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SOFT-BOTTOM MACROFAUNA: COLLECTION, TREATMENT, AND QUALITY ASSURANCE OF SAMPLES

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Our cover photo was taken by N. Penny Holliday aboard the RRS "Discovery" in rough seas in the Rockall Trough.

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This series presents detailed descriptions of methods and procedures relating to chemical and biological measurements in the marine environment. Most techniques described have been selected for documentation based on performance in ICES or other intercalibration or intercomparison exercises: they have been carefully evaluated and shown to yield good results when correctly applied. They have also been subject to review by relevant ICES working groups, but this is not to be construed as constituting official recommendation by the Council.

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Abstract

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These recommendations are intended to standardize the methods for benthos surveys used by different scientists to increase the comparability of results for different areas.

The results of ICES/HELCOM Quality Assurance workshops, intercalibrations, and ring tests have been incorporated into this set of recommendations to increase the quality, reliability and, therefore, comparability of benthos data before their final evaluation and storage in public databanks. These recommendations are timely, given the increasing number of researchers and institutions that are engaged in sorting and analysing benthos samples. This document covers all steps, from the design of the sampling programme, to considerations of which gear to use, as well as all shipboard methods, such as sampling with grabs, corers, dredges, and trawls. There is no single, standard sampling gear for benthos investigations. The choice of an appropriate sampler depends on the average living depth of the infauna of interest; this depth can range from the uppermost millimetre down to almost 1 m. When analysing the results, possible discrepancies between the penetration depth of the sampler and the actual living depth of the organisms must be considered. The choice of a suitable sampler is a compromise between specific sampling characteristics in different sediment regimes in the area to be sampled, good handling characteristics at sea in bad weather conditions, suitability for various ships, financial limitations, tradition, and scientific questions posed. Criteria for the rejection of samples are identified. Treatment of samples is described in detail, including sieving, transferring of the sample to the sample vessel, fixation, staining, and labelling. Laboratory procedures for sorting, taxonomic identification, and biomass determination are described. A list of items for in-house quality assurance is included, as well as details for a warp-rigged van Veen grab.

Keywords: macrozoobenthos, sampling strategy, infauna, epifauna, sampling methods, rejection criteria, treatment of samples, quality assurance, in-house quality-assurance manual

1 Introduction

These recommendations[1] are intended to standardize the methods used by scientists for benthos surveys, to increase the comparability of results for different areas and to allow, *inter alia*, detection of large-scale changes in the system, which might not otherwise be detected by scientists or groups working in isolation. *Large scale* refers to inter-European, latitudinal or similar scales, which are beyond the sampling scope of most individual research programmes. The recent activities of the Marine Biodiversity and Ecosystem Functioning EU Network of Excellence (MarBEF)[2] demonstrate the utility of data collected over large areas; most of these data have been obtained using comparable methodology, as outlined in this document or earlier versions (Rumohr, 1990, 1999). In these recommendations, soft bottoms are defined as those with sediments ranging from mud to sand. For descriptive surveys, macrofauna are defined as animals retained on a 1 mm sieve (mesh size 1 × 1 mm). However, if a finer sieve is used for some other purpose, the 1 mm sieve fraction should always be studied and reported separately, to permit comparisons. For a more comprehensive treatment of sampling design, procedures, and alternatives, the reader is referred to other relevant publications, e.g. Kajak (1963), Cochran (1977), Elliott (1977), Green (1979), Downing and Rigler (1984), Baker and Wolff (1987), Eleftheriou and McIntyre (2005), and Jennings (1999).

This revision of the previously published guidelines (Rumohr, 1999) was necessary because of the development of quality-assurance procedures for benthic measurements at a series of ICES/HELCOM Workshops on Quality Assurance of Benthic Measurements in the Baltic Sea (ICES, 1994, 1996). The results of these workshops, intercalibrations, and ring tests have been incorporated in this set of recommendations, to increase the quality, reliability, and hence comparability of benthos data. This happens at a time when an increasing number of researchers and institutions are engaged in sorting and analysing benthos samples before their final evaluation and the storage of information in public databases. This update has also been checked against the ISO 16665: 2005 (ISO, 2005) guidelines, to ensure consistency. The ISO guidelines are more detailed than this document, and should be consulted if more information on procedures is required.

[1] This is a revision of recommendations originally developed by the Baltic Marine Biologists (BMB; Dybern *et al*., 1976). Several revisions have been made since by the ICES Benthos Ecology Working Group, with the aim of harmonizing methods, as much as possible, for the environments of the Baltic and North seas and the North European Seas (Rumohr, 1990, 1999). H. Rees (CEFAS) was engaged in all stages of the discussions and provided valuable information and amendments to the manuscript.

[2] See http://www.marbef.org/.

2 Sampling strategy

The design of the sampling programme largely depends on the detailed aims of the study. Temporal and spatial scales are important for the sampling strategy, as are the local abiotic factors. An awareness of resource limitations, e.g. time, money, laboratory facilities, is of great importance (Saila *et al*., 1976; Bros and Cowell, 1987). The various options in designing a sampling programme can only be mentioned briefly, and the reader is referred to various other relevant publications for details, e.g. Cochran (1977), Elliott (1977), Green (1979), Frontier (1983), Rees *et al*. (1991), Gray *et al*. (1991), Van der Meer (1997), and Underwood and Chapman (2005). It must be emphasized that the sampling strategy influences the options for statistical analyses of the data. Therefore, the sampling strategy can only be designed after the initial working hypothesis has been formulated, along with the intended statistical tests.

Some basic sampling designs used in benthos investigations include:

- time-series sampling (equidistant, at biologically relevant time intervals, BACI design (Stewart-Oaten *et al*., 1986; Underwood, 1992));
- stratified sampling (according to strata, depth, sediment type, etc.);
- randomized sampling;
- single-spot (station) sampling;
- area sampling (grid sampling);
- transect sampling (usually along a biological, physical or chemical–contaminant or nutrient–gradient)

Related design details include:

- number of samples;
- sample size (surface area);
- precision of results.

2.1 Sampling

There is no single standard sampling gear for benthos investigations. The choice of a suitable sampler is a compromise between specific sampling characteristics in different sediment regimes in the study area, good handling characteristics at sea in bad weather conditions, suitability for various ships, financial limitations, tradition, and the purpose of the survey. The time required for processing the samples and the level of sampling precision required will also influence the choice of sampling gear (Jensen, 1981; Kingston, 1988).

2.2 Infauna

The choice of sampler also depends on the average living depth of the infauna of interest; this depth can range from the uppermost millimetre down to almost 1 m (and more) below the sediment surface. It also clearly depends on the ability of the chosen sampler to penetrate the sediment effectively. One should always be aware of a possible discrepancy between these factors, namely, the penetration depth of the sampler and the living depth of the organisms. It is also important to consider the spatial structure and the size of the infauna of interest when analysing the results.

2.2.1 Boxcorer sampling

The boxcorer is generally recommended for North Sea benthos, because of its generally superior sampling characteristics, especially in sandy sediments. Its advantages are good penetration capability, relative lack of seabed disturbance, and minimal distortion of the sample. The disadvantages are chiefly the need for reasonably calm weather and for large vessels to handle this heavy and expensive gear.

A variety of boxcorer designs have been successfully employed in benthos surveys. Most are based on the Reineck "Kastengreifer" design (Reineck, 1963), for example, the "spade corer" by Hessler and Jumars (1974), which has been increasingly used in European waters, because of its reliability and large sample volume (0.25 m^2). The special feature of this sampler is the removable spade from the lever arm, which reduces handling time on board and keeps the sample reasonably undisturbed during processing. A 0.1 m^2 version of this corer is also used, which has good penetration capabilities, as revealed by closed-circuit television observations. Boxcorers with round "boxes" have also been successfully employed; these include the NIOZ-type (NIOZ; Royal Netherlands Institute for Sea Research) of a modified Reineck boxcorer with a flat spade, and the "HAPS" design used by Danish research institutes (Kanneworf and Nicolaisen, 1973).

Despite a lack of information on comparative efficiencies of different box samplers, the following features have been found useful and are recommended.

1) A sufficient number of easily removable weights must be available. In silty sediments, the weight of the corer must be adjusted, so that the box does not penetrate beyond its own height.

2) Light-operating, flexible flaps, preferably on top of the box, should be provided, to reduce the bow-wave effect when the sampler contacts the sediment surface.

3) To minimize handling time once the sampler is on board, the spade should be removable from the lever arm. With some types of box sampler, a closing plate has to be fitted between the spade and the box. This operation, which could be difficult, becomes unnecessary when the box containing the sample can be removed together with the spade.

Some precautions that must be taken when using box samplers that are similar to those required when using grabs. These precautions are listed in the next section.

2.2.2 Grab sampling

When, for various reasons, a boxcorer cannot be employed, the widely used van Veen grab (van Veen, 1933, with the modifications described by Dybern *et al*., 1976; see also Ankar, 1977; Kingston, 1988) is recommended. This grab is comparatively reliable, and it is easy to handle at sea. The Day and Smith–McIntyre grabs are also widely used, for similar reasons (see Eleftheriou and McIntye, 2005, for descriptions). There is no statistical difference between the fauna of silty sediments sampled by the van Veen grab and by the boxcorer, as revealed by the Texel Intercalibration Workshop (Heip *et al*., 1985). However, there is no unequivocal evidence that any one grab performs consistently better than the others in all conditions. Therefore, to maintain the value of existing time-series, all such standard designs can continue to be used, but intercalibrations should be conducted, when comparisons between studies using different grab samplers are to be made. If the sampling gear in a long-term

programme is to be changed, parallel sampling with both gears over at least one year is recommended.

Accepting the above qualifications, there are some important features of a grab sampler (chiefly by reference to a van Veen design, for convenience). In general, the standard grab should have a sampling area of 0.10 m^2 and should weigh 35–40 kg (for mud/muddy sands) or 70–100 kg for sandy sediments. When empty, it should have the following technical features.

1) To reduce the shock wave caused by the grab, the windows on the upper side should be as large as possible (minimum 60% of the upper surface of the grab). These windows should be covered with metal gauze of 0.5 × 0.5 mm mesh size; the mesh size should be smaller when the sample is to be washed over a finer sieve. The windows must be easily opened for inspection and subsampling, before emptying the sample into a container or onto a washing table. In addition, when sediments are to be analysed for physical characteristics or chemical content, sealed flaps will be necessary, to avoid possible outwash of fine material during retrieval.

1) Means should be provided for attaching up to an extra 20 kg of weight. This is perhaps best done by fastening four equal pieces of weight on the upper edges of the jaws, or inside the grab. One could also, complementary to the standard weight grab, use a grab made of thicker sheet metal, weighing approximately 20 kg more.

2) Warp rigging of the long-armed grab gives significantly better results on hard and sandy bottoms (Kingston, 1988).

3) Special attention must be paid to the design of the grab, to prevent elevation of the grab during closure (see ICES, 1994).

4) There could be cases where the use of other gear with a smaller sampling area (e.g. modified Olausen grab, Ponar grab, or Ekman grab) might be advisable (e.g. when the fauna are very dense and uniform, or in conditions where larger grabs cannot be employed). In such cases, the comparability with other gears in use must be determined by intercalibrations.

The following precautions should be observed when using either a grab or box sampler.

1) The winching process is very critical for maintaining sample quality. Therefore, the winch operation should be standardized, including a complete stop, followed by a slow lowering (<0.5 m s^{-1}) for the last few metres. Gentle lowering and heaving of the sampler will (i) reduce the shock wave and, consequently, the risk of losing surface material, and (ii) reduce the risk of losing sediment as a result of raising the sampler before closure is completed.

2) The wire must be kept as vertical as possible, to guarantee that the sampler is set down and lifted up vertically.

3) In densely compacted sediments (e.g. fine sand), additional weights will be needed for the van Veen grab, to ensure adequate penetration. In general, this grab is unsuitable for sediments coarser than medium sand.

4) The exact sampling area, the volume, and the penetration depth of each sampler should be carefully recorded.

5) Special care is needed once the sampler is on board the ship, to keep the sample from spilling; the sampler should be rinsed thoroughly, to avoid loss of sample.

6) The volume of each sample must be measured. This can be done by grading the container or using a ruler.

Criteria for rejection of samples

Samples should be rejected and sampling repeated when possible if:

- Less than 5 l of sample volume is obtained by a 0.1 m^2 grab in soft sediments or less than 2.5 l in hard-packed sand (for the HAPS corer, less than 15 cm penetration).
- Incomplete closure is noted.
- Obvious uneven bite is noted.
- Spillage during transfer of samples is observed.
- Samples clearly deviate from the other samples taken in the same area, for example, there is an observed change from clean sand samples to *Mytilus* bank samples. Nevertheless, the samples should be kept, to record faunal patchiness, but another sample should be taken, to replace it in calculating the mean for the station. Synoptic samplings could be useful, to reveal extent and nature of patchiness.
- Washout or disturbed surface layer is noted.

2.2.3 Diver-operated samplers

Scuba diving is a very useful method for sampling soft sediments in shallow water. Nevertheless, when data from ship-collected samples and diver samples are combined, intercalibration is necessary. Scuba sampling can be done with tubes, for example, acrylic glass (Jensen, 1983), but diver-operated boxcorers (Rumohr and Amtz, 1982) or suction samplers, such as those described by Hiscock and Hoare (1973), can also be used on mud to sand bottoms. Further references to sampling by scuba diving can be found in Eleftheriou and McIntyre (2005).

2.3 Epifauna

The epifauna of marine sediments is a component of the benthic community generally not effectively sampled by grabs and corers. Many attempts have been made to sample parts of this fauna using methods differing markedly in efficiency.

2.3.1 Dredges and trawls

Dredges, epibenthic nets, and beam trawls could be valuable as a complement to grab or boxcorer samplers, because large sedentary and widely dispersed species of epifauna are seldom caught in sufficient numbers with grabs and corers. Descriptions of suitable devices for sampling epifauna can be found in Eleftheriou and McIntyre (2005). Standardized dredging should always be used when grab samples devoid of macrofauna are encountered. Dredge samples covering a greater area might reveal some of the remaining individuals in reduced (hypoxic) environments. Sampling of the epibenthos is described in detail in Rees (2008).

Considerable caution is required, however, in treating benthos data from trawls and dredges in a quantitative manner, owing to uncertainties of sampling efficiency. The problem is greater when combining data from different types of towed gear, for

example, marked differences in catch efficiency. Therefore, it is recommended that every effort be made to follow consistent sampling procedures when the data from different studies are to be combined or compared. Dredging can be useful for semi-quantitative sampling, for example, employing a five-point scale of abundance (none-single-few-many-large number).

To ensure a degree of comparability between studies, the following protocol is recommended. It should be noted, however, that although the following suggested practice has proven successful in the North Sea and other areas, modifications might be necessary if the gear becomes clogged or if insufficient material is caught.

A beam trawl with a minimum beam width of 2 m is recommended as a standard gear. It should be equipped with at least one tickler chain or a chain mat and the mesh size of the codend should not be larger than 1 × 1 cm. It is impossible to give exact guidelines on trawling speed and distance, but mesh size is very important in relation to those factors. A beam trawl fitted with a larger mesh net can be towed at greater speeds and over longer distances than a beam trawl with a smaller mesh size codend. For example, a 2 m trawl fitted with a 1 × 1 cm mesh size net could be towed for up to 1 nautical mile at a towing speed of approximately 2 knots. However, a 2 m trawl fitted with a 2 × 2 mm mesh net should be towed at speeds not exceeding 1 knot for a maximum of 5 min. However, it should be noted that increasing the distance of the tow might increase the likelihood of sampling different habitats, thereby complicating the interpretation of the data.

It must be emphasized that trawling distance be kept constant in a survey and be measured from the time when the gear reaches the bottom and starts to fish to the time when it leaves the seabed. The importance of maintaining a constant speed and direction with reference to any current, both within and between tows, should also be emphasized.

Samples should be processed as follows.

1) Sample volume should be estimated, the sample documented photographically, then the material washed in a sieve. As a minimum requirement, the material obtained should be washed in a sieve with a mesh size equal to the codend mesh size of the net. For larger samples, using a sieve with big meshes (e.g. 2 c) in addition to the sieve with the codend mesh size is recommended.

2) For general epifaunal surveys, only the material retained by the sieve with the mesh size similar to that of the codend should be referred to, because this is the only size class that will have been caught consistently. The large megabenthos[3] retained by such a sieve usually does not pose identification problems, and it can easily be processed on board ship.

3) If the survey has to be run with insufficient scientific work force and completed in a very short time, sample processing in the laboratory might be necessary. In this case, samples with a volume of less than 20 l should be fixed and taken back as a whole, whereas larger catches should be subsampled, approximately 20 l out of the middle of the sample, and fixed. It should be noted, however, that, whereas this procedure might be

[3] Kostylev *et al.*, 2001 define megabenthos as: organisms larger than 1 cm in linear dimensions.

acceptable for extrapolating densities of most species, it could not account for all species in a sample. A complete census will only be possible by sorting the entire sample contents. In the laboratory, the fixed (sub) samples should be sieved and evaluated, using the approach described above.

In recent years, epifaunal sampling has been conducted through the analysis of the bycatch from groundfish surveys that typically use larger beam trawls or otter trawls. The following procedure is recommended for the processing of bycatch samples.

1) Sort all fish out of the catch.

2) Collect the epifauna in appropriate containers (e.g. fishing baskets).

3) A standard protocol for sorting the catch cannot be given, because it depends largely on the catch composition. Sometimes it might be possible just to sort out the larger species and take a subsample of the rest. Sometimes it might be necessary to sort out the complete basket, to determine the species composition.

4) At a minimum, count all mobile specimens and register absence/presence of the sessile species. Weights and minimum and maximum length per species might be determined as well.

5) Document photographically.

In addition to the above procedures, the following information should be recorded.

- weather conditions
- windspeed and direction
- sea state
- start position of the tow
- end position of the tow
- time of day
- depth range
- volume of sample
- presence and nature of artefacts

It should be noted that gear that is more sophisticated, for example, epibenthic sledges, might be required for sampling hyperbenthic or bentho-pelagic species. Such gear is particularly valuable for studies of species that constitute an important component of the diet of fish, especially crustaceans. Epibenthic and hyperbenthic sledges (e.g. the Brattegard dredge; see Brattegard and Fossa, 1991; Sorbe sledge) are useful for the small mobile crustaceans and boundary fauna. If automatic closing mechanisms and dredge distance recorders are added, these instruments can be quantitative (cf. Gage deep-sea, epibenthic sledge). Special attention is drawn to the Triple-D dredge, which was designed for the quantitative collection of the large and rare epifauna and infauna (Bergman and Van Santbrink, 1994).

2.3.2 Remote techniques, underwater photography and television, and acoustics

Under certain circumstances, photographic and video records from drop frames, sledges, and remotely operated vehicles (ROVs) could provide reliable estimates of densities for conspicuous epifaunal species. Acoustic techniques, such as sidescan sonar and multibeam, are increasingly being used not only to detect ecologically relevant habitat features (e.g. sediment texture and topography), but also to directly

identify larger (epi-)fauna structures (e.g. mussel and oyster beds, aggregations of tube-building polychaetes, as well as seagrass meadows). With proper ground-truthing, remote techniques can be used reliably to determine epifaunal biomass, species, and numbers.

A major advantage of such methods over dredges and trawls is that they reduce the uncertainty associated with sampling efficiency, and data are more amenable to statistical analysis. In addition, such methods allow large areas to be surveyed, and they provide a means for assessing topographical and biological patterns, which might not be revealed by sampling at discrete stations. However, there are a number of limitations to visual (imaging) techniques.

1) The backscatter of light under turbid conditions results in poor images.

2) There is selectivity, because small, highly motile and cryptic species are not likely to be represented quantitatively in visual records. Such species could represent a substantial fraction of the epifauna.

3) In general, the resolution of high-speed still photography is superior to moving video images or frames extracted from the latter.

Equipment costs and maintenance requirements might be prohibitive. Unless automated, for example, via artificial-intelligence recognition systems, analysis of visual images can represent substantial costs in personnel effort. In view of the limitations of both TV/photographic and trawl/dredge sampling, a combination of both approaches is recommended, where possible. In general, all imaging with photographic and acoustic methods needs ground-truthing using trawl, dredge, and grab sampling. A review of the use of imaging in benthos monitoring can be found in Rumohr (1995), and Smith and Rumohr (2005).

3 Treatment of infauna samples

3.1 Separation of fauna from the sediment

The transfer of the sample to the sieve, the sieving procedure, and the transfer of the animals to the fixation jar are the steps during sample treatment most likely to introduce sources of error. To reduce the magnitude of these errors, the number of steps in the sampling and sieving procedures should be kept to a minimum and attention should be paid to the following.

3.1.1 Sieves

For descriptive surveys, sieves used for extraction of the macrofauna from sediments should have a mesh size of 1.0 mm. The use of an additional finer sieve of mesh size 0.5 mm, or even finer, is recommended for special purposes (see, for example, Section 3.8, below). The sieve mesh should be checked from time to time for damage and wear. If a finer sieve is also used, the sieve fractions should be treated separately and the results should be given for the individual and the summed fractions. If resieving of samples is done, a mesh size finer than that of the initial sieve should always be used. Small sieves can be cleaned with an ultrasonic bath. The use of brushes should be avoided, to prevent possible alterations of the mesh size. Distortion of woven mesh sieves happens with increasing frequency of use. This can introduce considerable errors in the collection of small organisms. The use of larger sieves is encouraged, because the risk of clogging is reduced, for example, sandy samples could rapidly fill or even overfill smaller sieves. Larger sieves also reduce the risk of spilling, when transferring samples from containers/buckets to the sieve. This risk can also be kept low using integrated sieve tables (see ICES, 1994).

It is noted that a growing number of institutes are changing to round mesh sieves, owing partly to a perceived improvement in the condition of the animals retained and partly to the theoretical improvement in mesh selectivity. The use of a square mesh introduces inaccuracies in collecting organisms in the size range of approximately the mesh size, because the mesh diagonal width is greater than the nominal mesh width. Further investigation is required to establish a basis for using either type of sieve. Errors associated with the use of different sieves are likely to be small in relation to other sources of sampling error.

There are new designs of sieving tables with hand-controlled water sprinklers, which help to reduce the physical stress on the analysts, while at the same time retaining the quality of the sampled specimens. In addition, tilting devices for the full sample container, providing the option to fix the container at a certain angle over the sieve, help reduce spillage and avoid the use of destructive tools.

3.1.2 Sieving procedure

Sieving should be conducted according to the following procedure.

1) Each grab and boxcore sample should be sieved, stored, and documented separately.

2) The grab or boxcore should be emptied into a container, then the sample should be transferred, portion by portion, onto the sieves as a sediment–water suspension. The use of sprinklers or hand-operated douches to suspend the sample is recommended. Very stiff clay can be gently fragmented by hand in the water of the container. The sieve must be

cleaned after each portion has been sieved, to avoid clogging and to ensure an equal mesh size throughout the entire sieving procedure.

3) To avoid damaging fragile animals, the best way to sieve a sample is to agitate the sieve gently under the water surface of a water-filled container, until all sediment that can pass the sieve has been washed through. On no account should water jets (e.g. deck hose) be used against the sieve surface.

4) Fragile animals, such as some polychaetes, should be picked out by hand during the sieving, to minimize damage. In addition, stones and large shells should be picked out, to avoid a grinding effect on the organisms and the sieve.

5) All material retained on the sieve should be carefully flushed off the sieve into an appropriate container with water from below and then fixed. The use of spoons or other scraping tools should be avoided (see Section 3.1.1).

6) When a 0.5 mm sieve is used, the 0.5 mm and the 1 mm fractions must be kept separate throughout all further processing.

3.2 Fixation

It should be noted that fixation and conservation (preservation) are two different steps in the treatment of a sample. The former procedure is employed to coagulate and harden the tissue of the organisms, whereas the latter prevents them from rotting and decaying. Improperly fixed specimens could create problems during further treatment, for example, through fragmentation of specimens or loss of appendages. Some zoological museums will only accept properly (formalin-) fixed specimens for further analysis and curation.

All the material retained on the sieves should be fixed in a buffered 4% formaldehyde solution (1 part 40% formaldehyde solution and 9 parts filtered seawater). For buffering, 100 g of hexamethylene tetramine (Hexamine, Urotropine) can be used per 1 l of concentrated formaldehyde (36–40%). Sodium tetraborate (Borax) in excess could also be used. Sponges are best preserved by putting them directly into absolute ethyl alcohol, to prevent fragmentation.

It should be noted that formaldehyde is a toxic compound, both acutely and chronically, and it is a known carcinogen. Individuals or laboratories using this chemical or handling samples fixed with this chemical should be aware of the associated safety issues as detailed on the Material Safety Data Sheets (MSDS). Appropriate means of laboratory air suction or ventilation must be provided for all procedures recognizing that formaldehyde is heavier than air and, therefore, air extraction at table level is recommended. For animal sorting, the samples must first be thoroughly washed with tap water and left to soak overnight, so that sorters are not exposed to formalin vapour.

Other fixation fluids that do not release formalin gas have been tested, such as formaldehyde depot chemicals (Dowicil 75 and Kohrsolin) used in clinics for sterilization purposes. The effects of these fluids on dry weight and ash-free dry weight are marked, and the effects on long-term storage are unclear; therefore, an unequivocal recommendation cannot be given (Brey, 1986).

In special cases, such as the study of the length distribution of polychaetes, the use of narcotizing agents before fixation might be advisable. For detailed information, see Steedman, 1976) and Lincoln and Sheals, 1979.

3.2.1 Staining

To facilitate sorting and to increase sorting accuracy, especially for small animals, staining the sample is recommended, for example, Rose Bengal and Eosin. However, in some cases, staining could cause problems with species identification. Zoological museums will not accept stained material for taxonomic purposes. The following procedure has been demonstrated to give good results.

1) Wash the sample free from the preservation or fixation fluid using a sieve with a mesh size smaller than 0.5 × 0.5 mm.

2) Allow the sieve to stand in Rose Bengal or other stain (1 g l^{-1} of tap water plus 5 g of phenol for adjustment to pH 4–5) for 20 min with the sample well covered.

3) Wash the sample until the tap water is no longer coloured.

As an alternative, Rose Bengal (4 g l^{-1} of 40 % formaldehyde) or Eosin could be added to the fixation fluid. Overstained specimens can be destained in alkaline (pH 9) fluids.

3.3 Sieving of fixed material

In instances where it is impossible to sieve material before fixation, it is possible to sieve the fixed material. However, it must be realized that the sorting characteristics of fixed material are different from those for live fauna and result in apparently greater abundance and biomass figures. An intercalibration of both procedures indicated that sieving procedure affects macrobenthos studies at the level of diversity, density, as well as community structure. This effect is particularly important when dealing with areas, such as nearshore environments dominated by small, interstitial and/or larger, slender polychaetes. Information that is more detailed is given by Degraer *et al*. (2007).

In publications and in databases, it should always be stated whether the sieved material was fresh (alive) or fixed.

3.4 Sorting

Sorting must be done using a magnification aid (e.g. magnification lamp, stereomicroscope). Any finer fraction (<1 mm) should always be sorted under a stereomicroscope. To reduce sorting time, a sorting aid, such as the one described by Pauly (1973) or a "fluidized sand bath" after P. Barnett, (see Holme and McIntyre, 1984) could be used, if its efficiency has been satisfactorily checked for the particular bottom material being sorted. The Ludox method (see Higgins and Thiel, 1988) has successfully been applied to meiobenthos study, and it might prove useful too for the extraction of soft-bodied macrofauna. In coarse sand, the following procedure is recommended. The sediment sample is placed on a PVC trough 5 m long, 20 cm wide, and 20 cm high; an ordinary rain gutter of the same length, with one open and one closed end, could be used. Water is poured over the sediment from one closed end and the extracted fauna are caught on a sieve on the other (open) end (Vanosmael *et al*., 1982). The residue should be checked for larger (heavier) animals. If samples are sorted alive, care should be taken, to avoid predation within the sample.

In general, sample splitting should be avoided. However, when taxa are present in large numbers (e.g. Polydora, phoronids, capitellids), it might be advisable to split the (entire) sample, to reduce the counting time. Different types of sample splitter can

be used. Rare species should be counted using whole samples. The accuracy of the sample-splitting device should be adequately assessed.

3.4.1 Taxonomic procedures

Taxa should be determined to the lowest possible level and any exceptions should be stated in the protocol. Fragments should be kept for biomass determinations, but only heads of partial organisms (e.g. of polychaetes) should be counted. Encrusting or colonial species should be mentioned (presence/absence) and their abundance can be reported in a five-level, semi-quantitative code, similar to that for dredge samples. If some species are excluded from the analysis, because they do not belong to the macrozoobenthos (e.g. large Nematoda), they should nevertheless be mentioned in the commentary section.

3.5 Biomass determination

The following measures of biomass can be used; wet weight, dry weight, and/or ash-free dry weight, either from fresh or fixed material. Moreover, energy content (J) and/or matter equivalents (C, N, P) can be determined using fresh material only. Fresh wet weight is to be preferred to formalin-wet weight, but if the latter has to be used, weighing should not be done until at least three months after fixation (Brey, 1986).

Wet weight is obtained after the excess fluid has been removed using filter paper. The animals are left on the filter paper until no more distinct wet traces can be seen. Animals with shells are generally weighed with their shells; the water should be drained off bivalves before weighing. When shell-free weights are given, the shell weight should also be included in the data. Echinoids should be punctured, to drain off the water before blotting on filter paper. As soon as the non-tissue water has been removed, the organisms are weighed with the accuracy required (e.g. for adult macrofauna, ±0.1 mg). In case tube-building animals have to be weighed together with their tubes, appropriate correction factors should be established.

The dry weight should be estimated after drying the fresh material at 60°C, or by freeze-drying, until constant weight has been reached. This will take at least 12–24 h, depending on the thickness of the material; large bivalves might require up to 96 h. Dry weights obtained by lyophilization (freeze drying) are slightly greater than those obtained by oven drying. For example, *Mytilus* lyophilized tissues weighed 10.9% more than oven-dried tissues (Gaffney and Diehl, 1986). Unlike heat-dried material, fresh lyophilized material is suitable for many types of proximate, detailed biochemical, and contaminant analysis. Chemical constituents are subject to far less danger of thermal or oxidative damage or evaporative loss of non-aqueous volatile components during lyophilization than during thermal drying (Jennings, 1999).

The use of ash-free dry weight is recommended, because it is the most accurate measure of biomass (Rumohr *et al*., 1987; Duineveld and Witte, 1987). However, it destroys specimens, and the consequences of this should be carefully considered. Ash-free dry weight should be determined after measuring dry weight. Samples are incinerated at 500°C in an oven until constant weight is reached; approximately 6 h, depending on sample type and size. The temperature of the oven should be checked with a calibrated thermometer, because there could be considerable temperature gradients (up to 50°C) within the burning chamber of a muffle furnace. Caution is advised to avoid exceeding 550°C, when a sudden loss of weight could occur, owing to the formation of CaO from the skeletal material ($CaC0_3$) of many invertebrates.

This can reduce the ash-free weight by 44%. Such loss happens very abruptly and within a small temperature range (Winberg, 1971). Before weighing, the incinerated material the samples must be kept in a desiccator, while cooling down to room temperature.

Conversion factors can also be used to estimate biomass from length or size measurements (Rumohr *et al*., 1987; Brey *et al*., 1988).

3.6 Preservation and storage

After sorting, weighing, and measuring, the animals (should be transferred to a preservation fluid, such as 70–80% alcohol or a saturated solution of propylene phenoxetol (for further information, see Lincoln and Sheals, 1979). If tap water is used, the pH of the final solution should be adjusted to 7.

3.7 Reference collection

It is advisable, even with routine samplings, to place some specimens of each taxon ("voucher specimens") under museum curatorship, to make later taxonomic checks possible. Laboratory reference collections should be validated by taxonomic experts.

3.8 Determination of production

For detailed production studies, routine samples might be insufficient, because survey data generally are inadequate for such studies. Therefore, the following additional recommendations are given, to cover the entire size or age range of the population of interest.

1) The use of finer sieves might be required, appropriate to the size of the bottom-living stages of particular species.

2) Sampling frequency might have to be increased to cover the seasonal variations in condition and population density over the entire life cycle.

3) Size/weight relationships have to be established for the species studied.

The computation of production is described in detail by Crisp (1984) and by Feller and Warwick (1988) for meiofauna. Attention is drawn to new techniques for analysing length frequencies using a computer (Brey, 1986; Brey and Pauly, 1986). For rough production estimates, production:biomass (PB) ratios can be used (Schwinghamer *et al*., 1986). A general and updated account on secondary production can be found in Brey (2001).

3.9 Integration with meiofauna studies

When sampling for both macrofauna and meiofauna at the same station, all sieving fractions from the meiofauna samples, including the 1.0 mm sieve, should be sorted and weighed, so that no size classes are omitted. The problem of the overlap between juvenile macrofauna/meiofauna (e.g. Oligochaeta, Ostracoda, Chironomidae, Nemertini, and Nematoda) can thus be avoided.

In general, grab samples are unsuitable for meiofauna studies, because the upper sediment layer could be flushed away during sampling. In addition, the vertical distribution of the organisms in the sediment is disturbed. Meiofauna samples should preferably be taken with diver-operated corers, tube corers, such as the Craib corer and with multiple corers, or as subsamples from boxcore samples. Corers are preferred, because they preserve the sediment surface, superficial detritus layers and associated fauna, meiobenthic densities and community composition (Bett *et al*.,

1994). Special extraction procedures are described by Eleftheriou and McIntyre (2005), Higgins and Thiel (1988), Platt and Warwick (1988), Somerfield and Warwick (1996), and Vincx (1996). Further information on the use of meiofauna in marine pollution monitoring can be obtained from Kennedy and Jacoby (1999), and Schratzberger *et al*. (2000).

4 Publication of abundance and biomass results

In investigations of soft-bottom macrofauna, the published results should include data for individual samples and/or average values per m^2 with standard errors or standard deviations (always stating which is reported) and number of samples, for both abundance and biomass for each taxon and the total fauna. When two or more sieve fractions are collected, these statistics should be given at least for the 1 mm fraction and the sum of the fractions. If the sample was split, this should be reported and the type of the splitter should be given. Whenever some taxon found on the sieves is excluded from the published results, this should be stated and the reasons given (e.g. *Piscicola geometra* not included, because it is a parasite).

It is advisable to store benthos data in public databanks (ICES, MarBEF). Coding by the individual researcher is no longer needed, because data conversion routines are available at the data centres. It is recommended that data and results be published in journals widely accessible to the scientific community.

5 Station data

Data recorded must include the following information: whether the ship was anchored or not, time of day, date, weather conditions during sampling, and a description of the sediment (Briggs, 1977). Near-bottom temperature, salinity, and oxygen measurements are desirable. For macrofauna study, the type and specifications of the sampler should be stated. If more than one sample is taken, the depth range of the samples should be stated.

The sediment description should encompass the following.

1) simple measure of grain-size distribution phi scale: silt/clay fraction <63 μm, 125 μm, 250 μm, 500 μm, 1000 μm, 2000 μm)

2) median grain size for the upper 5 cm

3) weight loss on ignition (500–520°C)

4) surface colour and colour change with depth as a possible indicator of redox state

5) smell (H_2S)

6) description of sediment types, including important notes, for example, the occurrence of concretions, loose algae, etc.

The use of stainless steel buckets or boxcorers is recommended in cases where the sediments are to be subsampled for trace metal and organic contaminant determinations. It is recommended that measurements of redox potential and shear strength be made in samples collected by a boxcorer rather than a grab, because the latter has a great chance of distorting the sample.

Precise position fixing during sampling is essential. The position and the depth should be controlled and documented for every single sample by track plotting during station work.

6 In-house quality assurance

It is essential that, at every phase of a monitoring or assessment survey, built-in controls be enforced to ensure the quality of data acquisition, collection, handling, and analysis, and of subsequent reporting (see also Rees, 2004). In-house quality assurance manuals should be developed in accordance with appropriate national and international standards and followed rigorously. Some of the items listed below have already been covered by this publication in more detail. Such manuals should at least include the following topics.

1) Formal listing of survey personnel.

2) Procedures for the handling and use of chemicals (i.e. formaldehyde and other reagents) in marine environmental surveys. Appropriate Material Safety Data Sheets (MSDS) can be found in the Internet.

3) Procedures for handling survey equipment.

4) Procedures for station selection and location, as well as navigational accuracy and documentation.

5) Procedures for the collection of biological material.

6) Procedures for the storage of biological material.

7) Procedures for sorting biological material.*

8) Procedures for the distribution of sorted biological material for taxonomic analysis. Signed protocols should be obligatory for all steps in analysis.

9) Procedures for identifying biological material. **

10) Procedures for the recording of biological and environmental data. **

11) Procedures for the analysis of biological and environmental data.

12) Procedures for survey report writing and documentation.

13) Details of the professional qualifications of survey and laboratory personnel.

*** These procedures should include the random check sorting and identification by experienced personnel. A common practice is to have 10 or 20% of the sorted samples be resorted by another sorter, to ensure 95%. This depends on the contract with the client.**

**** These procedures should include obligatory proofreading before entering the data into a computer, and before usage.**

Formal accreditation of the persons working with taxonomic identifications at the laboratories is desirable. Training should be offered by institutions possessing the appropriate level of expertise in the form of regular taxonomic workshops and ring tests. Participation in these workshops on a regional basis should be obligatory for all laboratories delivering data to public databanks. Taxonomic crosschecking between laboratories should be encouraged.

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