Soft bottom macrofauna: Collection and treatment of samples

compiled by

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INTRODUCTION

The aim of these recommendations is to standardize the methods used by different scientists for long-term benthos surveys, in order to increase the comparability of results for different areas and to enable, inter alia, detection of large-scale changes in the system that would not otherwise be detected by a single scientist.

In these recommendations, soft bottoms are defined as those with sediments ranging from mud to, and including, sand. For descriptive surveys, macrofauna is defined as animals retained on a 1 mm sieve (mesh size 1 x 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 allow comparisons. For a more comprehensive treatment of sampling design, procedures, and alternatives, the reader is referred to, e.g., Kajak (1963), Cochran (1977), Elliott (1977), Downing and Rigler (1984), Holme and McIntyre (1984), and Baker and Wolff (1987).

SAMPLING STRATEGY

The design of the sampling programme largely depends on the detailed aims of the study. The temporal and spatial scales are also of importance for the sampling strategy, as are the local abiotic factors. An awareness of resource limitations (time, money, laboratory facilities) is of the greatest 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, e.g., Cochran (1977), Green (1979), Elliott (1977), and Frontier.
(1983), to work out appropriate approaches. It must be stressed that the sampling strategy has a strong influence on the options for later statistical analyses. Thus, the sampling strategy can only be designed after the initial working hypothesis has been formulated, along with the intended statistical tests. Some basic sampling procedures used in benthos investigations are the following:

- time-series sampling (equidistance, at biologically relevant time intervals),
- stratified sampling (according to strata, depth, sediment, etc.),
- randomized sampling,
- single-spot sampling,
- area sampling (grid sampling), and
- transect sampling (usually along a biological/physical gradient).

Related problems include:

- number of samples,
- sample size, and
- precision of results.

2.1 Sampling

There is no single standard sampling gear for North Sea benthos investigations. The choice of a suitable sampler is - and always will be - 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. The time required for processing the samples and the level of sampling precision required will also influence the choice of sampling gear (Jensen, 1981; Riddle, 1984; Kingston, 1988).

2.2 Infauna

The choice of an appropriate sampler depends on the average living depth of the infauna in question, which can range from the upper millimetre down to almost one metre. 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, when analysing the results.

2.2.1 Box corer sampling

The box corer is generally recommended for sampling the North Sea benthos because of its generally superior characteristics, especially in sandy sediments. Its advantages are good penetration capability, and relative lack of seabed disturbance and distortion of the sample; the disadvantages are chiefly the need for relatively calm weather and for large vessels to use this heavy and expensive gear. There is no statistical difference between the fauna of silty sediments sampled by the Van Veen grab and by the box corer, as revealed by the Texel Intercalibration Workshop (Heip et al., 1985).
2.1.2

A variety of box corer designs have been successfully employed in benthos research. Most of them are based on the Reineck 'Kastengreifer' design (Reineck, 1963), as, for example, the 'spade corer' by Hessler and Jumars (1974), which has been increasingly widely used in European waters because of its reliability and the large sample volume (0.25 m$^2$). The special advantage is the removable spade from the lever arm, which reduces handling time on board and keeps the sample relatively undisturbed during further processing. This type is also used as an 0.1 m$^2$ version with good penetration capabilities, as revealed by TV observations. Box corers with round 'boxes' have also been successfully employed by different laboratories; these include the NIOZ type of a modified Reineck with a flat spade and the 'HAPS' design used by Danish institutes (Kanneworf and Nicolaisen, 1973).

Despite the lack of information on comparative efficiencies of different box samplers, the following features have proved to be useful and are suggested:

1) A sufficient number of easily removable weights must be provided. (In silty sediments, the box must not penetrate beyond its own height.)

2) Light-operating, flexible flaps, preferably on top of the box, should be provided so as to reduce the bow-wave effect.

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 samplers, a closing plate has to be fitted between the spade and the box. This operation, which may be difficult, becomes unnecessary when the box containing the sample can be removed together with the spade.

In general, some precautions that must be taken when using box samplers are similar to those required when using grabs; the latter are listed in the next section.

2.1.2 Grab sampling

When a box corer cannot be employed for various reasons, the already widely used Van Veen grab (Van Veen, 1933, with the modifications described by Dybern et al., 1976; see also Ankar, 1977; Riddle, 1984; and Kingston, 1988) is recommended as one standard sampling gear for benthic macrofauna research in the North Sea area, because of its comparative reliability and simplicity of handling at sea. Equally, the Day and Smith-McIntyre grabs are also widely used for similar reasons (see Holme and McIntyre, 1984, for descriptions). However, as yet there is no unequivocal evidence that any one grab performs consistently better than its counterparts, in all conditions. Therefore, in order not to decrease the value of existing time series, all such standard designs may continue to be used, but intercalibrations should be conducted when comparisons between studies 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.
Attention is drawn to a new design of a modified Smith-McIntyre grab with a hydrostatic/compressed-air closing mechanism, which overcomes the uncertainties of incomplete closure while heaving that are associated with some other designs (Kingston, 1988). Further results on its efficiency are awaited.

Accepting the above qualifications, some important features of a grab sampler can be listed as follows (chiefly by reference to a Van Veen design, for convenience):

The standard grab should have a sampling area of $0.10 \text{ m}^2$ 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) In order to reduce the shock wave caused by the grab, the windows on the upper side should cover as large an area as possible (minimum 60% of the upper surface of the grab). The windows should be covered with metal gauze of $0.5 \times 0.5 \text{ mm}$ mesh size. The mesh size should be smaller when the sample is to be washed over a finer sieve. The windows must be easy to open for inspection and sub-sampling prior to emptying the sample into a container. Nevertheless, when planning sediment analysis, one should be aware of a possible outwash of fine material during retrieval.

2) Means should be provided for attaching an extra 20 kg of lead weights. This is perhaps best done by fastening four equal pieces of lead on the upper edges of the jaws, or inside the grab. One may also, as a complement to the standard weight grab, use a grab made of thicker sheet-metal, weighing approximately 20 kg more, when needed.

3) Warp rigging of the long-armed grab gives significantly better results on hard and sandy bottoms (see Figure 1) (Kingston, 1988).

4) Special attention must be paid to the design of the grab to prevent elevation during closure. The shape of the buckets must be a quarter of a circle, but length $a$ must be slightly longer than length $b$ (see Figure 2) to provide optimal initial penetration.

5) There may be cases where the use of other gear with a smaller sampling area may be advisable (e.g., when the fauna is very dense and uniform); the comparability with other gears in use must nevertheless be proven by intercalibration.

The following precautions should be observed when using a grab (or a box sampler):

1) The grab should be set down on the seabed and closed as gently as possible. This will reduce the shock wave and the risk of loss of sediment by raising of the grab before closure is completed.
Figure 1. Warp rigging of the Van Veen grab, which increases digging efficiency.

Figure 2. Cross-section of a bucket which will fit into the hole it digs and permit an 8-cm initial penetration (Riddle, 1984).
2) The wire must be kept as vertical as possible to guarantee that the grab is set down and lifted up vertically.

3) In general, densely compacted sediments (e.g., fine sand) will not be penetrated by the Van Veen grab.

4) The exact sampling area and the volume or the digging depth of each particular grab should be carefully checked and the square-metre values calculated accordingly.

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

2.1.3 Diver-operated samplers

Scuba diving is a very useful method for sampling shallow soft bottoms, and will give more reliable data than the recommended grab method.

Scuba sampling can be done with tubes of, e.g., plexiglass (Jensen, 1983), but diver-operated box corers (Rumohr and Arntz, 1982) or suction samplers, such as that described by Hiscock and Hoare (1973), can also be used on mud to sand bottoms. Further references on sampling by Scuba diving can be found in Holme and McIntyre (1984).

2.3 Epifauna

The epifauna of marine sediments is a component of the benthic community which is never caught quantitatively in a strict sense. Many attempts have been made to sample parts of this fauna with various methods that differ markedly in efficiency.

2.3.1 Dredges and trawls

Dredge, epibenthic net, and beam-trawl hauls may be valuable as a complement to grab or box corer samples, since large sedentary, but comparatively rare, species are seldom caught in sufficient numbers with grabs and corers. Descriptions of suitable devices can be found in Holme and McIntyre (1984).

Considerable caution is required in treating benthos data from trawls and dredges in a quantitative manner owing to uncertainties about sampling efficiency. For example, marked differences in capture efficiency often result from changes in fishing practices. It is, therefore, recommended that every effort be made to follow consistent sampling procedures.

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 proved successful in the North Sea and other areas, modifications may be necessary if the gear becomes clogged or if insufficient material is caught.
A beam trawl with a minimum beam breadth of 2 m is recommended as a standard gear. It should be equipped with at least one tickler chain, and the minimum mesh size in the codend should not exceed 1 cm x 1 cm. For a 2-m trawl, a distance of 1 nautical mile is suggested; shorter distances may be appropriate for wider gear and other areas. It is important that the trawling distance be kept constant in a survey and be measured from the point at which the gear reaches the bottom to the beginning of recovery.

It is inappropriate to recommend a single towing speed owing to differences in vessel size, etc. (although for small vessels a speed of 2 miles/h over the ground may be suitable). However, the importance of maintaining a constant speed and direction with reference to any current, both within and between tows, should be stressed. The processing of the samples should be done as follows:

Sample volume should be estimated, documented photographically, and then sieved. As a minimum requirement, the material obtained should be sieved with a mesh size equal to the minimum one for the net.

Additional finer fractions may be collected and, in particular, the <1 mm fraction may usefully be retained as a reference for core or grab samples. (A 1-litre sample is normally sufficient for this purpose.)

For general epifauna surveys, only the material retained by the sieve with the minimum net mesh size should be referred to, as this is the only size class caught consistently. In larger samples, the use of a sieve with very large meshes (e.g., 2 cm) in addition to the sieve with the critical mesh size is recommended. Usually the very large megabenthos retained by such a sieve does not offer any problems of identification, and can easily be processed on board ship. If the survey has to be run with insufficient scientific manpower and completed in a very short time, sample processing in the laboratory may be required. In this case, samples with a volume of less than 20 litres should be fixed and carried back as a whole, while larger catches should be sub-sampled and about 20 litres should be fixed. In the laboratory, the fixed (sub-)samples should be sieved and evaluated in the same way as described above.

In addition to the above procedures, the following information should be recorded: weather conditions, wind speed and direction, sea state, start- and end-position of the tow, time of day, and depth range.

It should be noted that more sophisticated gear, such as epibenthic sledges, may be required for sampling hyper-benthic or benthos-pelagic species. Such gear is particularly valuable for studies of species (especially crustacea) which constitute an important component of the diet of fish.
2.3.2 Underwater photography and TV

Under certain circumstances, photographic and video records from drop frames, sledges, and remotely operated vehicles (ROV) may provide reliable estimates of densities for conspicuous epifaunal species. 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 provide a means for assessing topographical and biological patterns which may 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: highly motile and cryptic species are not likely to be represented in visual records. (Such species may represent a substantial fraction of the epifauna.)

3) Equipment costs and maintenance requirements may be prohibitive.

In view of the limitations of both TV/photographic and trawl/dredge sampling, a combination of both approaches is to be recommended, where possible.

3 TREATMENT OF 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, attention should be paid to the following procedures.

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, e.g., 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 single and the summed fractions. If resieving of samples is carried out, a finer mesh size than the initial sieve should always be used. Small sieves may be cleaned with an ultrasonic bath. The use of brushes should be avoided to prevent possible alterations of the mesh size.

It may be 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. Further work 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.

3.1.2 Sieving procedure

- Each grab sample should be sieved, stored, and documented separately.

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

- The grab should be emptied into a container, and 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.

- In order to avoid damaging fragile animals, the sample should not be sieved with a direct jet of water against the sieve net.

- Fragile animals, e.g., some polychaetes, should be picked out by hand during the sieving, to minimize damaging. Also, stones and large shells should be picked out, to avoid a grinding effect on organisms and the sieve.

- All material retained on the sieve should be carefully flushed off the sieve, with water from below, into an appropriate recipient and fixed. The use of spoons or other tools should be avoided.

- When the 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

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 sea water). For buffering, 100 g of hexamethylene tetramine (= Hexamine, = Urotropine) can be used per 1 litre of concentrated formaldehyde (36-40%). Sodium tetraborate (= Borax) in excess may also be used. Sponges are best preserved by putting them directly into absolute ethyl alcohol so as to prevent fragmentation.

It should be noted that formaldehyde is regarded as toxic and probably carcinogenic and should, therefore, be handled with great care; appropriate means of laboratory air suction or ventilation should be provided for all procedures. For animal sorting, the samples should first be thoroughly washed with tap water so that sorters are not exposed to formalin vapour.
In special cases, such as the study of the length distribution of polychaetes, the use of narcotizing agents before fixation may 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 with, e.g., Rose Bengal, is recommended. However, in some cases staining may cause problems with species determination. The following procedure has been shown to give good results:

- Wash the sample free from the preservation fluid by using a sieve with a mesh size smaller than 0.5 x 0.5 mm.

- Allow the sieve to stand in Rose Bengal stain (1 g/dm$^3$ of tap water + 5 g of phenol for adjustment to pH 4-5) for 20 minutes with the sample well covered.

- Wash the sample until the tap water is no longer coloured.

However, Rose Bengal (4 g/dm$^3$ of 40% formaldehyde) may be added to the fixation fluid. Overstained specimens may be destained in alkaline (pH 9) fluids (Thiel, 1966).

3.3 Sieving of fixed material

Samples may be sieved 'alive' or preserved. If they are preserved, it must be realized that the sorting characteristics are different from those for live fauna and result in apparently higher abundance and biomass figures. Intercalibrations of both procedures should be performed. In publications, it should always be stated whether the sieved material was fresh (alive) or fixed.

3.4 Sorting

Sorting must be done using some magnification aid (magnification lamp, stereo-microscope). Any finer fraction (<1 mm) should always be sorted under a stereo-microscope.

When taxa occur in great numbers (e.g., Polydora, phoronids, capitellids), it may be advisable to split the samples to reduce counting time. Different types of sample splitters can be used. Rare species should be counted from whole samples. The accuracy of the sample-splitting device should be adequately assessed. 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) may be used, provided that its efficiency has been satisfactorily checked for the particular bottom material studied. The Ludox method (see Higgins and Thiel, 1988) has successfully been applied to meiofauna work and may also prove useful for the extraction of soft-bodied macrofauna.
In coarse sand, the following procedure may be recommended: the sediment is fixed and placed on a PVC trough 5 m long, 20 cm wide, and 20 cm high (an ordinary gutter of the same length may also be used). Water is poured over the sediment from one closed side and the extracted fauna caught on a sieve on the other (open) side (Vanosmael et al., 1982).

3.5 Biomass determination

The following measures of biomass determination can be used: wet weight, dry weight, and/or ash-free dry weight, either from fresh or fixed material. Furthermore, energy content (J) and/or matter equivalents (C, N, P) may 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).

The wet weight is obtained by weighing after external fluid has been removed on 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 be included in the data list. 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 (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.

Dry weight should be estimated after drying the fresh material at 60°C, or by freeze drying, until constant weight is reached (at least 12-24 hours, depending on the thickness of the material). Dry weights obtained by lyophilization (freeze drying) are slightly higher than those obtained by oven drying. For Mytilus, lyophilized tissues weighed 10.9% more than oven-dried tissues (Gaffney and Diehl, 1986).

The use of ash-free dry weight is recommended in routine programmes, 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 estimated after measuring dry weight. It is determined after incineration at 500°C in an oven until weight constancy is reached (~6 hours, depending on sample and object size). The temperature of the oven should be checked with a calibrated thermometer, because there may be considerable temperature gradients (up to 50°C) in a muffle furnace. Caution is advised to avoid exceeding a certain temperature (>550°C), at which a sudden loss of weight may occur owing to the formation of CaO from the skeletal material of many invertebrates (CaCO3). This can reduce the weight of the mineral fraction by 44%. Such decomposition occurs very abruptly and within a small temperature interval (Winberg, 1971). Before weighing, the samples must be kept in a desiccator while cooling down to room temperature after oven drying or removal from the muffle furnace.
To estimate biomass from length or size measurements, conversion factors may also be used (Rumohr et al., 1987; Brey et al., 1988).

3.6 Preservation and storage

After sorting, weighing, and measuring, the animals (if still existing) 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 should be adjusted to 7.

3.7 Reference collection

It is advisable, even with routine samplings, to place some specimens of each taxon under museum curatorship to make later taxonomic checks possible.

3.8 Determination of production

For detailed production studies, routine samples may often be insufficient because survey data generally are inadequate for such studies. Therefore, the following additional recommendations are given in order to cover the entire size/age range of the population:

1) The use of appropriate finer sieves may be needed, depending on the size of the bottom-living stages of particular species.

2) Sampling frequency may 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 (P/B) ratios may be used (Schwinghamer et al., 1986).

3.9 Integration with meiofauna studies

When sampling for both macro- and meiofauna on 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 lost, and the problem of the overlap between juvenile macrofauna/meiofauna (e.g., Oligochaeta, Ostracoda, Chironomidae, Nemertini, Nematoda) can be avoided. In general, grab samples are unsuitable for meiofauna studies, since the upper sediment layer may be flushed away during sampling. Meiofauna samples should preferably be taken with diver-operated corers or as sub-samples from box core samples. Special extraction procedures are described by Holme and McIntyre (1984) and Higgins and Thiel (1988).
PUBLICATION OF ABUNDANCE AND BIOMASS RESULTS

In investigations on soft bottom macrofauna, the published results should include data for each individual sample and/or average values with standard errors of mean 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 sample splitting was done, this should be stated when reporting the data, 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 explicitly stated and the reasons given (e.g., *Piscicola geometra* not included because it is a parasite).

For coding purposes, the RUBIN species codes (Zetterberg, 1982) may be used, following the reporting formats issued by ICES and the Helsinki Commission. (The RUBIN codes do not cover all species of North Sea fauna at present.)

It is recommended that data and results be published in journals widely accessible to the scientific community.

STATION DATA

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

1) A simple measure of grain size distribution (φ-scale: silt/clay fraction <63, 125, 250, 500, 1000, 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 (H₂S);

6) Description of sediment type, including important notes, e.g., the occurrence of concretions, loose algae, etc.

When describing the sediment, the recommendations issued by ICES should be followed. The use of stainless steel buckets or box corers
is advocated in cases where the sediments are to be sub-sampled for trace metal and organic contaminants determinations.

It is recommended that measurements of redox potential and shear-strength be made in samples collected by a box corer rather than a grab because the latter has a great chance of distorting the sample.

REFERENCES

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