
**Water quality — Guidelines for
quantitative sampling and sample
processing of marine soft-bottom
macrofauna**

*Qualité de l'eau — Lignes directrices pour l'échantillonnage quantitatif
et le traitement d'échantillons de la macrofaune marine des fonds
meubles*

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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.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

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.

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Introduction

Analysis of macrofaunal communities in soft-bottom sediments is an integral part of marine environmental assessment. The faunal composition, in terms of both the species present and their relative abundance, reflects integrated environmental conditions in the survey area over a period of time. The composition and structure of soft-bottom macrofaunal communities therefore can be used to characterise environmental conditions and estimate the extent of environmental impact.

Characterisation of environmental conditions is usually based on quantitative methods, in this case by relating the numbers of species and individuals captured to a known area of sea floor. For accurate data interpretation, it is essential to add information on the geophysical/geochemical characteristics or properties of the water masses and bottom sediments, including nutrients, oxygenation and redox state where appropriate.

For effective data utilisation and quality assurance of the work carried out, it is essential that surveys are intercomparable temporally, spatially and between operators. This International Standard contributes to on-going work on quality assurance of data from soft-bottom macrofaunal surveys. These guidelines primarily aim assisting in standardising monitoring surveys carried out for commercial purposes or in connection with the EU Water Framework Directive. For this reason, detailed specifications are given in areas of consequence for data intercompatibility.

Where appropriate, cost-benefit issues have been taken into consideration, and accepted minimal requirements for general environmental impact assessment have been given. The cited minimum requirements for accuracy are not intended to satisfy research needs, or to provide a full ecological understanding of the sampling area. Designers of programmes for research or other studies requiring a detailed knowledge of soft-bottom macrofauna should consult the guidelines given in Reference [17] for decisions of survey design and sampling frequency.

This International Standard applies to all areas of the sea floor where it is possible to collect faunal samples by a grab or coring device. For practical reasons, this applies to animals retained on a mesh screen of 0,5 mm or 1 mm aperture size.

The sensitivity of the method, here defined as detection of faunal disturbance, change in taxon composition or faunal mapping, is dependent on the type of environmental influences present in the area and on the level of competence/standardisation of the personnel.

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Water quality — Guidelines for quantitative sampling and sample processing of marine soft-bottom macrofauna

1 Scope

This International Standard provides guidelines on the quantitative collection and processing of subtidal soft-bottom macrofaunal samples in marine waters.

This International Standard encompasses:

- development of the sampling programme;
- requirements for sampling equipment;
- sampling and sample treatment in the field;
- sorting and species identification;
- storage of collected and processed material.

This International Standard does not specifically address the following, although some elements may be applicable:

- bioassay sub-sampling;
- deep water (> 750 m) or offshore sampling;
- *in situ* faunal studies, e.g. recolonisation assays;
- nonbenthic organisms caught in the sampling device;
- estuarine sampling;
- intertidal sampling;
- meiofaunal sampling and analysis ^[3];
- sampling by dredge and sledge;
- Self-Contained Underwater Breathing Apparatus (SCUBA) sampling;
- statistical design.

Accuracy of position fixing is determined by the geographical area, equipment used and survey objective.

2 Terms and definitions

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

2.1
baseline survey
environmental impact assessment
survey with emphasis on characterisation and description of biotic and abiotic conditions in the survey area, and which forms the basis for future monitoring and/or follow-up surveys

2.2
benthic
associated with the sea floor

2.3
benthic macrofauna
bottom-dwelling animals retained on a mesh screen of 0,5 mm or 1 mm aperture size

2.4
receiving water body
water body which receives an input of material, of either natural or anthropogenic origin

NOTE The term often appears in the context of anthropogenic input, for example, effluent from municipal wastewater outlets or industrial processed water.

2.5
reference station
one or more sampling stations chosen to represent environmental conditions in a given area, i.e. free from direct anthropogenic influences

2.6
replicate sample
series of samples taken in the same time frame, at the same sampling station, in the same manner for statistical validity and comparison

NOTE Replicate samples may include sets of subsamples taken from a larger sample.

2.7
sampling station
precise location where samples are collected

NOTE A sampling station is defined by its geographical position (OS National Grid Reference, latitude, longitude), its depth (relative to chart datum and normalised to mean low water as given in tide tables) and any other invariant or physical conditions. The station is delineated using the given level of precision. In cases of doubt when revisiting sampling stations, emphasis should be placed on landmarks and water depth.

2.8
soft-bottom
areas of sea floor consisting of loose deposited particles including clay, mud, sand and gravel, shells and maerl, also including mixed substrata with gravels, small stones and pebbles scattered on a bed of finer material, but excluding cobbles

2.9
soft-bottom fauna
animals living on or completely/partially buried in soft-bottom sediments

2.10
sublittoral
portion of the shore which is either totally immersed or only uncovered by the receding tide infrequently and then for very short period (i.e. below the littoral zone)

2.11**subsample**

ideally representative portion removed from a sample, taken for separate analysis

NOTE See Annex A.

3 Quality and safety**3.1 Health and safety requirements****3.1.1 General**

All phases of benthic sampling and sample processing should adhere strictly to national and international health and safety regulations. The main points are listed below.

3.1.2 Laboratory safety facilities

A valid health and safety manual should be freely available in the institute or laboratory and the appropriate first-aid supplies and emergency facilities (such as eyewash station and shower) should be installed. The laboratory and storage areas should further be equipped with point-ventilation outlets and preferably have a monitor for chemical levels in the air.

3.1.3 Vessel safety and operation of field equipment

Vessels used for sampling should be certificated for safety and equipped with experienced crews and onboard machinery maintained and suited to the operating environment.

Many types of sediment samplers present a serious danger to personnel. All staff should be fully aware of the appropriate procedures to operate safely around each sampler. Only trained operators, or personnel under their supervision, should handle equipment on deck.

3.1.4 Behaviour and training

All personnel collecting and handling samples should be given training in the appropriate health and safety procedures and, where in force, have attained certification status. Refresher training should be carried out every three years or sooner. Staff should be trained in assessing risks to personnel or equipment and follow any documented procedures.

3.1.5 Handling of chemicals

Chemicals used for fixing or preserving faunal samples should be stored and handled with the proper precautions according to health and safety regulations, see 3.1.2 and 3.1.6. Non-drip dispensers should be used for liquid chemicals.

Common chemicals used in benthic work include the fixative formalin or substitutes, the preservative ethanol and biological stains such as rose Bengal or methyl green.

WARNING — Formalin is particularly hazardous to health, and prolonged or intense exposure can cause long-term allergies. A number of less hazardous, but expensive, alternatives to formalin are available and should be used where possible, especially when dealing with small sample volumes.

3.1.6 Equipment and protective clothing

Appropriate protective clothing should be made available. These include:

- in the field: helmet, safety boots, coveralls, life-jacket/floating suit (depending on the type of vessel), gloves;
- in the laboratory and store: aprons, gloves, goggles, gas-filters.

3.2 Quality assurance and quality control

Quality assurance and quality control measures should be incorporated during all stages of benthic sampling and sample processing programmes. These principles help to guarantee that all data produced are of a specified quality, and that all parts of the work are carried out in a standardised and intercomparable manner. All procedures should therefore be clearly described and carried out openly, such that all of the laboratory's activities can be audited internally and externally at any time.

NOTE The overall aim is to assure traceability and full documentation of samples and equipment from beginning to end from sampling, sample transport, offloading from survey vessel, placement within and retrieval from a sample store to sample processing, reporting and final archiving.

National and/or international accreditation should be sought if appropriate, required for most commercially-operative laboratories. Guidance from relevant accreditation bodies should be sought in developing specific in-house quality systems, work procedures and protocols. It is recommended that laboratories participate in intercomparative tests or learning schemes to develop expertise and maintain the appropriate skills. This ensures continued standardisation and reproducibility of results.

Further recommendations on quality assurance practices are given in Reference [17].

EXAMPLE Some examples of national guideline and/or audit schemes for marine benthos are given below:

- Germany (<http://www.umweltbundesamt.de/wasser/themen/q-bimp.htm>);
- UK - National Marine Biological Analytical Quality Control Scheme (<http://www.nmbaqcs.org/>).

Further, within the International Council for the Exploration of the Sea (ICES) are also two relevant Steering Groups on Quality Assurance of Biological Measurements in the Northeast Atlantic and Baltic Sea, respectively (see <http://www.ices.dk/iceswork/workinggroups.asp>).

A quality assurance/quality control scheme should encompass the following:

- training and training records;
- traceability of work and samples;
- standardised practices throughout;
- calibration of sampling and sample processing equipment;
- in-house and external audit, also referred to as Analytical Quality Control schemes;
- literature updates;
- reference or voucher collections.

Specific details on analytical quality assurance and quality control are given in 7.7.

4 Strategies and objectives for soft-bottom faunal surveys

4.1 Sampling programme and plan

The design of the sampling programme depends on the detailed aims of the survey and the required power of the data. The programme should be developed with regard to local topographical and hydrographic conditions in the survey area, information on local contamination sources and knowledge from previous surveys, if any. The number of sampling stations, their positions and numbers of replicate samples to be taken at each sampling station should be established prior to the initiation of the survey. The design of the programme has a strong influence on the options for data treatment and statistical analyses. Prior considerations about data treatment and reporting should therefore be made. Quality assurance procedures should be incorporated at this stage.

Guidance and considerations for sampling and statistical design may be found in Reference [17].

4.2 Positioning of sampling stations

4.2.1 General

Sampling stations should be located to satisfy predefined requirements, bearing in mind the objectives of the study and the likely scale of natural variability in the biota.

For monitoring purposes (except for biodiversity studies — see below), sampling stations should preferably be positioned in areas of even sandy/muddy bottom sediments. Certain bottom types where it is difficult to obtain good-quality samples, such as in sediments containing large amounts of stones, hard gravel, twigs and similar objects, should be avoided. However it may be possible for a diver to sample pockets of sediment in such areas. Alternatively, supplementary semiquantitative techniques may be used, e.g. underwater photography, video, remotely operated vehicle (ROV), or benthic dredging. In special cases where habitats within the sampling area vary strongly, different sampling techniques may be combined, but generally the same gear should be used for all sampling in one survey.

For biodiversity studies, various bottom types should be included, as appropriate to the aims of the programme.

Sampling stations can be positioned according to one, or combinations of, the following strategies:

- station network, see 4.2.2;
- randomly;
- stratified;
- transect;
- single-spot sampling, see 4.2.6.

4.2.2 Station network

Sampling stations are arranged in a regular grid-like pattern. This arrangement is appropriate for overview surveys and for mapping of distribution of factors of interest, for instance zone of influence around point source discharges. The survey area should be one of topographic homogeneity, but some adjustments can be made according to local conditions, for instance in fjords and coastal waters with smaller variations in depth.

4.2.3 Random or scattered sampling

In special circumstances, sampling stations may be positioned randomly or scattered. An example of this might be when no previous knowledge of the area is available as a guide to appropriate stratification, or when an unbiased value for a whole area is desired.

4.2.4 Stratified sampling

Sampling stations are arranged within locally homogeneous subdivisions of the survey area. The subdivisions (strata) may be delineated according to depth, sediment types or other factors that vary across the survey area. Stratification is appropriate in cases where habitat variability can confound patterns of interest. Within-strata stations may be placed in a network, for instance for zone-of-influence mapping, or randomised for description of "average" characteristics of the strata. Echo-sounders or appropriate ground discrimination tools should be used.

4.2.5 Transect sampling

Sampling stations are arranged along linear transects. One approach is to place stations along a known or anticipated gradient of a factor of interest in a sub-area of minimum habitat variability. This is applicable, for instance, to trace effects of point-source discharges by establishing the transect in the main current direction from the source. Another rather different approach is to place stations across possible habitat gradients when it is not feasible or appropriate to work in strata.

4.2.6 Single-spot (station) sampling

This applies when a small number of stations are placed according to individual assessment. In cases of known or suspected eutrophication or chemical contamination, sampling stations may be positioned in the deepest parts of the survey area (depressions, basins), where the earliest signs of disturbance are often seen.

However no formal statistical comparison among areas is possible based on single stations. This is regarded as an undesirable design, only to be used either when it is just the station in itself that is interesting, or when the limitation of available resources makes it impossible to sample several stations.

4.3 Reference stations

For surveys carried out in contaminated areas, or those believed to be contaminated, one or more reference stations should be chosen beyond the affected area. The reference stations should, as far as possible, be representative of conditions unaffected by effluent sources and allow assessment of natural temporal and spatial variations in the soft-bottom faunal communities. Reference stations should be used in surveys where special circumstances demand direct comparison of the fauna with that beyond the disturbed or affected area, or where knowledge of the extent of natural variation is required.

Reference stations should be located in conditions as similar as possible to those at the regular sampling stations, i.e. similar depth and sediment type. Multiple reference stations are particularly important in heterogeneous areas.

Statistical considerations and the required precision of results dictate the number of reference stations and sample replicates required.

NOTE Some surveys demand a higher number of sample replicates at reference stations than at "ordinary" stations.

4.4 Types of survey

4.4.1 General

Surveys may be divided into three main categories (see Table 1) according to the objectives.