 880 nm (ascorbic acid) using the molybdenum blue method;

(d) Silicic acid concentrations should be determined colorimetrically at 660 nm (stannous chloride) or 820 nm (ascorbic acid) as silica molybdate complex.

135. Data should be reported in mol/l (or nmol/l, µmol/l or mmol/l, depending on the specific concentration range of the constituent) and solid-phase data in mg/kg or weight percentage. Data should always be reported with blank information (if applicable), limits of quantification, and results with regard to certified reference material and/or reference material for nutrients in seawater. Each sample should be analysed in duplicate or triplicate measurements. Analytical precision for each sample should not exceed 5% relative standard deviation. Calibrations for each pore water nutrient constituent should be performed using IAPSO standard seawater with at least six standards. The coefficient of determination ($r^2$) for each calibration curve should be greater than 0.98. Average nutrient concentrations should be calculated from duplicate or triplicate measurements and displayed as depth plots. Information on the analytical quality (i.e. accuracy, precision) during measurement should be indicated.

136. The parameters that should be measured in both the water column and pore water are $NO_3^-$, $NO_2^-$ and $PO_4^{3-}$, with measurements for $NH_4^+$ and $Si(OH)_4$ taken in the water column only.

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

## E. Measured variable: oxygen

138. The methodology that should be used for measuring oxygen distribution in the water column is described in Langdon (2010), McTaggart et al. (2010), and Uchida et al. (2010). Bittig et al. (2018) should be consulted for a review of optodes. An automated laboratory method that may be used, with software support, is set out by the Oceanographic Data Facility at the Scripps Institution of Oceanography (https://scripps.ucsd.edu/ships/shipboard-technical-support/odf/chemistry-services/dissolved-oxygen).

139. Observations of oxygen on the sea floor should cover both measurements of oxygen consumption and the depth of penetration of the oxygen into the sediments. Consumption measurements are focused on the upper sediment layer and need to be carried out in situ (i.e. directly on the sea floor). Measurements of oxygen distribution along the sediment column should be obtained in the laboratory from retrieved cores obtained with multicorers (for the top decimetres) and with gravity cores to determine the penetration depth, (i.e. the depth at which the oxygen concentration drops to zero) (e.g. Mewes et al., 2014). Oxygen should be measured with sensors, either optical oxygen sensors (optodes) or Clark-type electrodes, to allow for measurements at the required spatial resolution and avoid the risk of contamination with atmospheric oxygen associated with sampling-based methods. Microsensors should be used (microelectrodes and fibre optic optodes) to record vertical profiles of oxygen concentration in pore waters. Larger and temporally more stable optical sensors should be used (macrooptodes) for time series measurements of oxygen in benthic chambers or bottom waters. Sensors should be thoroughly calibrated in the laboratory and recordings obtained in situ should be validated by comparing measurements taken above the sediments with bottom water concentrations determined with the methods mentioned previously.

140. Strong spatial and seasonal dynamics are expected in case of sea floor oxygen uptake, so in situ measurements carried out during expeditions with microprofilers and/or chambers should cover different time intervals relative to major productivity and export events (e.g. algal blooms, peaks in vertical fluxes and phytodetritus deposition incidents). To fully account for seasonal variability, those measurements should be supplemented by time series of oxygen uptake measurements performed

autonomously by repeated profiling and/or chamber incubations (see below) with mobile platforms (benthic crawlers) over longer periods of several months or throughout the year.

141. Oxygen uptake measurements should be determined in situ using benthic chambers and microprofilers (Boetius and Wenzhöfer, 2013). Chamber incubations determine total oxygen uptake, also referred to as sediment community oxygen consumption, and microprofilers measure diffusive oxygen uptake. For diffusive oxygen uptake measurements, oxygen microsensors are lowered into the sediments in small vertical steps by means of microprofilers. To fully address oxygen uptake, in situ oxygen measurements should generally include both total and diffusive oxygen uptake. If methodology and the quantity being addressed (i.e. total oxygen uptake or diffuse oxygen uptake) are consistent throughout the baseline observations, one of the two quantities are considered sufficient. If only one approach is selected, total oxygen uptake measurements are preferred, as they cover the entire sediment community and include the oxygen uptake taking place in the nodules and the respiration of nodule epifauna. However, diffusive oxygen uptake measurements, which mostly address microbial respiration, represent an acceptable alternative, as the contribution of fauna is typically low in deep-sea sediments and most of the respiration is expected to take place in the sediments rather than the nodules.

142. The deployment time for total oxygen uptake analysis should be long enough for a robust determination of the rate of decrease from the oxygen recordings on the basis of the sensor performance. Diffusive oxygen uptake should be calculated from the oxygen depth profile by matching the measurements with a one-dimensional diffusive transport and respiration model. As in situ profiles generally do not reach the oxygen penetration depth in deep-sea environments with low respiration rates, measurements should cover the sediment layer where significant oxygen uptake takes place (see next paragraph).

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

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

145. The parameter that should be measured is dissolved oxygen ($O_2$); the raw data should be provided as concentrations (mol/l)

146. From oxygen observations in the water column, the following should be determined: apparent oxygen utilization, net community production, net carbon

export flux, ocean oxygen inventories, and deoxygenation and oxidation consumption due to oxidation of reduced metals. For the sediments, the following should be determined: oxygen penetration depth, volumetric respiration of the different sediment layers, rates of sediment community oxygen consumption/oxygen uptake, carbon remineralization rates and net rates of organic matter flux to the sea floor. In addition, the redox zonation in the sediment should be characterized

## F. Measured variable: carbonate system

147. Instead of carbonate alkalinity (as described in ISBA/25/LTC/6/Rev.1 in conjunction with ISBA/25/LTC/6/Rev.1/Corr.1), total alkalinity should be used to characterize the carbonate system, as molecules other than carbonate and bicarbonate compounds, such as borate, hydrogen sulphide and dissolved organic carbon typically contribute to this variable.

148. Detailed information on the acquisition of data on the 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).

149. To constrain the full suite of the seawater carbonic acid system (i.e. [$CO_2$], [$H_2CO_3$], [$HCO_3^-$], [$CO_3^{2-}$], [$H^+$]), pressure, temperature and salinity should be used along with any two of the following: dissolved inorganic carbon, carbonate alkalinity, $pCO_2$ and pH (Millero, 2013). While total alkalinity is a robust variable of the carbonate system that can be measured ex situ without inducing artefacts, dissolved inorganic carbon, pH and $pCO_2$ are sensitive to changes in pressure and temperature as well as to induced degassing upon retrieval of samples from the deep-sea floor to the sea surface. Therefore, pH and $pCO_2$ should be measured in situ to avoid ex situ sampling artefacts that cannot be corrected during data processing.

150. To account for contributions to total alkalinity from other chemicals such as borate and hydrogen sulphide, additional measurements should be taken for sediment pore water. Since it is difficult to measure 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.

151. The GOOS essential ocean variables specification sheet should be consulted for further information on current global observing networks, including available sensor techniques (mainly for $CO_2$ content and pH, e.g. in the case of Biogeochemical Argo) and future observing capacity.

152. The carbonate system should be determined using total alkalinity and at least one of the following: dissolved inorganic carbon, pH and $pCO_2$ (Dickson et al., 2007; European Commission, 2011). Other variables, such as total boron concentration, total sulphide concentration and dissolved organic carbon, should be considered as well, if they contribute to total alkalinity (Luff et al., 2001; Zeebe and Wolf-Gladrow, 2001).

153. The methodologies for each of these are as follows:

(a) In pore water samples, total alkalinity should be determined in aliquots of extracted pore water by titration with a diluted HCl solution, observing the pH change spectroscopically, potentiometrically or optically (e.g. using a suitable pH indicator) and bubbling the solution in the titration vessel with nitrogen or argon gas to strip the produced $CO_2$ and $H_2S$ from the solution (e.g. Wallmann et al., 2006; Haffert et al., 2013);

(b) 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;

(c) Total dissolved inorganic carbon content should be determined coulometrically in aliquots of extracted pore water. Samples should be preserved against further microbial degradation by adding a $HgCl_2$ solution and should be stored in tightly closed vials that have been flushed with nitrogen gas to avoid an exchange of gas with the atmosphere. The dissolved inorganic carbon should be converted to $CO_2$ by treating the sample with phosphoric acid. For the measurement, the gas should be transferred to the coulometer with a purified helium carrier gas. 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 $\delta^{13}C$ isotopic signature of dissolved inorganic carbon should be determined by isotope ratio mass spectrometry. The stable carbon isotope signature of the dissolved inorganic carbon provides additional information that helps to discriminate organoclastic dissolved inorganic carbon production from methane oxidation pathways;

(d) pH profiles should be determined in situ using glass micro-electrodes (e.g. Wenzhöfer et al., 2001; Revsbech and Jorgensen, 1986);

(e) $pCO_2$ or dissolved $CO_2$ concentration should be determined in situ using micro-optodes (e.g. Wenzhöfer et al., 2001);

(f) Total boron concentration should be determined by inductively coupled plasma optical emission spectrometry or inductively coupled plasma mass spectrometry;

(g) Total sulphide concentration should be determined spectrophotometrically as methylene blue (Grasshoff et al., 1999; Haffert et al., 2013);

(h) Total dissolved carbon should be determined on the same sample as dissolved inorganic carbon, as described in section H.

154. As the marine carbonate system is constrained by measuring some of its variables to calculate the other species (e.g. Luff et al., 2001; Zeebe and Wolf-Gladrow, 2001), the propagated uncertainties of the calculated variables should be reported. The most important factor for uncertainty propagation of the marine carbon dioxide ($CO_2$) system is the choice of input uncertainties themselves (Orr et al., 2018). As samples can be preserved easily and the measurements made with low uncertainty, the measurement of the sum variables total alkalinity, dissolved inorganic carbon, total boron concentration, total sulphide concentration should be used, but other combinations, such as pH and dissolved inorganic carbon to calculate carbonate alkalinity and the carbonate species can be used if contributions from borate and hydrogen sulphide to total alkalinity can be neglected.

155. The use of certified reference material samples for both dissolved inorganic carbon and total alkalinity analysis is a critically important approach for assessing seawater chemistry over time, for accurately calculating $pCO_2$ and pH for seawater samples. In that regard, seawater reference material should be obtained from IAPSO or the Scripps Institution of Oceanography. Dickson et al. (2007) should be used as a guide for calculating standard deviation of measurements. For uncertainty and its propagation, the documentation in Orr et al. (2018) should be consulted. The same references should be used for links to and documentation on the add-on routines for the software packages with which carbonate chemistry variables can be calculated (seacarb, CO2SYS for Excel, CO2SYS for MATLAB, mocsy). In addition, there are open-access software packages for other acid-base systems, such as borate and sulphide, that contribute to pH and total alkalinity (AquaENV; Hofmann et al. 2010), and for pressure effects (SUGAR Toolbox; Kossel et al., 2013).

156. From those measurements, the following should be calculated: saturation states for carbonate minerals, such as aragonite and calcite, and for silicate minerals; carbonate compensation depth; lysocline; reaction rates for carbonate/silicate mineral dissolution; remineralization of organic matter; and oxidation of reduced metals. The redox zonation should be determined.

## G. Measured variable: trace metals

157. The publication *Sampling and Sample-handling Protocols for Geotraces Cruises*, also known as the "Geotraces cookbook", should be consulted for specific recommendations on appropriate sampling, cleaning procedures and sample handling for trace elements (particulates and total dissolved) and their isotopes in seawater, as well as on procedures to obtain accuracy and precision measures.

158. 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. No best practice guide has yet been published on this topic, so the most up-to-date literature should be consulted at the time of sampling.

159. For physical size fractionation of seawater and pore water, potential methods include:

(a) Sequential filtration resulting in different size fractions: > 0.2 µm (particulates), < 0.2 µm (total dissolved), 0.02–0.2 µm (inorganic colloids such as Fe oxyhydroxides, clays, Mn oxides), < 0.02 µm (soluble: small organic colloids, truly dissolved), on-board acidification of non-filtered samples (for total dissolvable concentrations);

(b) Ultrafiltration with a 1 kDa molecular weight cut-off (where a size pool of between 1 kDa and 0.2 µm contains all colloidal and nanoparticulate matter, and a size pool of less than 1 kDa is defined as a truly dissolved pool), conducted on board if sample volume availability allows, which is the main constraining factor when the aim is to conduct ultrafiltration for pore waters.

160. Other methods are available to assess chemical speciation, including:

(a) Voltammetric methods, home laboratory analysis;

(b) Diffusive gradients in thin film passive samplers for labile metal concentrations, on-board sampling, home laboratory analysis.

161. Samples should be adequately preserved (e.g. through acidification with ultrapure HCl to a pH ~1.8 for trace metal concentration analysis; also, see the Geotraces cookbook for details) or frozen (e.g. for chemical speciation analysis, ligand analysis).

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

163. Which analytical methods are the best for trace metals in seawater and pore water is subject to change because of technological developments and instrument availability, so various analytical methods are possible. The use of appropriate analyses and data processing should be proven with the requested metadata. Generally, metal concentration data should be obtained using inductively coupled plasma optical emission spectrometry and inductively coupled plasma mass

spectrometry. Prior to inductively coupled plasma analysis, sediment samples should be treated using acid pressure or microwave digestion with suitable acid combinations, for example HF + $HClO_4$ or HF + HCl + $HNO_3$ (Paul et al., 2018, Nöthen and Kasten, 2011). For trace metals in seawater and pore water, the use of a SeaFAST pre-concentration and matrix separation device is strongly recommended. Certified reference materials for trace metals and inorganic contaminants in solid phase (MESS-4, NIST-2702) and seawater (e.g. the NASS-7, CASS-6, SLEW-3 or Geotraces intercalibration standards) 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.

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

165. On the basis of these measurements, the following should be determined: trace metal fluxes, distribution between various physical and chemical species, labile concentrations, types and concentrations of nanoparticles and colloids, and the redox zonation in the sediment (including spatial and temporal variability).

## H. Measured variable: organic and inorganic matter

166. Baseline observations should address quantity, quality and lability of dissolved and particulate organic matter, as well as particulate inorganic carbon in the water column and on the sea floor, including their temporal and spatial variability, using measurements of appropriate proxies. Observations of particulate matter in the water column should include organic and inorganic particles.

167. The main emphasis of baseline observations should be on a well-replicated characterization of particulate inorganic carbon, particulate organic matter and dissolved organic nitrogen in the water column and in the uppermost decimetres of the sediment, where biogeochemical conversion rates are highest and where current knowledge suggests that the impact is likely to be the most pronounced. For sediment analysis, in addition to the resolution identified in section III.A, particulate inorganic carbon and particulate organic matter should be measured in deeper and older sediment at some sites to help to characterize the various settings found in the area, including past productivity and deposition regimes.

168. For seabed analysis, the distribution of the amount and characteristics of particulate inorganic carbon and particulate organic matter should be determined in subsamples taken from distinct depth layers of retrieved cores, while dissolved organic nitrogen should be analysed in pore waters extracted from distinct depth layers. In the top decimetres of the sediment, samples needed for analysis should be taken with state-of-the-art samplers that are able to recover the fluffy semi-liquid surface layer (e.g. multicorer, ROV-manipulated push corers). Deeper strata should be cored with a gravity corer or a piston corer.

### 1. Dissolved organic matter

169. The amount of dissolved organic nitrogen should be quantified in terms of dissolved organic carbon alongside with measurements of total dissolved nitrogen, typically by catalytic oxidation at a high temperature and after removal of inorganic carbon and volatile organic matter by means of acidification and purging with inert

gas. The ratio of dissolved organic carbon to dissolved organic nitrogen (calculated by subtracting the sum of $NH_4^+$, $NO_3^-$ and $NO_2^-$ from total dissolved nitrogen) provides a first indication of the chemical composition of the dissolved organic nitrogen; it should be used for a general characterization of dissolved organic nitrogen quality (i.e. potential availability to organisms as a food source). A general molecular characterization of dissolved organic nitrogen should be determined on the basis of optical analysis of the coloured and fluorescent pool. This can be done with off -the-shelf instruments that readily collect excitation emission spectra using fluorescence spectroscopy and combine that with absorption spectroscopy-based measurements.

170. Dickson et al. (2007) should be consulted for best practices in measuring dissolved organic carbon in the water column.

171. The parameters that should be measured in the water column are dissolved organic carbon and dissolved nitrogen.

172. The parameters that should be measured for the pore water are dissolved organic carbon, total dissolved nitrogen, dissolved amino acids and carbohydrate s, and dissolved organic nitrogen optical characteristics (coloured dissolved organic matter, fluorescent dissolved organic matter).

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

174. 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. They should be used in combination with trace metal complexation and bioavailability.

### 2. Particulate matter

175. With regard to particulate matter, a number of variables are used to describe the suspended particulates (total suspended matter) and particulate matter transport in the ocean, both the organic and inorganic fractions. Particles can be collected in the water column using several sampling techniques:

(a) By filtration of water from Niskin or Go-Flo bottles;

(b) With in situ pumps;

(c) With sediment traps.

176. Each of these sampling techniques has its advantages and disadvantages. Therefore, a combination of all should be used. While sampling techniques based on the filtration of water samples collected with water sampling devices such as Niskin or Go-Flo bottles are limited to relatively small volumes (< 12 l), in situ pumps, capable of filtering large volumes (in the hundreds of litres per hour) should be used to collect larger masses of particles, as they are required for certain investigations (such as of the activity 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 two to four hours. Particles obtained from filtering seawater from bottles and with in situ pumps should be used to determine particle concentrations, type and quantity; they are suitable for trace metal investigations. Whether particles sink and, if they do, how fast (i.e. their contribution to export fluxes) depends on their individual size, shape and density. Export fluxes should be deduced indirectly by measuring the activity of radiotracers (see sect. I). In addition, direct measurements of particle fluxes should be obtained with 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 assessed directly.

177. The Geotraces cookbook, Bishop et al. (2012) and Planquette and Sherrell (2012) should be consulted for guidance on best practices in sampling and sample processing methods for particulate matter investigations with the use of in situ filtration and on-deck filtration from Go-Flo bottles, with a special focus on trace metals. The Geotraces cookbook should also be consulted for recommended modifications to the method for determining particulate organic carbon and particulate nitrogen as originally published in JGOFS report 19 (Knap et al., 1996), which contains the recommendations for JGOFS and is a widely employed and cited method for small-volume samples of particulate organic carbon and particulate nitrogen (i.e. < 10 l)).

178. McDonnell et al., (2015) should be used for a review of 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, the 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 in addition be deduced from the guidelines on ocean observation published by the Oceanographic Society of Japan and in the International Ocean-Colour Coordinating Group protocol on particulate organic carbon sampling and measurements.

179. A review of optical techniques for the remote and in situ characterization of marine particles without collection and retrieval can be found in Boss et al. (2015), which covers techniques to assess bulk properties including particle mass, particle size distribution and particle shape information, together with single particle optical properties such as individual particle type and size. In addition, the authors review advances in imaging technology and its use in studying marine particles in situ. More details can be found in Giering et al. (2020) and Huffard et al. (2020).

180. The GOOS essential ocean variables specification sheet can be consulted for further information on current global observing networks and links to literature on autonomous data observation innovations.

181. The parameters that should be measured for the water column are particulate organic matter (particulate organic carbon, particulate organic nitrogen, particulate organic phosphorous), biogenic silica, particulate inorganic carbon, total organic carbon, total nitrogen, total suspended matter, particulate organic carbon flux, calcium carbonate ($CaCO_3$) flux, biogenic silica flux, lithogenic particles, iron and manganese oxides and oxyhydroxides, the concentration of particulate matter, carbon supply/carbon demand and particulate organic matter Redfield (C:N:P) stoichiometry.

182. The quantity and quality of sinking material varies seasonally and interannually, so particular emphasis should be placed on weekly to monthly sampling of primary production and monthly to annual resolution for export fluxes.

183. Organic matter observations in the sediments should address the quantity of particulate matter together with the amount of bioavailable organic matter and its quality (i.e. freshness/lability). Various approaches can be taken (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 measuring total organic carbon and total nitrogen, typically by means of an elemental analyser after removal of inorganic

carbon by acidification. The ratio of total organic carbon to total nitrogen (the C:N ratio) provides a first indication of the quality of the particulate organic matter. More specific information on the quality of the organic matter should be obtained by measuring chloroplastic pigment equivalents, including chlorophyll-a and its degradation products; by simple fluorometric analysis; by high-performance liquid chromatography; or by measuring biopolymeric carbon, including hydrolysable carbohydrates, proteins and lipids (using wet-chemical analysis). The freshness of the particulate organic matter should be determined using the ratio of chlorophyll-a to chloroplastic pigment equivalents (or the chlorine index, which is similar), or on the basis of analyses of the specific composition of biomolecule classes (e.g. the ratio of hydrolysable to total carbohydrates, proteins and lipids; the degradation index as based on amino acid composition; or the rates of fatty acid with different levels of saturation).

184. In conjunction with total organic carbon and total nitrogen, particulate inorganic carbon should be measured using a CNS element analyser. Particulate inorganic carbon is often reported as calcium carbonate ($CaCO_3$) content as a weight percentage of the dry sediment sample.

185. The distribution of particulate organic matter is expected to be heterogeneous, especially near the sediment surface. Because of their low density, the deposition of organic matter particles on the sea floor typically depends on small-scale patterns of currents and on sea floor morphology, which leads to patchy distributions and local accumulations, such as in small depressions. Appropriate statistical methods should be used to decide on the number of replications required and the appropriate resolution. This information should be provided along with the raw data. The number of replicates should never be lower than three cores per site and sampling campaign. Sea floor imaging surveys (cable-based imaging systems, AUVs) or time series (lander-based systems, benthic crawlers) should be used where possible to obtain semi-quantitative information on spatial and temporal variability in the supply, standing stock and processing of fresh particulate organic matter on the sea floor (semi-quantitative observations of greenish phytodetritus distribution in colour imagery, quantitative observations of chloroplastic pigment with fluorescence imaging or hyperspectral techniques).

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

## I. Measured variable: radioactive isotope tracers (radiotracers)

187. For sampling, sample processing and analysis for long-lived radionuclides and short-lived radionuclides in seawater (e.g. $^{230}Th$ and $^{210}Pb$, respectively), suspended sediment plume particles and sediments, the detailed recommendations in the Geotraces cookbook should be followed. The parameters that should be measured are

dissolved, colloidal and particulate $^{230}$Th, $^{234}$Th, $^{210}$Po, $^{210}$Pb, $^{231}$Pa, $^{224}$Ra, $^{226}$Ra, $^{228}$Ra, $^{227}$Ac and gross alpha radiation.

188. For the determination of the activity of short-lived radionuclide (e.g. $^{210}$Pb) in sediments:

(a) A few grams of dried, homogenized sediment samples should be sealed gas-tight and left for at least several weeks to ensure that the radioisotopes are in secular equilibrium (i.e. constant radioisotopic activity because production rate is equal to decay rate);

(b) Total $^{210}$Pb and $^{226}$Ra activity should be determined directly by gamma spectrometry (high-purity germanium (broad energy germanium) detector);

(c) In addition, total 210Pb can be measured indirectly by alpha spectrometry (PIPS detector) through its granddaughter isotope $^{210}$Po;

(d) External calibration should be performed using certified reference material such as IAEA-RGU-1 (uranium ore).

189. The activity of long-lived radionuclide in sediments ($^{230}$Th and $^{231}$Pa) and particles and in the water column (Ra series) should be determined by:

(a) Gamma spectrometry (Yokoyama and Nguyen, 1980);

(b) Alpha spectrometry (Lao et al., 1992);

(c) Mass spectrometry (Geibert et al., 2019);

(d) For sediment and particle analysis, IAEA-385 (Irish Sea sediment) (Pham et al., 2008) should be used as certified reference material;

(e) For water column analyses, IAEA-443 (Irish Sea water) (Pham et al. 2011) could be used as certified reference material.

190. The determination of gross alpha radiation can be replaced by measuring $^{230}$Th, $^{226}$Ra and $^{231}$Pa individually, then calculating expected gross alpha radiation on th e basis of equilibriums with their respective daughter isotopes.

191. Activity should be presented as total, dissolved and particulate activity, in dpm/g or Bq $kg^{-1}$. All radioisotope activity (except for their ratios) should be corrected for the interference of pore water salt during analysis (Kuhn, 2013; Geibert et al., 2019) and the exact procedure and corrections should be recorded.

192. On the basis of these measurements, the following should be determined: concentrations and activity, the $^{230}$Th deficit, radionuclide fluxes, sinking elemental fluxes and sedimentation rates. In addition, bioturbation depth, bioturbation activity, bioturbation mode (i.e. diffusive or non-local mixing), radiation level and pore water-mineral reactions (e.g. carbonate dissolution/precipitation) should be determined within the sediment.

193. Numerical transport-reaction models or analytical solutions are available for analysing the data. For example, the constant initial concentration model is a simple approach taken to calculate sedimentation rates for deep-sea sediments. Using the average activity of either $^{230}$Th or $^{231}$Pa within the bioturbated layer of undisturbed sediment (in which no significant depth trend for $^{230}$Th excess or $^{231}$Pa excess is seen), the depth at which the activity has decayed to one half of that level is determined. The difference between this depth and the bottom of the bioturbated layer, divided by the half-life in question, is a rough approximation of the sedimentation rate at that location.

## J. Data quality

### 1. Chemical oceanography

194. Five programmes working with oceanographic data, namely the Alliance for Coastal Technologies, the AtlantOS project, IMOS, the Joint Technical Commission for Oceanographic and Marine Meteorology and the United States Integrated Ocean Observing System Quality Assurance/Quality Control of Real-Time Oceanographic Data project, jointly published a review of existing best practices in quality assurance (Bushnell et al., 2019), which should be consulted for details on record-keeping for quality assurance purposes, check lists, maintenance recommendations, ways to improve measurement uncertainty and general quality assurance recommendations regarding oceanographic data. Also in that review, the recently created O cean Best Practices System is identified as a means of developing, sharing, documenting and curating more specific quality assurance processes.

195. In chemical oceanography, uncertainties associated with the sampling process, sample treatment and analytical measurements affect data values obtained from water samples. Those uncertainties can be reduced by increasing the number of observations. They need to be distinguished from another kind of uncertainty, namely the uncertainty or variability of 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 taken at similar but not identical locations within a radius of approximately 10 km). High analytical rigour (i.e. accuracy and precision) helps to distinguish between sources of uncertainty.

196. For trace metals, Geotraces states that two categories of replicates should be measured: field replicates and analytical replicates. Analytical replication is the repeated analysis of a single sample. It 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. It has an added component of variance owing to subsampling, 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 that the analyte is homogeneously distributed within the sampling bottle).

### 2. Biogeochemistry

197. The number of replicate samples or observations required to properly describe biogeochemical baseline conditions in the various physiographic units (see sect. III.A) depends not only on the existing natural variability, but also on relative changes occurring 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 mainly depends on the magnitude of change typically associated with mining-related impacts, along with the relevance of the variable to serve as indicator of ecosystem status, deterioration and recovery. As a guide, the chosen replication should allow for the detection of deviations of less than 30% compared with baseline conditions at a statistical power of at least 0.95 (Ardron et al., 2019). Statistics on the level of change that can be detected for the individual variables should be reported together with the baseline data.

198. To decide on an initial sampling effort, available information on natural variability should be collected, bearing in mind that three replicates should always be considered a minimum. The replication required for the variables should be regularly

revised 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.

### K. Data management

199. The technical notes of the International Ocean Discovery Program and its predecessor the Integrated Ocean Drilling Program contain details on data and on sample management and curation (as well as on biogeochemical and geological sampling and analysis) that should be followed.

200. Metadata are required to document whether the sampling and analyses have been undertaken appropriately and to trace the data provided back to their origin. They need to be provided for all chemical variables. Metadata related to sampling and sample logging as well as the resulting data should adhere to the guidelines defined by the Geotraces International Data Assembly Centre ( www.bodc.ac.uk/geotraces/), ICES and the Working Group on Marine Data Management. More information and metadata protocols can be found in the data management best practices guide compiled by the Biological and Chemical Oceanography Data Management Office on the basis of experience gained by the Globec and JGOFS ocean research programmes. It comprises a collection of best practice recommendations for the management of data obtained from research cruises. The guide can be downloaded from http://bco-dmo.org/resources. More guidelines for the management of data and metadata can be found in the Ocean Best Practices System repository and within the Argo programme community.

## VI. Geological properties

### A. Introduction

201. In combination with biogeochemical parameters (see sect. V), geological properties are targeted to characterize the habitat and to determine the heterogeneity of the sea floor and sub-sea-floor environment (bathymetry and geomorphology, geological setting, sediment and stratigraphy, diagenesis, weathering and remobilization, rock substrate geochemistry and mineralogy, mineral resource geochemistry and mineralogy) and to assist in the placement of suitable sampling locations to characterize the distribution and composition of faunal communities.

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

(a) Bathymetry: used to map large- and small-scale morphologic features of the seabed; can be used to plan other types of sampling;

(b) Sediment properties and habitat classification: important for characterizing the benthic habitat; in addition, the properties should be used to quantify deformation and changes of sea floor sediment physical properties during mining gear operations, and to design the mining system.

203. Resource properties are important for habitat characterization. They constitute the main target of any Exploration activity in the Area. Some resource characteristics may constitute information of commercial interest and may be subject to confidentiality under the contracts with the Authority. However, an assessment should be presented of the information needed to establish the environmental baseline.

## B. General methodology

204. Data and information on the geology and deep-sea floor morphology can be collected using the following:

(a) Multibeam echo sounding (with hull-mounted devices and/or systems towed by ROV or AUV);

(b) Side-scan sonar profiling (with devices towed by the vessel, ROV, AUV or by other means);

(c) Subbottom profiling (e.g. with a Chirp system);

(d) Photography and video recordings obtained by TV grab, sledge, ROV, AUV or submersibles.

205. Diverse methodological approaches exist to carrying out geological surveys and acquiring accurate, high-quality data on the geological variables; any of the commonly accepted practices should be used.

206. Sediment samples needed for analysis should be obtained using, for the top decimetres of the sediment, a multicorer, ROV-manipulated push corer or similar reliable equipment, and, for deeper samples, a gravity corer.

207. Specific methodologies for sediment sampling and bathymetry can be found in publications of the International Ocean Discovery Program (previously the Integrated Ocean Drilling Program 2003–2013) and in the Ocean Best Practices repository (https://repository.oceanbestpractices.org).

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

## C. Sampling resolution

209. Which sampling resolution is appropriate depends on whether the information is to be used for large-scale resource assessment or local habitat mapping; the resolution should be adjusted to the intended use. For large-scale surveys of the entire Exploration area, bathymetric and backscattered maps with resolutions greater than 80–100 m should be produced. In areas where other discrete sampling is undertaken or where conditions indicate higher variability, or in areas where an indirect impact from mining is predicted (sediment and discharge plumes), higher-resolution sampling should be conducted.

## D. Measured variable: bathymetry

210. For sea floor mapping, the following should be used to obtain high-spatial-resolution data on the physical status of sea floor habitats: multibeam bathymetry, backscattered mapping, side-scan sonar or synthetic aperture sonar methods using ship-based devices or devices deep-towed by ROVs or AUVs.

211. Suitable calibration is required to obtain reliable and consistent sea floor bathymetric and backscatter data (Lamarche and Lurton, 2018). Constancy of acquisition settings and specific design of backscatter-dedicated surveys are recommended; they 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). In

addition, references to publications on the standardization of undersea feature names are available at https://iho.int/en/bathymetric-publications and at www.gebco.net.

## E. Measured variable: sediment properties

212. To describe the sediment properties, the sediments' mineralogy and lithoclasts, particle size distribution, porosity and overall stratigraphy should all be studied. Lithology concerns the physical characteristics of a rock. Sedimentology concerns the origin, transport, deposition and diagenetic alterations of materials that make up sediments and sedimentary rocks. Stratigraphy concerns the investigation of the way in which sedimentary rocks are accumulated and distributed through time. Core samples should be taken using a suite of tools to sample the uppermost 30 cm of sediment (push core and multicore), the uppermost 50 cm (using box core) and several metres deep (using gravity core).

213. Physical oceanographic phenomena as well as mining can generate sedimentary structures on the deep-sea floor. Therefore, sea floor sedimentary structures should be identified and mapped, using optical imaging. Optical imaging acquired by deploying a variety of platforms, including ROVs, AUVs and towed or drop -down cameras, allows for a 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 types of structures should be described. Geographical Information System-based mosaicking approaches should be used to image complex or larger areas of the sea floor (Garcia et al., 2015), indicating the overlap percentage used.

214. 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).

215. Grain size is a fundamental physical property of sediment. It is correlated with the dynamic conditions of the Marine Environment and 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 Jaijel et al., (2021), a typical modern laser diffraction spectrometer has a size scale range of up to 2,000 μm, which covers the great majority of soft bottom sediments of the world's oceans. The grain size distribution of the bulk sediments should be determined using a standard methodology with appropriate handling (Jaijel et al., 2021, and references therein).

216. Sedimentological characterizations should be undertaken by examining samples under a loupe (unconsolidated sediments) and petrographic microscope (smear slides, thin sections) (e.g. Marsaglia et al., 2013, 2015a and 2015b). The mineralogical composition should be determined qualitatively and quantitatively. Several methods and combinations of methods are available, including detailed mineralogy, electron microprobe mineral analysis, X-ray diffraction and/or automated quantitative mineralogy using mineral liberation workflows and quantitative scanning electron microscopy techniques. They should be used to obtain a quantitative modal analysis and virtual petrography. In addition, quantitative measurements should be taken using the Rietveld analysis, in particular to fully characterize the sea floor surface of future mining areas and to define the clay fraction (particles < 2 μm in size) for modelling of potential environmental harm caused by plumes.

217. The chemical composition of sediments should be analysed in a laboratory equipped with quality systems in accordance with international standards, including by performing X-ray fluorescence, inductively coupled plasma mass spectrometry and inductively coupled plasma optical spectrometry measurements (see sect. V).

218. 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 2015b), Rothwell and Rack (2006), Mazzullo et al. (1988) and other resources available at https://repository.oceanbestpractices.org/ and http://publications.iodp.org/index.html.

219. The following parameters should be measured:

(a) Sediment occurrence: 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 microfaults, fluid escape structures and bioturbation), sediment colour (with the help of, e.g., a Munsell soil colour chart for classification);

(b) Composition of sediments: texture (sand, silt, clay), mineral composition, fossil composition, element content, concretions, biogenic material, identification of macroscopic biogenic and non-biogenic components;

(c) Early diagenesis: degree of diagenesis, lithification or cementation (presence of silicic or calcareous cements);

(d) Physical and mechanical properties: specific gravity, bulk density, sediment porosity, fluid saturation, shear strength and grain size;

(e) Oxidation-reduction status: depth at which sediment conditions change from oxic to suboxic.

220. The information collected should be used to determine seabed substratum characteristics and geomorphic features, thus gaining a detailed understanding of pre-mining conditions of claim areas.

## F. Habitat classification

221. To facilitate other sampling efforts, qualitative descriptions of basic geomorphic features, habitat classifications and non-biogenic disturbances resulting from coring should be mapped at a scale appropriate to the resource and habitat variability, using the terminology for standardization of undersea feature names provided by the International Hydrographic Organization (2019).

## G. Data quality

222. Details on quality assurance in oceanographic observations, including standards and guidance, can be found in Bushnell et al. (2019), among others. All methodology should be checked against quality assurance plans (Simpson and Batley, 2016). Guidance on quality control for hydrographic surveys and guidelines for data processing are available at https://iho.int/en/standards-and-specifications.

## H. Data management

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

224. All observations should be recorded in a worksheet following conventional data formats. They should be accompanied by high-quality close-up photographs with reference scale.

225. A best practices document template on data management can be found in Ocean Best Practices System (2020). The template can also be downloaded from https://repository.oceanbestpractices.org/handle/11329/1245.

# VII. Biological communities

## A. Introduction

226. The environmental baseline for biological communities should include spatial and temporal data on the pelagic and benthic communities and their ecosystem functions, as well as information on sea mammals, birds, turtles, fishes and large gatherings of surface nekton and plankton. The data collected will be diverse and should be extensive enough to assess the potential impact of mining on the sea floor and the water column.

227. The following variables should be determined to define the biological communities:

(a) Pelagic communities: the pelagic system comprises the entire water column from the sea surface down to the sea floor. Pelagic organisms range from bacteria to whales. The vast volume of water and the organisms within it move across potential mining sites, so sampling should extend beyond the zone of the immediate mining impact to include all the water and organisms entering, potentially interacting with and exiting the zone of the mining impact;

(b) Benthic communities: the benthos is the adult biota living in or on the sediment or near the sea floor. Benthic organisms range from bacteria and protists to metazoans. Mining has a direct impact on them in the form of habitat removal or habitat disaggregation, and an indirect impact in the form of increased turbidity and sediment redisposition;

(c) Connectivity: understanding the genetic diversity, biogeography, molecular connectivity patterns, habitat restrictiveness and endemicity, and turnover is essential to determining the potential recovery following a disturbance;

(d) Ecosystem functioning: knowledge of how the ecosystem functions is needed to understand how small-scale disturbances can lead to shifts in the structure of the food web and the cycling of organic matter by the resident benthic community;

(e) Ecotoxicology: metals and other contaminants released during mining operations may have an impact on organism physiology; it is therefore important to understand their potential toxicity;

(f) Marine mammals, sharks, turtles and surface nekton: it is important to record the presence of various species in the general contract area, in particular the presence of sensitive, threatened or endangered protected species, as their seasonal migration routes may pass through the area. Consideration should be given to making an assessment of their susceptibility to noise and to particular sound frequencies, the depths at which they may be encountered, and the impact that light and future mining operations may have on them;

(g) Seabirds: seabirds are one of the most threatened bird groups worldwide. Their behaviour is affected by marine installations. They are good indicators of the overall health of the ecosystem because they bioaccumulate heavy metals and toxic substances.

## B. General methodology

228. Temporal sampling is necessary to capture seasonal variability in biological parameters. Those include, but are not limited to, concentrations of metals and other contaminants in tissues and are used in ecotoxicology studies. Another factor to take into account are life history traits, such as migration patterns of pelagic species that might travel through the contract or reference area.

229. To document regional diversity and connectivity patterns, comparisons of specimens collected across a range of spatial scales may be required, for example from tens to thousands of kilometres,. Such comparisons may require sampling of distant sites as part of establishing the baseline, or they may rely on comparisons with third-party data sources.

230. All taxonomic identifications should be carried out at the best resolution possible. Molecular samples of the taxonomic units should be used to support identification.

## C. Sampling resolution

### 1. Pelagic sampling

231. In the pelagic realm, biological communities are partitioned by depth as follows: the photic zone, where there is sufficient light for photosynthesis by phytoplankton (0–200 m); the mesopelagic or twilight zone, which is dominated by animals of the deep-sea scattering layers (200–1,000 m); and the bathypelagic zone or ocean interior, which is inhabited by specialized organisms of the dark ocean depths (> 1,000 m). Finer layers occur within those depth zones. By contrast, the horizontal distributions may be quite homogeneous over hundreds of kilometres, punctuated by transitions at oceanic fronts or eddy systems. See subsection 5 below on microbiota for details on sampling and analysing microorganisms in the pelagic realm.

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

233. Particularly below 1,000 m, beyond the maximum range of ships' sonars, net sampling can be augmented with imaging systems. Those include underwater video profilers, which are lowered on a wire to take a vertical profile, submersibles used for oblique profiling as described by Robison et al. (2013), and various other systems used for bioluminescence profiling as described by Heger et al. (2008). ROVs and AUVs are likely to become important for such deep surveys.

### 2. Benthic sampling

234. Benthic sampling should span the range of size classes, different substrates (including sediments and nodules), biogeochemistry (see sect. V), ecosystem functioning and genetics. Details regarding specific variables are provided in the sections below.

235. Best practices such as the following should be followed when operating the sampling devices and handling the samples on board:

(a) Sediment sampling equipment should be landed on the sea floor gently to minimize the bow wave effect (deployment from the side of the ship, low wire speed, use of telemetry);

(b) Box cores for macrofauna should not be subsampled. Subsamples from a single box core, and separate cores from the same multicorer deployment, are pseudo-replicates and should not be regarded as true replicates (see sect. III.A);

(c) Samples and specimens should be kept as cold as possible to improve DNA quality (sieve the sample in a cold room, sorting on ice and preferably on board, preserve specimens and sieve residues in cold ethanol, maintain the cold chain during transport and storage of the samples).

236. The number of samples required should be determined using power analysis (Jumars, 1981) and rarefaction curves based on exploratory sampling. For macrofauna, exploratory sampling should include five to 10 cores per physiographic unit. Previous studies have indicated that at least 20 full box cores are needed, but that preferably more than 30 should be used, to obtain an adequate baseline for a statistical comparison of pre- and post-mining macrofaunal abundance in a physiographic unit. The actual number should be determined on the basis of a power analysis and rarefaction curves specific to the area of investigation. For megafauna, a power analysis is necessary to optimize design. Transects should be designed with the aim of encountering more than 500 individual organisms in each transect; at least five transects should be obtained (Simon-Lledo et al. 2019).

237. Sampling strategies should be focused on physiographic units that will be directly affected by mining (e.g. plains with dense nodule coverage), physiographic units that may be affected by secondary impacts as indicated by other variables (e.g. areas where plumes may settle), and suitable reference sites.

## D. Measured variable: pelagic communities

238. The vertical structure of the water column should be described on the basis of acoustic echo sounding using a ship-borne system (Simrad EK60 or equivalent) operating at multiple frequencies (18, 38, 70, 120 and 200 kHz), calibrated before the start of each voyage. Transects should be surveyed during both the day and night to estimate total biovolume or biomass, for example 10 line transects at each location, each 8 nautical miles long with the ship moving at 8 knots (Cox et al., 2013). The data should be processed to estimate biomass as a function of depth, and the total integrated biomass from the surface to a depth of 1,000 m (Irigoien et al., 2014). The sound scattering layers should be identified and classified using multifrequency analysis to discriminate fish, squid and crustaceans (Benoit-Bird et al. 2017). Acoustic echo sounder surveys using a Simrad EK60 or equivalent should be continued for at least three 24-hour cycles to quantify diel vertical migration as described by Klevjer et al. (2016).

239. Where possible, historic reference points should be used that are accessible by examining global sound scattering data available in various archives such as world data centres for oceanography and national data centres; global datasets such as Mesopelagic Biogeography (Proud et al., 2017)

240. The components of pelagic communities and the appropriate sampling methodology for each are as follows:

(a) Phytoplankton: primary production (chlorophyll-a) should be mapped across the sampling area from appropriate satellite multispectral imagery sources (AVHRR, SeaWiFS, MERIS and MODIS). Sampling is necessary to calibrate and verify satellite-based estimates of primary production. Replication is required to determine natural spatial and temporal variation. Water samples collected using Niskin bottles in a CTD device yield data on phytoplankton at various depths.

(b) Zooplankton (mero- and holo-): zooplankton should be sampled using nets to retrieve voucher specimens for identification and DNA sequencing with different sampling for each size class. Species of macro- and meso-zooplankton can be classified and quantified using high-definition video, active acoustic imaging (i.e. multi-beam cameras), photomultipliers (for measuring bioluminescence), high-definition and acoustic imaging, and bioacoustic sonars. To determine community composition, it is important to compute species distribution and assemblage structure per sampling zone and sum up the data for the whole area using high-definition and acoustic imaging. Complementary data may be obtained from the eDNA (DNA in situ sequencers), (Danovaro et al. 2020), as follows:

(i) Zooplankton: zooplankton should be sampled using nets, optical tools (e.g. underwater video profilers) and AUVs/ROVs to assess and retrieve voucher specimens for identification and DNA sequencing, with different sampling for each size class. The nets used for sampling should have a mesh size of less than 1 mm; Bongo nets or plankton pumps should be used in deeper waters and/or with a multiple opening and closing net enabling discrete depth samples to be taken on a single tow (see ISBA/25/LTC/6/Rev.1 in conjunction with ISBA/25/LTC/6/Rev.1/Corr.1). The nets should be equipped with flow meters to measure the volume sampled, as well as with depth and temperature sensors. Samples should be collected from 100 m above the sea floor up to the surface, with a minimum of two tows at each sampling station.

(ii) Mesopelagic nekton: a larger net should be used, such as the macro-zooplankton or "krill" net described by Wenneck et al. (2008), which is a pelagic trawl suitable for catching representative samples of scattering layer fishes, crustaceans and other organisms in discrete depth layers. It has five cod - ends, each equipped with a seven-litre bucket. Larger versions of the MOCNESS can also be used. Samples should be collected from 100 m above the sea floor up to the surface, with horizontal tows at the depth of each scattering layer, which should simultaneously be observed on the echo sounder to ensure correct targeting. Sample processing is described by Cook et al. (2013). Surface to 200 m sampled with nets, one net with a 350 µm mesh size cod end and one net with a 200 µm mesh size cod end are recommended. For multiple opening and closing net systems, mesh sizes can range from 64 µm to 3 mm, depending on the aim of the study and the target organisms.

(iii) Gelatinous zooplankton: gelatinous zooplankton constitutes a high proportion of the plankton biomass. It is abundant and diverse from the epipelagic to the abyssopelagic, including the benthic boundary layer. Optical tools (e.g. underwater video profilers) or AUV/ROV transects are the best way to survey for gelatinous zooplankton. Devices should be placed at depth intervals down the water column similar to the towed nets.

(iv) Benthopelagic plankton: the near-bottom layer can be sampled using plankton nets, but the nets require accurate pinger systems, depth meters or altimeters mounted on the gear to reduce the risk of damage from contact with the sea floor. The layer can also be sampled using plankton nets mounted on sledges that are towed across the sea floor (e. g. the "Brenke sledge"). Quantitative zooplankton samples can be collected using plankton pumps moored near the sea floor at very precise heights above it; moored sediment traps may be used to collect qualitative samples of zooplankton

(c) Microbiota: microbiota are organisms invisible to the naked eye. They are smaller than meiofauna. Operationally defined as less than 32 µm in size, they include nanoplankton, protistans, bacteria, archaea and viruses. Microbial communities in the

water column and near-bottom water layer may play a crucial role in biogeochemical cycles. See section E, subsection 5, for sampling and analytic guidelines.

(d) Nekton: nekton covers a large size range, from small micronekton (2 –20 cm) up to large fish and squid. Sampling is different for each size class:

(i) Small nekton should be collected using net samplers, i.e. MOCNESS;

(ii) Larger elements should be sampled using mid-water trawls to collect specimens, as well as acoustic methods to estimate biomass and categorize the deep scattering layer.

241. The various elements of the zooplankton should be characterized into the lowest taxonomic level possible. Holoplankton should be identified to the species level. With meroplankton, it may be necessary to identify to a more general grouping, for example, echinoderm larvae, polychaete trochophore, egg, etc. Molecular analysis can assist in the identification of both holo- and meroplanktonic taxa.

242. For all faunal groups, imaging and taxonomic information should be obtained; molecular techniques should be used to obtain genetic characters for taxonomic comparison between Contract Areas.

243. The parameters that should be measured are chlorophyll-a concentration ($\mu g\ l^{-1}$), phytoplankton composition and biomass, diel migration of micronekton and zooplankton, abundance, and composition and biomass of zooplankton and other faunal groups.

244. On the basis of these measurements and those collected for other parameters, primary productivity, density and diversity ( univariate and multivariate) of faunal groups, size classes and functional groups should be determined.

## E. Measured variable: benthic communities

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

(a) Megafauna: organisms visible in images, usually greater than 1 cm in size;

(b) Macrofauna: usually annelids, amphipod, tanaid and isopod crustaceans, molluscs, smaller echinoderms, usually retained on a mesh size of 250 –300 µm. Abyssal samples contain, in addition, numerous macrofauna-sized foraminifera (Bernstein et al., 1978) and large meiofaunal organisms such as nematodes, although those 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; that is the approach taken in the present Guidelines. Populations of the larger species among meiofaunal taxa may still be more accurately sampled in the larger sampling unit that is typically used for the macrofauna and may be considered as part of the macrofauna sensu lato. In the Clarion-Clipperton Zone, the macrofauna sensu stricto is dominated by two taxonomic groups: the polychaetes and the tanaids;

(c) Meiofauna: usually nematodes, harpacticoid copepods, ostracods, kinorhynchs and other small invertebrates (the metazoan meiofauna) retained on a 32-µm sieve. This size class also includes abundant smaller-sized foraminifera (the foraminiferal meiofauna). For practical reasons, they are typically limited to those retained on a 150-µm, 125-µm or 63-µm sieve;

(d) 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 organism;

(e) Microbiota: organisms invisible to the naked eye, smaller than meiofauna. They are operationally defined as less than 32 μm in size;

(f) Demersal fishes and scavengers: mobile animals that are often active predators in the benthic boundary layer; also includes species that exploit dead carcasses of, for example, fishes or whales, that fall to the sea floor.

### 1. Megafauna

246. The megafauna relevant, in the broadest sense possible, to mining operations should be assessed by means of imaging along straight line transects, replicated within specified strata or physiographic units. Image assessment on the basis of photographs (still images) rather than videos (moving images) should be used wherever possible, as that greatly facilitates analysis and quality control. Stills can be extracted from video images of very good quality, but the quality of photographs is almost always higher. Where possible, video should be included to assess the occurrence of rarer, highly mobile forms (e.g. fishes) and to provide multipl e view angles and behavioural observations.

247. The still cameras should have a resolution sufficient to reliably show megafauna greater than 10 mm in size in sufficient detail (e.g. each square of 10 x 10 mm on the sea floor is covered by 40 x 40 pixels in the image). In addition, the exposure settings on the still cameras should be capable of manual control. In addition, for the reliable characterization megafauna greater than 10 mm in size, video can be used if the resolution is sufficient (i.e. at least 720p high-definition; which has about 1 million pixels per image). Images should ideally be obtained in raw format, i.e. minimally processed data from the image sensor.

248. For seabed images, platform capable of acquiring well-lit, high-resolution images of a consistent scale and quality that allow for the reliable identification of megafaunal individuals of the determined size (usually 10 mm). It can be an AUV, ROV, crawler or towed camera platform. The survey altitude should be kept constant so that images are obtained at a constant altitude above the seabed. Navigation information for the platform should be obtained automatically at regular intervals (e.g. 1 Hz) using an acoustic transponder system .

249. The start positions and transect heading should be randomized. Transects should be replicated. The number of replicates should be determined and justified using statistical power analysis. At least five replicates should be obtained for each target stratum (Simon-Lledo et al., 2019). Transects should be independent of each other (i.e. a long line transect should not be split up into adjacent segments). Efficient strategies for obtaining independent transects are available; for example, multiple straight-line transects can be obtained in a zig-zag pattern. Transects should not cross physiographic units.

250. Transect length should be determined using existing data for the region to ensure that a sufficient amount of megafaunal organisms are encountered in each transect to make an effective and robust evaluation of the metrics of interest possible. For biodiversity assessment, transects should be designed with the aim of encountering more than 500 individual organisms in each transect (Simon-Lledo et al. 2019).

251. The transect width should be calculated on the basis of the actual imaging altitude and is typically around 2 m. If sufficient positioning information and spatially accurate sampling approaches are available, adjacent overlapping transects should be obtained to create mosaicked images and cover a wider area, as long as the mosaicked

image has sufficient resolution to make reliable identification of organisms of more than 10 mm in size possible.

252. Taxa that cannot be determined to be alive, such as invertebrates living in a shell or tube (most polychaete and gastropod taxa) should be listed. It may be necessary to exclude them from quantitative analysis.

253. Xenophyophores (protistan megafauna) should be analysed separately (Gooday et al., 2017, 2020b). Their numbers are typically several times higher than those of metazoan megafauna.

254. Image transects should be analysed as sample units (i.e. all organisms recorded in each transect should be summed to form a single sample unit) for the majority of analyses.

255. All images should be scaled following photogrammetric approaches using known optical properties of the camera, the camera position on the collecting device, altimeter records and vehicle pitch and roll data. The area of sea floor covered should be stated in the reporting.

256. Images should be annotated using specialist annotation software, such as BIIGLE (Langenkämper et al., 2017). Any of the range of highly suitable image-annotation tools available for seabed image analysis can be used (Gomes-Pereira et al., 2016; Schoening et al., 2016).

257. Images should be analysed in random order (to minimize any sequence- or time-related bias). All megafaunal individuals more than 10 mm in size should be detected and annotated. They should be identified to the highest taxonomic resolution possible, i.e. by morphotype (operational taxonomic unit) for consistent identification, typically at the genus or family level (Howell et al., 2019). The physical dimensions of each individual should be calculated on the basis of known image pixel sizes.

258. Where possible, observation of species made on photos or in video recordings should be verified by taxonomic and/or genetic analysis of several collected specimens. Recent studies have shown that some megafauna, such as ophiuroids, can comprise species complexes (e.g. Christodoulou et al., 2020).

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

260. The parameters that should be measured are numerical abundances of specimens per area sampled (individuals per $m^2$) for appropriate taxonomic/functional groups and for the whole metazoan/xenophyophore community. In addition, the size of each individual encountered and any observations of details of its location (such as whether it was attached to a nodule) should be recorded.

261. From those measurements, the density, statistics to describe community structure (univariate and multivariate diversity measures) and distribution patterns should be determined. The results should include maps or areas imaged, potentially including the extent of seabed habitats identified.

## 2. Macrofauna

*262.* Macrofauna should be sampled using the methodology outlined in the Authority's *Technical Study No. 13: Deep Sea Macrofauna of the Clarion-Clipperton*

*Zone*. Additional information can be found in ISBA/25/LTC/6/Rev.1 in conjunction with ISBA/25/LTC/6/Rev.1/Corr.1.

263. Both macrofauna living on nodules and those in the sediment should be collected. Once on board, the surface of the core should be photographed after the overlying water has been siphoned off over a sieve with the use of a plastic hose. The sieve residue from the overlying water should be processed together with the surface sediments.

264. For nodule-associated fauna, when box cores are recovered, obvious epifauna attached to the nodule surface should be identified. Nodule fauna should be imaged when still attached to the nodules in special small aquaria with cold filtered seawater (4°C); fauna should be removed, a snippet sample taken for DNA in a 2 -ml tube with cold 96% ethanol (-20°C) and the organism fixed in a separate tube. The nodule should be returned to the original container. All water that was in contact with the nodules should be sieved over a 32-µm sieve and the residue added to the original container. The size and weight of the nodules should be recorded before the nodules are preserved in formalin or cold ethanol.

265. For sediment fauna, all processing should be performed in a cold laboratory. Surface water in the corer should be siphoned off into a sieve (250 µm or 300 µm) and photographs taken of the intact core surface and cross-section, making note of any bioturbation and the depth of any changes in sediment colour to identify vertical changes in sediment type. The sediment should be divided into layers of the following depths: 0–3 cm, 3–5 cm and 5–10 cm. Each layer should be sieved with cold filtered seawater. The uppermost sample should be sorted immediately and the residue from the deeper slices should be kept in a cold lab in cold filtered seawater until those slices are processed. Increasingly, samples are needed for both morphological and molecular analysis; for that reason, the use of formaldehyde as a fixative should be carefully considered, as it may make molecular analysis of samples impossible. For morphological and molecular analysis, in the refrigerated laboratory (4°C), the 0 –3-cm and 3–5-cm layers of sediment should be sieved with cold filtered seawater and the residues preserved in 10% buffered formaldehyde or 96% ethanol. In the laboratory, the 5–10-cm layer of sediments should be sieved with cold filtered seawater and the residues fixed in 10% buffered formaldehyde or 96% ethanol. If there are large volumes of residue, stronger concentrations of formaldehyde may be needed to ensure the fixation of specimens. Formaldehyde solutions should not be used for fixing crustacean groups such as isopods and tanaids; for those taxa, preservation in 96% pre-cooled ethanol is advised. Samples should be fixed in a formaldehyde solution for at least 24 hours. As soon as practicable thereafter, all samples should be transferred from formaldehyde solutions into a 70–80% ethanol solution.

266. For molecular, morphological and biodiversity studies, the residues of the upper, 0–3-cm layer should be sieved and retained and the sample kept as cold as possible, sorting all metazoans into easily identifiable taxonomic groups over an “ice bed”. Live images of specimens should be taken before preserving them in ethanol. DESS (Yoder et al. 2006) can be used to preserve nematodes. Other layers should be sieved and the residues examined as described above or preserved in 96% ethanol. Polychaetes should be preserved in cold 80% ethanol, nematodes in DESS (and stored at 4°C) and all other groups in cold 96% ethanol. The ethanol should be changed after 24 to 48 hours and the samples stored at -20°C.

267. The parameters that should be recorded are taxonomic classification for each morphospecies, species by station matrices showing abundance (individuals per sampler) and gene sequences.

268. From those measurements, the density, species richness, statistics to describe community structure (univariate and multivariate diversity measures) and distribution patterns should be determined.

### 3. Meiofauna (including foraminiferal meiofauna)

269. Metazoan meiofauna should be sampled using the methodology outlined in the Authority's *Technical Study No. 7: Marine Benthic Nematode Molecular Protocol Handbook* (Nematode Barcoding).

270. For biodiversity analysis, meiofauna should be restricted to those taxa of the sediment fauna commonly recognized as meiofauna, such as nematodes harpacticoid copepods or kinorhynchs. Macrofaunal taxa (e.g. polychaetes and tanaids) captured in meiofaunal samples can be noted but should not be included in the meiofaunal abundance estimates.

271. At least one core per multicorer deployment should be dedicated to the morphological characterization of metazoan meiofauna and one core to the morphological characterization of foraminifera. Additional cores should be allocated to molecular characterization of those groups and other small-sized eukaryotes (e.g. small naked protists) (Gooday et al., 2020a) by means of barcoding and/or metabarcoding. Metabarcoding can be done on meiofauna extracted from the sediments or on sediment samples as such; the latter would constitute eDNA samples.

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

273. Once on board, all cores should first be photographed. Overlying water of the core for metazoan meiofaunal analysis should be siphoned off over a 32-µm sieve with the use of a plastic hose; the sieve residue should be processed together with the surface sediments. The slicing of the core should be determined on the basis of visual inspection. Typically, the presence of nodules prevents slicing, in which case the entire unsliced 0–5 cm section of the core may be preserved. Alternatively, nodules can be removed and cores sliced using a cutting plate into the following layers: 0 –1 cm, 1–2 cm, 2–3 cm, 3–4 cm, 4–5 cm (the depths identified in sect. III.A, but going down no farther than 5 cm into the sediment).

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

275. The temperature and chemical solution used for the preservation of meiofaunal samples should be mentioned explicitly (type and concentration). The intended analysis determines what type of sample preservation is needed. For example, samples for morphomolecular study (i.e. barcoding) should be preserved with a solution containing 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) wh ile keeping the possibility of extracting genetic material from the same specimen (i.e. DNA barcode) open, thus establishing a link between morphology and molecular identification (Bhadury et al., 2006). Samples for metabarcoding analysis should be frozen to at least -20°C immediately after sampling (Macheriotou et al., 2020). In addition, samples should be preserved with a borax-buffered 4–8% formaldehyde-seawater solution, but those specimens can only be used for morphological analysis. At least one core should be subsampled for metabarcoding of small-sized eukaryotes (protists and metazoans). From each core, three sediment subsamples should be taken

(approximately 2 ml in volume) using a sterile spoon, placed directly in plastic vials with 5 ml of a suitable soil preservation solution and stored at -20°C. Any nodules that are encountered should be preserved separately for further analysis of nodule -associated fauna.

276. Once in the laboratory, samples should be processed using any standard meiofauna extraction procedure. For metazoan meiofauna, flotation and centrifugation method should be followed (e.g. at 1,905 rcf), as that is known to yield up to 80% or more of the fauna (Mclntyre and Warwick, 1984). Because floatation yields 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 those are abundant and dominate foraminiferal diversity in samples from the Clarion-Clipperton Zone and the Indian Ocean. However, for monitoring purposes, analysis can be focused 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 so-called micropalaeontological approach).

277. 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 analyse finer-sized residues and 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 and Gooday, 2017, 2019). Ideally, all three fractions (> 150 µm, 125–150 µm, 63–125 µm) should be analysed, but if doing so is impractical, one fraction (> 150 µm, > 125 µm or > 63 µm) should be used consistently.

278. Sieve residues for morphological analysis should be stained in a rose bengal solution (e.g. 1 g in 1 l tap water), for example, by placing the sieve containing residue in a dish of stain solution overnight and then washing the residue on the sieve to remove excess stain. Foraminiferal sorting should be carried out in water, in a Petri dish, for example. Delicate monothalamids should be removed from the dish using a pipette and stored in glycerol on glass cavity slides, the slides being left uncovered so that specimens remain accessible. The more robust hard-shelled species should be stored on dry micropaleontological slides. For additional details on processing foraminiferal samples, including wet splitting and sediment sieving, distinguishing "live" from dead specimens and the problem of fragmentation, Goineau and Gooday (2017, 2019) and Gooday and Goineau (2019) should be consulted. Those papers and their supplementary materials include numerous photographs of common and mainly undescribed monothalamids. Schönfeld et al. (2012) and Alve et al. (2016) should be used for recommendations regarding the micropalaeontological approach to using multichambered foraminifera in monitoring studies.

279. The parameters that should be recorded are species/genus lists, species/genus by stations matrices showing abundance density per 10 $cm^2$, and gene sequences.

280. On the basis of those measurements, density and statistics to describe community structure (univariate and multivariate diversity measures) should be determined.

### 4. Fauna associated with polymetallic nodules

281. The fact that nodules have extremely slow growth rates means that, once removed, it will take millions of years before this hard substrate is re-established. Therefore, it is important to determine the extent to which species are shared between

soft sediments and nodules in abyssal nodule fields, and their functions or roles in that habitat.

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

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

284. All the epifaunal organisms attached to the exterior surface of the nodules should be photographed immediately, carefully removed from the nodule and stored in 96% ethanol for further microscopic and other laboratory analysis. The surface of each nodule should then be washed separately on a 32-µm-mesh sieve; the sieved material should be considered part of the ambient sediment fauna. The soft sediment on the nodule should be washed separately, preferably on a fine-mesh sieve (20–25 µm), and sieved material should be considered part of each fauna-containing sediment layer. For nodule-crevice metazoan meiofauna, nodules should be carefully washed to remove adhering sediments, and should be measured and weighed. The clean nodules should be broken down mechanically if needed and fixed in, for example, buffered formaldehyde or DESS for morphological and molecular investigations, bearing in mind that the fixation may affect the physical integrity of the nodule. The sample can then be processed using any standard meiofauna extraction procedure. However, it is recommended to use a flotation and centrifugation method (e.g. at 1,905 rcf), as that is known to yield up to 80% or more of the fauna (Mclntyre and Warwick, 1984). Next, the supernatant has to be washed onto a 20–32-µm-mesh sieve. The sieve residue should be carefully examined under a stereomicroscope (at 40× magnification). All the faunal organisms should be identified to the lowest taxonomic level possible, counted, sorted and stored separately in DESS at 4°C so that they can later be used for molecular identification.

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

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

287. From those measurements, the density and statistics to describe community structure (univariate and multivariate diversity measures) and distribution patterns should be determined.

### 5. Microbiota

288. Sediment samples should be collected with an ROV-manipulated push corer, manned submersible push corer, box corer, TV-guided box corer, multicorer or TV-guided multicorer, with the sampler sealed as close to the collection point as possible to prevent contamination during recovery.

289. Water samples should be collected using a CTD rosette equipped with a water sampler, or using an in situ unit for the filtration and extraction of particles, such as the McLane water transfer system, with the sampler sealed as close to the collection point as possible to prevent contamination during recovery. Samples should be collected at important water layers as defined by the water column sampling (see sect. V). The layers to sample include, but are not limited to, the surface layer, the subsurface chlorophyll maximum layer, the anoxic layer and the near-bottom layer.

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

291. A microbial count should be obtained using fluorescent staining method with DNA-specific dyes (e.g. DAPI) or following a real-time PCR method with groups-specific oligonucleotide primers (Labrenz et al., 2004). Where cultivation techniques are used, this should happen on board the sampling vessel.

292. Microbial DNA should be obtained according to the phenol chloroform DNA extraction method or with the help of DNA extraction kits; spectrophotometry (Qbit, nanodrop) and DNA agarose gel electrophoresis should be used to detect the DNA purity and integrity, respectively. Qualified microbial DNAs should be sequenced in high-throughput sequencing platform (e.g. Hiseq X, NovaSeq, Sequel II, MinION, GridION, PromethION, and MiSeq for metabarcoding). Additional amplicon sequencing should be performed for important marker genes (e.g. the 16S rRNA gene, functional genes).

293. Microbial RNAs should be obtained using RNA extraction kits or similar reagents as soon as possible after sample recovery; spectrophotometry and RNA agarose gel electrophoresis should be used to detect the RNA purity and integrity, respectively. Qualified microbial RNAs should be sequenced in a high-throughput sequencing platform. In addition, specific RNAs should be analysed with real-time PCR method with specific oligonucleotide primers.

294. There is currently no standard method for analyses involving high-throughput sequencing. The commonly accepted methods are FastQC for quality control; SPAdes for assembly of sequencing reads; MetaBAT 2 for contig binning; DADA2 for amplicon sequence variant generation; BLAST+ for sequence alignment and gene annotation; CheckM for assembly and binning quality assessment (Breitwieser et al., 2017).

295. Results of genome sequencing analysis or metagenomic binning of microbial population should be provided.

296. The parameters that should be recorded are identification, abundance and gene sequences.

297. From those measurements, the microbial diversity, community composition, abundance, functional differences of various groups should be determined.

### 6. Demersal fishes and scavengers

298. Two or more of the three main categories of sampling should be used: bottom trawls, baited systems and image transects. It should be noted that video transects and imagery collected by ROVs, AUVs or drop cameras are not ideal for sampling fish as they can attract or deter species and thus bias the species composition and abundance. For image transects, the approach outlined in subsection 1 should be followed. Bottom trawls can be towed independently or behind a camera sledge, the catch yields voucher specimens for taxonomy and DNA sequencing. Traps and long lines have the disadvantage that they are species-selective and should therefore not be used for biodiversity studies. Baited cameras mounted on landers result in unbiased sampling of the bait-attending fauna in any given area. For amphipods, small minnow-type traps can be attached to the legs of the camera lander to catch voucher specimens (Jamieson, 2015).

299. A disadvantage of camera systems is that species are often difficult to discriminate in images, but if utilized, a minimum of 10 replicate baited camera drops should be performed in each sampling area. A disadvantage of bottom trawl sampling is that survey trawls can have an adverse impact on vulnerable benthic species and habitats (Duran Munoz et al., 2020).

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

301. From those measurements, the density, species richness statistics to describe community structure (univariate and multivariate diversity measures) and distribution patterns should be determined.

## F. Measured variable: connectivity

302. Population connectivity studies should be undertaken for key species using samples from several geographic locations and/or habitats. For each species, the number of individuals in each population should ideally be relatively large (> 10 –20 individuals per site), so that only relatively abundant species are assessed and used as proxies for the wider assemblage. However, given the relatively low density of some species found in the Clarion-Clipperton Zone, even lower numbers (3–5 individuals per site) should still be enough to conduct connectivity studies (Taboada et al., 2018).

303. Depending on the setting, collecting enough individuals to undertake connectivity studies may require employing samplers in addition to those identified above. For example, collecting methods such as epibenthic sledges in benthic habitats may be necessary to ensure that enough macrofaunal individuals are collected. However, those methods should be avoided where they may have a negative impact on sensitive features. Samples for connectivity studies should be collected and stored in order to preserve DNA in its best condition, as detailed by Glover et al. (2016). When preserving large specimens or parts of larger specimens, 96% ethanol instead of 80% ethanol should be used.

304. For analysis, the reverse taxonomy approach should be used (Janssen et al., 2015). Vouchers of the specimens under study should be maintained, as further detailed examination of morphological characters is needed (for example using scanning electron microscopy techniques) to distinguish cryptic species identified molecularly.

305. The selection of appropriate molecular markers depends on the taxon selected. In some cases, standard approaches, such as using the most common molecular markers (e.g. COI, 16S rRNA gene) may not yield sufficient genetic variability to enable further analysis. A combined approach should be taken by using common molecular markers and microsatellite markers, including highly polymorphic microsatellites (Taboada et al. 2018), which can be used for small-scale studies.

306. In addition to microsatellites for population genetic studies, other molecular techniques should be explored, including using single nucleotide polymorphisms generated from reduced representation genome studies that can easily be applied to non-model organisms at a relatively low cost. For instance, with ddRADseq, hundreds to thousands of single nucleotide polymorphisms can be generated, which makes it possible not only to perform fine-scale population genomics studies, but also to investigate phylogenomics, adaptation strategies or introgression, among other population-level processes (Andrews et al., 2016).

307. Modelling approaches should be followed in which a range of available tools are used. Gene flow and migration patterns inferred from the genetic data should be compared with environmental factors such as oceanographic currents. The use of oceanographic models to estimate larval transport (see sect. IV, sect. D) may explain some of the patterns in the large-scale population differentiation and connectivity of the species (Taboada et al., 2018; Kenchington et al., 2019 ).

308. As a variety of software is being developed continuously, the results of baseline studies should include a clear indication of the tools used in the analyses and of the underlying assumptions.

309. From those studies, the connectivity and biogeography should be determined for key species in each functional grouping and should be inferred for the wider assemblages.

310. Specific metrics to be determined include:

(a) Minimum genetic distances, using haplotype networks, based on uncorrected p-distance and Kimura two-parameter models between and within species to establish the within- and between-species genetic distances;

(b) For genetic diversity, expected ($H_e$) and observed ($H_o$) heterozygosity and inbreeding coefficients ($F_{IS}$) should be calculated for each species, sampling station and region, using R packages or for example the Genodive program (Meirmans and Van Tienderen, 2004);

(c) For population structure, one of the following should be used:

(i) Clustering methods such as determined using the programs Structure (Pritchard et al., 2000) and DAPC; the latter is included in the adegenet R package (Jombart et al., 2010), which graphically represents the genetic affinities between samples;

(ii) Distance methods such as the fixation index statistic ($F_{ST}$) should be applied to measure the extent of genetic differentiation among populations, using pairwise $F_{ST}$ values to compare sampling sites and regions; analysis of molecular variance should be used to determine the hierarchical distribution of genetic variation;

(d) 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 be used to calculate migration patterns;

(e) 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; in addition, the occurrence of possible barriers to determining the genetic structure of populations should be evaluated using programs such as Barrier (Manni et al., 2004).

## G. Measured variable: ecosystem functioning

311. Infauna samples (a minimum of 10 to 12 randomly selected sites) for an analysis of natural isotope abundance for food web structure should be sampled at, for meiofauna, 0–1 cm, 1–2 cm and, for macrofauna, 0–1 cm, 1–5 cm and 5–10 cm. Megafauna should be sampled for natural abundance isotopes wherever possible so 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).

312. Meiofauna for stable isotope analysis should be sampled using megacorers or multicorers and sampled from the 0.5 cm layer. The sediments need to be stored frozen (at -20°C or colder) without any preservative until further analysis in the home laboratory. They should be sieved over a 32-µm sieve using cold filtered seawater. Macrofauna should be collected using a 0.25 $m^2$ box corer and sampled at 0–1 cm, 1–5 cm and 5–10 cm sediment depth; the sediment slices should be sieved on a 300-µm sieve using cold filtered seawater. Alternatively or in addition, the epibenthic sledge can be used to collect macrofauna samples for stable isotope analysis.

313. Samples for analysing the basic food web structure of infauna (e.g. the number of trophic levels) should be collected at the same sites as samples for analysing meiofauna and macrofauna community structure. The samples should be taken from at least 10 to 12 randomly selected sites. Where possible, megafauna (e.g. holothurians) should be collected by ROV during ROV transects or by trawling; efforts should be made to collect at least 10 animals of each major megafaunal taxon. Isotope labelling studies to quantify microbial and faunal activities and foo d web linkages should be undertaken in situ using benthic chamber platforms (ROVs or landers) at a minimum of 10 randomly selected sites, with replicate benthic chamber measurements made at each site (Sweetman et al. 2019).

314. Meiofauna and macrofauna sieve residues should be placed in a plastic bag, flash-frozen in liquid nitrogen and stored at -20°C. Alcohol-based fixatives should never be used when fixing samples for stable isotopes. Megafauna collected by ROV or trawl should immediately be transferred to a cold room; up to 10 individuals of each taxon should be sealed individually in plastic bags, flash-frozen in liquid nitrogen and stored at -20°C.

315. Meiofauna and macrofauna should be sorted once back in the laboratory, with care being taken to minimize sample warming. Fauna should be washed of attached organic debris in cold filtered seawater and placed in pre-weighed tin or silver (if calcareous) isotope analysis cups. Target tissues (e.g. body wall, muscle, ophiuroid arm) from megafauna should be removed in the laboratory, taking care to minimize tissue warming, and placed on foil. All samples should be dried for two to three days at 45°C and megafauna tissues ground by hand with a mortar and pestle. Calcareous megafauna tissues should be placed in silver isotope analysis cups. Calcareous animals and tissues (e.g. ophiuroid arms) should then be acidified with 10% HCl to remove carbonates and dried again at 45°C for three days, followed by an additional acidification step if not all the carbonates have been removed. Isotope samples should

then be prepared for isotope analysis (as specified by the laboratory that is analysing the samples) and sent away to be analysed as described in the literature (e.g. Hardy et al., 2008; Levin et al., 2009; Sweetman et al., 2013).

316. To quantify the dominant food types for the fauna, samples of particulate organic matter collected in sediment traps and sediment samples (see sect. V.H) should be prepared for stable isotope analysis; their isotope signatures should be corrected if the samples have been preserved in formaldehyde solution.

317. Isotope labelling studies to document food web activities and linkages should be undertaken in situ using ROV-operated benthic chambers or benthic chamber landers. To document heterotrophic microbial and faunal metabolic activity, $^{13}$C-labelled phytoplankton cultures should be used in the labelling studies (Sweetman et al. 2019), while autotrophic microbial activity can be determined using $^{13}$C labelled bicarbonate as a tracer. In addition, labelling studies in which $^{13}$C-labelled bicarbonate or $^{13}$C-labelled glucose is used allow for the further detection of food web linkages, such as identifying which fauna feed on microorganisms (Sweetman et al. 2019). In situ labelling studies should follow the methods of Stratmann et al. (2018) or Sweetman et al. (2019) and run for between 36 and 48 hours. Metabolism of organic C (from $^{13}$C labelled phytoplankton) into $CO_2$ can be quantified in these experiments if the chambers being used have syringe sampler capabilities. If so, samples should be collected at set times (e.g. every six to eight hours) during the experiment using the syringe sampler. In the lab, samples should be filtered (with an 0.45µm cellulose acetate filter) and fixed in exetainers with 5–10 µl of 6% mercury chloride for total dissolved inorganic carbon and $^{13}$C isotope-ratio mass spectrometry analysis (Sweetman et al., 2010). The depth of the water in the chamber and the area of the camber should always be noted to determine the volume of water in the chamber at the end of each experiment. At the end of the experiment, push/blade cores should be used to sample sediments for microbes and fauna from ROV-operated chambers, while benthic chamber landers, for the most part, automatically collect the sediment that has been exposed to the labelled substrate. Once on board, sediments should be transferred to a cold room and sampled for microbe samples at depths of 0 –1 cm, 1–5 cm and 5–10 cm, homogenized and flash-frozen in glass bottles (previously washed with methanol and dichloromethane in a 1:1 ratio and dried) using liquid nitrogen, and transferred to -20°C. Separate samples should be collected at the same depth horizons for sediment water content. Meiofauna should be sampled from a push corer (ROV-operated chambers) or syringe corer (benthic chamber lander) at depths of 0–1 cm and 1–2 cm, sieved on a 32-µm sieve and transferred to buffered 4% formaldehyde-seawater solution (i.e. 10% formalin). Macrofauna should be sampled from blade corers (ROV-operated chambers), or from the rest of the chamber in the case of a benthic chamber lander sample, sieved on a 300 -µm sieve and preserved in formalin. Samples for background microbial and fauna isotope signatures should be collected using ROV-manipulated push cores, box cores or megacores, and prepared and preserved in the same way. Although formalin preservation can affect $\delta^{13}$C signatures by 0.5–1 parts per thousand, the labelling of the fauna is likely to be significantly higher than that (500–1,000 parts per thousand), negating the need to freeze the samples. Moreover, the preservation of background samples in formalin cancels out the formalin preservation effect on the isotope signatures when calculating the faunal feeding rates. Once back in the laboratory, the amount of label uptake into microbial fatty acids and fauna biomass (i.e. metabolic/feeding activity) should be determined using the approaches described in Stratmann et al. (2018) and Sweetman et al. (2019).

318. Natural abundance isotope data from fauna ($\delta^{13}$C, $\delta^{15}$N), sediment trap samples and sediments should be generated using an isotope ratio mass spectrometer available at academic institutions and commercial laboratories. The data from samples

preserved in formalin should be corrected for formalin preservation. The corrected values plus the food web sources should be used determine the basal food sources that the sampled fauna is feeding on, using an isotope mixing model (e.g. MixSIAR) (Harbour et al., 2020), plus the number of trophic levels present within the benthic food web.

319. The parameters that should be recorded for natural isotope analysis are species lists, $\delta\ ^{13}C$ and $\delta\ ^{15}N$ signatures and biomass (in terms of µg C and N), together with the analytical methods, the number of samples and the appropriate error estimates.

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

321. In addition, isotope signatures ($\delta\ ^{13}C$, $\delta\ ^{15}N$) in tissues of benthic fauna, production of $^{13}C$-labeled dissolved inorganic carbon, $^{13}C$-signatures of microbial fatty acids and faunal biomass, depth distribution of $^{13}C$-labelled detritus through sediments should be recorded.

322. From those measurements, the following should be determined: the amount of carbon taken into the biomass of sea floor microbes and fauna per unit area per unit time (i.e. the metabolic or feeding activity), the number of trophic levels present within the food web, the dominant food sources being consumed, the contribution of various foods to the diets of different types of fauna, the trophic structure of meiofauna and macrofauna, microbial and faunal carbon cycling rates, rates of short-term sediment mixing and respiration rates.

## H. Measured variable: ecotoxicology

323. To establish the potential ecotoxicological risk of ore mining, multiple sources of data (or lines of evidence) should be used. The data sources can be compartmentalized into discrete components to build evidence of sufficient weight to establish the relative toxic risk for a particular resource and a particular mining operation (the weight-of-evidence approach) (Regoli et al., 2019). The practical guide on sediment quality assessment (Simpson and Batley, 2016) provides more details on relevant measures, but it must be borne in mind that none have yet been tested in the deep sea.

324. Weight of evidence should integrate data from the following lines of evidence:

(a) Sediment/mineral physicochemical properties;

(b) Laboratory ecotoxicological bioassays;

(c) Bioaccumulation of metals in indicator species;

(d) Sublethal effects/biomarkers in indicator species;

325. Each line of evidence should be analysed using the most suitable quantitative methods; each should be analysed during the baseline data collection.

326. Resource and sediment mineralogical characterization to determine the relative proportion of mineral and metals species should be used to identify the metal and metal mixtures that will contribute to the overall potential toxic risk to biological species.

327. In addition, biological specimens of key biomass or food web dominant species (from a minimum of three taxonomic groups, but see discussion in part R.10.3.2 of ECHA, 2008) for both benthic and pelagic (full water depth) compartments should be recovered on more than four occasions in at least one 12-month seasonal cycle in order to determine baseline concentrations of metals, other organic contaminants and the levels of biochemical and cellular biomarkers in key benthic, abyssopelagic and bathypelagic species. The biomarkers are the early warning signals of distress to ecosystem health (Andersen, 1997; Simpson and Batley, 2016; Mestre et al., 2017).

328. The activation of antioxidant detoxification pathways should be assessed using established biomarker assays (summarized in Simpson and Batley, 2016). Among those are biomarker assays of tissue superoxide dismutase activity performed by means of spectrophotometric determination of the reduction of cytochrome c by the xanthine oxidase/hypoxanthine system at 550 nm (e.g. McCord and Fridovich, 1969). Other antioxidant assays that could be performed include quantifying metallothionein protein concentration by means of differential pulse polarography (e.g. Bebianno and Langstone, 1989; Mourgaud et al., 2002), and enzymatic assays of catalase, glutathione peroxidase and glutathione-S-transferase activities (Auguste et al., 2016).

329. Next, the relative ecotoxicity of various bulk mineral/sediment phases (e.g. particulate and aqueous) to biological organisms should be established using proxy biological species in controlled, standardized laboratory experiments. The bulk toxicity of a resource can be established without a priori knowledge of the precise mineral composition. Using established laboratory protocols, the relative toxicity of the phases of the bulk resource (relative to known pure mineral standards that are expected to be present at that particular resource) may be assessed. Using established laboratory protocols, the relative toxicity of various phases of the bulk resource (relative to known pure mineral standards, e.g. $CuFeS_2$) should be quantified. Aqueous experiments (e.g. metal minerals leached from a freshly-exposed mineral surface) and solid-phase experiments should be conducted to mimic the intended mining operation, replicating fragment/particle size and duration/temperature of leaching (e.g. Brown and Hauton, 2018; Knight et al., 2018). Simpson and Batley (2016) and internationally recognized standard protocols should be consulted and employed to establish bulk resource ecotoxicity (e.g. ECHA 2008, ECHA 2016).

330. The potential toxicity of sediment discharge plumes from dewatering processes to proxy biological species at the intended depth of discharge should be assessed on the basis of the operator plan for recovery, riser transfer to surface, dewatering and shipment. Model biological species may include cultures of cyanobacteria (e.g. Prochlorococcus, Synechococcus or Cyanobium) in the epipelagic zone, zooplankton (e.g. calanoid or cyclopoid copepods) or cnidarians (or similar gelatinous zooplankton) (e.g. Aurelia or Nematostella) for discharge plumes in the meso- and bathypelagic zone, as well as fish (e.g. Oryzias melastigma) (Bo et al., 2011; Kong et al., 2008).

331. The lethal concentration ($LC_{50}$) or lethal dose toxicity ($LD_{50}$) of potential sediment discharge plumes from dewatering processes to relevant macrofaunal proxy species should be determined, together with chronic or sublethal toxic effects of exposure to the solid or aqueous phases of the bulk mineral or the dewatering plume, and the activity of the most relevant biomarkers.

## I. Measured variable: marine mammals, sharks, turtles and surface nekton

332. To gather information on marine mammals, sharks, turtles and surface nekton, a combination of ship-borne visual line transects should be used following the

standard methods described in Buckland et al. (2001), Barlow and Forney (2007), Verfuss et al. (2018) and on the website of the SCANS II project. This should be done at each station during daylight hours with the ship moving at a constant speed of 9 to 10 knots along a grid pattern, supplemented by the use of towed hydrophones to detect marine mammal vocalization. The information thus collected should be supplemented by data from passive acoustic monitoring stations deployed on oceanographic moorings to continuously monitor the vocalizations of marine mammals over several complete annual cycles.

333. The parameters that should be recorded are group size, species encountered (for sea mammals it may be possible to identify specific individuals) and the abundance of those species. Photographs should be taken where possible.

## J. Measured variable: seabirds

334. To obtain a thorough understanding of the distribution and abundance of seabirds and impacts of any human activity on them at sea, information should be obtained from several sources. Seabird attraction to infrastructure and ships (both transiting and stationary) and seabird collisions with infrastructure and ships should be monitored, systematic seabird censuses should be carried out, previously collected seabird tracking data should be compiled and analysed, including readily available layers of GIS mapping such as marine important bird and biodiversity areas and key biodiversity areas, and monitoring programmes on relevant breeding sites should be analysed (e.g. breeding numbers, demographic parameters or breeding success). In addition, where possible, relevant species and populations should be tracked.

335. Seabird abundance and attraction should be studied from stationary platforms or ships using visual surveys, imaging or radars. Visual surveys from stationary ships should be conducted using instantaneous counts of birds, also referred to as “snapshot counts”, within a semicircle radius (usually up to 300–500 m) for 10 to 15 minutes at regular time intervals (e.g. 20–60 minutes) (Gjerdrum et al., 2012; Bolduc and Fifield, 2017). Marine radars should be used to estimate seabird abundance and collision risk (Gauthreaux and Belser, 2003; Desholm and Kahlert, 2005; Bertram et al., 2015; Assali et al., 2017). In addition, seabird abundance and attraction should be assessed by censusing seabirds using line transects from ships or aeroplanes (Camphuysen et al., 2004; Ronconi and Burger, 2009; Gjerdrum et al., 2012).

336. Whenever possible, carcasses of seabirds killed by collisions should be collected in systematic searches, preserved frozen in permanent infrastructure for future reference with regard to emerging contaminants, and analysed for contaminants in various tissues (Gochfeld, 1973; Barbieri et al., 2010; Amélineau et al., 2016). The purpose is to create a baseline against which to compare tissue content in carcasses collected during operations. A wide range of contaminants should be analysed, particularly those that may be released during mining activities.

337. Relevant data sets should be requested and used for assessing the importance of a specific area for seabirds (among other marine predators). At-sea tracking data exist for many marine top predators. Several global initiatives currently exist under which compilations of information on marine migratory species are regularly collated and analysed to identify important areas at sea, including important bird and biodiversity areas (https://maps.birdlife.org/marineibas) and key biodiversity areas (www.keybiodiversityareas.org). Among those initiatives are the Seabird Tracking Database (www.seabirdtracking.org/), Migratory Connectivity in the Ocean (https://mico.eco) and the Movebank for Animal Tracking Data (www.movebank.org/cms/movebank-main).

338. Thanks to tracking data, the origin of the seabirds occurring in a specific area can be identified and thereby their population of origin can be identified and monitored. Tracking data also allow for obtaining precise estimates of the population size and identification of the species visiting a specific area (some of them difficult to identify at sea from a ship or a platform), breeding status, seasonal variation, specific populations visiting that area and even the age and sex structure of the visiting animals. The information should be used to identify the source breeding colonies. Monitoring programmes conducted in those breeding colonies supply additional baseline data that should be reviewed.

339. The parameters should be recorded throughout the year, as follows:

(a) From visual surveys, censuses, imaging or radar counts: relative and absolute abundances of seabirds identified to the lowest taxonomic level possible, usually at the species level, and, whenever possible, by sex, age, seasonal and morph plumage variations; diversity indices; and the use made of the area and shipping route over time;

(b) (b) From tracking data: the estimated proportion of birds from each colony in a defined area and along a defined shipping route using that area or shipping route over time, identified by species, population, breeding colony, breeding status, sex and age;

(c) From monitoring programmes: population size, breeding success, juvenile, immature and adult survival, recruitment age, population trends and estimates of population viability and time to extinction;

(d) From collisions and collected carcasses: numbers of deaths per day over time disaggregated by species, sex, sexual maturity, moulting and body condition. Tissue should be collected from the liver, muscle, fat and feathers, and the concentration of contaminants (Stockholm Convention list) in those tissu es determined; stomach content should be analysed and the amount of microplastics and microfibres in the stomach determined.

## K. Data quality

340. For temporal sampling, the same general area should be revisited as for previous surveys wherever possible. Samples for temporal analysis should be of sufficient size for a robust determination of the parameters of interest. To improve comparability, sample size should be kept constant between surveys.

341. Data sets collected or analysed by different researchers should be standardized to enable comparison. This is particularly important in time series investigations or investigations for which multiple operators are used. Where inconsistencies are found, further quality control is necessary.

342. Comparisons between megafaunal surveys can be made even if the acquisition methodologies are not identical. However, robust comparison relies on having accurately quantified (scaled) images and as much consistency in image quality as possible (with regard to resolution, lighting, colour balance and others). For any subsequent comparisons, the possibility of methodological bias between surveys should be carefully evaluated; for example, key taxa controlling patterns should be evaluated to ensure that they are clearly distinct in different data sets. The assumption should be that there is methodological bias until proven otherwise.

343. For the quality of the images to be suitable, lighting should be sufficient to maintain near uniform coverage of the entire sea floor image at the target altitude, imager settings such as zoom and exposure should be kept constant throughout the

survey and the camera should not be moved relative to the camera platform for any transect (e.g. by using a pan-and-tilt unit on an ROV).

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

345. Many organisms can be identified to the species level only by examining features not visible on photographs (e.g. hidden, internal or microscopic features). Molecular and other approaches (e.g. genomics, transcriptomics or population genetics) require specimen material. Therefore, precise samples of individual specimens should be obtained that are linked to in situ images, ex situ images, tissue samples and a sample for morphological analysis from the same individual. Such samples are best obtained by remotely operated or human-occupied vehicle. This is particularly important for the many taxa, particularly soft-bodied forms (e.g. anemones), that look very different alive on the seabed than they do on the surface, after recovery.

346. All identifications should be at the lowest taxonomic level possible. In addition, taxonomic keys and references used to determine the designations should be provided in order to ensure equivalence between identifiers.

347. Molecular identification through barcoding (Sanger sequences) and metabarcoding (amplicon sequence variants) should yield a species or genus list obtained by matching the genetic data acquired to those available in public reference databases such as GenBank (www.ncbi.nlm.nih.gov/genbank). This can be achieved by means of the Basic Local Alignment Search Tool (Blast) or the Ribosomal Database Project classifier.

348. The appropriate method for evaluating biomass is by means of an ecological material cycle model; in that regard, classification on the basis of size is better than classification on the basis of taxonomy.

349. When larger samples are needed than can be collected with precise approaches, trawl or epibenthic sledge sampling may be appropriate. Care should be taken, as these techniques have the potential to disturb relatively large areas of the sea floor their use may require an Environmental Impact Assessment (see ISBA/25/LTC/6/Rev.1 in conjunction with ISBA/25/LTC/6/Rev.1/Corr.1) and may affect other sampling efforts.

350. To determine whether a sufficient number of individuals have been collected to characterize the communities, a collector's curve, also referred to as a Chao analysis, should be made. This is likely to be required given the low numbers of individuals and the high diversity.

351. To ensure statistical robustness, a sufficient number of replicates should be sampled. The number of replicate samples depends on the density or richness of the taxon of interest and its variance. In order to demonstrate statistical robustness, the determined power of a before/after control impact analysis of variance should be reported on the basis of actual data provided by the baseline. The power analysis should be presented giving consideration to 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 95% should be provided (Ardron et al. 2019).

352. 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 approximately 25 nodules should

be collected randomly for the benthic biodiversity study. For better spatial sample coverage, samples from at least three box cores should be collected per physiographic area during the baseline data generation and monitoring study.

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

354. Numbers of seabirds are specific for a given site; it will not be possible to establish 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 (such as Harrison, 2000; Howell and Zuflet, 2019). Most seabird tracking data are biased or are limited to certain species (certain small, but mostly medium- to large-size species), certain periods of the annual cycle and certain life stages (usually adult breeders).

## L. Data management

355. Metadata should be generated for all the specimens collected, including depth, latitude, longitude and substrate where they occur (e.g. nodule, infauna, association with other organisms). On the basis of the metadata, catalogues of species should be created using the Darwin Core layout.

356. Vouchers for all specimens should be deposited in museums or national collection facilities to make them available to the scientific community. This should be done using a storage method that is appropriate for the analysis (e.g. formalin or ethanol for morphologic identification, ethanol or freezing for molecular analysis). Some analysis methods (e.g. ecotoxicology) do not allow for storing the specimen in its entirety; in such cases, several tissue samples should be taken (at least muscle, feathers, intestinal fat and liver) and stored individually.

357. DNA extractions should be preserved in cryofacilities of museums. Genetic sequences should be deposited in free repositories such as GenBank (www.ncbi.nlm.nih.gov/genbank) or the Barcode of Life Data System (www.boldsystems.org). Genotypes should be deposited in free repositories such as Dryad (https://datadryad.org/stash) or Pangaea (www.pangaea.de). RADseq data should be deposited in free repositories such as the Sequence Read Archive database of the National Center for Biotechnology Information ( www.ncbi.nlm.nih.gov/sra). Sanger and high-throughput sequencing data should be archived in publicly available databases along with all relevant metadata, in particular georeferencing information. GenBank should be used for Sanger data and the Sequence Read Archive should be used for high-throughput sequencing data; note that high-throughput sequencing data should be uploaded demultiplexed, i.e. two read files per sample.

358. Wherever possible, identifications should be documented using photographic evidence, in case the information needs to be revisited.

359. Ideally, the images should be stored as obtained by the camera (in the raw file format) and as processed for analysis (in another file format). Both the raw and the processed image files should be linked to the survey metadata by assigning them a unique image name, so that the data sets can be combined with ease.

360. Raw data and information on the place and method of storage of the specimens should be submitted to the Authority as part of the annual reports and as metadata in the contractor’s data submissions to the Authority’s DeepData database.

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# IX. Abbreviations and acronyms

| | |
|---|---|
| ADCP | acoustic Doppler current profiler |
| ADP | acoustic Doppler profiler |
| ADV | acoustic Doppler velocimeter |
| AUV | autonomous underwater vehicle |
| AVHRR | advanced very high-resolution radiometer |
| CNS | carbon, nitrogen and sulphur |
| CTD | conductivity, temperature and depth |
| ddRADseq | double-digest restriction-site-associated DNA sequencing |
| DCP | Doppler current profiler |
| DESS | dimethyl sulphoxide, disodium ethylenediamine tetra-acetic acid and saturated salt |
| DNA | deoxyribonucleic acid |

| | |
|---|---|
| ECHA | European Chemicals Agency |
| eDNA | environmental deoxyribonucleic acid |
| EPA | United States Environmental Protection Agency |
| ESA | European Space Agency |
| EuroGOOS DATA-MEQ | European Global Ocean Observing System Data Management, Exchange and Quality Working Group |
| FAU | formazin attenuation units |
| FNU | formazin nephelometric unit |
| GIS | geographic information system |
| GOOS | Global Ocean Observing System |
| Go-Ship | Global Ocean Ship-based Hydrographic Investigations Program |
| IAEA | International Atomic Energy Agency |
| IAPSO | International Association for the Physical Sciences of the Ocean |
| ICES | International Council for the Exploration of the Sea |
| IMOS | Integrated Marine Observing System |
| IOC | Intergovernmental Oceanographic Commission of the United Nations Educational, Scientific and Cultural Organization |
| IODE | International Oceanographic Data and Information Exchange |
| IRZ | impact reference zone |
| ISO | International Organization for Standardization |
| JAXA | Japan Aerospace Exploration Agency |
| JGOFS | Joint Global Ocean Flux Study |
| LADCP | lowered acoustic Doppler current profiler |
| MERIS | medium-resolution imaging spectrometer |
| MOCNESS | multiple opening/closing net and environmental sensing system |
| MODIS | moderate-resolution imaging spectroradiometer |
| NASA | National Aeronautics and Space Administration |
| NOAA | National Oceanic and Atmospheric Administration |
| NTU | nephelometric turbidity units |
| PCR | polymerase chain reaction |
| PIPS | passivated implanted planar silicon |
| PRZ | preservation reference zone |
| RADseq | restriction site-associated DNA sequencing |

| | |
|---|---|
| RCM | rotor current meter |
| rcf | relative centrifugal force |
| RNA | ribonucleic acid |
| ROV | remotely operated vehicle |
| rRNA | ribosomal ribonucleic acid |
| SeaWiFS | sea-viewing wide field-of-view sensor |
| TEOS-10 | thermodynamic equation of seawater (2010) |

# I – Members

## China

Para.22 (a) and (b), A. Spatial and temporal variability, III. Sampling and data acquisition

III. Sampling and data acquisition
A. Spatial and temporal variability
22 (a) For water column sampling (including physical measurements, unless indicated otherwise in sect. IV.B), a higher resolution should be used for sampling in the 200 m below the surface (~~three or four samples at depths determined on the basis of local variability~~ e.g. at 0 m, 25 m, 50 m, 75 m, 100 m, 125 m, 150 m and 200 m) and in the 500 m above the seabed (e.g. at ~~5 m,~~ 10 m, 25 m, 50 m, ~~75 m,~~ 100 m, ~~150 m,~~ and 200 m ~~and 500 m~~ above the seabed), keeping in mind that surface weather conditions and localized topography may affect the resolution that is possible very close to the seabed;

22 (b) …Where deeper measurements are required, samples should be taken every 5 cm between a depth of 20 cm and a depth of 50 cm, ~~and every 20 cm in deeper layers over a sediment column of up to 5 m~~…

E. Measured variable: benthic communities, VII. Biological communities
VII. Biological communities
E. Measured variable: benthic communities

None

Rationale

On Para.22 (a), A. Spatial and temporal variability, III. Sampling and data acquisition, from the long-term deep-sea practice, China found that the environmental parameters in the 200 m below the surface vary greatly, three or four samples at depths are not enough to describe the vertical variability, it is suggested that more samples at depths should be taken. While the environmental parameters in the 500 m above the seabed are stable, there is no need for such intensive sampling, however, the above layers can be used during the impact monitoring period.

On Para.22 (b), A. Spatial and temporal variability, III. Sampling and data acquisition, according to China's long-term deep-sea practice, it seems unnecessary to take samples every 20 cm in deeper layers over a sediment column of up to 5 m for analyzing pore water chemical parameters of sediments, as deep-sea mining does not affect such deep sediments.

On E. Measured variable: benthic communities, VII. Biological communities, China notes that some terms and definitions in the guidelines are inconsistent with the environmental guidelines previously issued by the ISA, and lack continuity. Fox example, in 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), Macrofauna means “animals retained on a 250- or 300-µm mesh, typically sorted and identified with a microscope, that include taxa such as polychaetes, bivalves, isopods and tanaids”. Whereas, in the Draft guidelines, Macrofauna is defined as “usually annelids, amphipod, tanaid and isopod crustaceans, molluscs, smaller echinoderms, usually retained on a mesh size of 250–300 µm”. It is suggested that the LTC should pay attention to this problem and adopt a uniform definition of Macrofauna.