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Marine environmental impact assessment (MEIA) — Technical specifications for marine biotic surveys in the international seabed area — General principles

Évaluation d'impact sur le milieu marin — Spécifications techniques pour les relevés biotiques dans la zone internationale des fonds marins — Principes généraux

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Foreword

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This document was prepared by Technical Committee ISO/TC 8, *Ship and Marine technology*, Subcommittee SC 13, *Marine technology*.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Introduction

In accordance with the United Nations Convention on the Law of the Sea (UNCLOS)[6] and the 1994 Agreement relating to the Implementation of Part XI of the UNCLOS, the International Seabed Authority (ISA) has developed regulations such as those contained in References [7], [8] and [9]. These regulations include provisions for contractors working in mineral exploration in the area to gather oceanographic and environmental baseline data and to establish baselines. These data and baselines are then used to assess the likely effects of the programme of activities under the plan of work for exploration on the marine environment.

Since high-quality environmental baseline data are a prerequisite for the correct assessment of the environmental impacts from deep-sea mining, the ISA Legal and Technical Commission issued *Recommendations for the guidance of the contractors for the assessment of the possible environmental impacts arising from exploration for polymetallic nodules in the Area*[10] in 2001, one year after the approval of the *Regulations on Prospecting and Exploration for Polymetallic Nodules in the Area*.[7] With the publication of two additional regulations on exploration[8,9] in 2010 and 2012, it was deemed necessary to develop an environmental guideline applicable to the exploration for various deep-sea resources.

Therefore, in 2013, ISA published *Recommendations for the guidance of contractors for the assessment of the possible environmental impacts arising from exploration for marine minerals in the Area*.[11] This guidance was updated in 2020[12] and 2022.[13] However, the technical specifications and recommendations for environmental baseline surveys remain unclear, especially for marine biotic surveys.

In the context described above, this document provides general provisions and technical recommendations for conducting marine biological surveys, mainly for marine biological baseline surveys in the exploration of deep-sea solid mineral resources in the international seabed area.

Marine environmental impact assessment (MEIA) — Technical specifications for marine biotic surveys in the international seabed area — General principles

1 Scope

This document provides general technical recommendations for components of marine biotic surveys in the international seabed area, including station and survey line design, sampling strategies, survey items, equipment for survey and analysis, and sample preservation and analysis.

This document is applicable to marine biotic surveys in the international seabed area.

2 Normative references

There are no normative references in this document.

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

ISO and IEC maintain terminology databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at https://www.electropedia.org/

3.1
international seabed area
seabed, ocean floor and subsoil thereof, beyond the limits of national jurisdiction

[SOURCE: UNCLOS, 1982, Article 1.1, modified — "the" has been removed from the definition.]

3.2
environmental baseline
sufficient information collected from the exploration area to describe the natural values of environmental factors and biocompetence succession without being directly affected by intense human activities, such as exploration and exploitation of deep-sea resources

[SOURCE: ISBA/25/LTC/6/Rev.1, Annex II, 2020, modified — the definition has been shortened.]

3.3
chlorophyll *a*
pigment in the cells of autotrophic plants, the main substance that absorbs and transmits light energy during photosynthesis in plants

3.4
sediment chlorophyll *a*
chlorophyll a (3.3) in the phytoplankton debris and humus that settle on the seabed, providing indicative information on the output flux, mixing and degradation of the active components of the particulate organic component of the seafloor

3.5
primary productivity
ability of autotrophic organisms to produce organic matter through photosynthesis

Note 1 to entry: Primary productivity is usually calculated as the mass of the organic matter (usually expressed in organic carbon) per unit area (or volume) per unit time (year or day), corresponding to the primary production in the same area (or volume) over that time.

3.6
microorganism
group of tiny unicellular or multicellular primary organisms with simple structures and a variety of physiological characters,

EXAMPLE 1 Prokaryotes, such as bacteria and archaea.

EXAMPLE 2 Eukaryotes, such as fungi (yeasts and moulds), protozoa and microscopic algae.

EXAMPLE 3 Noncellular organisms, such as viruses, viroids and prions.

3.7
plankton
group of organisms lacking advanced locomotive organs, with no or weak mobility, floating in the water layer and often moving with the flow, including phytoplankton and zooplankton

Note 1 to entry: According to individual size, plankton can be divided into the following types:

— megaplankton: plankton with a diameter larger than 20 cm;

— macroplankton: plankton with a diameter of 2 cm to 20 cm;

— mesoplankton: plankton with a diameter of 200 µm to 20 mm;

— microplankton: plankton with a diameter of 20 µm to 200 µm;

— nanoplankton: plankton with a diameter of 2 µm to 20 µm;

— picoplankton: plankton with a diameter less than 2 µm, including heterotrophic bacteria and autotrophic organisms, here referring to photosynthetic picoplankton.

3.8
megafauna
fauna clearly visible in photographs of the seabed

Note 1 to entry: Since the resolution of the camera varies, the benthic fauna larger than 1 cm are generally considered megafauna.

3.9
macrofauna
animal retained on a 250-µm sieve, typically sorted and identified with a microscope

EXAMPLE Polychaetes, bivalves, isopods and tanaids.

[SOURCE: ISBA/25/LTC/6/Rev.1, Annex II, 2020]

3.10
metazoan meiofauna
small invertebrate retained on a 32-µm sieve (except foraminifera), typically sorted and identified with a microscope

EXAMPLE Nematodes, harpacticoid copepods, ostracods, kinorhynchs, tardigrades and gastrotrichs.

[SOURCE: ISBA/25/LTC/6/Rev.1, Annex II, 2020]

3.11
nodule fauna
fauna attached to the surface and crevices of polymetallic nodules

3.12
demersal scavenger
animal that eats waste products and dead remains of other animals and plants that it did not kill itself

[SOURCE: ISBA/25/LTC/6/Rev.1, Annex II, 2020, modified — the term has been made singular.]

3.13
marine mammal
viviparous vertebrate, with the characteristics of lactation, pulmonary respiration, constant body temperature, streamlining, and forelimbs specialized as fins

3.14
nekton
fish, squids, crustaceans and marine mammals that are active swimmers in the open ocean environment

[SOURCE: ISBA/25/LTC/6/Rev.1, Annex II, 2020]

3.15
environmental DNA
eDNA
DNA molecule in the environment, including water and sediment, or exfoliated tissues and excreta released from organisms into the environment, that can reflect their current and past biological activities and existence in the environment

3.16
seabird
bird that is fully adapted to the marine environment in terms of morphology and behaviour and can forage in salt water

3.17
human-occupied vehicle
HOV
self-propelled submersible with its own energy, life support and accessory system

Note 1 to entry: A HOV can carry marine scientists into the deep ocean to investigate the seabed. It also includes two manipulators that can be operated to collect samples.

3.18
remote operated vehicle
ROV
underwater vehicle that is remotely controlled by the connected cable transmitting signals and power from the support vehicle

3.19
autonomous underwater vehicle
AUV
unpiloted and cableless submersible that can operate according to predetermined procedures or adapt to environmental changes

3.20
conductivity, temperature and depth profiler
CTD profiler
system for measuring conductivity (an indicator of salinity), temperature and depth (defined from pressure measurements)

3.21
conductivity, temperature and depth rosette water sampler
CTD rosette water sampler
rosette sampler with *CTD profiler* (3.20) attached and at least 12 Niskin bottles to sample larger volumes of sea water

3.22
lander system
system equipped with camera and trap deployed to the seafloor to observe animals in situ or recover specimens to the surface

3.23
deep towed camera system
imaging system that consists of still camera and video camera within a frame and is towed over the seabed

Note 1 to entry: A deep towed camera system can be used for collecting high quality video and still images over relatively large areas from the seabed.

3.24
box-corer
sediment sampler that has a detachable, square, open-ended steel sample box attached to a weighted column with a removable spade closure for the bottom of the box

Note 1 to entry: According to whether it is guided by underwater television, it can be divided into the following two types:

— box-corer not guided with underwater television;

— box-corer guided with underwater television (TV box-corer).

3.25
multicorer
sediment sampler that consists of an outer framework and weighted collecting head of plastic core tubes hanging from a water-filled hydraulic damper

Note 1 to entry: According to whether it is guided by underwater television, it can be divided into the following two types:

— multicorer not guided with underwater television;

— multicorer guided with underwater television (TV multicorer).

3.26
epibenthic sledge
sled equipped with a camera and environmental sensor system to collect epibenthic fauna and larvae by towing on the seabed

3.27
plankton net
sampler used for collecting plankton samples, equipped with either multiple nets or single

3.28
multinet
sampler equipped with multiple nets for sampling planktons from multiple water layers by opening and closing the nets in succession

4 Specifications and recommendations

4.1 Station and survey line design

The recommendations for station and survey line design are as follows:

a) Biological sampling strategy: High-resolution bathymetric and seabed topographic maps as well as a robust statistical design should be used when preparing the biological sampling strategy, taking into account variability in the environment.

b) Representative samples: Sample collection should include fauna which is representative of the variability of habitats, such as different bottom topography, depth, seabed and sediment characteristics, targeting the water column and the mineral resource.

c) Polymetallic nodules: For each block of polymetallic nodules, at least four stations should be surveyed. Surveys of chlorophyll *a*, primary productivity and plankton should be carried out with marine chemical and hydrological surveys synchronously. In benthic surveys, the stations for box-corer and multicorer sampling should be the same. For each station, box-corer and multicorer sampling should be performed at least twice to generate replicates for the analysis of macrofauna and meiofauna. A benthic survey should be carried out with a sediment survey synchronously.

d) Cobalt-rich crust: A survey of the abundance and diversity of megafauna in each seamount of the cobalt-rich ferromanganese crust region should be initially based on at least four transects (located on different sides of the seamount), which should include the summit, slope and base of the seamounts. The box-corer and multicorer sampling stations should include the summits and bases of four different sides of the seamount. Chlorophyll *a*, primary production and plankton should also be investigated on four different sides of each seamount, and three sampling stations should be set on the summit, slope and base of each seamount.

e) Polymetallic sulfides: The sampling station should include an active hydrothermal vent area and an adjacent inactive hydrothermal vent area.[15] In the active hydrothermal area, 5-10 sampling stations should be arranged along the gradient of temperature and hydrothermal fluid. In the inactive hydrothermal vent area, the sampling sites should include mineralized sites, nonmineralized sites and hydrothermal deposition sediment areas.

f) Application of underwater vehicles: If a remote operated vehicle (ROV) or a human-occupied vehicle (HOV) is used for the investigation, a push-corer can be used to collect sediment samples, a manipulator arm can be used to collect nodule fauna and megafauna, and a pump can be used to collect demersal fish and scavengers. If an autonomous underwater vehicle (AUV) is used for the investigation, it is recommended to carry out a cruise along the survey line at a set height off the bottom (optimally up to 3 m off the bottom, with a maximum limit of up to 5 m off the bottom) and to take photographs and video.

4.2 Sampling strategy

4.2.1 Interannual variation

For most environmental baselines with interannual variability, observations at the same site during similar seasons or under similar environmental conditions should be conducted for at least three years to assess interannual variability and increase the chance of capturing periodic events, especially events that can cause periodic changes in environmental baselines, such as the El Niño-Southern Oscillation (ENSO).[16-19] Data collected in years prior to the publication of this document can be used to assess interannual variability.

4.2.2 Intra-annual variation

In each block, at least one station for time-series measurement should be established to observe the temporal variability of environmental parameters, such as chlorophyll *a*, primary productivity, particle flux and hydrodynamics, at different times of the year to cover seasonal and monthly changes. The

seasonal and monthly changes in chlorophyll *a* and primary productivity can be measured by ocean colour remote sensing.

4.2.3 Seasonal variation

For parameters that are not expected to show obvious seasonal variations, such as sediment characteristics and the biogeochemical environment of deeper sediment layers, the observation results of different seasons should be verified at least once at the same site.

4.2.4 Diurnal variation

For zooplankton and other animals with obvious diurnal movement ability,[20] samples should be collected at the same station during the day and at night.

4.3 Sample types and sampling methods

4.3.1 Water samples

Water samples for surveys of chlorophyll *a* (above a 200 m depth), microorganisms, picoplankton, microplankton, and environmental DNA (eDNA) should be collected using the conductivity, temperature and depth (CTD) rosette water sampler. The vertical sampling resolution can be followed as in Table 1. For water samples to be used for the measurement of primary productivity, the sampling depth should be set according to the recommendations for the measurement of primary productivity.

Table 1 — Water sampling depth

Dimensions in metres

Type		Sampling depth
Area for polymetallic nodule exploration		2, 25, 50, 75, 100, 125, 150, 200, 500, 800, 1 000, 2 000, 3 000, 4 000 (5 000), 100 from seabed, 50 from seabed, near-bottom layer
Area for cobalt-rich crust exploration	Depth <3 000 m	2, 25, 50, 75, 100, 125, 150, 200, 500, 800, 1 000, 2 000, 100 from seabed, 50 from seabed, near-bottom layer
	Depth ≥3 000 m	2, 25, 50, 75, 100, 125, 150, 200, 500, 800, 1 000, 2 000, 3 000, 4 000 (5 000), 100 from seabed, 50 from seabed, near-bottom layer
Area for polymetallic sulfide exploration		2, 25, 50, 75, 100, 125, 150, 200, 500, 800, 1 000, 2 000, 300 from seabed, 200 from seabed, 100 from seabed, 50 from seabed, near-bottom layer

NOTE During water sampling, the data of conductivity, temperature, depth and dissolved oxygen can be obtained synchronously. The sampling depth can be adjusted appropriately, especially for the layers of the thermocline, subsurface chlorophyll *a* maximum depth and oxygen minimum depths. For areas with water depth less than 5 000 m, the sampling depth can be adjusted accordingly.

4.3.2 Sediment samples

4.3.2.1 A box-corer or TV box-corer with an opening area of 50 cm × 50 cm should be used for sampling macrofauna to meet statistical requirements.[21,22] Polymetallic nodules on the surface of the sample are used for the analysis of nodule fauna. Before collecting biological samples from nodules, the attached organisms on the nodules should be photographed, described and preserved on site, and the overlying water and sediment samples of the whole box-corer should be used for species identification and quantitative analysis of the macrofauna. The sediment sampling procedure should follow the recommendations in ISO 23040:2021, 16.4.

4.3.2.2 Sediment samples for surveys of metazoan meiofauna, foraminifera, microorganisms, sediment chlorophyll *a* and eDNA can be collected by (TV) multicorers and push corers. (TV) multicorers should be installed with more than 8 sampling tubes, with lengths greater than 60 cm and diameters

no less than 9,5 cm.[23,24] The sediment sampling procedure should follow the recommendations in ISO 23040:2021, 16.4.

4.3.3 Net samples

Net samples are collected for surveys of megafauna, nekton, and plankton. Megafauna can be sampled by an epibenthic sledge or bottom trawls with a transducer. Nekton should be collected by using appropriate nets to retrieve reliable specimens. Plankton in different water layers can be collected with multinets, which should have at least 5 layers of nets, with a net hoop area of 0,5 m^2 and multiparameter CTD profiler and flowmeters installed.

4.3.4 Megafauna and demersal scavenger samples

The samples of megafauna, demersal scavengers, demersal fish and nodule (crust) fauna can be collected by lander system, HOV and ROV.[25-27]

4.3.5 Video and photography data

4.3.5.1 Profile (survey line) video and photography data can be obtained by a video or camera device installed on an HOV, an ROV, an autonomous underwater vehicle (AUV), a benthic sled or a deep-tow system, which should be equipped with a high-definition camera system with a resolution of at least 1 080 pixels.[14,25,26]

4.3.5.2 Long-time series video and photography data at fixed sites can be collected by a free-fall platform for seafloor observation, on which the camera and lighting can be set with reference to ISO 23731.

4.3.6 Acoustic data

As described in ISO 23730:2022, 3.3, the marine acoustic environment consists of natural sounds, ranging between 1 Hz and 100 kHz. These sounds can originate from animals, weather and waves, or humans, and provide a baseline for acoustic ecology. Marine acoustic data should include the sound emitted by marine animals and underwater noise generated by anthropogenic activities, which can be collected by towed, onboard or moored hydrophones.[28,29]

4.4 Survey items

4.4.1 Measured parameters

The measured parameters are shown in Table 2.

Table 2 — Main parameters of marine biological surveys

First-level basics	Second-level basics	Parameters/index
Pelagic communities	microorganisms	abundance, biodiversity
	picoplankton	abundance, biodiversity
	nanoplankton	abundance, biodiversity
	microplankton	abundance, biodiversity
	mesoplankton megaplankton	biomass, abundance, biodiversity, vertical migration
	ichthyoplankton	abundance, biodiversity
	nekton	biomass, abundance, biodiversity
Benthic communities	megafauna	abundance, biodiversity
	macrofauna	biomass, abundance, biodiversity
	metazoan meiofauna	biomass, abundance, biodiversity
	foraminifera	abundance, biodiversity
	nodule fauna	abundance, biodiversity
	microorganisms	abundance, biodiversity
	demersal fish and scavengers	biodiversity
Other organisms	marine mammals, seabirds, turtles, sharks	biodiversity
Ecosystem functioning	primary productivity	chlorophyll *a*, primary productivity
	food web structure	$\delta^{13}C$ and $\delta^{15}N$ ratios of main dominant groups of organisms and sediment, biological nutrition level and food source contribution rate

4.4.2 Environmental parameters

Auxiliary parameters include:

— parameters of physical oceanography: pressure, current direction and velocity, temperature, salinity and turbidity;

— parameters of water column chemistry: pH, dissolved oxygen, nutrients, total organic carbon, methane, carbon dioxide, alkalinity, trace metals, etc.;

— parameters of sediment chemistry: trace metals, Fe^{3+}/Fe^{2+} ratio, Eh, etc.;

— parameters of sediment characteristics: sediment type, grain size, mineral composition, fossil composition, and element content etc.,

— sinking particle flux and composition of materials: sinking flux, particulate organic matter etc.

4.5 Research vessel facilities

4.5.1 General laboratory

The general laboratory should support the treatment and analysis of chlorophyll *a*, plankton, benthos, and fish samples and should be equipped with ventilation facilities. External or internal pollution and mechanical, noise, heat, light and electromagnetic interference levels should have no significant effect on the measurement results.

4.5.2 Radioisotope laboratory

The radioisotope laboratory should enable the analysis and determination of ^{14}C in the samples for the measurement of primary productivity, requiring ventilation and radioactive protection facilities. Radiation laboratories and instruments in contact with ^{14}C should have clear signage and should be periodically examined for radioactivity.

4.5.3 Microbiological laboratory

The microbiological laboratory should support the treatment of microbial samples and should be equipped with sterile facilities.

4.5.4 Storage for samples and reagents

Refrigerators and freezers at 4 °C, −20 °C, and −80 °C and liquid nitrogen tanks should be available to store biological samples for different analytical purposes. The storage area for reagents should be cool (≤30 °C) and ventilated.

4.5.5 Other facilities

There should be a CTD profiler winch, trawl winch, and coaxial cable (or optical fibre cable) winch to adapt to the requirements for on-site sampling. In the laboratory, there should be seawater, fresh water, and a power supply. At the rear deck, there should be seawater and fresh water for rinsing the biological samples, plankton net and instruments. For nekton surveys, vessels should have a fish detector, and the stern should be suitable for trawling operations. Trace metal surveys should be conducted with an airtight clean bench with cleanliness that meets the level 5 requirements specified in ISO 14644-1:2015, 4.3.

4.6 Equipment for survey and analysis

4.6.1 Main survey gear

Table 3 shows the main survey equipment. Any combination of this equipment may be used, as best suited to the collection/survey tasks.

Table 3 — Main survey gear and types of samples and data collected

Survey gear	Types of samples and data collected
HOV	megafauna, nodule fauna, microorganisms, seawater, sediment, eDNA seafloor video and photography, CTD data
ROV	megafauna, nodule fauna, microorganisms, seawater, sediment, eDNA seafloor video and photography, CTD data
AUV	seafloor video and photography, CTD data, turbidity data, and side-scan Sonar data used for habitat mapping.

Table 3 *(continued)*

Survey gear	Types of samples and data collected
Lander system	demersal fish, scavengers seafloor video and photography, sea current, dissolved oxygen, and CTD data
Deep towed camera system	seafloor video and photography
(TV) box-corer	sediment and nodule samples, samples for surveys of macrofauna and nodule fauna seafloor video
(TV) multicorer	sediment samples for surveys of meiofauna, foraminifera, microorganisms, sediment chlorophyll *a*, and eDNA seafloor video
CTD rosette water sampler	seawater samples for surveys of chlorophyll *a*, primary productivity, microorganisms, picoplankton and microplankton, and eDNA CTD data
Multinet	samples for surveys of mesoplankton, megaplankton, ichthyoplankton CTD data
Planktonnets	samples for surveys of microplankton, mesoplankton, megaplankton, ichthyo-plankton
Epibenthic sledge	samples for surveys of megafauna, macrofauna, nodule fauna

4.6.2 Main analytical instruments and equipment

Table 4 lists the main analytical instruments and equipment.

Table 4 — Main analytical instruments and equipment and their parameters/use of measurement

Analytical instruments and equipment	Parameters/use of measurement
stereomicroscope with digital photomicrographic system	morphological observation, identification, photography and counting of macroplankton, mesoplankton and benthic organisms
optical microscope with digital photomicrographic system	morphological observation, identification, photography and counting of nanoplankton and microplankton
fluorescence microscope	microbial observation and counting
differential interference phase contrast microscope	morphological observation, identification and photography of meiofauna
fluorometer	chlorophyll *a*, other pigments
liquid scintillation counter	primary production
flow cytometer	cell counting and sorting of microorganisms and picoplankton
sequencer	DNA/RNA sequences

4.7 Sampling

4.7.1 Recommendations for sampling

4.7.1.1 Position of deployed samplers

Deployment of a plankton net and CTD should avoid sewage outfall of the survey vessel.

4.7.1.2 Sampling position

The position for lander system, (TV) box corer and (TV) multicorer sampling should be determined by a device with precise positioning (e.g. transponder).

4.7.1.3 Water sampling

The vessel should be positioned so that sampling is upwind of the exhaust generated by the vessel. The water sampler should be checked to determine whether the lids are open and ensure that the outlets remain closed before deployment. The sampler should be deployed into the intended water layer, and the dwelling time should be strictly maintained. Sampling and treatment should be performed as needed.

4.7.1.4 Net sampling for plankton

Nets may be used for sampling according to professional requirements. When a vertical tow is used to collect plankton samples, the speed of vertical towing should be controlled between 0,5 m/s and 1,0 m/s during deployment and retrieval. When a horizontal or oblique tow is used, the speed of the vessel should be controlled between 0,926 km/h to 2,778 km/h (0,5 kts to 1,5 kts). A flowmeter should be installed in the plankton nets. The net status should be checked to ensure it is normal, and effective actions should be immediately taken once abnormal circumstances are detected. After retrieving the net, it should be rinsed carefully, and samples should be collected, especially those attached to the net and bottom tube.

4.7.1.5 Sediment sampling

The sampler should be used according to the survey item and strictly following the operating procedure. If leakage of overlying water from the sediment sample is observed and the sample is seriously disturbed, the sediment should be resampled. Box-corers for macrofauna should not be subsampled.

4.7.1.6 Sampling at active hydrothermal vents

Biological samples from active hydrothermal vent systems should be collected only using non-destructive sampling by ROV/HOV technology according to the subhabitat and placed into discrete sample boxes.

4.7.1.7 Sampling at seamounts

Megafauna, demersal fishes and other nekton living over the seafloor in the seamounts should be assessed using benthic landers and/or ROV/HOV observations and photographs.

4.7.1.8 Collection of seabed video and image data

a) For the collection of video and photography data for profiling (survey line), the operating procedures of acquisition equipment for seabed video and image data should be strictly followed. The survey altitude should be kept constant so that images and videos can be obtained at a constant altitude above the seabed. The resolution of video and images should be sufficient for reliable characterization of megafauna.

b) Requirements for the collection of long-time series video and photography data at fixed sites can be found in ISO 23731:2021, 5.4.

4.7.2 Sample preservation

The collected samples should be processed within 12 hours to obtain high-quality specimens. The samples should be handled and preserved immediately on board or stored in a cold room, and the storage time before preservation should not exceed six hours. According to different purposes, a variety of preservation methods can be used, such as formalin or 75 % ethanol for taxonomic research and

cryopreservation, 100 % ethanol for molecular research, drying for the analysis of stable isotopes, and freezing for the analysis of trace metals and biochemistry.

4.7.3 Records

Recommendations for records include:

a) All collected specimens should be labelled with relevant information, at least including the date, time, sampling method, longitude and latitude, and depth. The collected samples and sample derivatives (photos, videos, gene sequences, etc.) should be filed.

b) The original photos of organisms should be recorded whenever possible.

c) The formats for records of sampling, analysis, identification and determination are given from A.2 to A.7 following the specifications. In the case of unusual phenomena or novel discoveries, photographs or videos should be taken in addition to record collection.

4.8 Sample analysis

4.8.1 Taxonomic identification

Generally, all identifications should be made at the lowest taxonomic level possible. Molecular identification through barcoding and eDNA should be used to support morphological identification. An example procedure for molecular analysis can be found in ISO 23040:2021, 16.5 and ISO 23732:2021, Annex B.

4.8.2 Count and quantitative analysis

The concentration of chlorophyll *a* and primary productivity can be determined in the laboratory by fluorescence and liquid scintillation counting. The abundance of picoplankton and microorganisms can be analysed by flow cytometry. The abundance of nano- and microplankton can be measured by the concentration method and sedimentation method for counting. Zooplankton, ichthyoplankton, macrofauna and meiofauna samples can be counted under a stereomicroscope and then converted into abundance. The biomass of zooplankton, swimming animals and macrofauna can be measured by wet mass, and the biomass of metazoan meiofauna can be obtained by converting volume.[30,31]

4.8.3 Food web

After pre-treatment, an appropriate number of samples can be taken into tin bags and used for mass spectrometry of the stable isotope ratio to determine the stable isotope composition of carbon and nitrogen. The carbon and nitrogen isotopic compositions use as references the Vienna Pee Dee Belemnite (VPDB) and atmospheric nitrogen international reference standards, respectively, represented by the δ value. The stable isotope ratio of carbon and nitrogen and the contribution rate to the food source should be calculated by appropriate formulae.[32]

4.8.4 Sample preservation

After analysis, measurement, and identification, it should be determined whether all or part of the samples should be reserved, according to the demands of data analysis, application and academic value. The type specimens and typical specimens used for species identification should be preserved permanently.

4.9 Analysis of video and photography data

Taxonomic analysis of the obtained video and photography data can be performed with regard to References [14], [26] and [33-37]. A deep-sea taxon reference image database can be established with methods described by References [38,39] to support image-based analyses.

4.10 Quality control

The recommendations for quality control include the following:

a) All equipment and instruments for on-site investigation and analysis at sea should be tested/calibrated and self-examined with record maintained according to the requirements for measurement. Calibration certificates for the conductivity, temperature, depth and turbidity sensors should be valid.

b) The standard materials used for analysis should be within the validity period.

c) Investigators should have intensive training before expedition and training records should be maintained for future examination/audit.

d) On-site sampling and analysis records should have audit records (see Annex A).

Annex A
(informative)

Examples of sheets for samples and data collection

A.1 General

This annex provides examples of records used for sample and data collection. These sheets are intended to be used for recording information on sample and data collection.

A.2 Summary sheet of samples and data collection

Page of

Survey area_____ Research Vessel_____ Cruise_____

Station_____Sampling date (UTC)_____Year_____Month_____Day to_____Year_____Month_____Day

	Number of station/ Length of survey line (km)	Sample type and quantity			Data type and quantity		
		SW	SE	OR	PH	VI	OD
HOV							
ROV							
AUV							
Lander system							
Deep towed camera system							
(TV) box-corer							
(TV) multicorer							
CTD rosette water sampler							
Multinet							
Plankton nets							
Epibenthic sledge							
Key SW = sea water SE = sediment OR = organism PH = photo VI = video OD = other data							

Sampler_____Recorder_____Proofreader

A.3 CTD profiler observation and water sampling

Page of

Survey area_____ Research Vessel_____ Cruise_____

Station_____ Sampling date (UTC)_____Year_____Month_____Day

Time_____Hour_____Minute to_____Hour_____Minute

Equipment		Serial No.	
Starting time (UTC)		Release time (UTC)	
Starting longitude		Starting latitude	
Starting water depth (m)		Water depth of touchdown (m)	
Landing longitude		Landing latitude	
Landing time (UTC)		Ending time (UTC)	
Minimum distance above bottom (m)		Maximum wire rope (m)	

Sampling depth (m)												
Bottle	1	2	3	4	5	6	7	8	9	10	11	12
Depth												
Fire												
Bottle	13	14	15	16	17	18	19	20	21	22	23	24
Depth												
Fire												
Remark												

CTD file name		Acoustic doppler current profiler file name	
CTD computer watchkeeper		Acoustic doppler current profiler operator	
Winch watchkeeper			

Sampler_____Recorder_____Proofreader

A.4 Record of multicorer sampling

Page of

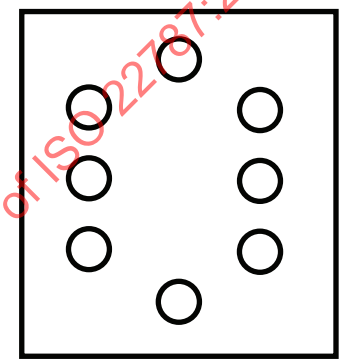
Survey area_____ Research Vessel_____ Cruise_____Station_____

Longitude (E/W)____°____′____″ Latitude (N/S)____°____′____″ Depth____m Sampling No.____

Diameter of corer (ψ)____cm

Sampling date (UTC) ____Year____Month____Day_to ____Year____Month____Day_

	Release	Bottom contact	Back on deck
Local time			
Latitude (N/S)	° ′ ″	° ′ ″	° ′ ″
Longitude (E/W)	° ′ ″	° ′ ″	° ′ ″
Depth/m			
Wire rope length/m	——		——



Corer No.	Thickness of overlying water layer (cm)	Thickness of sediment layer (cm)	Total thickness (cm)	Note
1				
2				
3				
4				
5				
6				
7				
8				
Description:				

Sampler _____ Recorder _____ Proofreader

A.5 Record of box-corer sampling

Page of

Survey area_____ Research Vessel_____ Cruise_____ Station_____

Longitude (E/W)____°____′____″ Latitude (N/S)____°____′____″ Depth____m

Sediment sampler_____ Sampling area____m^2 Sampling sequence No.____

Sampling time (UTC)____Year____Month____Day____Hour____Minute

to____Year____Month____Day____Hour____Minute

Bottom touch time (UTC)____Year____Month____Day____Hour____Minute

Layer	Subsamples		Organisms from the surface	
	Sample No.	Mass	Taxa	Individual
Overlying water				
0 cm ~ 2 cm				
2 cm ~ 5 cm				
5 cm ~ 10 cm				
Notes:				

Sampler_____Recorder_____Proofreader

A.6 Plankton sampling record

Page of

Survey area_____ Rearch Vessel_____ Cruise_____ Station_____

Longitude (E/W)____°____′____″ Latitude (N/S)____°____′____″ Depth____m

Sampling time (UTC)____Year____Month____Day____Hour____Minute

to____Year____Month____Day____Hour____Minute

Sampling type		Bottle No.	Length of rope (m)	Angle (°)		Flowmeter read		Remarks
				Start	End	Start	End	
Net	Net							
	Net							
	Net							
	Net							
	Net							
Water						Water volume (cm^3)		
	m							
	m							
	m							
	m							
	m							
	m							
	m							
	m							
Sea conditions:								

Sampler_____Recorder_____Proofreader