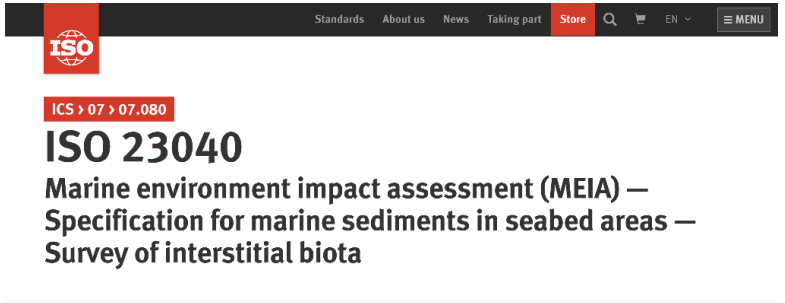
Sixth Marine Instrumentation Workshop for Asia-Pacific Region
“Ensure high quality procedure we take, deliver the ocean data we need”
13-17 December 2021



Development and Technology of International Standards for Marine Survey in Seabed Area: A Case Study of ISO 23040

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INTERNATIONAL STANDARD

ISO 23040

First edition
2021-12

Marine environment impact assessment (MEIA) — Specification for marine sediments in seabed areas — Survey of interstitial biota

Évaluation de l'impact environnemental marin — Spécifications relatives aux sédiments marins dans les zones de fonds marins — Étude du biote interstitiel

Reference number
ISO 23040:2021(E)

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ICS › 07 › 07.080

ISO 23040

Marine environment impact assessment (MEIA) — Specification for marine sediments in seabed areas — Survey of interstitial biota

- the first ISO international standard in the field of Marine surveys developed by China and jointly formulated by eight countries
- an important breakthrough in the internationalization of China's Marine survey technical standards.

Process of development of ISO 23040:2021

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- In 2017, in the absence of an ISO standard in the field of marine surveys, China proposed a New Proposal (NP) for this international standard, which was approved by the ISO project
- This project received positive responses from 8 countries (United States, the United Kingdom, Russia, Germany, Iran, South Korea, Singapore and Panama) , they appointed experts to participate in the formulation of this international standard.
- the Working group Draft (WD) was adopted in May 2019
- the Committee Draft stage (CD) was adopted at the International Standards Conference in September 2019
- the inquiry Draft International Standard stage (DIS) international ballot was adopted in October 2020.
- It lasted for four years. ISO 23040：2021 is officially published and promulgated by ISO .

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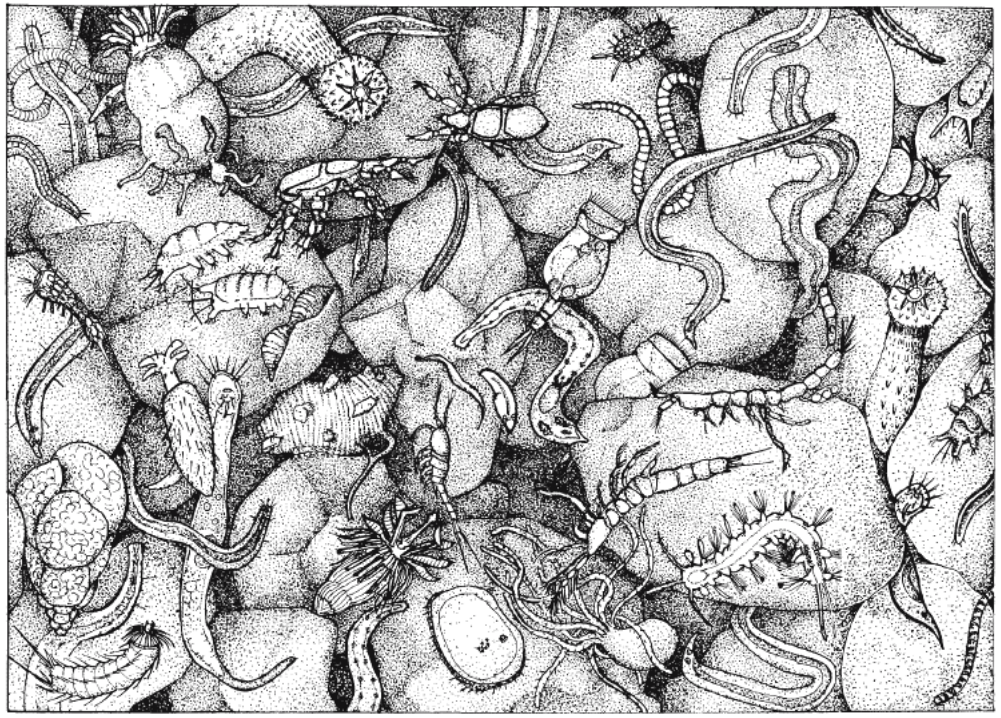
OUTLINE

1. Introduction of ISO 23040
2. Contents and techniques of ISO 23040
3. International collaboration, Promotion and training

Background__

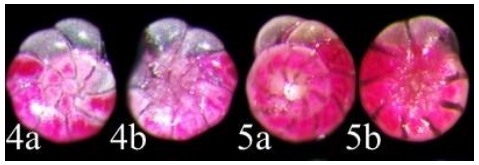
Significance of sediment interstitial biota

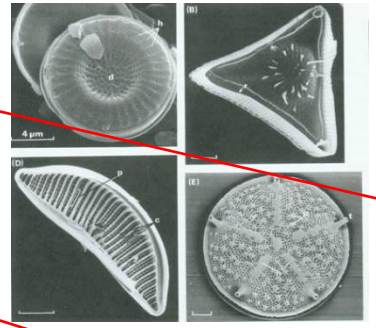


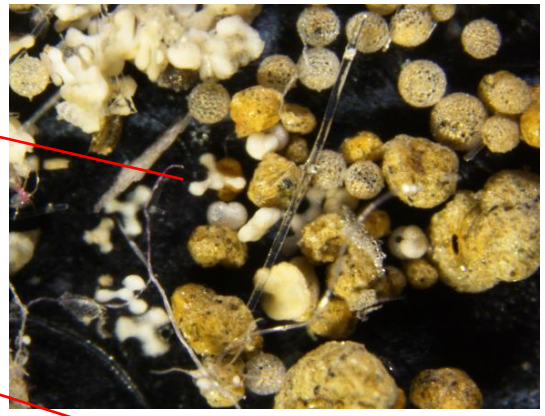
- Benthic life forms inhabited or deposited in the interstitial spaces between sediment particles

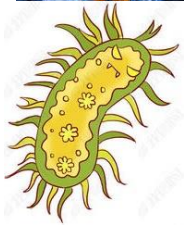
Foundation of sediment ecological ecosystem

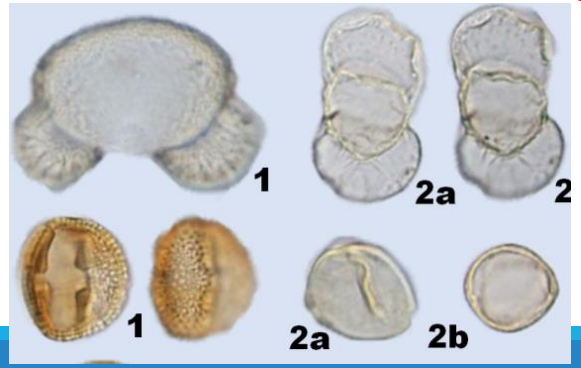
- Foraminifera
- Ostracods
- Radiolaria
- Sediment diatom
- Coccolithophore
- Sedimentary sporopollen
- Benthic viral
- Benthic microalgal
- Benthic protozoa
- Benthic meio-metazoa

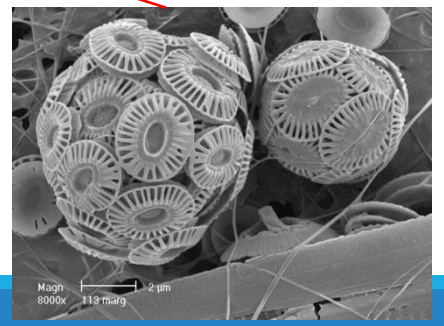






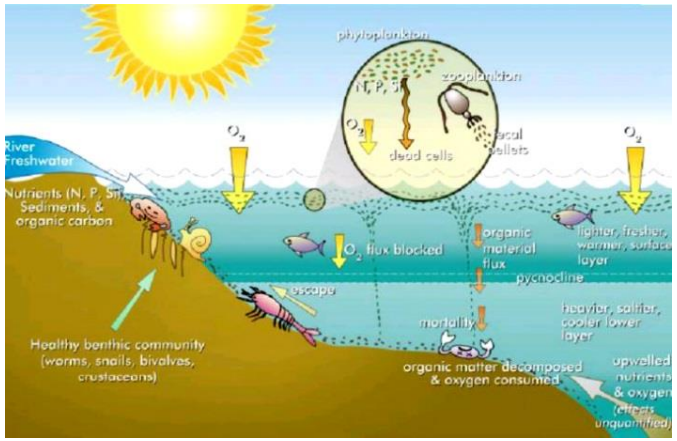






Marine interstitial Biota Survey:

important biological organism in Marine ecological system



- Microbial food web: support material circulation and energy flow in benthic microbial food web

- Seed bank of Harmful Algae Bloom:

  toxic cysts released from interstitial sediment to water column, caused disaster to human being

Recorders for marine environment to reveal earth history

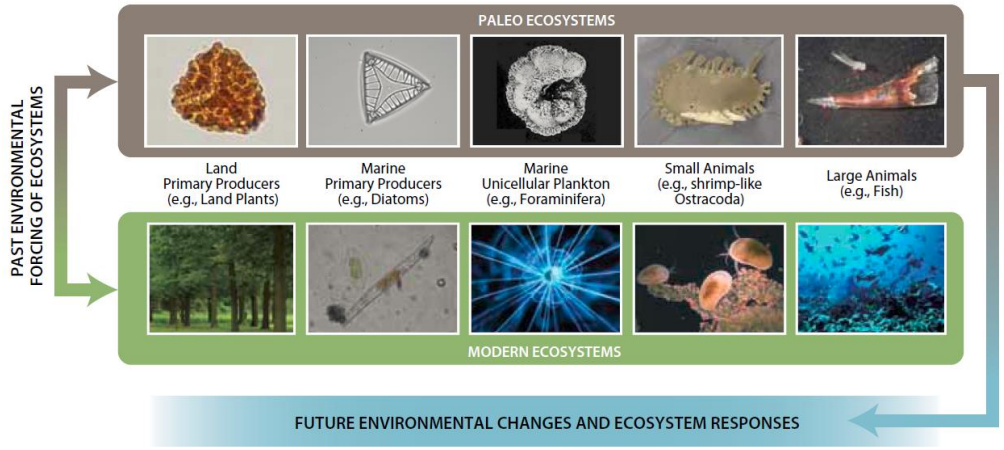
Investigation

Geologic record

Biology and environment

Evolution and extinction

Evolution rate



- Sediment Interstitial Biota Survey provided us history evidences to serve future earth

Help in biostratigraphic division

Neogene

Paleogene

Cretaceous

Jurassic

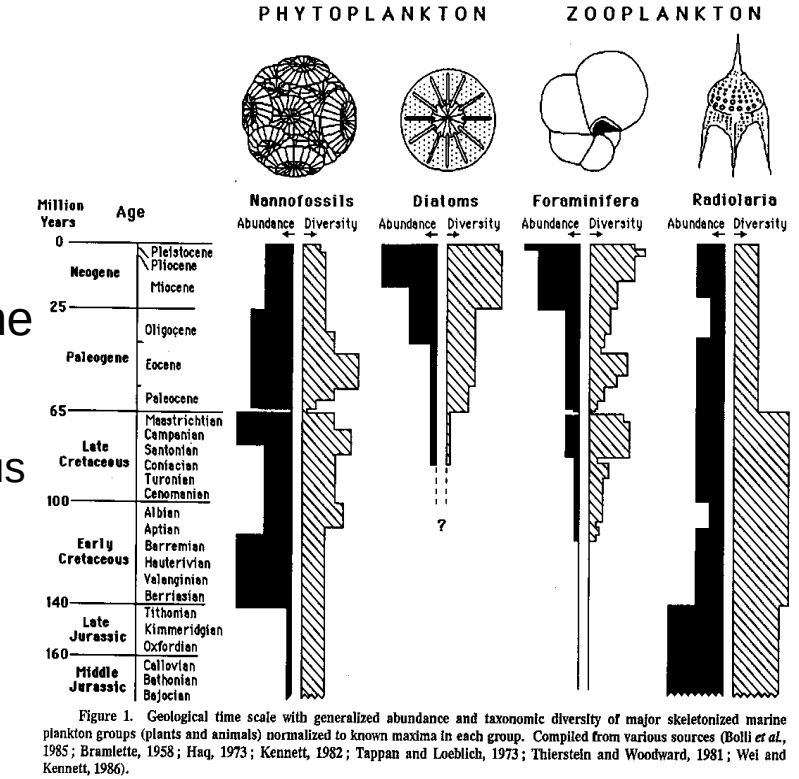


Figure 1. Geological time scale with generalized abundance and taxonomic diversity of major skeletonized marine plankton groups (plants and animals) normalized to known maxima in each group. Compiled from various sources (Bolli *et al.*, 1985; Bramlette, 1958; Haq, 1973; Kennett, 1982; Tappan and Loeblich, 1973; Thierstein and Woodward, 1981; Wei and Kennett, 1986).

International Ocean Research programs

"Understanding the history is to predict the future"

- **DSDP:Deep Sea Drilling Program (1968~1983)**
- **ODP: Ocean Drilling Program (1985~2003)**
- **IODP: Integrated Ocean Drilling Program (2003-2013)**
- **IODP: International Ocean Discovery Program (2013-2023)**

In the International Seabed Area, a number of large international research programs have been carried out.

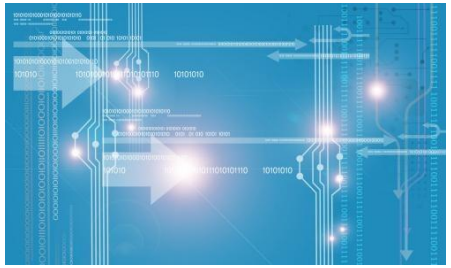
- Marine sediment survey is the main body subject in many large international programs

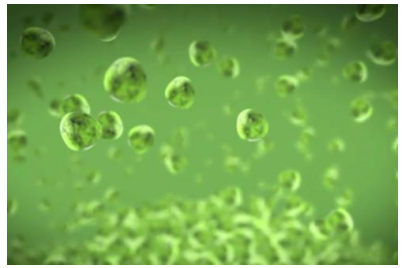
Scopes and Purpose of ISO 23040:2021

- In the International Seabed Area, marine sediment interstitial biota survey is indispensable content of a number of large international research programs.

- - Provide basic provisions for sample collection, experimental procedure, tools, sample analysis and data management, etc.

- - Meet the needs of different countries for conducting marine surveys in the International Seabed Area, facilitating international cooperation and data comparison.



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Content

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Website: www.iso.org

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Contents

Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular, the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

For an explanation of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT), see www.iso.org/iso/foreword.html.

This document was prepared by Technical Committee 8, *Ships 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

Interstitial biota in marine sediments refers to the benthic life forms inhabited or deposited in the interstitial spaces between sediment particles, including marine microorganisms, benthic virus, microbenthos and meiobenthos. They cover the six "kingdoms" of life in the three-domain taxonomic system: Archaea, Bacteria, Fungi, Protista, Plantae and Animalia. Interstitial biota in marine sediments are so small that cannot be obtained and analysed by conventional methods for marine biological survey; they are numerous and complex; they have diverse functions, remarkable ecological significances and rich gene resources; they are ubiquitous and make up the basic components of the life system in marine sediments. Sediment interstitial biotas are the most abundant and complex life groups in the estuaries, intertidal zones, shelf shallow seas and deep sea. They play key roles in the regulation of material and energy flows in benthic ecosystems.

In seabed areas, a number of large international research programs have been carried out, such as the ocean drilling program (ODP) and the international ocean discovery program (IODP). Interstitial biota in marine sediments surveys have been key to solve scientific problems in relevant fields, such as marine biodiversity, oil and gas resource exploration, marine carbon cycle, global change, monsoon rainfall, ice melting, ocean acidification and deep-sea biological resources. But so far the lack of an International Standard leads different countries to use different regulations and technologies on the investigations, resulting in barriers to comparing research results in international cooperation.

This document provides relevant technical approaches for the investigation of sediment interstitial biota in seabed areas. Its purpose is to reflect the recent developments of modern marine science and technology to facilitate international cooperation. It is applicable to investigations and evaluations of marine sediment biodiversity in seabed areas, favouring the development and utilization of marine biological resources, the comprehensive environmental exploration, ecological environment assessment, protection and management, etc. The specifications in this document incorporate technical advances and technological key points reflecting current state-of-the-art and international practice.

Marine environment impact assessment (MEIA) — Specification for marine sediments in seabed areas — Survey of interstitial biota

1 Scope

This document provides requirements and recommendations for conducting marine surveys of interstitial biota in marine sediments. It includes the specification of technical methods for the investigation of marine sediments, foraminifera, ostracoda, radiolaria, diatoms, coccoliths, sedimentary sporopollen, benthic viruses, benthic microbes (including bacteria, archaea and fungi), benthic microalgae, benthic protozoa and metazoan meiobenthos.

This document is applicable to marine surveys in diverse benthic habitats at any seabed, such as benthic sediments of coastal zones, shallow seas, or deep-sea waters.

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
marine sediment
substances under the action of crustal surface geology, where the original products such as weathered rocks, metamorphic rocks and pre-existing *sedimentary rocks* (3.2) of the parent rocks (i.e. magmatic rocks, metamorphic rocks and sedimentary rocks) are transported, settled or precipitated by biogenic, volcanic and cosmic phenomena as loose unconsolidated deposits on the sea floor

3.2
sedimentary rock
one of the three major types of rocks that make up the lithosphere (the other two are magmatic rocks and metamorphic rocks), which are formed from the weathering products of a parent rock (or any pre-formed rock), biogenic materials, volcanic material, cosmic material and other original material, and sedimentation after the formation of rock diagenesis

3.3
interstitial biota
benthic life forms that inhabit or are deposited in the interstices between sediment particles

Note 1 to entry: It includes *marine microorganisms* (3.6), benthic viruses, *microbenthos* (3.4), and meiobenthic organisms. In terms of individual sizes, interstitial biota in *marine sediments* (3.1) cover femto-level with a size of less than 0,2 µm, pico-level (0,2 µm to 2 µm), nano-level (2 µm to 20 µm) and micro- and meio-level benthic organisms of more than 20 µm.

3.4
microbenthos
unicellular prokaryotic and eukaryotic microbes living on the surface, and within the interstices, of sediments, which can be trapped by 0,2 µm membrane filtration

Note 1 to entry: Mainly benthic bacteria, benthic microalgae and *benthic protozoa* (3.5). See Figure A.1 for examples of major groups. In terms of sizes of individuals, the microbenthos covers the pico-level of less than 2 µm, the nano-level (2 µm to 20 µm) and the micro-level of more than 20 µm.

3.5
benthic protozoa
unicellular eukaryotes whose life history is entirely or mostly associated with sedimentary environments

Note 1 to entry: It includes heterotrophic flagellates, ciliates, amoebae, etc.

3.6
marine microorganism
microeukaryotes and metazoans included in sedimentary investigations and marine geological surveys, including extant and fossil species of various groups

Note 1 to entry: It includes foraminifera, ostracoda, radiolaria, diatoms, calcareous fossils, sporopollen, pteropoda, ichthyoliths, etc.

3.7
benthic microbe
unicellular and small acellular organism with simple structure and a variety of physiological types that inhabits sedimentary environments

Note 1 to entry: It includes bacteria, archaea and fungi.

3.8
metazoan meiobenthos
metazoan meiofauna
small metazoa and larvae of large metazoans living in sedimentary environments that can pass through a 500 µm aperture mesh but are retained on a 42 µm to 31 µm aperture mesh

Note 1 to entry: The main groups include nematodes, copepods, tardigrades, ostracods, gastrotrichs, priapulid worms, bivalves, arthropods, acarina, polychaetes, kinorhyncha, rotifers, etc. Several major groups are shown in Figure A.2.

4 General

4.1 Technical design

Surveys of interstitial biota in sediments should be designed in terms of survey-related items, including survey section, station, object, detail, method, date, frequency, device, personnel quality, ship, equipment, expected results and survey plan. The establishment of the investigation plan shall refer to the requirements of the related survey plan.

4.2 Basic recommendations for the surveys

4.2.1 Survey object

The survey object can include marine sediments, foraminifera, ostracoda, radiolaria, sedimentary diatoms, coccoliths, sporopollen, benthic viruses, benthic microbes, benthic microalgae, benthic protozoa, and metazoan meiobenthos. Specific objects may be adjusted or designed according to the survey plan.

Techniques

4.7 Data archiving

The following data should be archived:

a) survey contract or survey plan;

b) reports, technical design, program report and statements of approval;

c) executable plan and sampling stations;

d) original record of the survey, experiments undertaken and analysis;

e) report and explanation of the results;

f) tables, figures (including base map), photographs with explanatory legends;

g) voyage report and objects summary report;

h) investigation report and acceptance of the results;

i) tables of objects members and reconciliation of budget.

Related requests concerning data archiving, file quality and acceptance of the results can refer to the related clauses in this document.

4.8 Program and quality control

The institution executing the objects can provide the quality prospectus, including quotations of specifications and articles, summary of survey plan, quality target, organization and responsibilities of the execution institution and assurance measures of quality prospectus. Quality control can refer to the related clauses in this document.

Measures for ensuring quality control include the following.

a) Establish a quality control system: In addition to accepting supervision from administrations and technical supervision agency, a process of self-checking or quality control can be adopted. Formulate the quality control systems. Define the duty of quality control and programs of quality supervision and examination. Execute provisions of quality control strictly.

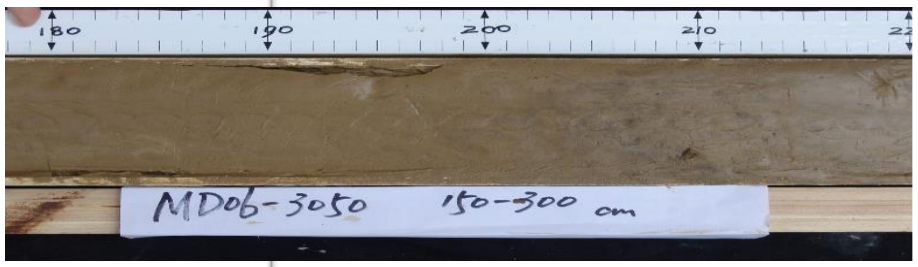
b) Execute quality control: There shall be clear quality requirements in the survey plan. Analyse specific quality of articles and data. Instruments, equipment, tools and materials can conform to the quality standards. Take specific field records for samples and data obtained at sea. Check original samples and data after the survey. Analysis and identification of samples, data consolidation and counts can be based on facts. Archiving of documents, data and results shall fulfil these requirements.

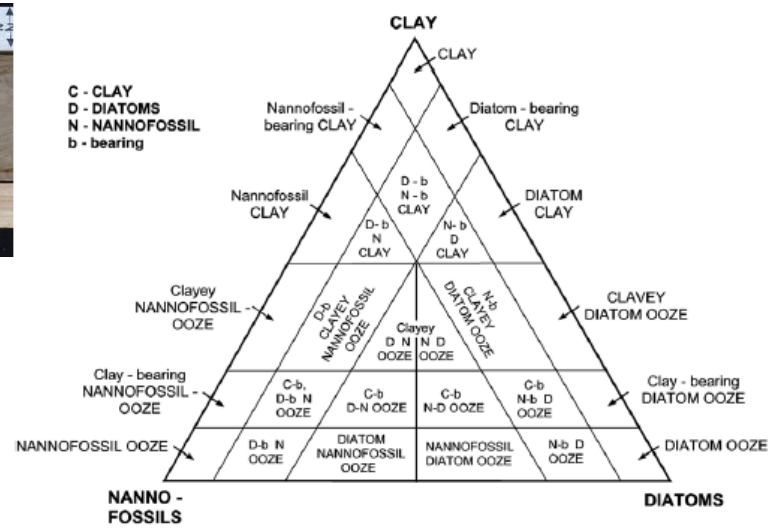
c) Full participation in quality control: Staff participating in the survey can have relevant professional skills. It is the duty of all staff to maintain quality control requirements.

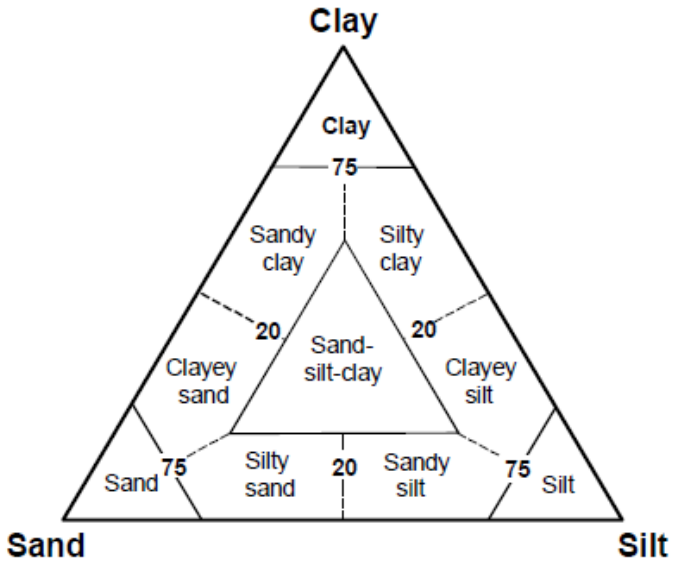
5 Survey of the sediment

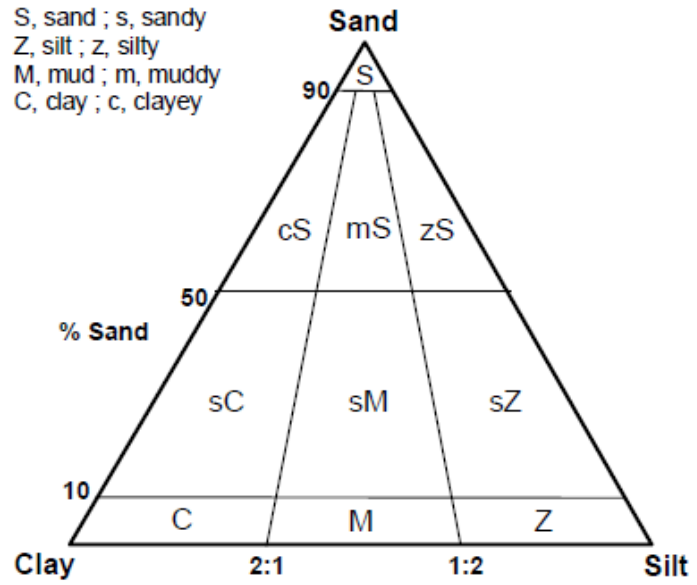
5.1 Principle

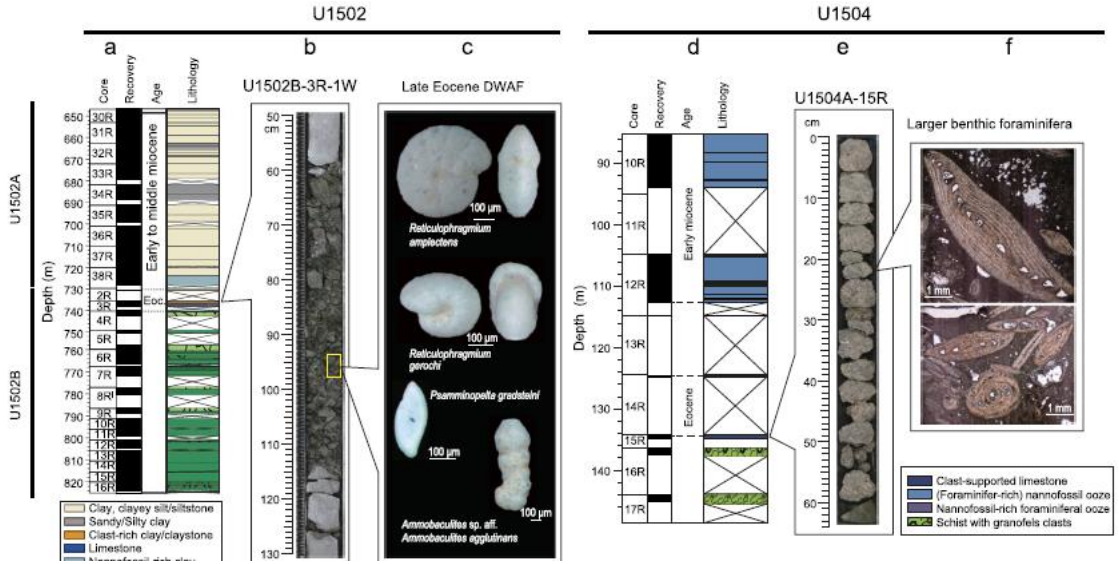
Analyses of sediment characteristics, including sediment classification, physicochemical characterization and granulometry, to obtain information on the substrate environment for surveying the interstitial biota.











5.11.3 Collection and preservation of the samples

According to the requirements for the different objects, seafloor sediments, columnar or undisturbed drilling cores can be selectively collected, sealed on site, and refrigerated or frozen for preservation.

5.12 Organization of data

The organization of data from the surveyed sediments should follow the specific recommendations of this document.

6 Survey of foraminifera

6.1 Principle

Based on the collection, preparation, preservation, identification and analysis of living specimens and tests of the foraminifera, the distribution and preservation of foraminifera in the sediments are investigated to reflect the hydrological and environmental changes in the area.

6.2 General provisions

The general provisions include the following.

a) Design the sampling method and the sampling process according to the survey plan. For sea floor surface sediments, collect from the top 0 cm to 2 cm layer; for deeper sediments, collect at 2 cm intervals from core samples, or at different intervals according to the survey plan.

b) Determine the sampling volume according to the survey plan. Generally, use 20 g to 50 g samples for continental shelf and shallow water depths, and 2 g to 10 g samples for the slope and deeper water depths, because the abundances of foraminifera differ according to depth.

c) Ensure that the samples are not mixed or contaminated. Record the station and sample information

d) Observe the foraminifera specimens (>0,150 mm) under a microscope. Generally, identify the planktonic foraminifera and the dominant species of benthic foraminifera to species level, and identify the others can be to genus level or as ecological categories. Record the abrasion, breakage and dissolution of foraminifera specimens.

e) Count all the specimens if the number of specimens is less than 300. Divide the samples by the riffle or diagonal sample method if the sample volume is too great and identify at least 300 foraminifera for each subsample. About 300 planktonic foraminifera specimens and 150 benthic foraminifera specimens are recommended for microscopic examination.

f) Record the relative abundance of each species, showing its percentage (%), or record its absolute abundance as individuals in per gram dried sample (individuals/g), or record its abundance as individuals in per square centimetre dried sample (individuals/cm^2) for surface sediment sample.

6.3 Collection and preservation of the samples

6.3.1 Sampling and sample processing

Based on different marine sediment types, divide the sampling and processing methods into two types, as follows.

a) Offshore and shoal water type: these sediments are composed mainly of terrigenous clast that have fast deposition rates and low foraminifera content. For obtaining foraminifera specimens, the sediment is placed in a beaker and soaked in clean water. Sodium hexametaphosphate is added for dispersing the sediment particles. After heating and disaggregation, the organic matter is dissolved by adding a moderate amount of hydrogen peroxide. The sample is then washed using

c) carry out the classification and biocoenosis statistics (species number, abundance, diversity and group ratio, etc.) according to the survey plan;

d) for offshore sediment samples, carry out the classification and statistics of 0,063 mm specimens selectively according to the survey plan;

e) fill in the tables for the identification and statistics of foraminifera (tables are designed according to the survey plan).

7 Survey of ostracoda

7.1 Principle

Based on the collection, preparation, preservation, identification and analysis of living specimens and tests, the distribution and preservation of ostracods in sediments are investigated to reflect the hydrological and environmental changes in the area.

7.2 General provisions

The general provisions include the following.

a) Design the sampling method and the sampling process according to the survey plan. For sea floor surface sediments, collect from the top 0 cm to 2 cm layer; for deeper sediments, collect at 2 cm intervals from core samples, or at different intervals according to the survey plan.

b) Determine the sampling volume according to the survey plan. Generally, use 20 g to 50 g samples for continental shelf and shallow water depths, and 2 g to 10 g samples for the slope and deeper water depths, because the abundances of ostracods differ according to depth.

c) Ensure that the samples are not mixed or contaminated. Record the station and sample information.

d) Identify ostracods with a stereomicroscope, where possible to species level but otherwise to genus level. Record any abrasion, breakage or dissolution of ostracod specimens.

e) For statistical analyses of biocoenosis, identify all the specimens if the number present is less than 100. If the volume is large, divide the samples by the riffle or diagonal sample method and identify at least 100 specimens for each subsample.

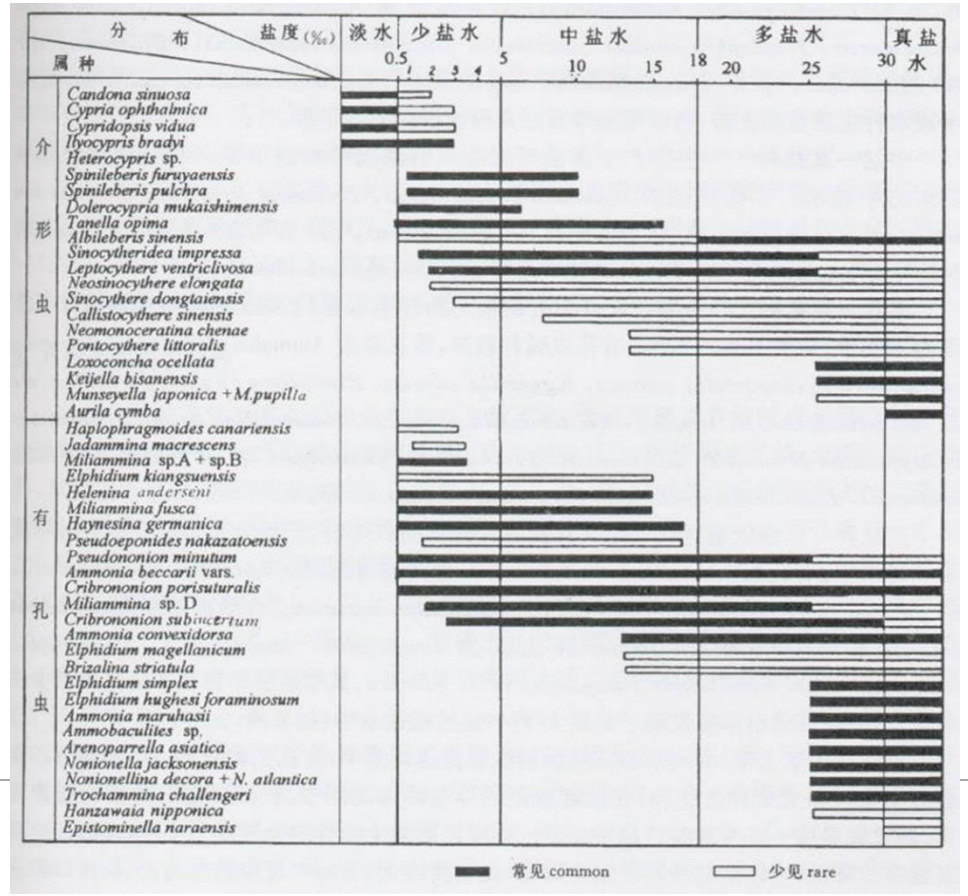
f) Record the relative abundance of each species, showing its percentage (%), or record its absolute abundance as individuals per gram dried sample (individuals/g), or record its abundance as individuals per square centimetre dried sample (individuals/cm^2) for surface sediment sample.

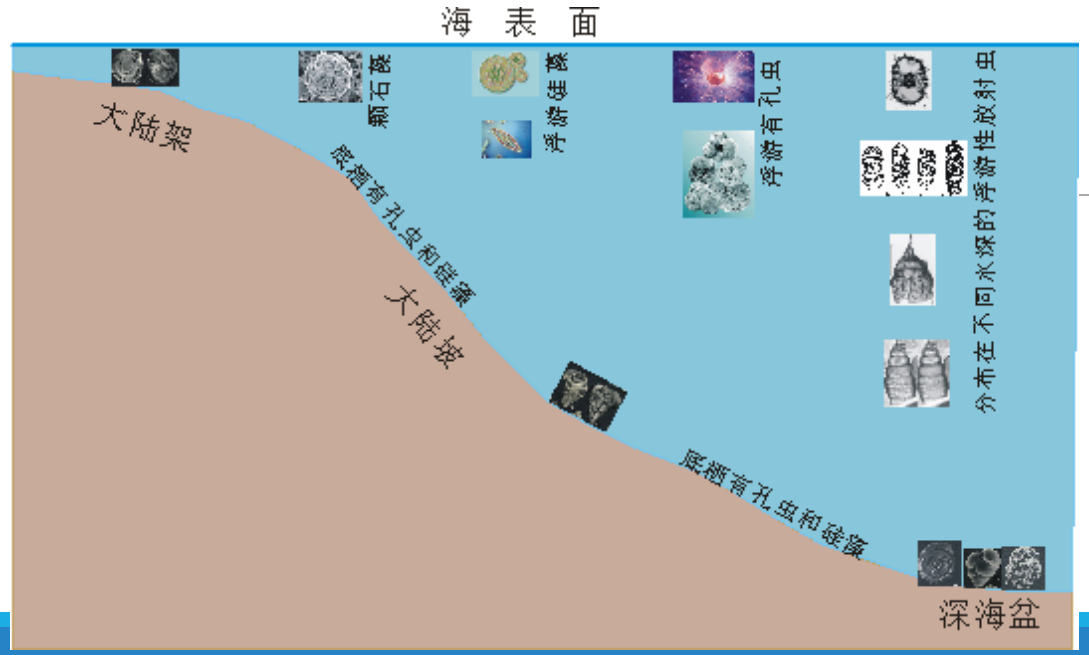
7.3 Collection and preservation of the samples

7.3.1 Sampling and sample processing

Based on different marine sediment types, divide the sampling and processing methods into two type as follows.

a) Offshore and shoal water type: these sediments are composed mainly of terrigenous clast that ha fast deposition rates and low ostracod content. For obtaining ostracod specimens, the sediment placed in a beaker and soaked in clean water. Sodium hexametaphosphate is added for dispersi the ostracods and the sediment particles. After heating and disaggregation, the organic matter dissolved by adding a moderate amount of hydrogen peroxide. The sample is then washed usi a 0,063 mm sieve. Clean ostracod specimens are separated from coarse particles by flotation tetrachloromethane. Ostracods are suspended by stirring and collected by passing through 0,063 mm filter paper.





8 Survey of radiolaria

8.1 Principle

Survey of the preservation status, abundance and community structure of planktonic radiolarians deposited in the sediment of related sea area.

8.2 General provisions

The general provisions include the following.

a) Design the sampling method and the sampling process according to the survey plan. For sea floor surface sediments, collect from the top 0 cm to 2 cm layer; for deeper sediments, collect at 2 cm intervals from core samples, or at different intervals according to the survey plan.

b) Determine the sampling volume according to the survey plan. Generally, use 5 g to 10 g samples for continental shelf and shallow water depths, and 1 g to 2 g or 5 g samples for the slope and deeper water depths.

c) Identification of radiolarian specimens is carried out using diascoptic lighting under a biomicroscope. In general, identify to species level for the 60 dominant or common species, other specimens can be enumerated only for statistical analysis. But if the survey plan requires species diversity data, identify all specimens to species level.

d) For biocoenosis statistics, all samples should be counted if the total number of shells is less than 300. If the number of shells is large, sample can be divided by a sampler or a diagonal sampler.

e) Calculate the relative abundance of each species and record its percentage (%) or record the absolute abundance as individuals in per gram dried sample (individuals/g).

8.3 Collection and preservation of the samples

8.3.1 Sample processing

Based on the sediment type, samples should be processed in one of the two following ways.

a) Shelf and shoal water type: these sediments are composed mainly of terrigenous clast and organisms with calcareous shells; they have a fast deposition rate and the radiolarian content is low. For obtaining radiolarian specimens, sediment is soaked in a beaker with clean water. Tetrasodium pyrophosphate is added for dispersing the particles. After heating and disaggregation, organic matter and calcareous fractions are eliminated and dissolved by adding moderate amounts of hydrogen peroxide and hydrochloric acid. The sample is then washed over a 0,063 mm sieve and oven-dried. In order to separate the clean radiolarian specimens from coarse particles, flotation in tetrachloromethane while stirring is carried out. Radiolarian shells in suspension are poured onto a boulting cloth of 0,063 mm mesh or a filter paper.

b) Slope and deeper sea type: these sediments have less terrigenous clast, fine sediment particles, relatively slow deposition rates, and a higher radiolarian content. The sediment is soaked in a beaker with clean water. Tetrasodium pyrophosphate is added for dispersing the particles. Based on the content of calcareous particles, superadd moderate amounts of hydrogen peroxide and hydrochloric acid in order to dissolve organic matter and calcareous fractions. Continue until the reactions stop. The remnant is washed through a 0,063 mm sieve and oven-dried. The dried material may be directly used for making radiolarian specimen slides for species identification and enumeration.

c) The temperature of the hot plate or thermostatic drier box should be maintained at about 60 °C to 80 °C.

d) Allow the slide to dry naturally, or place in a thermostatic drier box (60 °C to 80 °C, being careful to avoid bubbling). Remove surplus balsam from the slide by using xylene after balsam consolidation and hardening.

e) Label the slide.

9 Survey of sedimentary diatom

9.1 Principle

Based on the collection, identification and analysis of community composition of diatom shells in the sediment, the distribution and preservation of diatoms are investigated to reflect the hydrological, climatic and environmental information in the area.

9.2 General provisions

The general provisions include the following.

a) Design the sampling method and the sampling process according to the survey plan. For sea floor surface sediments, collect from the top 0 cm to 2 cm layer; for deeper sediments, collect at 2 cm intervals from core samples, or at different intervals according to the survey plan.

b) For wet samples, take about 5 g to 10 g, for dry samples take about 1 g to 5 g.

c) Diatom identification is carried out using transmitted light microscope. Each sample is observed by random row number. It is suggested to identify the species or variant, otherwise identify to genus level. For incomplete diatom frustules, if more than half of the central diatom is complete, or if the longitudinal furrow side of feathery diatoms is complete, the specimen should be identified. Dominant or common species can be selected for identification with other specimens only being enumerated, however, if the survey plan requires details of species diversity, all species in each sample should be identified.

d) For statistical analyses, at least 300 specimens per gram of dry sample or per square centimetre should be identified for every sample. If there are fewer than 300 diatom specimens in the sample, all should be identified. The relative abundance of each species should be recorded.

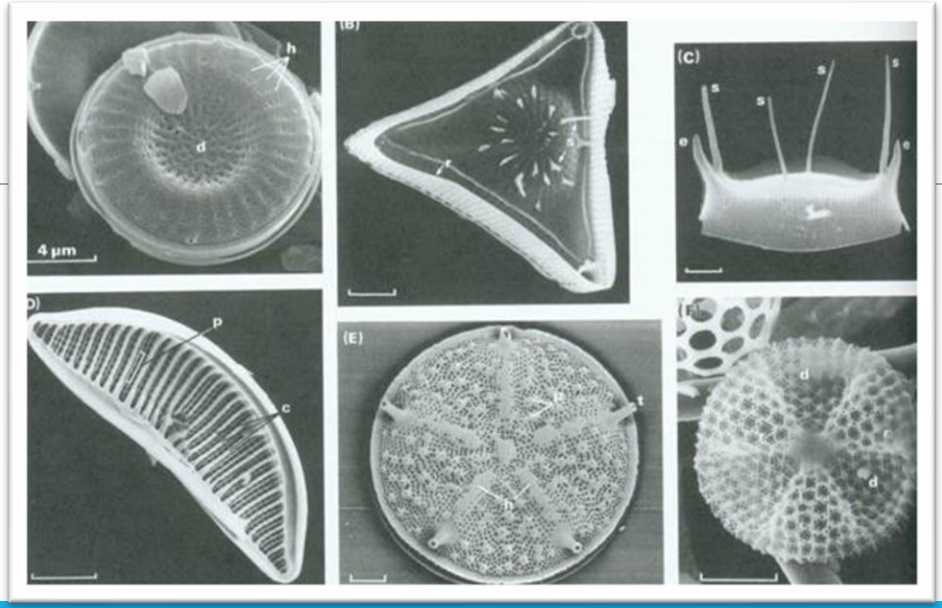
9.3 Collection and preservation of the samples

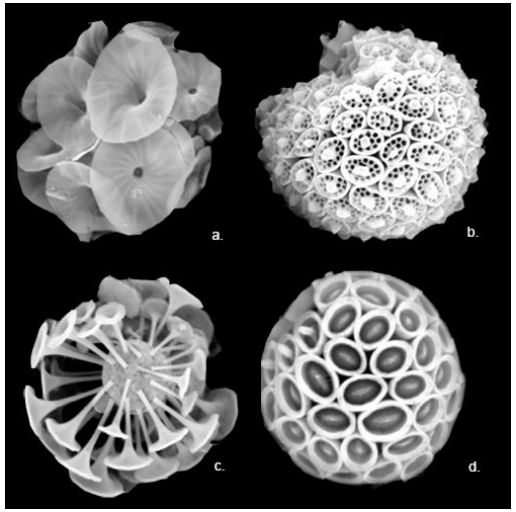
9.3.1 Sampling and treatment

According to differences in diatom content in different types of seabed sediments, sample collection and processing are divided into two types, as follows.

a) Continental shelf and shallow water type: these sediments are composed mainly of terrigenous clast and organisms with calcareous shells; they have a fast deposition rates and the diatom content is relatively low. Dispersion of diatom specimens is by soaking the samples in distilled water. Appropriate amounts of hydrogen peroxide and hydrochloric acid are added in order to remove calcareous particles and organic matter. Allow to stand until the reaction stops. Add zinc bromide or other heavy liquids with a specific gravity of about 2,4 in order to separate the diatoms from other particles by flotation. Stir to keep the diatoms in suspension. These can be used directly for making specimen sheets.

b) Slope and deep-sea type: these sediments have less terrigenous detritus, fine sediment particles, relatively slow deposition rates, and a higher diatom content. Diatoms are dispersed by soaking in distilled water. Based on the calcium content, appropriate amounts of hydrogen peroxide and





d) Wash 3 times with distilled water.

If the sample is dry, it is advisable to take about 1 g to 5 g dry sample, put it into 300 ml beaker, add 200 ml of distilled water and 0,5 g to 1 g of sodium pyrophosphate and allow to stand for about 10 h. After the sediment has dispersed, continue as above.

9.5.2 Preparation of microscope slide specimens

The procedure for the preparation of microscope slide specimens includes the following.

a) Immerse coverslips in a volume fraction of 10 % to 20 % HCl solution for at least 24 h, and then immerse in alcohol to remove HCl, before use.

b) Spread diatom suspension evenly on coverslip with a glass rod and allow to dry. Add 1 or 2 drops of neutral resin onto the glass slide. Place the coated coverslip gently onto the resin drop, avoiding air bubbles.

c) Slide can be dried naturally or in an oven (45 °C to 55 °C, temperature should not be too high so as to avoid bubbling) for 48 h.

d) Label the slide and store it in a sample box.

e) Prepare replicate slides for each sample for identification and analysis.

9.6 Organization of data

Identification of diatoms is made by observing under a microscope at 1 000× magnification. Enumeration can be carried out at 400× magnification. At least 300 diatom frustules (not including resting spores and auxospore) per sample should be identified. The relative abundance (%) of each species relative to the total number of diatom frustules (i.e. not including resting spores and auxospore) should be recorded for each sample.

Data processing should meet the relevant provisions of this document. For the calculation of biocoenosis analysis, refer to Annex E.

10 Survey of coccoliths

10.1 Principle

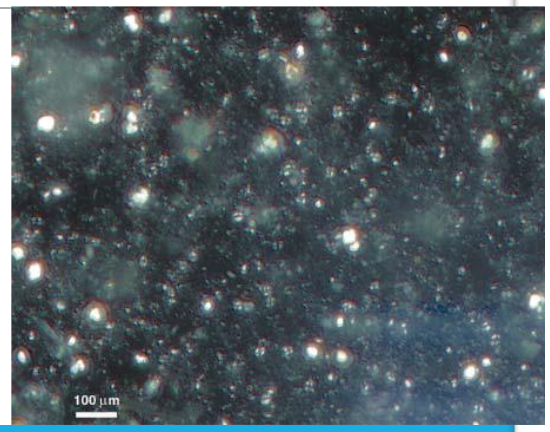
The objectives of investigating coccoliths in sediments are: to meet the need of chronostratigraphy and to date geological age of sediments by analysis and identification of coccoliths; to explore their oceanographical/palaeoceanographical significance through analysis of characteristics of coccolith assemblages; to infer sedimentary environments (including sediment sources) or diagenesis based on analysis and evaluation of coccolith preservation status.

10.2 General provisions

The general provisions include the following.

a) Sampling quality: the sample shall not be contaminated for example by cross-sampling, mixing with another sample, re-using disposable tools for sampling.

b) Sampling depth or depth interval: the topmost 0 cm to 2 cm layer of sediment should be collected for seafloor surface samples; depth intervals between 2 cm to 10 cm, or other intervals in accordance with the requirements of a survey plan can be selectively collected from box-corer, multicorer or gravity sampler, for subsurface sediment samples. For undisturbed sediments and well-preserved sediment sequences, a grab sampler can be used from which vertical tube subcores can be taken. Sampling can then continue in the same way as by multicorer.



b) Evaluation of coccolith preservation status: provide a qualitative and quantitative estimate of the preservation status of coccoliths, e.g. if they have been damaged by chemical etching, physical breakage, or by secondary crystallization/recrystallization (Table G.1). Relative coccolith abundance in sediments is the proportion of coccoliths relative to the sediment clastic components and is expressed as a percentage (Table G.2). It is determined by referring to sediment particle abundance charts[50]. The evaluation should be made based on observations of at least 10 fields of view at a magnification of 1 000×. Observation of at least 100 fields of view is needed if sediments contain very few coccoliths.

c) Enumeration of coccoliths. There are two minimum requirements; at least 300 coccoliths shall be observed and counted for each sample; and at least more than 5 fields of view (at a magnification of 1 000×) randomly located on the slide should be observed. Very commonly, the sediment samples analysed contain abundant coccoliths, and the number of coccoliths within one field of view is more than 100. In this case, a subdivision of one field of view into 4 parts can be made by using the cross-hairs in the eyepiece, and coccoliths within 1/4 field of view can be counted. In this case, at least 5 fields of view shall be observed. If a sediment sample contains very few coccoliths, at least 100 fields of view shall be observed.

d) Analysis of coccolith assemblage abundance: for basic marine geological survey objects, it is recommended to estimate the relative abundance of coccoliths.

e) There are several semi-quantitative estimation methods. For example, the method suggested by Reference [5], it is to obtain the number of coccoliths per area on a sample slide, with the purpose of analysing some selected coccolith species that have significance for ecology or geological age diagnosis. There are also several absolute quantitative methods of analysis to obtain the number of coccoliths per mass in the sample. For example, the random settling method that was suggested by Reference [7] and by Reference [55], the spray method suggested by Reference [8] and the microbeads method suggested by Reference [47]. These methods are commonly applied in palaeoceanographic studies, however, they need more time for sample preparation than simple smear slides. These methods can be applied if necessary in the marine survey objects.

11 Survey of sporopollen

11.1 Principle

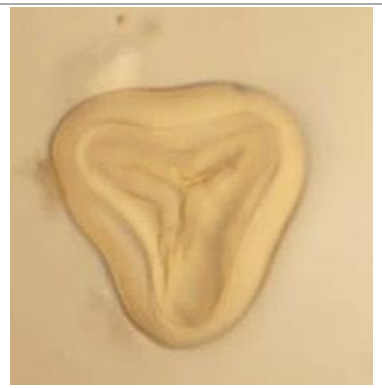
The outer coat (exine) of pollen and spore is mainly composed of tough, resistant organic compounds, namely sporopollenin $(C_{10}H_{16}O_3)_x$ and chitin, which protect the sporopollen from desiccation and oxidation. The sporopollen assemblage in sediments reflects the community characteristics of the original vegetation and provides information on the temperature and humidity of terrestrial habitats around sedimentary basins. The methods employed for investigating sporopollen in sediments include sample collection, sample processing by both physical and chemical treatments in order to extract the sporopollen by removing organic materials, carbonate and siliceous minerals, the identification and enumeration of sporopollen and data analysis. Based on the sporopollen data, it is possible to reconstruct past climate change in the terrestrial habitats surrounding sedimentary basins.

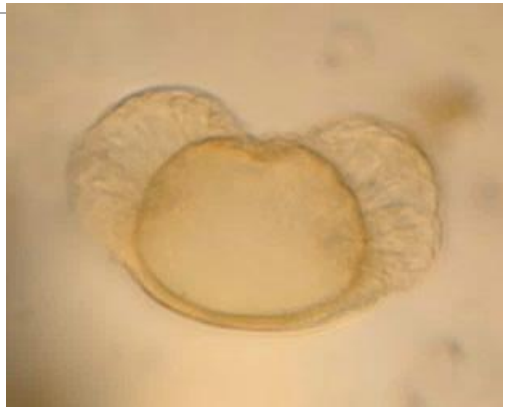
11.2 General provisions

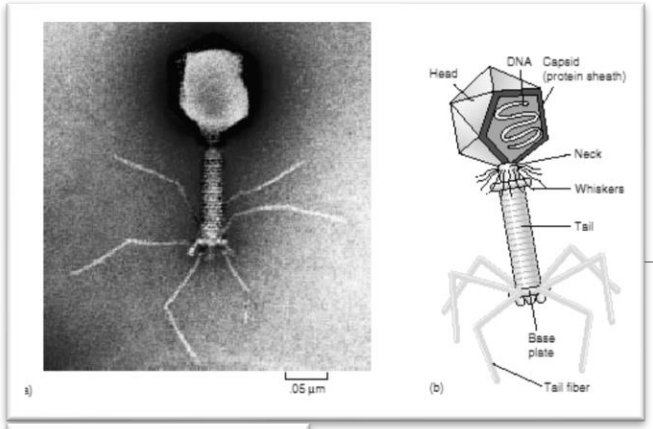
The general provisions include the following.

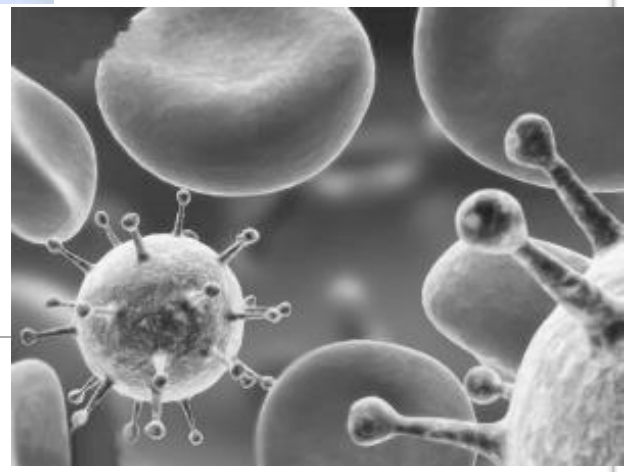
a) All samples should be collected and processed without contamination; only filtered or distilled water can be used; laboratory windows should be kept closed when the pollen filtration system is in operation; there should be no other sporopollen source in the laboratory. Detailed information of the field site and samples shall be recorded.

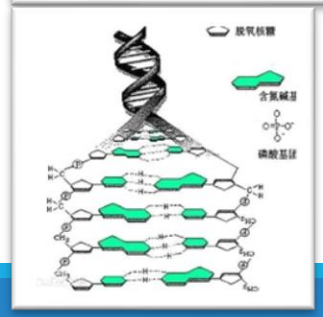
b) Samples should be collected from the upper 2 cm layer of the sea surface sediments, and at 2 cm intervals for the core sediments, or as required by the investigation program.











or polypropylene ware in this step. The acetolysis solution can be explosive when it comes in contact with water. Be familiar with the property of the chemicals and the specification for experimental procedures, such as using protective clothing, gloves and an eye shield. Note the location of the first aid kit, the shower, the eye-wash station and the burn blanket, and know how to use them. All procedures shall be carried out in a fume hood.

11.6 Organization of data

Data of the sporopollen investigation in sediments shall conform to the requirements in this document. The sporopollen concentrations can be calculated using the exotic *Lycopodium* tablet technique and be expressed in numbers of grains per gram dry mass of sediment (N/g), or per millilitre of the sediment (N/ml). The relative abundances of individual sporopollen taxa can be estimated on their group totals (arboreal and herbaceous pollen) and expressed as percentages of the total.

12 Survey of benthic viruses

12.1 Principle

Sediment samples are frozen directly without fixation or fixed with 0,02 µm-filtered seawater containing 2 % formalin or 2 % glutaraldehyde (for fluorescence microscopy) or 0,5 % glutaraldehyde (for flow cytometry). Benthic viruses are enumerated by epifluorescence microscopy (or flow cytometry) after extraction, centrifugation, dilution, filtration, staining and slides preparation (see flow diagrams in Figure H.1).

12.2 General provisions

The general provisions include the following:

a) seawater and MilliQ®[1] water should be filtered with 0,02 µm membrane after sterilization;

b) reagents and solutions should be filtered with 0,2 µm membrane;

c) individual abundance should be presented as individuals per gram of sediment dry mass;

d) biomass should be presented as micrograms of carbon per 10 square centimetre or microgram of carbon per cubic centimetre;

e) investigation elements should include determining the abundance (or biomass) of viruses.

12.3 Collection and preservation of the samples

12.3.1 Samples for epifluorescence microscopy

Samples for epifluorescence microscopy include the following.

a) Fixed samples: three replicate samples each of 0,5 ml are collected from the top 1 cm layer of undisturbed sediment core and transferred into 5 ml storage tubes. To each replicate, add 3 ml of 2 % formalin (or glutaraldehyde) made using 0,02 µm-filtered seawater. Shake gently and place in the dark at 4 °C for 15 min to 30 min. Samples are then quick-frozen in liquid nitrogen and stored at -80 °C.

b) Non-fixed samples: three replicate samples each of 0,5 ml are collected from the top 0,5 cm or 1 cm layer of undisturbed sediment core and transferred into 5 ml storage tubes. To each replicate, add 3 ml of 0,02 µm-filtered sterile seawater. Samples are then quick-frozen in liquid nitrogen, and then stored at -80 °C.

1) MilliQ® water is an example of a suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this product.

j) add an equal volume of chloroform-isoamyl alcohol (24:1 volume fraction), mix and centrifuge at 12 000 r/min at 4 °C for 10 min;

k) collect the supernatant and add 0,6 volume of precooled isopropanol and place at room temperature for 1 h;

l) centrifuge at 16 000 *g* at 4 °C for 20 min and discard the supernatant;

m) add 70 % ethanol, mix and centrifuge at 16 000 *g* at 4 °C for 10 min, and then discard the supernatant;

n) repeat step m), and invert the tube on a lint-free paper to remove the ethanol;

o) add 50 µl of sterile deionized water and store at -20 °C for the subsequent analysis.

12.5.3.5 Linker-amplified shotgun library

The procedure includes the following steps:

a) randomly shear the obtained viral DNA;

b) carry out end-repairing of the DNA fragments;

c) ligate the dsDNA linkers;

d) randomly amplify the fragments using high-fidelity DNA polymerase;

e) ligate the resulting fragments into the pSMART vector;

f) electroporate into MC12 cells;

g) cloning and culture;

h) sequence.

12.5.3.6 Data analysis

Data analysis includes the following:

a) remove low-quality sequences;

b) annotate the sequences against the NCBI database;

c) construct phylogenetic trees to reveal taxonomic affiliations.

13 Survey of benthic microbes

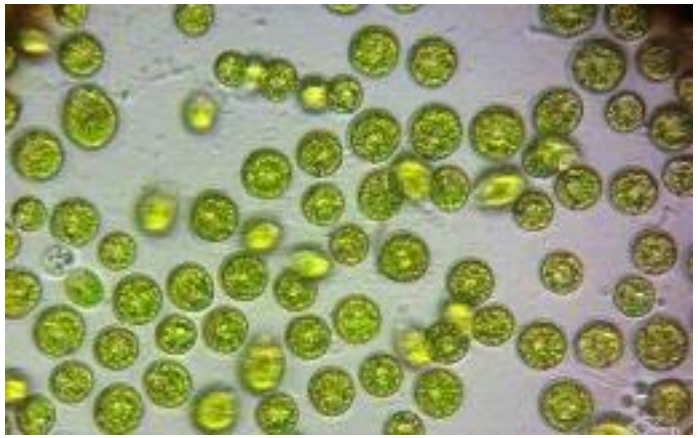
13.1 Principle

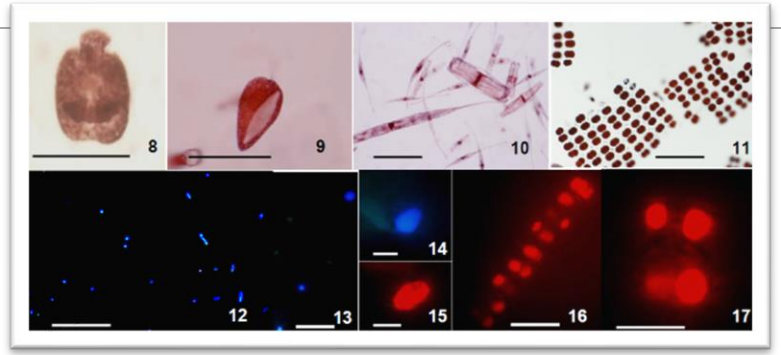
The survey can include bacteria, archaea and fungi from the sediments. Genomic DNA is extracted directly from oceanic sediments. The benthic microbial diversity and community structure are determined using molecular biological techniques such as polymerase chain reaction (PCR), clone library, and high-throughput sequencing. The abundance of benthic microbes are determined by fluorescence microscopy and real-time PCR.

13.2 General provisions

The general provisions include the following:

a) sediment samples should be collected with minimal disturbance;





f) collect the fluorescence signal at 85 °C for 10 min.

13.5.2.2.5.3 Real-time PCR reaction cycle for fungi

The procedure includes the following steps:

a) pre-denaturation: 95 °C 10 min;

b) denaturation: 95 °C 20 s;

c) annealing: 55 °C 20 s;

d) extension: 72 °C 20 s;

e) repeat steps b) to d) for 40 cycles;

f) collect the fluorescence signal at 85 °C for 10 min.

13.5.2.2.6 Real-time PCR and data analysis

The procedure includes the following steps.

a) Perform a real-time PCR reaction with standard plasmid and examine the sample at the same time.

b) Determine the baseline of the real-time PCR reaction. Usually, this refers to the signal level during the initial cycles of PCR, usually cycles 3 to 15, in which there is little change in the fluorescence signal.

c) Set the threshold at the exponential phase of the real-time PCR reaction. Usually, real-time PCR instrument software automatically sets the threshold at 10 times the standard deviation of the fluorescence value of the baseline.

d) Determine the threshold cycle (C_t) of standard plasmid and the examined samples. C_t is the cycle number at which the fluorescence signal of the reaction crosses the threshold.

e) The log of each known copy number in the dilution series of the standard plasmid (X-axis) is plotted against the C_t value for that concentration (Y-axis) to generate a standard curve.

f) The C_t values of the examined sample are compared to the standard curve to determine their copy number. The abundance of the benthic microbes in the sediments is expressed in copies/g (of wet mass).

14 Survey of benthic microalgae

14.1 Principle

The objects of qualitative investigation are mainly microalgae, and microalgal cysts, in the surface sediment with full body, bright pigments, and detectable pigment content. The objects also can include microalgal cells and cyst that can reproduce normally. The sea areas of the quantitative survey are limited to the shallow waters and areas with a shallow euphotic layer. (See Figures J.1 for experimental facilities. For key technical processes, see Figure J.1.)

14.2 General provisions

The general provisions include the following.

a) Determine the technical requirements for qualitative or quantitative investigations according to the needs of the survey plan.

14.5.6 Algal toxins determination

14.5.6.1 Technical considerations

Many benthic dinoflagellates contain toxins, and the content of toxins in the dinoflagellate cysts is higher. Technical considerations include the following:

a) based on the results of the pre-experimental analyses of the species identification and the enumeration of cells, the decision is made whether or not to investigate the algal toxins in the sediment;

b) if the number of toxic algal cell or cysts is above 10^5 cells in sample, the toxins can be investigated.

14.5.6.2 Sample collection

Collect the sediment samples qualitatively or quantitatively according to the needs of the survey plan. Only surface sediments are collected.

14.5.6.3 Sample treatment

The procedure for sample treatment includes the following.

a) Weigh 50 g sediment, add filtered sea water and mix well.

b) Ultrasonic oscillation for 15 min to 30 min.

c) Use a sieve to wash the sample (mesh-sizes of a series of sieves, see 15.3.1). Wash the sediment on the upper sieve carefully with filtered seawater using a wash bottle in order to wash out the adsorbed cysts. Transfer the mixture that passes through a 20 µm sieve into a watch glass.

d) Turn the watch glass to use centrifugal force for separating the cysts and algae cells from sand grains. Transfer the cysts and cells into a centrifuge tube with a pipette. The cysts and algae cells in the tube are the sample for toxin extraction.

14.5.6.4 Determining the type of algal toxin

The benthic toxic dinoflagellates are mainly *Prorocentrum* and *Gambierdiscus*. *Prorocentrum lima* contains diarrhetic shellfish poison (DSP). *Gambierdiscus* spp. contain ciguatera. Planktonic dinoflagellate cysts such as *Alexandrium* spp. contain paralytic shellfish poison (PSP).

15 Survey of benthic protozoa

15.1 Principle

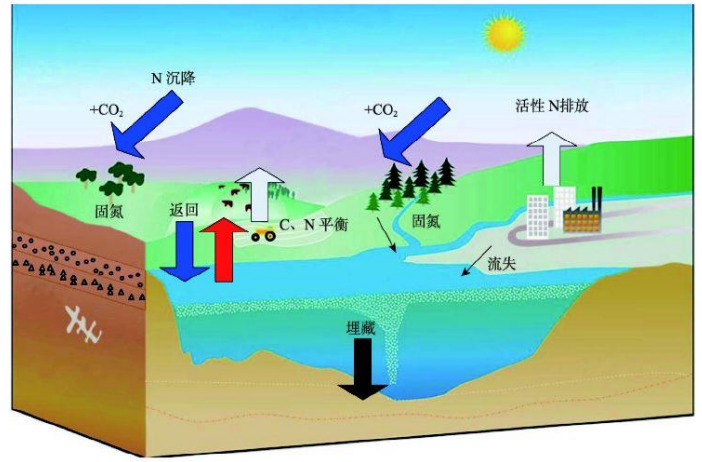
Due to the different densities of organisms and sediment particles, microbenthos (e.g. small-density protozoa) and meiobenthos can be separated from sediment particles by silica sol density centrifugation: micro- and meiobenthos float in the silica sols whereas sediment particles sink to the bottom of the centrifuge tube. The target organisms are harvested and enumerated using either a standard or an inverted light microscope. If possible, the analysis combines the quantitative protargol stain and molecular diversity (Figures J.2, J.3, J.4, J.5).

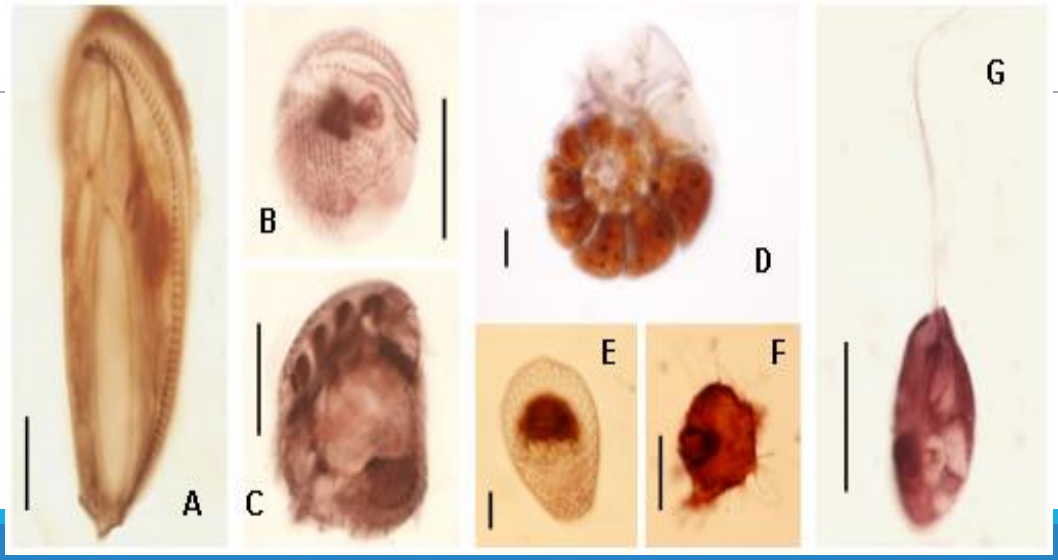
15.2 General provisions

The general provisions include the following:

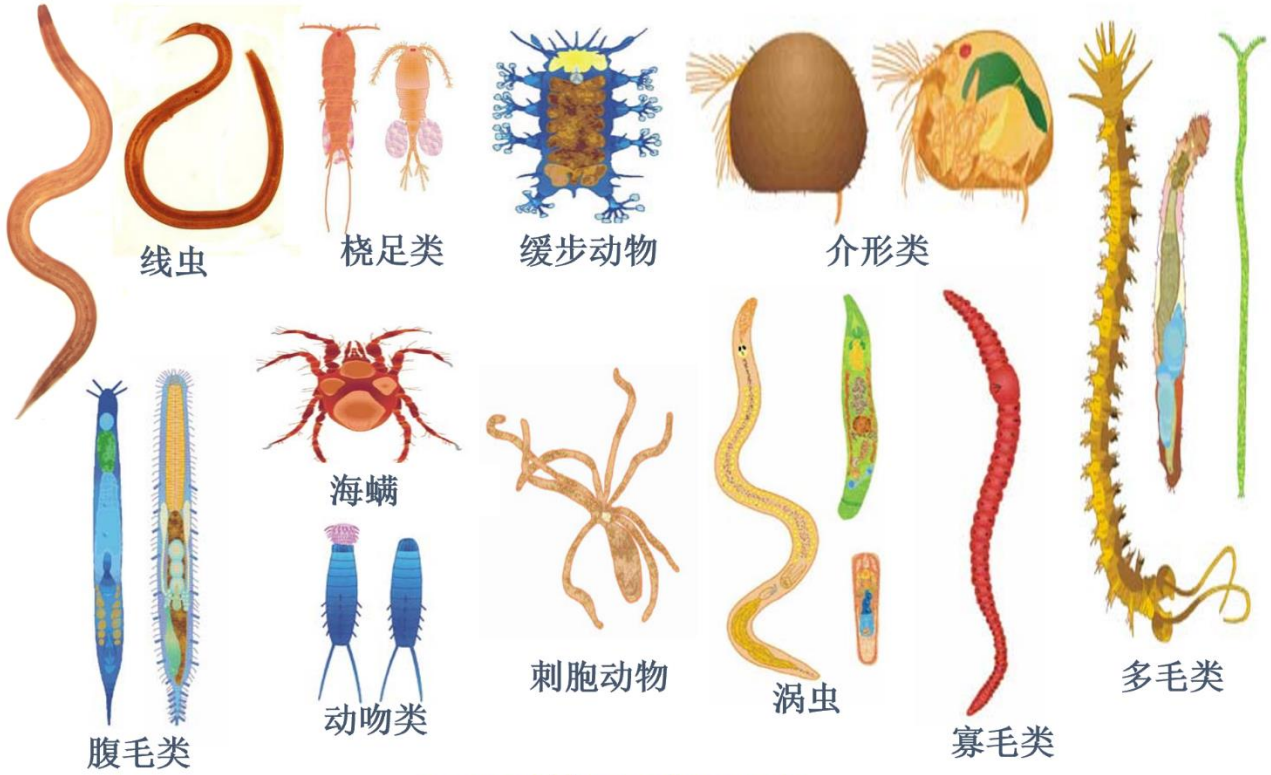
a) sampling sediments are undisturbed;

b) the survey contents should include the composition, abundance and dominant species;





Meiobentos



h) incubate at 37 °C for 1 h with continuous shaking;

i) add 50 µl to 200 µl of LB to the culture medium;

j) incubate at 37 °C for 16 h.

15.5.3.5.4 Detection of positive clone

The procedure for detection of positive clone includes:

a) collect the white colonies of bacteria for the PCR following the program described in 15.5.3.3;

b) PCR products are analysed on a 1 % agarose gel for detection;

c) collect 100 to 500 colonies for sequencing.

15.5.3.5.5 Data analysis

The procedure for data analysis includes:

a) check for chimeras using the Check-Chimera method;

b) cluster OTUs using the software DOTUR;

c) calculate the alpha diversity index for samples using software DOTUR;

d) construct Neighbor-Joining (NJ) trees to identify taxonomic affiliations.

15.5.3.6 High throughput DNA sequencing

15.5.3.6.1 Preparation for sequencing

The procedure for the preparation for sequencing includes:

a) prepare the ice box;

b) transfer the PCR products into the ice box;

c) send the PCR products to a sequencing company.

15.5.3.6.2 Data analysis

The procedure for data analysis includes:

a) cluster the OTUs according to their sequence similarities;

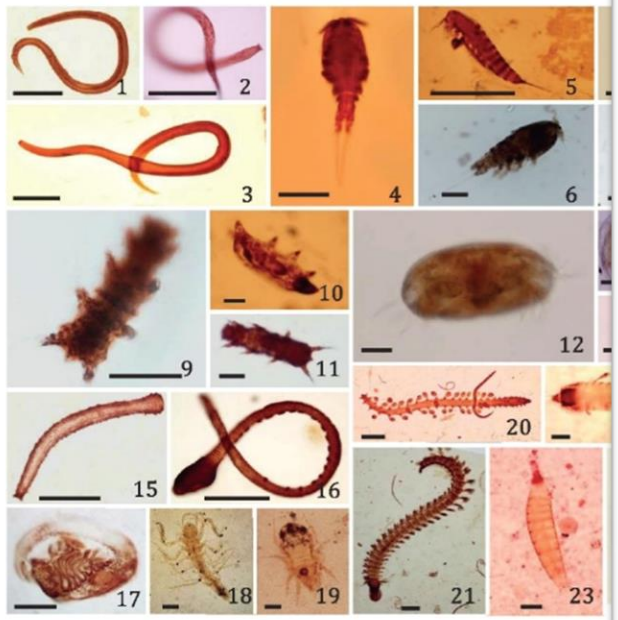
b) annotate the representative sequences of each OTU and estimate the community composition.

16 Survey of metazoan meiobenthos

16.1 Principle

Sediment samples pretreated on site are centrifuged through silica sol and the metazoan meiobenthos floated on the silica gel column. The biological samples are collected in Petri dishes and placed directly under a stereomicroscope to isolate, identify and enumerate the metazoan individuals. Permanently sealed specimens and quantitative silver staining are used for high-precision qualitative and quantitative analysis of major metazoan groups according to the needs of the survey plan (see Figure A.2). In addition, molecular biology tools are employed to assist in the classification and determining the diversity of the metazoan meiobenthos.

Figure A.2 shows pictures of several representative metazoan meiobenthos. These a
photographs.



Key

1, 2, 3	nematodes	17	bivalve
4, 5, 6, 7, 8	copepods and copepod nauplius	18	arthropod
9, 10, 11	tardigrades	19	mite
12, 13, 14	ostracoda	20, 21	polychaetes
15	gastrotricha	22, 23	kinorhyncha
16	priapulid worm	24	rotifera

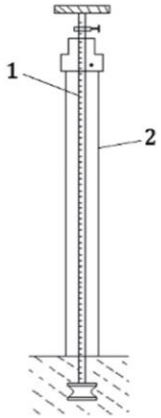
NOTE All pictures are after quantitative protargol staining.

Figure A.2 — Example of representatives of major groups of meiob

Annex B
(informative)

Several stratified sampling devices for the survey of interstitial biota

Figure B.1 and Figure B.2 show example of quantitative stratified sampling devices for interstiti
survey.



Key

1 stratified sampling device
2 cylindrical sampling tube

Figure B.1 — Push-type stratified sampling device

Annex J
(informative)

Technical flowcharts for the investigation of microbenthos

Figures J.1, J.2, J.3, J.4 and J.5 show several key technical flowcharts for the investigation of microbenthos.

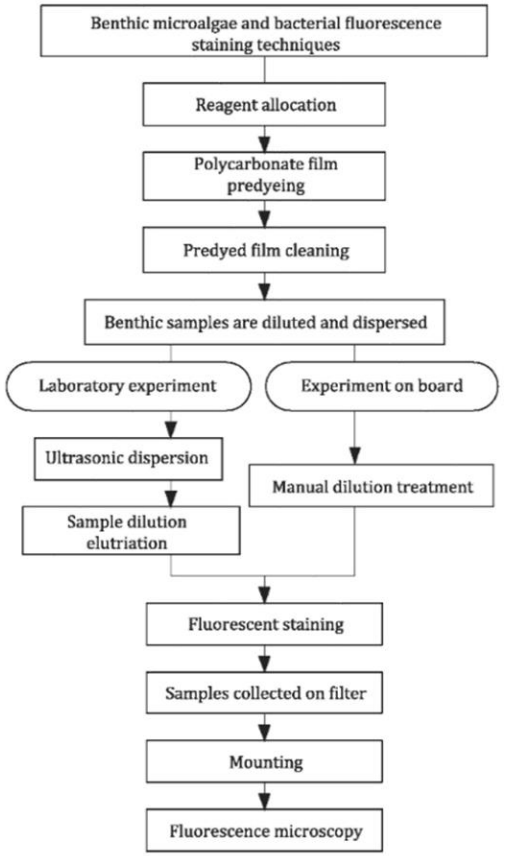


Figure J.1 — Flowchart for fluorescence staining of microalgae and bacteria

Annex C
(informative)

Tables for sample labelling and sampling record — Exam

See Tables C.1, C.2, C.3 for examples of sample labels and sampling records for interstitial bio

Table C.1 — Sample label

Station:_____	Date:_____	Time:_____	Weather:_____
Water depth:____m	Longitude:_____	Latitude:_____	Layer:_____
Sample condition:			
Water temperature:	Air temperature:		
Sediment texture:	Sediment colour:	Sediment smell:	
Column sediment core:	Surface layer sediment:		
Note:			

Table C.2 — Sampling record table

Page_____Total pag

Sea area_____Vessel_____Cruise_____Station_____

Longitude of the measured station (E/W)_°_'_" Latitude (N/S)_°_'_" Water depth___m Sampling

Inner diameter of sampling tube___cm Sampling date (UTC)___Year___Month___Day to___Year___Mo

	Deploying into water	Touching the bottom	Retrieving onto deck
Local time			
Latitude (N/S)	° ' "	° ' "	° ' "
Longitude (E/W)	° ' "	° ' "	° ' "
Water depth, m			
Cable length, m	——		——

Core number	Height of overlying water (cm)	Length of sediment (cm)	Length of sampling tube (cm)	Note
1				
2				
3				
4				
5				
6				
7				
8				

Annex K
(informative)

Solutions and regents

K.1 Fixatives

Glutaraldehyde: 2 % (mass/volume) glutaraldehyde, or formaldehyde paraformaldehyde.

K.2 Extraction sols

Percoll®, or Ludox ™, or Ludox® HS 40.

K.3 Fluorochrome

K.3.1 4', 6-diamidino-2-phenylindole, DAPI: 1 µg/ml to 5 µg/ml final concent

K.3.2 Acridine orange: 10 µg/ml final concentration.

K.4 Reagents for black filters

Sudan black B or Irgalan black: 2 g of Sudan black B or Irgalan black dissolve 70 % ethanol to give a 2 % final concentration.

K.5 Embedding and curing agents

K.5.1 Agar: 0,7 g agar dissolved in 20 ml of distilled water with a 90 °C v concentration in the range of 3 % to 4 %.

K.5.2 Formaldehyde: 36 % (mass/volume) formaldehyde.

K.6 Solutions and regents for the quantitative protargol stain

K.6.1 Potassium permanganate

0,08 g of potassium permanganate dissolved in 40 ml of distilled water with 0

K.6.2 Oxalic acid

2 g of oxalic acid dissolved in 80 ml of distilled water with 2,5 % final concent

K.6.3 Silver proteinate

Peptone 50 g, silver nitrate 20 g, acetone 500 ml, absolute ethanol 300 ml, am

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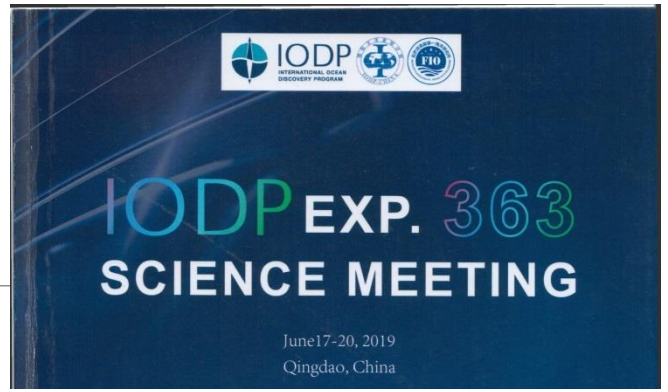
OUTLINE

1. Introduction of ISO 23040

2. Contents and techniques of ISO 23040

3. International collaboration, Promotion and technical training

International collaboration technical exchange



2019 June 17-20, Qingdao, China

Meeting with German, USA, UK experts to make a face-to face discussion and technical justification and to revise WD

Technical exchange and training

2014 Technology Exchange Conference in China

2019 National publicity and implementation

Technical exchange and training

Marine biodiversity monitoring and conservation, Nov 2018 Beihai

ASEAN (Association of Southeast Asian Nations)

A total of 20 trainees from 6 ASEAN countries: Singapore, Myanmar, Thailand, Malaysia, Indonesia and the Philippines

Thanks for your attention!

December 14-17, 2021