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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

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

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

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

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

ISO 16665 was prepared by Technical Committee ISO/TC 147, *Water quality*, Subcommittee SC 5, *Biological methods*.

Introduction

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

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

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

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

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

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

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

1 Scope

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

This International Standard encompasses:

— development of the sampling programme;

— requirements for sampling equipment;

— sampling and sample treatment in the field;

— sorting and species identification;

— storage of collected and processed material.

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

— bioassay sub-sampling;

— deep water (> 750 m) or offshore sampling;

— *in situ* faunal studies, e.g. recolonisation assays;

— nonbenthic organisms caught in the sampling device;

— estuarine sampling;

— intertidal sampling;

— meiofaunal sampling and analysis [3];

— sampling by dredge and sledge;

— Self-Contained Underwater Breathing Apparatus (SCUBA) sampling;

— statistical design.

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

2 Terms and definitions

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

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

2.2
benthic
associated with the sea floor

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

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

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

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

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

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

2.7
sampling station
precise location where samples are collected

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

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

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

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

2.11
subsample
ideally representative portion removed from a sample, taken for separate analysis

NOTE See Annex A.

3 Quality and safety

3.1 Health and safety requirements

3.1.1 General

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

3.1.2 Laboratory safety facilities

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

3.1.3 Vessel safety and operation of field equipment

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

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

3.1.4 Behaviour and training

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

3.1.5 Handling of chemicals

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

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

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

3.1.6 Equipment and protective clothing

Appropriate protective clothing should be made available. These include:

— in the field: helmet, safety boots, coveralls, life-jacket/floating suit (depending on the type of vessel), gloves;

— in the laboratory and store: aprons, gloves, goggles, gas-filters.

3.2 Quality assurance and quality control

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

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

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

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

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

— Germany (http://www.umweltbundesamt.de/wasser/themen/q-blmp.htm);

— UK - National Marine Biological Analytical Quality Control Scheme (http://www.nmbaqcs.org/).

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

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

— training and training records;

— traceability of work and samples;

— standardised practices throughout;

— calibration of sampling and sample processing equipment;

— in-house and external audit, also referred to as Analytical Quality Control schemes;

— literature updates;

— reference or voucher collections.

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

4 Strategies and objectives for soft-bottom faunal surveys

4.1 Sampling programme and plan

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

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

4.2 Positioning of sampling stations

4.2.1 General

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

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

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

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

— station network, see 4.2.2;

— randomly;

— stratified;

— transect;

— single-spot sampling, see 4.2.6.

4.2.2 Station network

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

4.2.3 Random or scattered sampling

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

4.2.4 Stratified sampling

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

4.2.5 Transect sampling

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

4.2.6 Single-spot (station) sampling

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

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

4.3 Reference stations

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

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

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

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

4.4 Types of survey

4.4.1 General

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

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.

Table 1 — Overview of main categories of survey type

Survey type	Objectives	User group	Precision of results
Pilot survey	Gives a general overview of bottom and faunal conditions. Used for 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/ environmental impact assessment	Characterises conditions in a given area. Also maps or identifies the impact of point-source discharges (spatial extent and intensity). Faunal composition is compared with specified assessment criteria or simply with other representative areas	Mainly regulatory authorities and consultancies. Research use for mapping, succession/ recolonisation or gradient studies	Medium to high, depending on individual requirements
Temporal trend monitoring	Describes 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

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/ 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.

4.4.2 Pilot survey

This is an initial assessment of faunal conditions in the bottom sediments in an area where the contamination source 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, such as baseline/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.

Pilot surveys can have another important use, namely to help design the size and calculate statistical power for future monitoring programmes. For this purpose, it is desirable to have the pilot study 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 summarised in 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 semi-quantitative 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 semi-quantitative 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 5.1.).
Reference station requirements	Should also be sampled, unless previous data exist to assess the status of reference areas.

4.4.3 Baseline survey/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 characterised 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 5.4 for comments on supplementary nonquantitative sampling.

Strategy and design for baseline surveys/environmental impact assessment are summarised in Table 3.

Table 3 — Strategy and design for baseline surveys/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	At least one, 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 5.1.).
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).

4.4.4 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 standardised 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 summarised in Table 4.

Table 4 — Strategy and design for temporal trend monitoring surveys

Sampling devices	Usually grab or box corer, supplemented if appropriate by semi-quantitative 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/ environmental impact assessment.
Minimum requirements for faunal assessment	As for baseline survey/ environmental impact assessment.
Optional sampling	As for baseline survey/ environmental impact assessment.
Field documentation required	Field log of sampling conditions and sediment description (see 5.1).
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 production.

The timing of sampling varies geographically. In certain areas, winter sampling is not possible due to ice cover, subzero temperatures or other unfavourable conditions (e.g. the Arctic and Baltic Sea). In these cases, spring and/or autumn sampling are the only alternatives. Where climate is not an issue, the preference is usually for winter sampling, with additional sampling in autumn, if appropriate.

4.5 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.

5 Sampling

5.1 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:

— person responsible for sampling;

— project or contract identification code;

— geographical co-ordinates for each sampling station (also for each replicate sample if required, e.g. in case of boat drift during sampling, see 5.2.2);

— 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, weight 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. The sample may be photographed, if wished:

— 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;

— main groups of large, easily visible animals present;

— anthropogenic debris, rubbish, sanitary products, plastics;

— other particular characteristics, for example, presence of oil, drilling mud, fish food pellets, stones, dead shells, terrestrial material, fruit pips/seeds as sewage indicator, etc.;

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

5.2 Sampling and sample processing in the field

5.2.1 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/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 hauler with boom or crane of sufficient height and correct lifting specification to allow unhampered retrieval of the sampling device on deck;

— wire 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;

— seawater hose with adjustable pressure and preferably sprinkler devices.

5.2.2 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/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.

Differential Global Positioning System (DGPS) with monitor should be used in all sea areas if possible. If the differential is not available, accuracy should be assessed and a minimum of Global Positioning System (GPS) without differential receiver used. Unless in open seas, sampling stations should also be defined using the direction and distance from landmarks or fixed points of reference, in addition to geographical coordinates.

When revisiting sampling stations poorly-defined in terms of geographical coordinates, the normalised 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 $\pm$ 50 m and $\pm$ 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 normalised to mean low water according to tide tables.

5.2.3 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.

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 hinged inspection ports covered with metal gauze (0,5 mm aperture size), extending over a minimum 60 % of the top surface, to reduce the bow-wave effect. 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. Weights can be added to adjust according to the sedimentary conditions (25 kg to 40 kg in mud/muddy sands and up to 70 kg to 100 kg in coarser sediments). 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 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 B. Further guidelines on choice of equipment in relation to sampling conditions and requirements are given in References [8], [17] and [18].

5.2.4 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 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 (see 5.1).

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. Any necessary sample splitting, e.g. into vertical sedimentary layers for vertical faunal distribution analyses, may be carried out 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.

5.2.5 Guidelines for approval/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.

5.2.6 Sieving of samples in the field

Approved samples should be sieved in the field using seawater to remove the fine sedimentary material. The sample is emptied onto 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. However, as a minimum, a laboratory should be consistent in its 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 recognised 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 onto the sieve mesh.

The samples should be washed and sieved with seawater. 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. 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/ jars and fixed (see 5.3) before being placed in the container along with the rest of the sample. Large stones, shells, sticks, 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, using a water stream applied to the underside of the sieve. 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/project code, station number/code, replicate number and date). An additional label on waterproof paper

containing the same information may 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. The use of spoons and other hard tools in the transfer of material should be avoided.

Should more than 5 % of the sample material be lost during processing, the entire sample should be discarded and a new sample collected.

5.3 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 formalin. Formalin is the common name for a dilution of formaldehyde (between 37 % and 41 % mass fraction formaldehyde). 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.

For small sample volumes, where no particularly large animals or dominance of tube-dwellers and most of the sediment passed has through the sieve, a 10 % formalin:seawater 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 concentration should be increased to 20 %. Extreme cases may even require 30 %. See 3.1 for health implications.

Buffer should be added to neutralise acidity, e.g. a 2 g/l solution of borax powder. 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 at least the same amount of solution in the sample container as solid material. 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 12 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 can seriously reduce the value of the material for taxonomic and analytical purposes by masking certain features. The recommended stain is rose Bengal. Sufficient strength of colour is achieved with approximately 1 mg per ml formalin, i.e. 1 g rose Bengal powder to 10 l formalin. 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. Rose Bengal should be handled according to appropriate health and safety procedures (see 3.1).

5.4 Background environmental descriptors

Background environmental descriptors can provide sedimentary data at the sampling stations, and physical and chemical properties of the water masses in the survey area. The selected background parameters represent the most influential environmental conditions. An understanding of these is essential for correct interpretation of the faunal data, or as required as part of any set programme. Table 5 lists the main descriptors used in benthic surveys.

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/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/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). 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 subsamples, 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.

Table 5 — Main environmental descriptors, methods and information value

Matrix	Environmental descriptor	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 characterisation	Qualitative
	Assessment of sediment oxidation state	1 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_2S smell can be scaled. 2 Standardised colour classification according to Munsell® Soil Color Charts [10]. 3 Redox – electronically : standardised against reference cell. 4 Interstitial DO – gas-sensitive probes.	General characterisation	Qualitative Semi-quantitative Quantitative Quantitative
Sediment: laboratory measurements	Sediment granulometry (fractions expressed as % dry mass)	Dry sieving, laser granulometry or coultercounter, according to "Wentworth grade classification" [2] and [23]. (Silt/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/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 larger than 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 normalised for sediment particle size composition.	Quantitative
	Total organic matter (C and N)	Loss on ignition (LOI). 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 larger than 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
	Interstitial 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 or HF.	Useful in describing anthropogenic impacts around discharges.	Quantitative
Aquatic: field measurements	Salinity/ Temperature/pH/ DO	Meter analyses calibrated against known standards.	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/autumn (see ISO 5813 and ISO 5814).	Quantitative

6 Sample processing in the laboratory

6.1 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, such as 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 for commercial institutes 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 subsample, see Annex A for further description. The method of subsampling used is recorded in the sample log.

All fauna should be sorted into the main taxonomic groups, which are placed in separate sample vials with identification label. Large forms, such as large shells, starfish and sea urchins, should be kept in separate vials/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 and Pogonophora, should also be kept in separate vials. The sorted material should be placed in 75 % to 80 % alcohol (IMS or ethanol). If wet mass analyses are to be carried out, this should be done before transfer to ethanol. For long-term storage, glycerol may to advantage be added to the alcohol (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 preprinted on the label using alcohol-proof black ink or soft lead pencil as appropriate on waterproof paper. In case of preprinted 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/project code;

— sampling station number;

— replicate number;

— date of sampling;

— animal group;

— initials of sorter.

6.2 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 (for example fruit pips/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.

7 Taxon determination and quantification

7.1 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. 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 [4]. 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;

— Nemertini;

— Nematoda including the larger macrobenthic forms;

— Oligochaeta unless important to the survey aims, and where good identification literature exists, in which case identification to “genus” is appropriate;

— Harpacticoid copepods;

— Chironomidae and insects in general;

— Hemichordata.

7.2 Quantification

In the case of 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 subsampled before quantification, see Annex A. 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 subsampled.

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 standardised 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.

7.3 Reference collection (see also 7.7.8)

It is recommended that a reference and voucher collection be kept of all taxa identified by the institute/laboratory. Interlaboratory validation of the reference collection is recommended. Wherever possible, relevant taxonomic experts such as museum personnel should be asked to check specimens that are difficult to determine. Nomenclature should follow standard taxon lists, e.g. national lists, European Register of Marine Species [4].

The collection should contain at least the following information:

— species name;

— name of identifier and supervisor, if appropriate;

— sampling location/ station code;

— date of collection;

— verification date, if any, and name of verifier;

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

— 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 7.7).

7.4 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.

7.5 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 (such as 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:

— complete list of taxa recorded and numbers of individuals within each taxon (if identifications based on damaged/incomplete specimens, this shall be stated);

— number of taxa and number of individuals in each sample and at each sampling station;

— biomass as appropriate to the individual survey aims;

— derived statistics of faunal diversity as appropriate to the individual survey aims;

— reference to national and/or international habitat or biotope classification schemes.

7.6 Storage and archiving

For long-term storage, the biological material should be kept in 75 % to 80 % alcohol, with 10 % to 20 % glycerol added, if appropriate.

NOTE Further guidance on long-term storage is given in ISO 5667-3.

Processed material should be stored in facilities suited 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.

7.7 Analytical quality control and quality assurance

7.7.1 Auditing

Where available, laboratories should participate in internal or external audits and 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/fail tag be applied to the data.

It is especially useful if the external auditor can identify particular problems which the laboratory can then address, thus putting in place a review system for improvement. Training is required for internal audits but these can then be completed by appointed scientific staff within the organisation.

7.7.2 Equipment calibration and operating safety

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

— 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.

7.7.3 Training

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

Staff should participate in appropriate workshops and courses whenever possible.

7.7.4 Check-lists, sample log and anomaly reporting

To ensure sample traceability (see also 3.2), a system of check-lists 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 normally required as part of laboratory accreditation procedures.

7.7.5 Sample sorting

A recommended minimum of 10 % of the processed samples, randomly selected, should be subjected to quality control (QC) on the processes of sorting, quantification and identification. 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.

7.7.6 Taxon identification

In practice, there are three levels of quality assurance and quality control for taxonomic characteristics:

— the identifications should be scientifically correct and follow updated taxonomic knowledge and faunal nomenclature;

— the identification practices carried out should be consistent at least within a single survey, and in particular where several identifiers are involved in processing a batch of samples;

— the quantifications should be accurate or within a specified level of accuracy for each individual sample in the case of very abundant taxa. For example if results vary by more than approximately 10 % or 10 individuals, then all the samples, or at least those taxa in the batch, should be requantified.

An appropriate target for taxonomic identification might be to remain within 10 % or $\pm$ 2 individuals, whichever is greater. This target may be related to the abundance of individuals, e.g. misidentified taxa should not account for $>$ 10 % of total abundance of all individuals.

Each identifier should have a proven level of relevant taxonomic competence. Where available, identifiers should participate in national/international ring tests and other efforts towards taxonomic standardisation.

NOTE It is useful for the laboratory to send a selection of identified specimens to an auditor if available, or willing expert/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 standardisation 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.

7.7.7 Identification literature

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. The list should be arranged taxonomically rather than by author, to quickly identify appropriate keys.

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

Annex A
(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 low magnification (×2 to ×3), while the finer material (from the 1 mm mesh screen) is sorted under a magnification of at least ×3 to ×6. 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 subsampled, but at least 1/4 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 subsampled.

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

For samples containing taxa that are represented by particularly large numbers of individuals, these taxa may be subsampled before quantification. A minimum of 1/10 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.