Manual for Marine Monitoring in the

COMBINE

Programme of HELCOM

Part C

Programme for monitoring of eutrophication and its effects

Annex C-8

Soft bottom macrozoobenthos

ANNEX C-8 SOFT BOTTOM MACROZOOBENTHOS

1. INTRODUCTION

The species composition of benthic communities generally depends on the substrate, depth, wave exposure, oxygen availability and salinity, etc.

Macrobenthic communities are an appropriate target for monitoring since:

1. an important component of benthic communities is formed by species which are long-lived and which therefore integrate environmental change over long periods of time;
2. they are relatively easy to sample quantitatively;
3. they are well-studied scientifically, compared with other sediment-dwelling components (e.g. meiofauna and microfauna) and taxonomic keys are available for most groups;
4. community structure responds in a predictable manner to a number of anthropogenic influences (thus, the results of changes can be interpreted with a degree of confidence);
5. there may be direct links with commercially valued resources, e.g. fish and for wintering birds (via feeding).

The so-called positive effects of nutrient enrichment/eutrophication may increase the food supply to the benthos and, therefore, may give rise to changes in species composition and numbers, increased biomass, shifts in functional groups and changes in community structure. Evident negative effects of eutrophication, as wide range anoxia, leads to impoverished benthic communities or even bottoms depleted with macrofauna.

This Manual is based on the previous HELCOM guideline (HELCOM, 1988) as well as on the OSPARCOM Guidelines for JAMP (OSPARCOM 1997). Much information exists on methodology for benthos investigations. The most relevant reports are those by Rumohr (1990) - available now in www.ices.dk/pubs/times/times08.doc - , which deals largely with methodology for the collection and treatment of samples of the soft-bottom macrofauna, and by Rees *et al*. (1991), which focuses on the monitoring of benthic communities around point-source discharges. The latter also deals more generally with the role of benthos studies in investigations of human impact and includes guidance on approaches for the sampling of different substrate types.

Both reports refer to a range of earlier documents which are of value in the planning and conduct of marine benthos sampling programmes. The most valuable of these is that by Holme and McIntyre (1984) which is a standard reference for anyone working in this field. Guidelines which have been published since the two sets of ICES guidelines (i.e. Rumohr 1990 and Rees *et al* 1991) include Gray*et al.* (1992) concerning approaches to marine pollution assessment and which provides practical examples of applying the PRIMER ('Plymouth Routines in Multivariate Ecological Research') package for univariate, graphical and multivariate data analyses.

2. PURPOSE

The monitoring of benthic communities is carried out for, *inter alia*, the following purposes:

1. to monitor the spatial variability in species composition, abundance and biomass within the maritime area resulting from anthropogenic nutrient inputs;
2. to monitor temporal trends in species composition, abundance and biomass within the maritime area (at a timescale of years) in order to assess whether changes can be related to temporal trends in nutrient inputs;
3. to support the development and implementation of a common procedure for the identification of the status of the benthic communities;
4. to understand the relationship between nutrient concentrations and temporal trends in species/community characteristics.

3. SAMPLING STRATEGY

Sample sites should be representative of the whole monitoring area and therefore, characteristic habitat structures and substrates must be sampled. Prior to temporal trend analysis, checks must be made to ensure that sample sites are inhabited by a homogenous benthic community rather than non-comparable, heterogeneous benthic communities.

Establishment of the baseline community structure and variability at the site under consideration is important. Sample points must be spread out over the extent of the habitat studied to ensure an adequate consideration of spatial variation. It cannot be assumed that one point is representative of the habitat as a whole. When measuring anthropogenically induced change a control reference site is required for each test site. It is important that similar habitats are selected for comparison.

Guidance on the design and implementation of field sampling programmes around waste discharges (Rees *et al*., 1991) may usefully be applied to eutrophication-related studies. The strategy comprises five stages, as follows:

Stage 1: desk study;
Stage 2: planning a sampling programme;
Stage 3: analysis and interpretation of data;
Stage 4: rationalisation of sampling design for regular monitoring;
Stage 5: establishment of routine.

4. SHIP-BOARD ROUTINES

4.1 SAMPLING

Sampling on shallow stations (70 m or less) is recommended to be conducted during daytime, since some benthic species have semipelagic activity during the night.

The following information should be recorded in the field:

- type of positioning system and its accuracy;
- whether or not a buoy was used;
- whether or not the ship was anchored;
- the time of day;
- the weather conditions and state of the sea during sampling;
- the depth from which the sample was taken;
- a description of the sediment, including:

1. surface colour and colour change with depth (as a possible indicator of redox state);
2. depth of the oxygenated surface layer
3. smell (H_2S);
4. a description of sediment type (e.g. caly, sand, mud, etc), including important notes, e.g., the occurrence of concretions, loose algae, etc.

- the type and specification of the sampler.

Near-bottom temperature, salinity and oxygen have to be measured. If more than one sample is taken at a station, the depth range of samples should be recorded. An estimate of the volume of sediment retained should be made for each sample taken, as a measure of sampler efficiency. Criteria for rejection of samples collected by grabs are given by Rees *et al.* (1991) and in the QA part of this guideline.

The widely applied 0.1 m^2 Van Veen grab should be used as the standard gear for benthic macrofauna sampling in the Baltic Sea, because of its very good reliability and simplicity of handling at sea. The emptied grab should weigh about 25-35 kg when used for fine grain size and up to 80 kg in sandy bottoms. In order to reduce the shock wave caused by lowering the grab, the windows on the upper side shall cover an area as large as possible, in practice around 60% of its upper surface. The windows shall be covered with metal gauze of 0.5 x 0.5 mm mesh size.

There may be cases where the use of other gear with smaller sampling area is advisable, e.g. if the fauna is very dense and uniform. When other gears than the standard grab are employed, intercalibrations have to be done on a regional basis and on specific sediments on which these samplers will be used. When a change of gear is intended, it is recommended to sample parallel with both gears for a period of 3-5 years.

Precautions that must be taken when using the grab:

- The settling down and the closing of the grab must be done as gently as possible. Winch operation should be standardized (complete stop and slow lowering (< 0.5 m/s) for the last few meters). This will reduce the shock wave and the risk of sediment loss as a result of lifting the grab before completed closure;

- The wire angle must be kept as small as possible to ensure that the grab is set down and lifted up vertically.

If, as often happens on sandy bottom or erosion sediments, less than 5 l of sediment is collected, the sample should be regarded as not quantitative, and a new sample should be taken after loading the grab with an extra weight. This may as much as double the effective sampling depth of the grab. If less than 5 l of sediment is still collected, the sample may be used, but the low sample volume should be stressed when results are given (Dybern *et al*. 1976). The evidence of this problem may be different in different parts of the Baltic Sea, depending on, e.g., how deep in the sediment the species live.

The choice of sample size and number of samples is always a compromise between the need for statistical accuracy and the effort which can be put into the study. One way to do this is to calculate an index of precision. The ratio of standard error to arithmetic mean may be used (Elliott, 1983), i.e. (x = arithmetic mean, s = standard deviation, n = number of samples). A reasonable error would probably be 0.2 ± 20%.

On the representative stations, at least 3 to 5 samples should be taken, depending on area and species composition, to enable the investigator to reach a certain level of precision by sorting as many samples as necessary. The same procedure is strongly recommended for all other benthos stations unless another sampling strategy (area sampling) is employed in national/coastal monitoring programmes.

Each laboratory shall carefully check the exact sampling area of its grab at several occasions in order to make possible a correct calculation of the number of individuals per square metre. (The area of the grab has a tendency to increase, especially when sampling in stiff clayey sediments.)

4.2 SIEVING

The standard sieve for the Baltic Monitoring Programme shall be of metal gauze (stainless steel, brass or bronze) and have a mesh size of 1.0 x 1.0 mm. In order to collect quantitatively developmental stages of the macrofauna and abundant smaller species it is, however, recommended to use an additional sieve with mesh size of 0.5 x 0.5 mm. This sieve must have the same material as the 1 mm sieve. The mesh size of the sieves has to be checked from time to time for damage and wear. The kind of mesh should be stated.

Attention must be paid to the following points:

- Each sample must be sieved, stored and documented separately;
- The volume of each unsieved sample must be measured. This can be done by grading the container or by using a ruler;
- The grab has to be emptied into a container and should be brought portion by portion onto the sieve as a sediment-water suspension. The use of sprinklers and hand-operated douches to suspend the sample is recommended. Very stiff clay can be gently fragmented by hand. Between the pourings the sieve must be cleaned to avoid clogging and thus to ensure an equal mesh size during the whole sieving procedure;
- The sieving of the sample has to be done carefully in order to avoid damage of fragile animals. Therefore, a direct jet of water against the sieve should be avoided;

- Visible fragile animals, e.g. some polychaetes, shall be hand-picked during the sieving; stones and big shells should be picked out to avoid the grinding effect;
- All residues retained on the sieves should be carefully flushed off the sieves with water from below. Spoons and other tools for sample transfer should be applied carefully. The minor residues in the sieve should be transferred with water;
- When the 0.5 x 0.5 mm sieve is used, the 0.5 and 1.0 mm sieve fractions must be kept separate throughout all further processing;
- Fixed samples should never be sieved.

4.3 OTHER SAMPLING METHODS

Dredge hauls are a valuable complement to grab samples, since mobile as well as large but comparatively rare species are more easily caught by dredging. Dredging is not a quantitative sampling method, but can be useful for qualitative sampling with a five-point scale of abundance. Standardized dredging should always be used when van Veen grabs are likely without fauna, if not done on a routine basis at every sampling. When done so, a visual documentation by video or photography is recommended. Video control and track plotting of dredging actions is recommended as that is the only suitable way of estimating the dredge effort. Descriptions of suitable dredges can be found in Holme & McIntyre (1984) and Bergman & van Santbrink (1994).

In areas where the burrowing depth of the fauna are beyond the penetration depth of the grabs (or that type of gear cannot be used), core samplers may be advisable to use, provided that their efficiency has been satisfactorily proven by intercalibrations to the standard grab.

Photographic and video and records are recommended as a complement to traditional sampling methods. Sediment profile imaging (see e.g. Rhoads and Germano (1982), Rumohr (1995)) may provide a useful means for rapid surveys and classification of sediment structure and bioturbation depth. Side-scan sonar images will provide information on bottom topography and substrate type, which can be useful in the planning of benthos monitoring programmes or in the interpretation of the data. Images should be complemented with 'ground-truth' measurements by underwater video recording and/or grab sampling of sediments.

4.4 FIXATION

The hand-picked animals and the sieving residue shall be fixed in buffered 4% formaldehyde solution (1 part 40% formaldehyde solution and 9 parts water). All necessary measures should be taken to avoid health damage by formalin. For buffering, 100 g of hexamethylenetetramine (Hexamine = Urotropin) shall be used per 1 dm^3 of 40% formaldehyde. Sodiumtetraborate (= Borax) in excess may also be used.

5. LABORATORY ROUTINES

5.1 STAINING

In special cases, i.e. samples from sandy bottoms, it may be advisable to stain the 1 mm sieve samples to facilitate the sorting process. However, in some cases staining may cause problems with species determination.

The staining shall be done before sorting by:

- wash the sample free from the preservation fluid by using a sieve with a mesh size smaller than 0.5 X 0.5 mm;
- allowing the sieve to stand in Rose Bengal stain (1 g/dm^3 of tap water + 5 g of phenol for adjustments to pH 4-5) for 20 minutes with the sample well covered.

However, Rose Bengal (1 g/dm^3 of 40% formaldehyde) may be added already to the fixation fluid.

5.2 SPLITTING OF SAMPLES

Splitting and pooling of samples should be avoided. Instead of splitting use a smaller core sample obtained using a standardized method, which should be fully documented.

5.3 SORTING

Small portions of the unsorted material shall be put on a 0.5 mm mesh size sieve and washed with tap water, so that sorters are not exposed to formalin vapour. Sorting should always be done using magnification aid (magnification lamp, stereo-microscope). Any finer fraction (< 1 mm) should always be sorted under a stereo-microscope.

Broken animals shall only be counted as individuals by their heads (e.g. polychaetes) or hinges of bivalves with adhering pieces of tissue.

5.4 BIOMASS DETERMINATION

Samples should be stored for at least three months before weighing. The biomass shall be determined as dry weight and ash-free dry weight.

The biomass determination shall be carried out for each taxon separately.
All polychaetes should be removed from the tubes, other methods have to be explicitly stated (e.g. for large numbers of polychaetes).
The dry weight shall be estimated after drying the formalin material at 60^{o}C to constant weight (for 12-24 hours, or an even longer time, depending on the thickness of the material).

5.5 SUPPORTING MEASUREMENTS

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). Ash-free dry weight should be determined after measuring dry weight. It is measured after incineration at 500-520^o C in an oven until weight constancy is reached, 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°), at which a sudden loss of weight may occur owing to the formation of CaO from the skeletal material of many invertebrates ($CaCO_3$). 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.

As a simple measure of grain size distribution for the upper 5cm the following sieves should be used: 63µm, 125 µm, 250 µm, 500 µm, 1000 µm and 2000 µm together with weight loss on ignition (500° C - 520° C), total organic carbon and pigments (recommended).

6. RECOMMENDATIONS FOR QUALITY ASSURANCE

Most of the recommendation given here are based on the outcome of two ICES/HELCOM workshops on Quality Assurance of Biological Measurements in the Baltic Sea (ICES, 1994; ICES, 1996).

6.1 GENERAL REMARKS

Experienced and well-trained personnel is a prime basis for maintaining quality standards on a high level. Allocation of resources for proper training and education of field and laboratory personnel is important.

Ring tests and intercalibration exercises at least on a regional basis should be undertaken regularly basis and be obligatory for institutions delivering data to HELCOM. They should be open to all institutions including private industry. Technicians who carry out the actual procedures rather than managing scientists should take part in the exercises.

Exact positioning and correct depths when sampling should be noted in the protocols to avoid comparisons between samples taken at different localities (although noted as the same station in the protocols). If exact positioning due to weather or technical problems is impossible, then fix station work to the correct depth.

Track-plotting during sampling (especially when dredging) is highly recommended, since it both gives information on the size of the area sampled and, if the track-plots are saved, after some time they can provide a detailed depth map of the station.

The number of steps in the sampling and sieving procedures must be kept as small as possible.

Decks hoses are not suited for washing subtle benthos samples. Washing samples on sieves by hand in water-filled containers is recommended as the most gentle way of washing samples.

The use of large sieves is encouraged because:

- the risk of clogging is kept low;
- on sandy bottoms so much sand might be collected that small sieves are filled or even overfilled;
- they reduce the risk of spilling when transferring samples from containers/buckets to the sieves.

Only suspended matter must reach the sieves. The use of water jets directly onto the sieving nets is forbidden.

Rejection criteria for samples are that the samples should be rejected if:

- less than 5 litres is obtained (van Veen, for Haps less than 15 cm penetration);
- incomplete closure is noted;
- obvious uneven bite is noted;
- spillage during transferring of samples is observed;
- samples clearly deviate from the other samples, if noted during sampling, (they should be kept though, but another sample should be taken to replace this in calculating the mean for the station).

If samples are sorted alive, care should be taken to avoid predation within the sample.

It is advisable to stain the samples to facilitate sorting, if this does not hamper species identification.

6.2 CONTROL ROUTINES

To check whether animals are lost when sieving because of using too high water pressure, an extra 1 mm sieve should be placed below the main sieve which should be checked for the number of animals found there after sieving the sample.

The sorting efficiency of the personnel sorting the samples should be checked by an experienced technician. At least 5 % of the samples should be checked for sorting efficiency. Also the species determination should be checked in the same way.

All data lists must be proof-read after input to the computer, before usage. Any spread sheet can be proof-read by the computer with a Sound Card, so you do not need two persons to do it.

One way to check the quality of numbers in the database is to compare individual mean weights. If they are abnormally high or low, the figures need verification.

6.3 TAXONOMY

Lists of taxonomic literature in use should be reported with the data.

Regional taxonomical workshops should be held on a regular basis and be attended by every laboratory.

A checklist of species in the area should be developed, distributed to the participating laboratories and updated regularly.

If the dry weight and ash-free dry weight are determined by drying-burning, an extra sample should be taken and kept preserved unsorted for some years as a reference, in order, for example, to be able to go back to check for the presence of new species.

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

6.4 IN-HOUSE QUALITY ASSURANCE

All laboratories should develop programmes for in-house QA, including the appliance of a quality assurance manual.

Signed protocols should be obligatory for all steps in the analyses.

Taxonomic certification of the persons responsible at the laboratories is recommended.

7. REPORTING REQUIREMENTS (Updated 25/11/2021)

The data should be reported to the HELCOM Combine database hosted by ICES (https://dome.ices.dk/) in accordance with the Environmental Reporting Format (see ERF3.2.doc and Simplified_Format_ Communities_PP-ZP-PB-ZB.xlsx at https://www.ices.dk/data/Documents/ENV/Environment_Formats.zip), to be available and included in HELCOM assessments.

Data should be reported at sample level (actual counts/weights in the sample) together with the sampled area (grab size). Aggregated count/weight values (e.g. average values of replicates or m-2 -values) should not be used.

The reported taxonomical information should follow WoRMS taxonomy

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