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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

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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, www.iso.org/directives.

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For an explanation on the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the WTO principles in the Technical Barriers to Trade (TBT) see the following URL: Foreword - Supplementary information

The committee responsible for this document is ISO/TC 147, *Water quality*, Subcommittee SC 5, *Biological methods*.

This second edition cancels and replaces the first edition (ISO 16665:2005), which has been technically revised.

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 characterize environmental conditions and estimate the extent of environmental impact.

Characterization 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 geophysicochemical characteristics or properties of the water masses and bottom sediments, including nutrients, oxygenation, and redox state where appropriate.

For effective data utilization and quality assurance (QA) of the work carried out, it is beneficial and may be essential (depending on the individual survey aims) that surveys be intercomparable temporally, spatially, and between operators. This International Standard contributes to ongoing work on QA of data from soft-bottom macrofaunal surveys. These guidelines primarily aim to assist in standardizing 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 [13] for decisions on 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 survey design, the type of environmental influences present in the area and on the level of competence or standardization of the personnel.

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WARNING — Persons using this document should be familiar with normal laboratory practice. This document does not purport to address all of the safety problems, if any, associated with its use. It is the responsibility of the employer or user to establish appropriate safety and health practices and to ensure compliance with any national regulatory conditions.

IMPORTANT — It is absolutely essential that tests conducted in accordance with this document be carried out by suitably trained staff.

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:

a) development of the sampling programme;

b) requirements for sampling equipment;

c) sampling and sample treatment in the field;

d) sorting and species identification;

e) 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. recolonization assays;

— non-benthic organisms caught in the sampling device;

— estuarine sampling;

— intertidal sampling;

— meiofaunal sampling and analysis (see Reference [9]);

— 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 Ecological or biological concepts

2.1.1
benthic
dwelling at the bottom of an aquatic environment

2.1.2
benthic macrofauna
bottom-dwelling animals retained on a mesh screen of 0,5 mm or 1 mm aperture size

2.1.3
receiving water body
recipient
recipient water body
water body which receives an input of material of either natural or anthropogenic origin

Note 1 to entry: The term often appears in the context of organic enrichment by, for example, effluent from municipal waste water outlets or industrial processed water. The macrofaunal part of receiving water body surveys describe the state of organic enrichment in a given area.

[SOURCE: ISO 5667-19:2004,[3] definition 3.4, modified — in the Note to entry, "contamination" has been replaced by "organic enrichment", and it is further specified that the terms apply to the macrofaunal part of receiving water body surveys]

2.1.4
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

2.1.5
soft-bottom fauna
animals living on, or completely or partially buried in, soft-bottom sediments

2.1.6
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)

2.2 Surveys and samples

2.2.1
baseline survey
environmental impact assessment
survey with emphasis on characterization and description of biotic and abiotic conditions in the survey area, which provides the basis for future monitoring and/or follow-up surveys

[SOURCE: ISO 5667-19:2004,[3] definition 3.2, modified — "classification" changed to "characterization"; "biotic and abiotic" added]

2.2.2
reference station
one or more sampling stations chosen to represent environmental conditions in a given area, i.e. free from direct anthropogenic influences

2.2.3
reference collection
collection of identified specimens, used for reference purposes

Note 1 to entry: Institute reference collections are usually verified by an appropriate and approved taxonomic specialist. In addition, individual identifiers may keep a personal collection and/or some surveys require contract- or area-specific reference collections.

2.2.4
replicate samples
series of samples taken in the same time frame, at the same sampling station, in the same manner for statistical validity and comparison

[SOURCE: ISO 5667-19:2004,[3] definition 3.6, modified — "simultaneously" has been changed to "in the same time frame"; "for statistical validity and comparison" added]

Note 1 to entry: Replicate samples can include sets of sub-samples taken from a larger sample.

2.2.5
sampling station
precise location where samples are collected

Note 1 to entry: A sampling station is defined by its geographical position (latitude, longitude), its depth (relative to chart datum and normalized 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.

2.2.6
sub-sample
ideally representative portion removed from a sample, taken for separate analysis

[SOURCE: ISO 5667-19:2004,[3] definition 3.7, modified — "ideally" and "taken for separate analysis" added]

3 Strategies and objectives for soft-bottom faunal surveys

3.1 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 (QA) procedures should be incorporated at this stage.

For guidance and considerations of sampling and statistical design, see Reference [13].

3.2 Positioning of sampling stations

3.2.1 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.

Sampling stations should, for monitoring purposes (except for biodiversity studies — see the following), preferably be positioned in areas of homogenous sandy or muddy bottom sediments. Certain bottom types where it is difficult to obtain good quality samples, e.g. 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. Consult EN 16260[6] for guidance on visual seabed surveys.

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 3.2.2;

— randomly, see 3.2.3;

— stratified, see 3.2.4;

— transect, see 3.2.5;

— single-spot sampling, see 3.2.6.

3.2.2 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, e.g. 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, e.g. in fjords and coastal waters with smaller variations in depth.

3.2.3 Random or scattered sampling

In special circumstances, sampling stations may be positioned randomly or scattered. An example of this is 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.

3.2.4 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, e.g. for zone-of-influence mapping, or randomized for description of “average“ characteristics of the strata. Echo-sounders or appropriate ground discrimination tools should be used.

3.2.5 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. Such sampling is, for example, applicable 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.

3.2.6 Single-spot (station) sampling

This applies when a small number of stations are placed according to individual assessment. In fjordic or sill-influenced systems, where eutrophication or chemical contamination is suspected or investigated, 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.

3.3 Reference stations

For surveys carried out in disturbed areas or those believed to be impacted in some way, 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.

3.4 Types of survey

3.4.1 General

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

Table 1 — Overview of main categories of survey type

Survey type	Objectives	User group	Precision of results
Pilot survey	To give a general overview of bottom and faunal conditions. To provide a simple rapid assessment or to give basic information for designing more detailed sampling programmes	Regulatory authorities and consultancies. Research use as precursor to larger programme	Low
Baseline survey or environmental impact assessment	To characterize conditions in a given area. To map or identify the impact of point-source discharges (spatial extent and intensity). To compare faunal composition with specified assessment criteria or simply with other representative areas	Mainly regulatory authorities and consultancies. Research use for mapping, succession or recolonization, or gradient studies	Medium to high, depending on individual requirements
Temporal trend monitoring	To describe changes in benthic fauna over time, either for detecting change in biodiversity or as applied to environmental conditions	Mainly regulatory authorities and consultancies. Research use for environmental and biodiversity changes over time (also applied to climate monitoring)	Medium to high, depending on individual requirements

Precision of results refers to the expected accuracy of the data obtained, i.e. how representative the samples are of the environmental conditions. Precision of results is less in heterogeneous relative to homogeneous sediments or water depth across a sampling area. Therefore, to achieve the same

precision, heterogeneous sediments require higher numbers of sampling stations and/or replicate samples relative to homogeneous sediments. In addition, precision varies depending on whether the samples are processed quantitatively or semiquantitatively. The required precision and therefore the sampling and processing intensity is determined by the individual aims of the survey.

Note that the different survey types may supplement each other. For example:

— a pilot survey may provide information needed to design a sampling programme for a baseline survey or environmental impact assessment;

— any of the surveys when repeated in the same manner and at the same time of year may provide temporal trend data.

3.4.2 Pilot survey

This is an initial assessment of faunal conditions in the bottom sediments in an area where the source of the impact is not known or where there are no existing data from the area. The survey allows a coarse assessment of conditions and can provide the basis for development of a sampling programme for applied surveys, e.g. baseline or environmental impact assessment surveys as well as long-term surveillance by temporal trend monitoring. The requirements for equipment, sampling methodology, and repeatability are usually relatively simple, see Table 2.

Table 2 — Strategy and design for pilot surveys

Sampling devices	Usually grab or box corer, preferably supplemented by use of a benthic dredge. If appropriate, also other semiquantitative techniques may be used (such as underwater photography, ROV, video or acoustic ground discrimination tools).
Strategy for sampling stations	May be one or a combination of strategies outlined in 4.2
Minimum requirements for faunal assessment	Minimum requirements depend on purpose of survey. If carried out to identify best sampling stations for future programme, a minimum of semiquantitative assessment of benthic fauna should be done at all stations (at least presence and relative abundance of the major animal taxa), preferably also identification of large, abundant or otherwise prominent organisms. If pilot survey required to make firm statements about environmental disturbance, quantitative sampling is recommended.
Optional sampling	Additional samples from priority stations (as assessed by visual observations or physico-chemical data obtained during sampling or other documented or anecdotal information) may be retained for later quantitative processing.
Field documentation required	Field log of sampling conditions and sediment description (see 4.1.)
Reference station requirements	Should also be sampled, unless previous data exist to assess the status of reference areas.

Pilot surveys can have another important use, namely to help design the size and calculate statistical power for future monitoring programmes. For this purpose, the pilot study should resemble the planned monitoring programme as much as possible in terms of the spatial and temporal arrangement of samples.

A pilot survey generally requires relatively few samples. For applied purposes, the sampling area is chosen in accumulation areas rather than where net erosion takes place. Sampling stations may be positioned at random or in a grid. If the objective is to assess the faunal assemblages across an area at large, samples should be taken in both deep and shallow water. The sampling area should cover as much of the survey area as possible.

In addition to quantitative faunal sampling, dredging should be carried out to collect the rare, large and more mobile taxa not adequately sampled by remote quantitative methods. Especially in regions with varying sea floor topography and open to wind and currents, an ROV or sledge-mounted video reconnaissance is recommended to determine the extent of sediment and faunal patchiness (can occur

in areas of both coarse and fine sediments). If appropriate, acoustic ground discrimination techniques may also be used to provide additional information.

Strategy and design for pilot surveys are summarized in Table 2.

3.4.3 Baseline survey or environmental impact assessment

This is a survey widely carried out for applied research or commercial surveys, generally either where a known source of impact exists or before effluent discharge is established. Such surveys may also be carried out for biodiversity research or where an area needs to be characterized biologically. The aim is to document faunal conditions and/or map the spatial extent of biological impact. Such surveys can be carried out using relatively simple methodology, but usually there are specified requirements for the methodology and procedures to be used.

Where external reference or survey data exist, these should be used to help plan the survey programme and to assess overall impact, where appropriate. See also 4.4 for comments on supplementary non-quantitative sampling.

Strategy and design for baseline surveys or environmental impact assessment are summarized in Table 3.

Table 3 — Strategy and design for baseline surveys or environmental impact assessment

Sampling devices	Usually grab or box corer, preferably supplemented by a benthic dredge. If appropriate, also other semiquantitative techniques may be used (such as underwater photography, ROV, video or acoustic ground discrimination tools).
Strategy for sampling stations	Sampling stations positioned according to aims of survey Grid or transect sampling; stations positioned in relation to known discharge points if applicable. Stratified random sampling may also be applied according to the knowledge of expected distribution of impacts. Likely impact distribution can be determined by assessing the degree of impact in relation to local hydrography and bottom topography. If intended to detect diffuse effluent or to monitor environmental change, one station may be placed in the deepest part of the survey area (where impacted conditions often first appear). In some cases, a follow-up survey can be carried out using fewer sample replicates or sampling stations than the initial thorough environmental description. If the samples are used for legislative purpose, the required precision of results (or statistical power) should be determined, and the number of replicate samples taken adjusted as appropriate. If necessary, the number of replicate samples to be used for the analyses can be determined by calculating taxon-area curves
Minimum requirements for faunal assessment	Usually three or more, replicates are processed quantitatively, depending on statistical requirements. Faunal assessment may focus on individual taxa, groups of taxa or community-based assessment. For impact assessment, larger-scale effluents demand a more extensive station network and statistical power than small-scale effluents
Optional sampling	Contingency replicates may be collected from priority stations (assessed as for pilot survey) to be processed later if required
Field documentation required	Field log of sampling conditions and sediment description (see 4.1.)
Reference station requirements	Reference station(s) should be established in cases where environmental impacts are expected. In areas of strong impact gradients, one reference station may be sufficient. Where there is much natural variation in conditions (heterogeneous bottom) and/or only low to moderate impacts, two or more reference stations are recommended. If the end-points of transects are demonstrated outside the zone of impact, these may act as reference stations. Where standards of “pass/fail” have already been established for the area, reference stations may not be required. To assess possible overall impact in the area studied, external reference data across a wider area are recommended (can encompass new or existing data)

3.4.4 Temporal trend monitoring

This is a survey of the benthos in response to temporal changes in the chemical and/or physical conditions in the sediments to document either contamination or natural variation over time. The surveys should be carried out using standardized methodology according to an established programme. Sediments that are physically disturbed by human activities (e.g. trawling) are generally not suitable for retrospective trend monitoring purposes.

Strategy and design for temporal trend monitoring surveys are summarized in Table 4.

Table 4 — Strategy and design for temporal trend monitoring surveys

Sampling devices	Usually grab or box corer, supplemented if appropriate by semiquantitative assessment techniques (such as benthic dredging, remote underwater photography, ROV, video or acoustic ground discrimination tools)
Strategy for sampling stations	Sampling stations positioned according to aims of survey, but positions fixed and resampled at regular intervals. A high level of documentation and replicability is required. Statistical power is assessed as for baseline survey or environmental impact assessment
Minimum requirements for faunal assessment	As for baseline survey or environmental impact assessment
Optional sampling	As for baseline survey or environmental impact assessment
Field documentation required	Field log of sampling conditions and sediment description (see 4.1)
Reference station requirements	Reference station(s) appropriate only if monitoring effluent impact (in which case strategy as for zone of impact mapping)

Seasonal sampling can have an important influence on the results of temporal trend monitoring. Surveys should always be carried out during the same season to ensure continuity. The minimum is one sampling per survey year, but two or more samplings during the same year are advantageous. For monitoring surveys, sampling during known recruitment periods (e.g. summer) generally is avoided, except where there is an express interest in recruitment and productivity.

The timing of sampling varies geographically. In certain areas, winter sampling is not possible due to ice cover, sub-zero temperatures or other unfavourable conditions (e.g. the Arctic and Baltic Sea). In these cases, spring and/or autumn sampling are the only alternatives.

3.5 Change in sampling programme and intercalibration

The issue of reproducibility should be given due concern. If changes or modifications are to be made in a running sampling programme, care should be taken to ensure comparability of old and new data. In particular, if the sampling gear in a long-term monitoring programme is to be changed, appropriate validation of the new techniques should be carried out. Intercalibration should be conducted when comparisons between studies carried out with different techniques are to be made.

4 Sampling

4.1 Documentation and field log

A field log should be kept for recording information pertaining to the sampling, sampling stations and the individual samples.

A minimum of the following information concerning sampling and the sampling stations should be recorded in the field log:

— people involved in sampling or sample processing in the field;

— unique survey, project or contract code;

— geographical co-ordinates for each sampling station and datum used (also for each replicate sample if required, e.g. in case of boat drift during sampling, see 4.2.2);

— track-plot of stations sampled, if required by the relevant protocols;

— water depth (in metres) and tidal state, especially coastal or shallow waters, at each sampling station and for each replicate sample;

— sampling programme for each sampling station (number of replicate samples, sampling of background parameters, etc.);

— date and time for each sample and/or sampling station;

— sampling equipment used, mass and bite area;

— sieve mesh aperture sizes and number of replicates collected;

— other comments such as rejected samples, delays and other problems experienced, together with the causes.

The following additional information should be recorded for future retrospective assessment of any sample anomalies:

— estimated wind strength and direction;

— estimate of wave height or assessment of state of sea (e.g. Beaufort scale).

The following information should be recorded for each sample replicate before and after sieving, as appropriate.

Before sieving:

— sediment volume or bite depth measured by means of, for example, a calibrated rod or volume markers on the inside of the sampling device;

— visual sediment description (sand, silt and/or clay, giving relative proportions of each for mixed sediments);

— description of the sediment profile and thickness of surface layer, if visible;

— colour, surface and down the sediment profile; see Table 5;

— smell, e.g. presence and severity of H_2S; see Table 5.

After sieving:

— main groups of large, easily visible animals present;

— anthropogenic debris, rubbish, sanitary products, plastics;

— other particular characteristics, e.g. presence of oil, drilling mud, fish food pellets, stones, dead shells, terrestrial material, fruit pips or seeds as sewage indicator;

— number of sample containers used for each replicate, after sieving and fixing.

The sample may be photographed, if desired.

4.2 Sampling and sample processing in the field

4.2.1 Sampling requirements

Sampling may be carried out from a wide range of survey vessels, from fully equipped research or supply vessels to small inshore workboats and inflatable crafts, depending on the requirements of the survey. The use of small boats may be an advantage in shallow inshore areas or around aquaculture installations. The choice of sampling equipment should be scaled appropriately and used within the operational safety limits of the individual vessel.

All survey vessels should comply with local sea-going safety regulations and carry all required certificates and equipment. Trained crews or scientists with experience in sampling techniques, position fixing or recording, and necessary seamanship should man the vessel. The survey vessel should be appropriately and adequately equipped for bottom sampling, with sufficient deck space. The following equipment is required:

— winch or haulier with boom or crane of sufficient height and correct lifting specification to allow unhampered retrieval of the sampling device on deck;

— wire or rope of the appropriate dimensions depending on equipment type and safety requirements, rigged to a meter wheel or marked with depth markers;

— echo-sounder;

— satellite navigation global positioning device: differential global positioning system (DGPS); reference system universal transverse Mercator (UTM 32 or 33) — if no GPS signal can be obtained, a minimum requirement of radar in conjunction with admiralty charts is recommended;

— sufficient sea water hoses with adjustable pressures to supply each stage in the sample collection and sample processing — sprinkler devices may also be beneficial.

4.2.2 Defining the position of sampling stations

The position of the sampling stations should be defined unambiguously, such that they can be relocated by other operators. The system used for plotting should be stated explicitly in any report or work procedure.

Positions should be defined using geographic co-ordinates, e.g. latitude and longitude to at least two decimal points with reference to the appropriate system for graticules (such as European datum: ED-50; world geodetic system: WGS-84), and also using the universal transverse Mercator (UTM) system, if so desired. Accuracy of sampling station positions should be defined by the aims of the survey. Track-plotting during longer sampling stations may be advantageous.

DGPS with monitor should be used in all sea areas if possible. If the DGPS signal is not available, accuracy should be assessed and a minimum of global positioning system (GPS) without differential receiver used. Sampling stations should also be defined using the direction and distance from landmarks or fixed points of reference, in addition to geographical co-ordinates, if the accuracy of the GPS positions is low or in question.

When revisiting sampling stations poorly defined in terms of geographical co-ordinates, the normalized water depth, known landmarks as well as sediment features should be used as the main criteria for relocating the sampling station. DGPS should then be used to relocate for future reference.

A minimum accuracy of ±50 m and ±20 m in open waters and estuarine areas, respectively, should be attained. A greater accuracy is desirable and achievable if survey aims dictate.

Water depths should be given to the nearest metre, relating to chart datum and normalized to mean low water according to tide tables.

4.2.3 Choice of sampling equipment

Remote sampling is most commonly carried out using a grab or corer. The various sampling areas of different equipment should be taken into account when selecting gear. Sampling devices should be constructed of appropriate rust-free material.

Grabs are widely used for routine sampling purposes, and operate best in sandy sediments, as well as fine mud with an admixture of gravel and stones. An appropriate type of corer should be used in cases where a completely undisturbed sediment surface is required, e.g. for sediment-water interface studies or in cases where the infauna lives deeper than the penetration depth of a grab to ensure that organisms inhabiting sediment layers deeper than 20 cm are adequately sampled. Corers are also useful where information on the vertical distribution of organisms is sought.

Grabs should be equipped with inspection ports covered with metal gauze or sieve plate (0,5 mm or 1,0 mm aperture size depending on sieving size), extending over a minimum 40 % of the top surface, to reduce the bow-wave effect. If for practicable reasons the mesh size is fixed, then this should be to the smallest size used in the survey designs, e.g. 0,5 mm. Similarly, corers should be open at top and bottom during descent. The apparatus should close completely during hauling, e.g. by rubber flaps on top of inspection ports and a “lip“ around the jaws, in the case of a grab, such that the sample material is not washed out, and so that the supernatant water is kept stationary relative to the sediment surface.

The biting depth of grabs can vary with sedimentary conditions. Masses can be added to adjust weight according to the sedimentary conditions (e.g. 25 kg to 40 kg in mud or muddy sands and 70 kg to 100 kg in coarser sediments, dependent on initial grab mass). Similarly, most corers can be weighted according to sampling conditions. This is usually carried out before commencing the sampling. If required by national accreditation schemes, the precise sampling area of the grab should be verified at regular intervals, particularly after prolonged use or sampling in hard packed or silty sediments, which may distort the bite action.

A grab type with a benthos fauna sampling area of 0,1 m^2 should be used in the majority of surveys. If a long-term investigation has been carried out using a different sampling area, it is permissible to continue its use. However, for large-scale integration, it is highly recommended that new faunal investigations use 0,1 m^2.

Grabs and corers with smaller sample volumes are used in certain types of surveys, and are representative if the faunal density is high and homogeneously distributed. In special cases, such as in shallow or enclosed waters, or where a vessel with the appropriate heavy rigging for benthic sampling equipment cannot be used, smaller hand-operated grabs and coring devices may be used. Small grabs should have a minimum sampling area of 0,02 m^2 and top flaps to prevent loss of or disturbance to sediment (essential for most background parameters). On the other hand, coarse sediments or sampling in deep water may require the use of a larger type of grab or box-corer.

A brief overview of grabs and box-corers is given in Annex A. Further guidelines on choice of equipment in relation to sampling conditions and requirements are given in References [11][13].

4.2.4 Sampling procedure

The apparatus should be lowered vertically towards the sea floor, at an even rate, at a speed that avoids triggering the mechanism. Care should be taken to keep the survey vessel in position and the wire vertical during lowering. Between approximately 5 m and 10 m above the sea floor, the lowering speed should be reduced to a maximum of 0,2 m/s in order to reduce further the bow-wave and water turbulence in front of the apparatus. Contact with the sea floor is observed by the slack on the wire, after which the apparatus is gently raised for approximately the first 5 m. Then the apparatus should have closed, left the bottom and can be raised with maximum safe speed. Appropriate equipment for receiving and processing the samples should be ready on deck. The samples should be inspected for approval via the top opening flaps immediately upon retrieval on deck. Sediment characteristics and background information should be recorded before sieving.

Any sampling for background sediment descriptors is carried out at this point. The rest of the sample is then discarded. Only complete samples should be kept for faunal analysis. If a splitting of the samples is

necessary, e.g. into vertical sedimentary layers for vertical faunal distribution analyses, this shall be done before sieving. The sample can be transferred to a barrel to await sieving, but sieving should commence as soon as possible. When air temperature is less than 12 °C, sieving can, if necessary, be postponed for up to 8 h to 10 h. It is recommended that sampling on shallow stations (<70 m) be conducted during daytime, because some benthic organisms have semipelagic activity during the night.

The apparatus should be rinsed thoroughly between each sample. In areas where there is a risk of transfer of infectious agents, e.g. near aquaculture sites, the apparatus should be disinfected before moving to a new area, e.g. between cage groups. Disinfections should be carried out in accordance with the appropriate regulations. If sampling is for bioassays and/or sediment chemistry in conjunction with benthic faunal sampling, the appropriate guidelines should be followed for cleaning the sampling device.

4.2.5 Guidelines for approval or rejection of samples

Samples from different sampling apparatus require different rejection criteria. To be approved, a sample from a large grab should contain the upper layer of sediment, and a volume of at least 5 l with sand or 10 l with sediment mud. Alternatively, bite depth may be measured, 5 cm for sand and 7 cm for mud. The sediment surface should be undisturbed. Samples from a smaller grab should contain the upper layer of sediment and an appropriate volume or bite depth (depending on the grab size). Samples collected by corer should include the sediment surface and be at least 7 cm to 10 cm in length. Samples for which the apparatus has not closed properly and the draining supernatant water has damaged the sediment surface should be discarded, as should those where the bite is obviously uneven. Any samples discarded, together with the cause, should be noted in the field log.

If sediment characteristics make it impossible to collect approved samples, the best available samples should be retained, and the circumstances noted in the field log.

4.2.6 Sieving of samples in the field

Approved samples should be sieved using sea water to remove the fine sedimentary material. The sample is emptied on to a washing table, hopper, autosiever or other container, where the sample material can be washed out gradually into the sieves as a suspension. National and individual requirements for mesh size vary. For most routine environmental monitoring surveys, a mesh screen of 1 mm aperture size should be used as the finest sieve, but in particular cases, e.g. studies of juvenile recruitment or estuarine studies, sieves of 0,5 mm and 1 mm aperture sizes can be used in series. In the latter case, the 1 mm and the 0,5 mm fractions should be processed separately for comparability, e.g. with other surveys using only 1 mm sieves. This separation procedure is best carried out in the field, because live and fixed materials behave differently during sieving. If there is any fractionating of samples in the laboratory, it should be done using a consistent procedure. The sieve aperture size(s) used should be recorded in the field log. Sieves should be quality controlled, each carrying a calibration certificate and conform to recognized quality standards.

Samples that contain large amounts of coarse material can, if appropriate, be passed through a sieve stack, usually with aperture sizes of 5 mm and 1 mm. During sieving, the sieves should be placed in a water bath deep enough to cover the mesh screen and “puddled“ to remove remaining fine material, unless using an autosiever. The use of a water bath greatly reduces damage to the organisms caused by direct hosing on to the sieve mesh.

The samples should be washed and sieved with sea water. Polychaetes, amphipods, and oligochaetes in particular are very fragile. During sieving, the sieve may be agitated gently, and in the event of blocking, the mesh screen can be rinsed gently from the underside. Sieving is complete as soon as the fine material is washed out of the sample. Long sieving times should be avoided because small animals may actively pass through the sieve and there is greater risk of damaging the fauna. Appropriate characteristics should be noted in the field log.

If the sediments contain a large component of firm glacio-marine clay, processing should be divided into two phases. Firstly, the soft upper layer of sediment that is rich in animal life should be sieved and the sieve residue carefully transferred into a separate sample container. Thereafter, the more compact clay should be given a firmer treatment during washing. If necessary, the clay may be broken up manually, in

search of burrowing organisms. Should it take an unreasonably long time to dissolve the clay, clay lumps that appear not to contain animals may be put into separate sample containers and fixed for subsequent washing in the laboratory.

If the sample contains animals that produce slime (e.g. *Chaetopterus*, *Cerianthus*, *Myxine*) or large, heavy molluscs and echinoids (*Arctica*, *Brissopsis*), these should be placed in separate plastic bags or jars and fixed (see 4.3) before being placed in the container along with the rest of the sample. Large stones, shells, stick, etc. which can cause damage to the sample material during transport, should be kept in separate containers or discarded if devoid of encrusting fauna. If the sample is not processed immediately, obvious predators should be separated from the rest of the sample.

After completion of sieving, the sample should be carefully washed to the edge of the sieve and carefully flushed into appropriate sample containers, e.g. plastic jars or small plastic buckets equipped with watertight lids/delete. It is recommended that this be carried out over a mesh screen or sieve, as a safety measure in case of accidental spillage.

The sample containers should be indelibly marked with the unique sample information externally (field name or project code, station number or code, replicate number and date). An additional label on waterproof paper containing the same information should be added internally. The sieving equipment should be washed clean between each sample to ensure that material is not transferred between samples.

Fragile animals may be carefully washed or picked out of the sample during sieving, using soft forceps to prevent damage. Large objects, such as stones or empty shells, should be discarded if no attached fauna or shell borers are present or stored separately to prevent grinding during transfer and storage. If practicable, the use of spoons and other hard tools in the transfer of material should be avoided.

If any sample material is lost during processing, the entire sample should be discarded and a new sample collected.

4.3 Sample fixation

Sample fixation is the act of stopping the life functions of the organism tissues. Fixative solutions are not suited for long-term storage. Preservation is accomplished by the transfer of the fixed organisms to ethanol, wherein they can be stored almost indefinitely.

Samples should be fixed as soon as possible after sieving using diluted formalin. If specimens are not required for long-term storage, fixation in ethanol should be considered. Ethanol-fixed specimens can be further used for analysis of genetic material. There is no fixed rule for the appropriate amount of formalin to apply to “bulk” samples, because this depends on the type of sample, relative proportions and forms of animals, sediments and organic debris in the sample.

NOTE 1 Formaldehyde (CH_2O) is an organic compound which is available in liquid form. Formalin is a commercially sold aqueous saturated solution of formaldehyde at ~40 % volume fraction or ~37 % mass fraction. However, the precise contents may vary slightly between producers. A small amount of stabilizer, such as methanol, is usually added to limit oxidation and polymerization. A typical commercial-grade formalin solution cay contain a volume fraction of 10 % to 12 % methanol.

NOTE 2 Due to the health risks associated with formalin, a number of alternative compounds are available or under development. Use of formalin alternatives should be considered, if appropriate to requirements (sample quality, cost–benefit etc.).

For small sample volumes, where there are no particularly large animals or a dominance of tube-dwellers and most of the sediment has passed through the sieve, a 10 % volume fraction formalin sea water solution should be appropriate. Where the sample contains organic debris, tube-dwelling polychaetes, particularly large animals or a lot of residual sediment, especially in compact clay sediments, the formalin volume fraction should be increased to around 20 %. Extreme cases may even require 30 % volume fraction.

Buffer should be added to neutralize acidity, e.g. a 2 g/l solution of sodium tetraborate ($Na_2B_4O_7{\cdot}10H_2O$; common name borax). The pH of the formalin solution should not be below 7. Because the pH level decreases over time, additional buffer should be added as required to samples kept in storage prior to

sorting. This should be determined by periodic checks on pH levels in the stored samples. More buffer should be added if the pH drops below 7.

There should be approximately the same amount of solution or more than solid material in the sample container. Large shells may, if deemed necessary, be opened or slightly perforated to allow the fixative to penetrate to the animal tissues. The sample container should be gently inverted to mix in the fixative, which should remain in the sample for at least 2 h. The samples should be protected from frost during all stages of storage and transport.

The samples may be stained as required. Staining increases sorting efficiency, but too much stain can reduce the value of the material for taxonomic and analytical purposes by masking certain features or causing visual discomfort during binocular microscopy work. The recommended stain is rose Bengal. Typical rose Bengal concentrations in formalin range from 0,1 mg/ml to 0,4 mg/ml, i.e. 1 g to 4 g rose Bengal powder to 10 l formalin, although this is highly dependent on the nature of the sample and user colour intensity preferences (see previous).

Alternatively, stain can be applied in the laboratory, where the sample should be washed to remove formalin and then immersed in stain for 20 min. Handle rose Bengal according to appropriate health and safety procedures.

NOTE 3 In certain circumstances, it is possible or desirable to process the samples before fixation (live specimens). Examples include where specimens are to be examined for taxonomic purposes and/or photographed. Live sample processing can also be carried out where the use of chemicals is undesirable, e.g. in protected environments or in cases of allergy. See Annex B for comments on processing samples prior to fixation (live specimens).

4.4 Background environmental descriptors

Background environmental descriptors can provide information on the sediments at the sampling stations, and physical and chemical properties of the water masses in the survey area.

An understanding of these is essential for correct interpretation of the faunal data or as required as part of any set programme.

The background parameters selected should reflect the most influential environmental conditions. Table 5 lists some of the main descriptors used in benthic surveys.

Table 5 — Main environmental descriptors, methods and information value

Matrix	Environmental descriptor[a]	Method	Information value	Data quality
Sediment: field measurements	General description of bottom sediments	List the dominant sediment types (clay, sand, pebbles, boulders etc.). For mixed sediments, estimate the relative abundance of each type	General characterization	Qualitative
	Assessment of sediment oxidation state	Assess the sediment colour and smell. Black sediments or patches of sediment indicate anoxic conditions. Depth of anoxic layer and intensity of e.g. methane/H_2O smell can be scaled	General characterization	Qualitative
		Standardized colour classification according to *Munsell® soil color charts* (Reference [12])	General characterization	Semi-quantitative
		Redox — electronically: standardized against reference cell	Measure of sediment oxygenation; often carried out along a depth profile in the sediment to assess redox potential discontinuity (RPD) depth	Quantitative
		Interstitial dissolved oxygen (DO) — gas-sensitive probes	Measure of sediment oxygenation	Quantitative
[a] Procedures should be carried out in accordance with relevant International Standard guidelines, where these exist.				

Table 5 *(continued)*

Matrix	Environmental descriptor[a]	Method	Information value	Data quality
Sediment: laboratory measurements	Sediment granulometry (fractions expressed as % dry mass)	Dry sieving, laser granulometry or coultercounter, according to Wentworth grade classification, References [8][14] Silt or clay (<63 µm), very fine sand (63 µm to 125 µm), fine sand (125 µm to 250 µm), medium sand (250 µm to 500 µm), coarse sand (500 µm to 1 mm), very coarse sand (1 mm to 2 mm), gravel (2 mm to 64 mm) and pebbles (>64 mm). Additionally, assessment of the <16 µm sediment fraction is recommended in estuarine areas	Indicates sediment homogeneity or heterogeneity and sorting. Important in determining faunal community composition and relationship to organic or trace metal/contaminant content. Gives useful indication of bottom current conditions.	Quantitative Method used shall be quoted in any report, as results can vary.
	Total organic carbon (TOC) of the sediment	Use of element analyser on dried sediment, excluding particles >2 mm	Gives an indication of food availability to benthic fauna. Note that not all TOC is bio-available. Can also indicate degree of organic enrichment. Should preferably be normalized for sediment particle size composition	Quantitative
	Total organic matter (C and N)	Loss on ignition (Lol). Sediments dried in muffle furnace	Simple surrogate for TOC indicating degree of organic enrichment	Quantitative
	In coastal areas also total nitrogen (TN) and total phosphorus (TP) if applicable	Use of element analyser on dried sediment, excluding particles >2 mm	TOC: TN ratio indicates extent of terrestrial input. TN and TP useful in areas at risk of eutrophication	Quantitative
	Sediment water content or equivalent dry substance	Calculated from mass loss after drying	Indicates degree of compactness of the sediment. Particularly useful where this is altered by anthropogenic impact	Quantitative
	lnterstitial water salinity (in estuaries)	Draining or squeezing	Assists in explaining species distribution in areas of high surface run-off	Quantitative
	Total sulfide	Titration against iodine	Particularly useful around aquaculture installations. Relates to degree of reduction in sediments due to organic deposition and deoxygenation	Quantitative
	Trace metal or. other known contaminants	Flame AAS	Useful in describing anthropogenic impacts around discharges	Quantitative
Aquatic: field measurements	Salinity Temperature pH DO	Meter analyses calibrated against known standards. Refractometer if lower accuracy and precision is needed or acceptable	May be recorded from the bottom water or through a depth profile. These should be carried out as close as possible to the end of the stagnation period, normally in late summer or autumn (see ISO 5813[4] and ISO 5814[5])	Quantitative

[a] Procedures should be carried out in accordance with relevant International Standard guidelines, where these exist.

When using a grab or box-corer, the sediment sample for background environmental descriptors should be taken out through the inspection ports on the top of the sampling device. The surface of the sediment should be undisturbed. The sediment samples should be put into plastic jars or bags and frozen at –20 °C for storage.

If a high level of precision is required for the sediment analyses or if sectioning of the sediment profile is to be carried out, a coring device should be used in preference to a grab, because the latter can distort the sediment. However, most well-designed grabs, appropriately weighted, provide an adequate sample for the analyses used to support faunal data.

Samples to be analysed in either the field or laboratory for environmental descriptors should not be taken from the faunal samples but from separate or additional replicate samples. This avoids loss of material from the faunal sample. However, in heterogeneous areas, intersample variation should be borne in mind, and it may be advantageous to take several replicates for environmental descriptors

as well as for faunal analyses. In addition, the faunal biomass can be measured in the laboratory (see Annex C).

Descriptors appropriate to the aims of the survey should be agreed during the design of the survey strategy, i.e. they are not all necessary in every case. Field descriptors are very useful, and such data should be recorded in the field log: these should include qualitative sediment descriptions. It may be appropriate to use video surveillance to assist in this process.

It is, however, customary to carry out quantitative laboratory measurements of at least sediment granulometry and a measure of carbon or organic content. In aquaculture surveys it is usual, in addition, to measure total sulfide, redox, trace metals (Cu and Zn) and often pH. Separate samples may also be taken for contaminant analyses (e.g. trace metals) as required (see ISO 5667-19[3]). This is useful in areas where gradients of impact are suspected.

Samples for element analyses in the laboratory should be determined from the surface 0 cm to 1 cm layer of sediment. If sediment mixing by bioturbation is an issue, element analyses may be carried out on vertically sectioned sub-samples, usually at 1 cm intervals down to 5 cm. Granulometric analyses should usually be carried out on the 0 cm to 5 cm layer, especially in areas where the top layer is extremely flocculent and not representative of the sediments as a whole, but may also be vertically sectioned if appropriate to the survey aims.

5 Sample processing in the laboratory

5.1 Sorting

A sorting log should be compiled, either as a continuation of the sample log used in the field or a separate but compatible document. Any general notes on the sample, e.g. its general appearance or any anomalies in fixation, may be recorded here, together with the name of the sorter for traceability purposes. Additional useful information includes the time taken for sorting and, if deemed excessive, the reason for this.

The formalin solution should be rinsed from the sample in the laboratory in a ventilated sink or under a fume extractor, using a mesh screen with aperture size the same or smaller than that used in the field. Provided biomass measurements are not going to be carried out, samples which should be stored for more than one to two months before sorting may, to advantage, be washed and transferred in their entirety to ethanol, particularly if the material is for taxonomic investigation.

The sample material should be sorted under suitable magnification. As a general rule, all fauna should be extracted from the residue, but in cases where there is a large volume of sample material or faunal densities, it may be acceptable to sub-sample, see Annex D. The method of sub-sampling used is recorded in the sample log.

All fauna should be sorted into the main taxonomic groups, which are placed in separate sample vials each with an identification label. Large forms, such as large shells, starfish and sea urchins, should be kept in separate vials or jars. Tubes should not be removed from polychaete worms at this stage because of the risk of specimen damage and also because these are informative for identification, see Annex C for issues with biomass measurements. Animals attached to stones and organisms that could easily be confused with abiotic material such as Foraminifera, should also be kept in separate vials. The sorted material should be placed in alcohol of volume fraction 75 % to 80 % (ethanol is preferable, but industrial methylated spirits may be used if the specimens are not for long-term storage). If wet mass analyses are to be carried out, this should be done before transfer to ethanol, to avoid osmotic mass loss. For long-term storage, glycerol may to advantage be added to the alcohol (at a volume fraction of 10 % to 20 %) to protect specimens from drying out.

A consistent sample- and vial-labelling system assuring sample integrity and traceability is required. Sample information should be written or pre-printed on the label using alcohol-proof black ink or soft lead pencil as appropriate on waterproof paper. With pre-printed labels, particular care should be paid

to the choice of ink type and printing process to ensure durability. The following information should be recorded on the labels:

— field name or project code;

— sampling station number;

— replicate number;

— date of sampling;

— animal group;

— initials of sorter.

5.2 Sample residue

The volume and composition of the sample residue, i.e. the material remaining after extraction of benthic macrofauna, should be recorded in the sorting log (e.g. fruit pips or seeds, mineral sand, shell remains, wood fibres, slag). These notes may in future help to interpret any anomalies in faunal composition. Unless otherwise specified, the residue is retained until completion of control sorting and up to the limits stated within any quality procedures and/or national guidance.

6 Taxon determination and quantification

6.1 Level of identification and taxon lists

The fauna should be identified to the lowest taxonomic level possible or that appropriate to the aim of the survey.

NOTE Taxonomic level used is not always a direct measure of accuracy. In some cases, species level identification is not appropriate or possible due to taxonomic confusion, lack of specialist competence, time constraints (according to the aims and required precision of the survey) or lack of intact specimens. In such cases identification to a taxonomic level higher than species becomes the most appropriate solution.

The nomenclature used should be in accordance with recent editions of general faunal works and an agreed regularly updated literature checklist or relevant catalogues of benthic fauna, such as the *European register of marine species* (ERMS, Reference [10]), and/or *World register of marine species* (WoRMS, Reference [8]). References to useful faunal lists are given in the bibliography. Where a taxon is not listed in such a catalogue, the reference to the original description should be given together with any additional identification literature used. Where a taxon has changed its name since list publication, then the new reference should be cited.

Unless otherwise specified in the survey programme outline, the following animal groups may be identified to a higher taxonomic level for routine monitoring purposes:

— Platyhelminthes;

— Nemertea;

— Nematoda including the larger macrobenthic forms;

— Oligochaeta unless important to the survey aims (see Note 2 below), and where good identification literature exists, in which case identification to genus is appropriate;

— harpacticoid copepods;

— Chironomidae and insects in general;

— Hemichordata.

In certain coastal sea areas, where oligochaetes, insects and chironomids are essential in status assessment (e.g. the northern Baltic), it is recommended that the taxonomic identification of the aforementioned groups should be done to the lowest feasible level.

6.2 Quantification

For fragmented specimens, body parts that can be identified unequivocally, such as the head in annelids or the mouthparts of brittle stars, should be counted. Unless otherwise specified in the survey programme outline, the following animal groups are not quantified, but their presence should be noted:

— Foraminifera;

— Nematoda;

— Cirripedia;

— colonial Porifera, Cnidaria and Bryozoa;

— planktonic organisms.

Taxa represented by a particularly large number of individuals may be sub-sampled before quantification, see Annex D. If a taxon is represented by a large number of juvenile individuals, i.e. newly settled larvae, the adult and juvenile specimens should be recorded separately.

Identification of encrusting organisms on stones should be carried out in a manner appropriate to the survey aims. Where very high numbers occur, e.g. *Filograna* or newly settled barnacles, these may be sub-sampled.

Particular in-house guidance is required to define the size limits or stage of maturity used to make a practical definition of juveniles in the relevant area. For example, suitable size-controlled objects or images attached to the bottom of a Petri dish may be used to help identifiers quantify juveniles and adults in a rapid and standardized manner.

Measurements of shell size in molluscs or measurements of length–width in other taxa may be carried out if appropriate to the survey aims.

6.3 Reference collection

At least one specimen, but preferably as many as practical, of each identified taxon should be placed in separate vials in the reference collection. Nomenclature should follow standard taxon lists, e.g. national lists, ERMS (Reference [10]), and/or WoRMS (Reference [8]).

The collection should contain at least the following information:

a) species name;

b) name of identifier and supervisor, if appropriate;

c) sampling location or station code;

d) date of collection;

e) verification date, if any, and name of verifier;

f) any nomenclatural changes when these differ from the standard taxon list;

g) depth and sediment type if possible; this is time-saving for future reference to the specimens.

The collection should be updated continually during the course of surveys carried out, and be easily available for users. Particular attention should be paid to sealing of the vials. For particularly important specimens, the “double-vial“ practice carried out by museums is recommended. The reference collection also serves as part of quality control procedures (see 7.9).

6.4 Biomass

Biomass measurements give additional information and may be carried out as required. Wet mass analyses are preferable for routine and monitoring surveys. Analysis of dry mass and ash-free dry mass may be carried out in certain circumstances, but is not generally recommended for faunal studies as the material is destroyed in the process. Annex C gives further information on wet mass analyses.

6.5 Data reporting

The taxon lists should reflect the complete faunal content of the samples. Pelagic organisms, colonial forms and groups which, due to small size, are not quantitatively represented in the samples (e.g. Nematoda) should be marked as such in the taxon lists.

The data should be recorded as a taxon by individuals by station matrix in electronic spreadsheet format or a relational database.

The following information and analyses are carried out as a minimum for scientific reporting.

a) Complete list of taxa along with numbers of individuals within each taxon, specified to unit sampling area. If identifications are based on damaged or incomplete specimens, this shall be stated. If mean abundances are used, the standard deviation or standard error shall be given.

b) Number of taxa and number of individuals in each sample and at each sampling station.

c) Biomass as appropriate to the individual survey aims.

d) Derived statistics of faunal diversity as appropriate to the individual survey aims.

e) Reference to national and/or international habitat or biotope classification schemes.

f) Information on any sub-sampling and the volume used.

6.6 Storage and archiving

For long-term storage, the biological material should be kept in alcohol of volume fraction 75 % to 80 %, with glycerol at a volume fraction of 10 % to 20 % added, if appropriate. Further guidance on long-term storage is given in ISO 5667-3.[1]

Processed material should be stored in facilities suitable for long-term storage. The storage area should be fireproof and preferably lockable. The material should be easily accessible for periodic checks and refilling of preservative.

In principle, all material is retained for as long as is practicable. However, certain quality procedures specify a fixed storage time. For particularly valuable material, an agreement should be made for permanent storage of the material, e.g. at an appropriate natural history museum.

All documentation for stored material should be properly archived and retained for at least as long as the samples are stored.

Data should be stored in an appropriate system with backup, at the appropriate level of access (ranging from delivery of data to public national or international data repositories or databases, or kept confidential to client or owner). With emerging needs for understanding large-scale, long-term trends in benthic macrofauna as part of studies on climate-change and multiple pressures, possibilities for retrieval of both historical and new data sets is of increasing importance. Synergy between consultancy, monitoring and research is advantageous.

7 Quality assurance and quality control

7.1 General

QA and quality control (QC) measures should be incorporated during all stages of benthic sampling and sample processing programmes. These principles ensure that all data produced are of a specified quality, and that all parts of the work are carried out in a standardized and intercomparable manner.

All procedures should be clearly described and carried out openly, such that all of the activities of the laboratory can be audited internally and externally at any time.

It is particularly important that adequate resources be allocated for these purposes when co-operative studies involving several institutes are to be conducted or when the data are to be centrally archived.

A laboratory meeting the requirements of ISO/IEC 17025[16] can be accredited by an accreditation body.

Further recommendations on QA practices are given in Reference [13].

QA or QC schemes for soft sediment macrofauna surveys should encompass the following:

— participation in interlaboratory comparisons where available;

— training on a regular basis and training records;

— traceability of work and samples;

— standardized practices;

— standardization and calibration of sampling and sample-processing equipment;

— in-house and external audit on a regular basis;

— literature updates;

— reference collections.

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

7.2 Auditing

Laboratories should participate in internal or external audits and where available ring tests.

External audits can take a variety of forms. A full or partial audit on a random selection of processed samples, including sample residue, by an appointed person outside the laboratory is recommended. The frequency and level of detail should be determined as appropriate, but is often dictated by national requirements. Agreed standards can be applied and a pass or fail tag applied to the data.

Training is required for internal audits, but these can then be completed by appointed scientific staff within the organization.

7.3 Equipment calibration and operating safety

The technical quality of the equipment used should be verified at appropriate intervals. The most important of these are:

— operational safety should comply with health and safety requirements/regulations;

— accuracy of depth and position fixing equipment;

— grab bite area;

— sieve mesh apertures (most sieves have manufacturer certification);

— microscope maintenance, including periodic recalibration of eyepiece graticules, if used.

Any other laboratory equipment should also be included in a regular checking system.

7.4 Training

It is necessary for all participating staff to be given the appropriate training and that a minimum level of competence be achieved and documented. This includes all parts of the process, from sample collection, processing and documentation.

Staff should participate in appropriate workshops and courses when possible.

7.5 Checklists, sample log and anomaly reporting

To ensure sample traceability, a system of checklists for samples should be developed, with a means of noting the progress of the sample through the various stages from sampling to data reporting. The worker associated with each stage in the process should be noted. These combined checklists and notes therefore form a detailed sample log.

This should ensure that, at any time, the whereabouts and status of the sample or its individual components are documented and readily available for internal use as well as for external audits.

A reporting system for anomalies found or operational errors should also be developed. Immediate action should be taken to reduce the risk of re-occurrence.

NOTE Such checklists and reports are usually required as part of laboratory accreditation procedures.

7.6 Quality of sample sorting

An appropriate portion of the processed samples, randomly selected, should be subjected to QC. The person who carries out the original analyses should always be different from the one who carries out the QC. Targets can be set for each part of the process or as an overall target.

The appropriate proportion of samples which should be quality controlled depends on various factors including the required precision of results for the survey in question and the documented expertise of the sorting personnel. National guidelines, where available, should be followed.

An example of national guidelines is the UK NMBAQC scheme (Reference [15]). These state that a recommended minimum of three samples (randomly selected) if more than 100 samples are processed should be subjected to QC on the process of sorting (as well as quantification and identification). Note that other countries or laboratories may operate to more rigorous QC practices for sorting, e.g. control sorting of 10 % or more of samples, depending on the survey type.

7.7 Quality of taxon identification

In practice, there are the following principles of QA and QC which shall be carefully observed.

— The identifications should be scientifically correct and follow up-to-date taxonomic knowledge and faunal nomenclature.

— The taxonomic level of identification should be appropriate to the specimens in the sample. Specimens which are grouped to a higher taxonomic level due to inability to identify them (e.g. damaged or particularly time-consuming taxa) should be distinguished from those which are recognized as belonging to a discrete taxon (e.g. species), but which remains unnamed either due to lack of literature or knowledge or because it may be new to science.

— The identification practices carried out should be consistent at least within a single survey (or within a set of surveys where spatial and/or temporal trends are analysed), and in particular where several identifiers are involved in processing a batch of samples.

Each identifier should have a proven level of relevant taxonomic competence. Where available, identifiers should participate in national or international ring tests and other efforts towards taxonomic standardization (see, for example, Reference [15]).

NOTE It is useful for the laboratory to send a selection of identified specimens to an auditor if available, or willing expert or external colleague, for checking. In regions where a formal taxonomic auditing scheme is established, the auditor selects the taxa required for checking. If appropriate, entire vials of identified material can be reprocessed by an internal or external auditor to check accuracy of taxon identification and quantification.

For in-house standardization among a team of identifiers, and/or among individual surveys, the respective taxon lists should be checked against each other to ensure consistency in:

— taxonomic level used, especially for difficult or time-consuming taxa;

— taxonomic conventions followed;

— distinctions between juveniles and adults;

— quantification accuracy.

7.8 Identification literature

The latest taxonomic literature should be used. Name changes and literature used shall be recorded. A list of literature used for taxonomic identification of the different faunal groups should be compiled by each institute. The list should be updated at regular intervals and should reflect recent advances in the taxonomic literature.

7.9 Reference and collection

It is recommended that a reference collection be kept of all taxa identified by the institute or laboratory. Interlaboratory validation of the reference collection is recommended. Wherever possible, relevant taxonomic experts, e.g. museum personnel, should be asked to check specimens that are difficult to determine.

Any identifier who is not formally associated with the institute or laboratory concerned, or who does not have access to the reference collection while working, should make a separate reference collection. In certain circumstances, a separate reference collection may be required for individual surveys.

Annex A
(informative)

Sampling devices

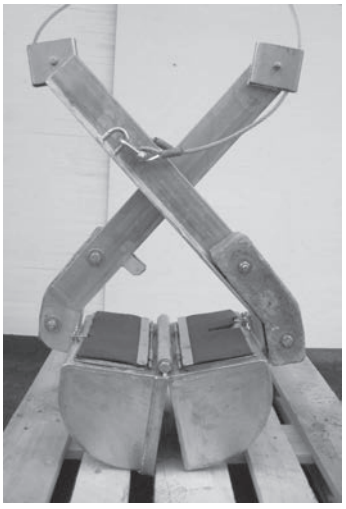
A.1 General

Because of the continual development and refinement of sampling devices, this annex is not intended to be comprehensive. A wealth of information on the construction and utility of the various types of sampling devices can be found online.

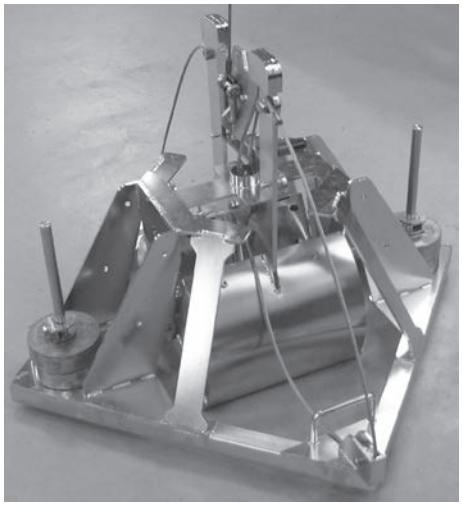
A.2 Grabs

A.2.1 General

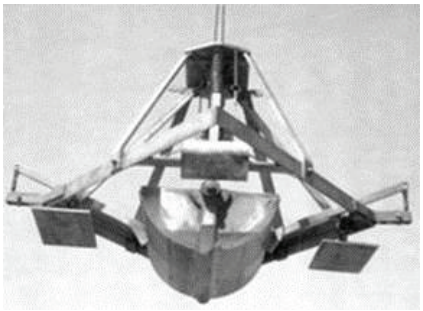
The recommended types of grab are the van Veen grab, the Day grab and the Smith-McIntyre grab (see Figure A.1). The Hamon grab is also widely used in gravelly deposits. The top of the grab should be equipped with inspection ports, covered with fine mesh netting (pore aperture the same or smaller than sieve pore dimensions) and/or an overlying rubber, metal or plastic flap. The flap is hinged and flexible and attached so as to allow flow-through of water while the grab is being lowered to the bottom, and so that it closes while the grab is being raised, to prevent the surface sediment from being washed out of the grab or disturbed (important for background sediment samples). The mass of the grab is adjusted by assembling additional masses as required.



a) van Veen grab; image source: Akvaplan-niva, reproduced with permission



b) Day grab; image source: KC Denmark, reproduced with permission



c) Smith-McIntyre grab; image source: Geo Seabed Instruments AS, reproduced with permission

Figure A.1 — Examples of commonly used grabs

A.2.2 Hamon grab

This is a frame-mounted grab with a lever-driven scoop action, widely used in gravelly sediments around the United Kingdom and English Channel (Figure A.2). The sampling area is about 0,25 m^2 and the grab can be weighted according to sediment consistency.

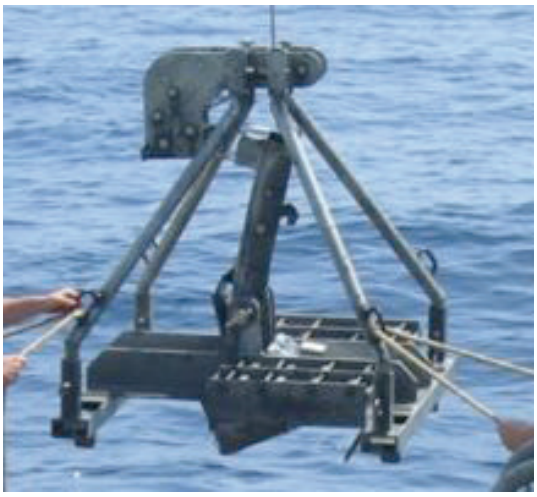


Figure A.2 — Example of a Hamon grab; image source: VLIZ (Flanders Marine Institute), reproduced with permission

A.2.3 Ponar grab

The Ponar grab sampler consists of two opposing semi-circular jaws that are normally held open by a trigger mechanism (Figure A.3). The sampler is lowered to the bottom where contact with the bottom sets off the trigger and a strong spring snaps the jaws shut, trapping a sample of the bottom inside. A fine copper screen covers the top of the jaws so that the trapped material does not wash out as the sampler is retrieved.

Figure A.3 — Example of a Ponar grab; image source: Rickly Hydrological Company, USA, reproduced with permission

A.3 Corers

A.3.1 General

Typical coring devices are the box-corer (large sample volume), HAPS and Craib single corers or various multiple corers based on the Craib principle, such as the OSIL[1)] series (small sample volumes).

A.3.2 Box-corers

Box-corers are described in Reference [11]. Light box-corers are the most appropriate for routine sampling purposes, but in general these have limited utility in sandy sediments. In special circumstances, larger box-corers with a sampling area of up to 0,25 m^2 [such as the Kastengreifer] may be used (Figure A.4). These devices may be appropriate for sampling in deep water (>750 m). Use of these large sampling devices requires a large vessel with strong rigging and the sampling generally is time-consuming.

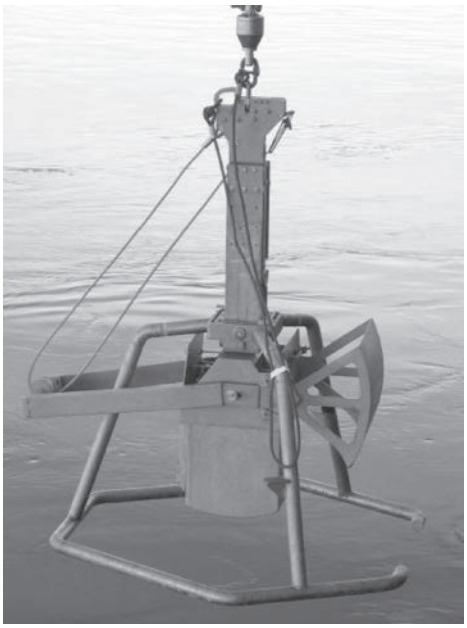


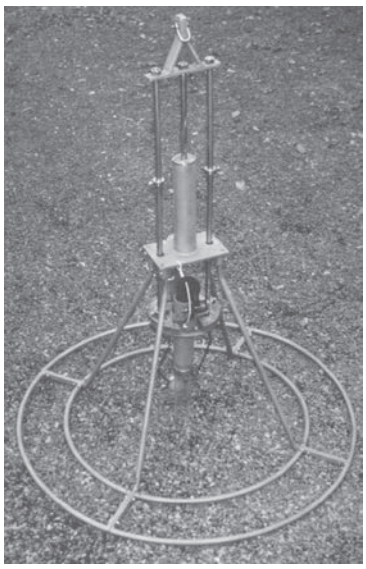
Figure A.4 — Example of a box corer of the Kastengreifer type, here as used onboard the research vessel *Polar Stern*, of the Alfred Wegener Institute, Germany; image source: Marine Geoscience Data System, USA, reproduced with permission

A.3.3 Craib corer and multiple corers

The Craib corer comprises a metal frame and a single cylindrical core that is pushed gently into the sediment by means of masses and a simple hydraulic mechanism [Figure A.5 a)]. The top of the core is open during descent, almost eliminating the bow wave. After penetration, the top of the core is sealed with a rubber bung and the core raised, drawing the sample out by suction. Once the bottom of the core tube emerges from the sediment, another rubber bung swings into place so that the core tube is sealed at both ends. Multiple-tube corers have been developed using the same principle [OSIL range of multiple corers; Figure A.5 b) and c)]. Various models are available with between 4 and 12 tubes, with a choice

1) The OSIL multiple corer is the trade name of a product supplied by OSIL. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of the product named. Equivalent products may be used if they can be shown to lead to the same results.

of two tube-lengths and diameters (64 cm or 110 cm). The smallest models are hand operated and are suitable for small boat work.



a) Craib corer; image source: Spartel, reproduced with permission

b) OSIL[1] multiple corer, model 1; image source: OSIL, reproduced with permission

c) OSIL[1] multiple corer, model 2; image source: OSIL, reproduced with permission

Figure A.5 — Examples of cylindrical sediment corers

Craib-type corers function extremely well in soft muddy to sandy sediments, but are less suitable in gravelly or stony areas.

Further information is given in ISO 5667-12,[2] Annex J, and Reference [11].

A.3.4 HAPS corer

The HAPS corer is a winch-operated cylindrical single-core sampler mounted within a robust square-based frame (Figure A.6). The core tube is pushed into the sediment by means of masses, after the device has settled on the bottom. The masses can be adjusted to compensate for sediment and weather conditions (up to 150 kg total mass). The HAPS corer is suitable for sampling in both soft, and mixed and hard sediments. Commonly manufactured core tube diameters are 133 mm (stainless steel) or 140 mm (polycarbonate). The HAPS corer is sealed during ascent by a plane shovel that is pressed under the core during the onset of the hauling process.

Figure A.6 — Example of a HAPS corer; image source: KC Denmark, reproduced with permission

A.4 Wilson Autosiever

The Wilson Autosiever is a framework supporting the sieve containing the sediment sample (Figure A.7). Underneath is a chamber of water within which a spinning spray head (dishwasher principle) is mounted, with the spray directed upwards. Waste sieved sediment is transported away via a pipe mounted on the underside of the water chamber.

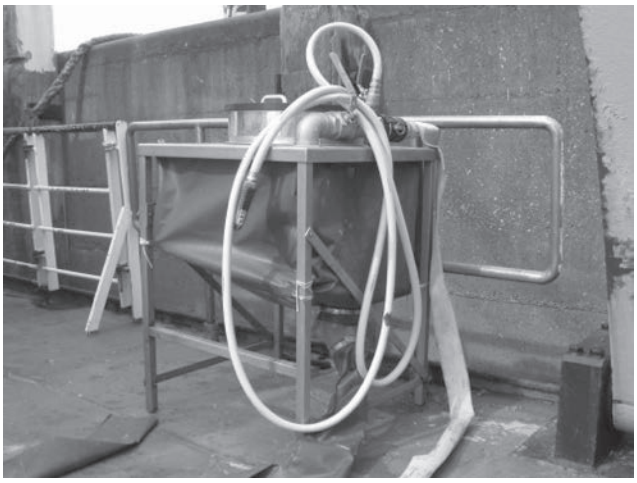


Figure A.7 — Example of a Wilson Autosiever; image source: VLIZ (Flanders Marine Institute), reproduced with permission

Use of a Wilson Autosiever is recommended in areas where the sediment does not readily pass through the sieves. Its construction and instructions for use are given in Reference [11].

Annex B
(informative)

Processing of samples with live specimens

Because many live specimens are almost transparent under low magnification, and are more vulnerable to damage by handling, it may not be possible to achieve the same level of quality of sample sorting (including control-sorting). Processing of live material is therefore not recommended for fully quantitative surveys, where a high level of QA is required for recording numerical abundance and numbers of taxa.

The advantage of using live organisms is that a range of anatomical features is visible, which are destroyed in preserved material, such as body form, pigmentation (including eyespots), gonads, and other internal structures as well as typical movement patterns that can support the identification of the correct species.

Processing of live material requires that only a short time elapses between sampling and analysis, and that the organisms arrive at the laboratory well preserved. Particular care should be exercised when handling live specimens, generally avoiding the use of forceps. Pipettes and fine artists' brushes can be helpful for moving specimens. Appropriate measures should be taken to avoid overheating of the specimens (cold light source, refrigeration, etc.).

In general, sampling should be carried out as described in 4.2, up to the point of completion of sieving and transfer to appropriate sample container. Thereafter, appropriate procedures should be adopted for sample transport from field to laboratory (including amount of water, ventilation, and cooling facilities). The specimens should be further processed according to the aims of the survey, for example first sorted into higher taxa (see 5.1), and then further handled according to fragility (e.g. annelids first, followed by crustaceans, echinoderms, and molluscs) or other appropriate priority (cannibalism, slime production, etc.).

Material may be fixed or preserved after processing, for reference purposes, biomass determination and/or analyses of genetic material, using appropriate dilution(s) of formalin, formalin-substitutes or ethanol, as appropriate to the aims of the survey (see 4.3).

Annex C
(informative)

Biomass measurements

C.1 General

A discussion of wet versus dry mass analyses, together with techniques is given in Reference [13]. For faunal analyses where the emphasis is on community structure and species composition rather than sediment energetics, non-destructive wet-mass analyses are recommended. Approaches for dry-mass and ash-free dry-mass biomass determination, see C.3 and C.4, respectively, are also given.

For wet-mass analyses, animals should be weighed before transfer to ethanol, complete with shell, tube, etc. where appropriate. Biomass measurements can be carried out at various levels of detail. The following alternatives may be appropriate.

a) The animals are weighed at the taxonomic level required by the survey objectives, for each replicate. For example, if information on the production of each individual species is required, this is to the lowest identified taxonomic level.

b) Key taxa are selected on the basis of prior knowledge of the dominant organisms. This gives some information on the production of species of ecological importance.

c) The major taxonomic groups are weighed collectively after sorting. This can reveal broad trends in environmental disturbance.

There are arguments for and against removal of tubes from polychaete worms before biomass analyses. Including them can inflate the recorded masses. Completely removing all parts of the tube can cause undue damage to the specimens (depending on the expertise of the worker). Further, in some cases (e.g. mucus or calcareous tubes), the tube is entirely created by the animal, thereby using energy resources. The decision whether to include or exclude tubes, or whether to distinguish types of tubes or even weigh them separately, should therefore be dictated by the individual survey aims and the fauna present.

C.2 Wet-mass biomass determination

Recently collected material is kept in buffered fixative for a recommended period of 3 months before wet mass analysis, to stabilize the mass. Practical issues relating to survey demands may dictate earlier analysis, in which case absolute values may be unreliable, but spatial information can still be informative. The type of fixative or preservative used should be specified.

A scale with at least 0,1 mg accuracy should be used for weighing, and porcelain or aluminium dishes should be used as tare containers. The tare mass should be determined, i.e. the mass of the dish being used without any sample material in it. This value should be recorded in the relevant section of the log. The organisms should be weighed at room temperature. Biomass values less than 0,1 mg shall be recorded as <0,1 mg, unless a higher level of accuracy is required.

The specimens should be removed from the sample container using appropriate tools. The sample should then be placed on absorbent paper to remove excess liquid, and then transferred to the tare container and placed on the scale. The animals are weighed at room temperature after blotting with filter paper to remove residual fluid. Specimens of Echinoidea (such as *Echinocardium cordatum*) and the shells of large bivalves should be punctured to drain excess fluid. Unless otherwise specified, organisms should be weighed with their shells on. See C.1 for comments on tube removal, particularly for Polychaeta.

The specimens should be returned to the sample container immediately after weighing, unless the sample is required for other purposes.

C.3 Dry-mass biomass determination

See C.1 for comments on destructive biomass analyses.

The sample should be dried at 60 °C in a drying cabinet for at least 12 h, to constant mass. Shells should be dried in a desiccator. After drying, the dry biomass is recorded using a scale with the appropriate level of accuracy.

C.4 Ash-free dry-mass biomass determination

See C.1 for comments on destructive biomass analyses.

The samples should be heated at 485 °C to 500 °C in a muffle furnace for at least 3 h to constant mass (the exact heating time depends on sample and organism size). During the drying process, the furnace temperature should be monitored using a calibrated thermometer, because muffle furnaces may show temperature fluctuations of ±50 °C. The temperature shall not exceed 550 °C because this results in undue mass loss. The samples should be cooled in a desiccator at room temperature for at least 1 h prior to weighing, using a scale with the appropriate level of accuracy.

Annex D
(informative)

Processing particularly large samples

In general, the sample material retained on the sieves should be processed in full. If both coarse and fine mesh screens (usually 5 mm and 1 mm) have been used, the material from the coarse sieve is sorted under 2× to 3× magnification, while the finer material (from the 1 mm mesh screen) is sorted under at least 3× to 6× magnification. In special cases, where samples contain large amounts (>2 l) of sieve residue, such as shell-sand or plant material, the methodology for processing may be amended as appropriate.

For samples containing large amounts of sand, flotation techniques may be used, in which the low-density material is separated from the rest of the sample during washing and sorted separately. The remaining material may be sub-sampled, but at least a quarter of the material should be sorted.

Samples containing large amounts of plant material may be stained, if appropriate depending on the type of plant material present, and sorted under low-power magnification. In the case of extremely large sample volumes, these may be sub-sampled.

If the entire sample is dominated by very high numbers of individuals of very few taxa, e.g. *Capitella capitata* in enriched areas, sub-sampling can be carried out prior to sorting, using appropriate equipment or strategy. When sub-sampling, to avoid bias, it is desirable to take several small sub-samples rather than one large sub-sample (if possible).

For samples containing taxa that are represented by particularly large numbers of individuals, these taxa may be sub-sampled before quantification. A minimum of one-tenth of the material should be counted. The remaining taxa are quantified in the usual manner.

If the procedures are thus modified, all taxa found in the sample should be recorded, even if the subsequent quantification is not entirely accurate. The procedures used are described and documented in detail.

Bibliography

Due to the large volume of literature available online and continual developments in the field, the references listed here are limited to those explaining the scientific basis of macrobenthic surveys or those specifically mentioned in the text (including annexes). Faunal check-lists for specific geographic areas or taxa are not given, but source literature is given in the cited web-based taxonomic checklists.

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[2] ISO 5667-12, *Water quality — Sampling — Part 12: Guidance on sampling of bottom sediments*

[3] ISO 5667-19, *Water quality — Sampling — Part 19: Guidance on sampling of marine sediments*

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