OSPAR COMMISSION

Protecting and conserving the North-East Atlantic and its resources

JAMP Eutrophication Monitoring Guidelines: Benthos

(OSPAR Agreement 2012-12)[1]

Contents

[1] Replaces Agreement 1997-6

1. Introduction

Benthic communities (including hard-bottom and soft-bottom macrophytobenthos and hard-bottom and soft-bottom macrozoobenthos) generally occur in recognisable states, depending on the substrate, depth, wave exposure and salinity etc. Macrobenthic communities are an appropriate target for monitoring since:

a) an important component of benthic communities is that formed by species which are long-lived and which therefore integrate environmental change over long periods of time;

b) they are relatively easy to sample quantitatively;

c) they are well-studied scientifically, compared with other sediment-dwelling components (e.g. meiofauna and microfauna) and taxonomic keys are available for most groups;

d) community structure responds in a predictable manner to a number of anthropogenic influences (thus, the results of change can be interpreted with a degree of confidence);

e) there may be direct links with commercially valued resources, e.g. fish (via feeding) and edible molluscs;

f) the floral part integrates long-term change of water quality (turbidity).

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, a shift from k-selected to r-selected species, shifts in functional groups, changes in community structure and an impoverishment of benthic communities due to anoxia. These guidelines are intended to support the minimum monitoring requirements of the Monitoring Programme. [2]

Much information exists on methodology for benthos investigations. The most relevant reports are those by Rumohr (2009) which deals largely with methodology for the collection and treatment of the soft-bottom macrofauna, and by Rees et al. (1991) and Rees (2009) which focus on the monitoring of benthic communities around point-source discharges and epibenthic studies, respectively. These accounts also deal more generally with the role of benthos studies in investigations of human impact, including guidance on the sampling of different substrate types. The HELCOM 'COMBINE' manual for monitoring in the Baltic Sea is another important reference source (see www.helcom.fi).

A range of other documents are of value in the planning and carrying out of marine benthos sampling programmes. The most useful is that by Eleftheriou and McIntyre (2005) which is a standard reference for work of this type. Gray et al. (1992) report on approaches to marine pollution assessment and provide practical examples of applying the PRIMER ('Plymouth Routines in Multivariate Ecological Research') package for univariate, graphical and multivariate data analyses (see Clarke and Gorley, 2001 for further details). Kramer et al. (1994) have produced a manual for the sampling of tidal estuaries. An account of survey methods employed by a team of scientists undertaking a review of marine nature conservation in UK inshore waters together with a rationale for such work is given by Hiscock (1996), Davies et al. (2001) and Connor et al. (2004). A monitoring programme and monitoring guidelines have been prepared for the Wadden Sea 'Trilateral Monitoring and Assessment Programme' (TMAP, 2000). The last update of this document was mainly to harmonize it with the EN ISO 16665 (2005) a European and International Standard on quantitative sampling and sample processing of marine soft-bottom macrofauna. For marine biological surveys of hard-substrate communities the EN ISO 19493 (2007) gives advice. These EN ISO guidelines are mandatory regulations which have to take over in national regulations and should be consulted when detailed questions on sampling and sample processing are to be cleared.

[2] The Nutrient Monitoring Programme as adopted by OSPAR 1995 (OSPAR 95/15/1, Annex 12).

2. Purposes

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

a) to monitor the spatial variability in species composition and biomass within the Maritime Area resulting from anthropogenic nutrient inputs;

b) to monitor temporal trends in species composition 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;

c) to support the development and implementation of a common procedure for the identification of the status of the benthic communities;

d) to understand the relationship between nutrient concentrations and temporal trends in species/community characteristics.

3. Quantitative objectives

The patchy distribution of benthic communities together with the many taxa involved means monitoring programmes are very dependent on the design of the field programme. It is very difficult to formulate a general monitoring model suited to a wide variety of organisms, particularly for epilithic habitats. Furthermore, great care must be taken when transferring techniques developed in less complex systems (e.g. the Baltic Sea) to more complex systems (e.g. the North Sea). Taking into account these precautionary notes, the objectives of benthic monitoring are as follows:

a. to assess the influence of eutrophication on changes in community composition and function, biomass and community structure;

b. to assess the influence of eutrophication on increase in the abundance of ephemeral/annual algae such as *Cladophora*, *Ulva* and *Ectocarpus* and a decrease in perennial algae such as *Laminaria* and *Fucus* and the angiosperm *Zostera marina* (eelgrass);

c. to assess the influence of eutrophication levels on a decreased depth distribution of the macrophytes (e.g. due to increased turbidity).

The minimum requirements for surveys on hard-substrate according to the main aim of a survey are given in EN ISO 19493 (2007). Prior to monitoring, it is necessary to determine the number of sample replicates required to describe the species spectrum (this may be done using a species area curve or a comparable advanced technique. Alternate methods can be used when fixed frames or transects are utilized). Before sampling begins, levels of acceptable variability must be set and followed for all parameters measured. The effects of organic matter inputs on benthic communities are adequately described by the empirical “enrichment” model of Pearson and Rosenberg (1978) and examples of studies which have postulated links between changes in the benthos and eutrophication are given by ICES (1995). The model, which is equally applicable to trends in space and time, describes cyclical (i.e. non-linear) changes in numbers, densities and biomass of benthic species along an enrichment gradient. Multivariate analytical methods may be used to examine between-station differences and temporal trends in the data. Univariate measures amenable to statistical testing include:

- a count of species (coverage of plants and colonial animals included);
- a coverage of plant species and colonial forms;
- measurement of densities and biomass;
- quantification of species in terms of functional groups e.g. feeding types;
- categorisation into r-selected and K-selected species.

The natural patchiness of benthic communities must be accounted for in the analysis. Hierarchical statistical methods may be used. Sophisticated computer packages for the statistical analyses of benthic data are now widely available. Use should be made of at least one established diversity index and one multivariate analytical technique. A consideration of trends in the “primary” variables (i.e. numbers of individuals, taxa and biomass) should also be undertaken in relation to physical/chemical measures derived from sediment sub-samples. The statistics for these evaluations may be undertaken using appropriate software packages.

4. Sampling strategy

Sample sites should be representative of the whole monitoring area and 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. It is important to establish the baseline community structure and variability at the site under consideration, in relation to area sizes to be assessed separately (e.g. WFD types). 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 changes control/reference sites (preferably at least three) are required for each test site. It is critical that similar habitats are selected for comparison. There are several sources of guidance on the design and implementation of field sampling programmes, including Elliot (1971), Cohen (1977), Green (1979), Andrew and Mapstone (1987), Skalski and Robson (1992), Rees et al. (1991 and 2009), Underwood (1997) and Underwood and Chapman (2005). An eutrophication-related monitoring programme would typically include a desk study and survey planning stage, followed by pilot, baseline and ongoing surveys, which should be available already from ongoing monitoring.

The sampling strategy for macrophytobenthos and hard-bottom macrozoobenthos is described at Technical Annex 1. The sampling strategy for soft-bottom macrozoobenthos is described at Technical Annex 2. Rejection criteria for insufficient grab samples are:

- the penetration depth is too low;
- the grab is (almost) empty, has obviously not closed properly;
- the grab has penetrated diagonally into the ground (very different stack height);
- loss of material during the screening or the placing of the animals in the sample vessels.

Is the minimum depth of penetration not reached at a station, to at least 5 attempts, the grab samples with the greatest penetration depth should be taken and the procedures and reasons for the deviations from the standard method must be recorded in the sampling protocol.

All steps in the sampling and analytical procedure must be documented in written form.

5. Sampling equipment

The sampling equipment for macrophytobenthos and hard-bottom macrozoobenthos is described at Technical Annex 1. The sampling equipment for soft-bottom macrozoobenthos is described at Technical Annex 2. For all activities the health and safety rules requirements have to be enforced strictly.

6. Storage and pre-treatment of samples

The storage and pre-treatment of macrophytobenthos and hard-bottom macrozoobenthos samples is described at Technical Annex 1. The storage and pre-treatment of soft-bottom macrozoobenthos samples is described at Technical Annex 2.

7. Analytical procedures

Analytical procedures for macrophytobenthos and hard-bottom macrozoobenthos are described at Technical Annex 1. Analytical procedures for soft-bottom macrozoobenthos are described at Technical Annex 2.

The data generated should be stored in a database. The database should be of a type capable of storing and/or generating information of the following type:

- the spatial distribution and size of epilithic communities, particularly concerning mats of green macroalgae, eelgrass meadows and mussel beds;
- sketch illustrations showing the distribution of substrate types and the dominant species associated with the substrates;
- the depth distribution of plant and animal biomass by species, functional group and any other arbitrary selection, as well as the relative quantities of the primary functional groups such as dominant, annual and perennial organisms;
- temporal trends concerning changes in depth distribution, percentage cover, biomass, species composition and distribution etc.;
- a statistical evaluation including explanatory power;
- correlations of specific types of benthos data against supporting information (e.g. Secchi depth, salinity, oxygen, nutrients, pelagic primary production, other types of benthos data)
- characterisation of the variability in space and time, sediment qualities, slope of shallow banks and tidal stages, oxygen concentrations, tidal level, stratification of overlying waters (extension, frequency) and salinity.

The description of the sediment character is valuable supporting information. As a measure of grain size distribution for the upper 5 cm of the sediment the following sieves should be used: 63 µm, 125 µm, 250 µm, 500 µm, 1000 µm and 2000 µm. Other more advanced methods such as Laser diffraction, sedimentation columns etc. may also be used. Further parameters like weight loss on ignition (500 °C – 520 °C), total organic carbon, particulate nitrogen and pigments should also be measured, as supporting/interpretation variables (recommended).

8. Quality assurance

The quality assurance (QA) programme should ensure that the data are fit for the purpose for which they have been collected. The objectives on assuring the quality of biological and ecological assessments are described in EN 14996 (2006). Appropriate QA schemes based on EN ISO/IEC 17025 (2005) should be established before the onset of survey work. An accreditation by a recognized accreditation authority is recommended.

It is particularly important that adequate resources are allocated for these purposes when co-operative studies involving several institutes are to be conducted, or when the data are to be centrally archived. It is essential that the QA also includes the explanatory power and the experimental design. Thus, the QA must take into account as many steps of the analytical chain as possible in order to determine the contribution of each step to the total variation.

Quality assurance methods are still under development for some activities, e.g., biomass determinations. If the abundance estimates are to be carried out by different workers, an intralaboratory comparison of their cover estimates in the field must be performed. This can be accompanied by comparing in situ survey data with digital and point sampling estimates of underwater photo documentation. Underwater photography

and/or video may provide an additional means of obtaining cover estimates but these techniques are more appropriate where foliose phytobenthos does not obscure underlayers. Animals that can be counted often provide a better basis for estimates of cover than subjective assessments or point sampling.

National and international Cross checks between separate sampling programmes (estuaries/coastal waters) are recommended.

The latest taxonomic literature should be used. Name changes and literature used must be recorded. Quality assurance for soft-bottom macrozoobenthos should take account of Rees (2004) and Rumohr (2009) (see also ICES 1994, 1996). Each Contracting Party which intends to deliver data to a common data pool should take part in regular quality control audits such as interlaboratory comparisons, ring tests and associated taxonomic workshops. The compilation of reference collections is recommended. Voucher specimens should be deposited regularly at museums to make later taxonomic checks possible.

9. Reporting requirements

For data reporting the Integrated Reporting Formats for Environmental Data of ICES should be used. Data for the common pool will have to be submitted via the national data centres in order for them to keep in touch with progress of the work, including the availability of data from each Contracting Party. This procedure should help to guarantee data quality, since the national data centres will be ultimately responsible for the timely submission of completed data sets to the common pool.

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Technical Annex 1
Hard-bottom macrophytobenthos, soft-bottom macrophytobenthos and hard-bottom macrozoobenthos

1. Sampling strategy

An overview of the methods available for monitoring has been given by ICES (1996), Hiscock (1996), Davies et al. (2001), Eleftheriou and McIntyre (2005), Rees (2009) and EN ISO 19493 (2007). Diver operated methods in shallow water and remote underwater photography in deeper areas, are the most suitable options.

Monitoring should take place annually at a particular time within the four summer months (June–September) for the first three years of the monitoring programme. Subsequent sampling frequency then depends on the expected rate of change in species composition. In areas where large changes are expected sampling should take place on an annual basis. In areas where little change is expected sampling every 5 to 10 years would be sufficient. Three main sampling techniques are available for hard-bottom and soft-bottom macrophytobenthos and for hard-bottom macrozoobenthos: aerial surveillance (in tidal areas), diving transects (in sub-tidal areas) and quantitative (destructive) sampling. Voucher specimens should be deposited regularly at museums to make later taxonomic checks possible.

2. Aerial surveillance

Aerial surveillance can be used as an optional method to determine the size and distribution of epilithic communities, including mats of green macroalgae, eelgrass meadows and mussel beds. High-wing monoplanes flying at low altitude (150 m) are an appropriate platform for the relevant sensors. Positions should be located by means of satellite navigation (i.e. GPS). Aerial surveillance can cover large areas and results should always be validated by means of quantitative field inspections (ground truthing) at selected locations (see section entitled “Quantitative sampling”). When applied, aerial surveillance of green algae should take place during May–October at four-week intervals during low tide. One flight should be carried out at the end of the winter for mapping the distribution of mussel beds.

3. Diving transects

Diving transects are used to provide a description of the depth distribution and abundance of the dominant plant and animal communities. The length of transects should extend to at least the maximum depth of the algae, but should not be deeper than 30 meters (for diver safety). Depth limits of kelp, dense foliose algae or the deeper foliose algae may be measured using digital instruments, recorded and corrected for tidal amplitude. Abundance and/or coverage should be determined at sites within the main assemblages or within sub-habitats, if these are distinct. The coverage should be used for plants and animals in colonies or high abundance. Reconnaissance surveys, which may include remote sensing (see section entitled “sampling equipment”) are also useful in helping to choose transect locations. Transect surveys should be undertaken at the beginning of the monitoring programme and should be repeated regularly, for example every 5 to 10 years. As estimates of distribution and percentage cover are carried out in situ, a cord with meter marks should be placed along the transect. Progressing along this cord, divers should note the distribution and type of substrate as well as the degree of cover for the main plant and animal species in a strip 5-10 m wide. Divers should estimate abundance using an appropriate scale (Hiscock 1990, Kautsky 1993, Krause-Jensen et al. 1994, Karlsson 1995, Pedersen et al. 1995, EN ISO 19493). This may be time consuming under water, but gives a good estimate over the whole depth zone, which is much harder to achieve using frames. An alternative approach would be to apply the abundance estimation scale at fixed sites within the main zonal

biotopes. Species/categories that are not immediately obvious may warrant the use of more time-consuming techniques such as quadrat counts (see section entitled “Quantitative sampling”).

The following information should be recorded in the field:

- the exact position of the transect (using for example a map, photography, a permanent mark on the shore, GPS
- the distance from the shore (using a meter marked line along the transect);
- the depth (according to a calibrated depth gauge and corrected for tidal amplitude);
- substrate type (rock, boulders, stones, gravel, sand, mud, glacial clay, etc.);
- the presence of loose sediment deposited on plants and substrate (in terms of “none”, “little covered”, “heavily covered”);
- an estimate of the abundance of different plant and animal species;
- the maximum depth of dominant sub-littoral species and the lower limit of vegetation;
- the degree of wave exposure, Secchi disk depth (i.e. light transmission) and salinity (if possible).

A photographic and/or video documentation (video/photographic profiles of the transects, panoramic views and, at fixed marked sites if possible, stereo photographs) is recommended. The use of satellite image based software is suggested to visualise the exact location of a diving transect.

4. Quantitative sampling

Depending on the time spent on analysing a transect, direct observations by divers may overemphasise the importance of particular eye-catching species. Quantitative sampling by divers gives unbiased information about plant and animal communities but is extremely time-consuming. Quantitative samples, obtained via stratified random sampling, are required in order to determine species composition and biomass. At least three parallel quantitative samples of key species/communities should be collected at different pre-selected depth intervals. Sample locations at each depth are chosen by random placement of a quadrat, or by sampling at random distances along the transect from the shore. Tests should establish the number of parallel samples and the minimum sample area, and this will vary according to the type of community/species being sampled and its distributional characteristics (Elliott, 1983). For example small but patchy distributed species may require large quadrats, whereas it may be possible to use relatively small quadrats for small but evenly distributed species. Rocky habitats are usually architecturally very complex and care is needed to specify slope, aspect and exposure. These methods follow recommendations by Anon. (1991) Dybern et al., (1976), Hiscock (1987), Hiscock and Mitchell (1989), Jespersen et al. (1991), Kautsky (1993) and Davies et al. (2001).

The following data should be recorded in the field whenever possible:

a) the exact distance of the sample site from the shore;

b) water depth (according to a calibrated depth gauge and corrected for tidal amplitude);

c) a photographic image of the site;

d) the number of individuals of each taxon;

e) the coverage of plant and animal taxa;

f) the size structure of some animals (mainly molluscs);

g) tidal level, salinity, temperature, Secchi depth, size of sampled area etc.

Biological material should also be collected for biomass determinations, as reference specimens for herbaria etc. and for algal toxins (in conjunction with other monitoring programmes sampling should be carried out at the same time). The selection of appropriate fixation and preservation media depends on the purpose and has to be agreed with the receiving institutes.

5. Sampling equipment

Submarine video in combination with GPS is useful for sampling transects and for surveying large areas for approximate species composition and the depth distribution of the vegetation as a whole. Larger areas may be scanned using remote-sensing techniques (e.g. by satellite or aircraft), but only for communities close to the surface. For visual inspections during low tide in intertidal areas, manual mapping is sufficient. For aerial surveillance vertical images and video recordings are generally cost-effective techniques.

Surveys estimating abundance should be sampled within a large area containing the same biotope in order to reduce edge-effects or effects resulting from irregular species distribution. Quadrate frames with a side length of 0.10 m to 0.50 m are suggested for quantitative sampling (the smaller frames should be used in the littoral zone for small species such as barnacles).

6. Storage and pre-treatment of samples

Sampled material should be preserved by freezing (- 20 °C) or by using formaldehyde (2 – 4 %). It should be emphasised that thawing may cause leakage and thus underestimate biomass, and that species may react differently depending on their morphology. The same also applies for preservation with formaldehyde. Fixation using formaldehyde should be avoided for samples which will be analysed for nutrients and for further genetic analysis. Samples for biomass determination must be free of overgrowth and rinsed with freshwater before drying. Sampled animal material should be stored in alcohol (70 %) after biomass (wet weight) determination.

7. Analytical procedures

Macrozoobenthos measurements should comprise individual length, width, volume etc. Macrophytobenthos determinations should normally be accompanied by the co-monitoring of relevant macrozoobenthos and vice versa.

Samples obtained using quadrate frames (see section entitled “Quantitative sampling”) may be analysed to determine plant and animal species composition and biomass. In areas where species numbers are low biomass may be expressed per species.

The degree of accuracy required for taxonomic sorting depends on the purpose of the monitoring programme. Normally, taxa should be determined as precisely as possible. Some times it should be sufficient to identify organisms, whose taxonomic specification is difficult or time-consuming, to the genus level rather than to the species level. (e.g. *Cladophora* spp., *Ulva* spp.). Rare species may be sensitive and thus indicative species. They should be determined with higher taxonomic resolution if possible. All functional groups should be covered as far as possible.

The following measures of biomass [g/m²] should be used: dry weight and/or ash-free dry weight. For dry weight results the biological material should be dried at + 60 °C until constant weight (this can be up to one week depending on volume of sample). For ash-free weight the samples should be combusted at + 500 °C until constant weight (at least 6 h). Biomass expressed as volume (e.g. using water displacement) should be measured in the field whenever possible.

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Technical Annex 2
Soft-bottom macrozoobenthos

1. Sampling strategy

An initial spatially extensive “baseline” survey will facilitate the selection of representative stations within and in adjacent to areas perceived to be vulnerable to the effects of eutrophication. It will be necessary to repeat the baseline survey periodically to check the continued validity of representative stations and to ensure that no unexpected effects are occurring beyond the region predicted to have been affected by eutrophication. Full use should be made of historical information in the planning of surveys and for assessments.

Large-scale sampling of the macrozoobenthos community in offshore subtidal soft-bottoms should comprise as many stations as needed for an e.g. 10 % resolution and an adequate replication number per station. A large-scale sampling grid (preferably stratified random) covering the whole area of investigation should be sampled at intervals of 10 years and this should be sampled by a variety of methods in order to cover the full range of the species spectrum. This large-scale sampling every 10 years is necessary to confirm the representativeness of annual temporal trend monitoring stations. For temporal trend monitoring, sampling at a frequency of once per year (at the same time of year) should be adequate, although locally severe effects of nutrient enrichment (such as hypoxia) may dictate a higher sampling frequency. If the sampling frequency is twice per year, then sampling should take place in late winter/early spring to establish the stable community conditions and in late summer/autumn to detect possible effects of nutrient and biomass enrichments (such as hypoxia) on the macrozoobenthos.

The sampling strategy for macrozoobenthos communities in coastal soft-bottom areas needs site-specific adaptations of site selection, choice of sampler and sampling frequency (see, e.g., Trilateral Monitoring and Assessment Program, 2000). For example: estuaries should be sampled from the limnic to the marine area, backwaters and lagoons should be sampled twice a year at representative stations (a large-scale sampling programme should be performed every 5 years) and fjords should be sampled along a transect ending at the outer edge of the sill. The sampling strategy should take into account also the hydrochemical conditions (e.g. sampling along salinity gradients should be considered to get reliable data on number of species, species composition, biomass etc.).

The following information should be recorded in the field:

- whether or not the ship was anchored;
- depth and position of each replicate; a GPS track plot would be desirable;
- date and time;
- the weather conditions during sampling and sea state (e.g. wave amplitude);
- a description of the sediment, including:
    - surface colour and colour change with depth (as a possible indicator of redox state);
    - smell (H_2S);
    - a description of sediment type, including important notes such as the occurrence of concretions, loose algae;
- the type and specification of the sampling device (e.g. weight and sampled area);
- mesh size of the sieve;
- tidal level, salinity, temperature, Secchi depth, size of sampled area etc.

Near-bottom temperature, salinity and oxygen measurements are desirable. If more than one sample is taken at a station, the depth range of samples should be recorded. All samples must be treated separately, i.e. must not be pooled. An estimate of the volume of sediment retained should be made for all samples taken, as a measure of sampler efficiency and penetration depth. Criteria for rejection of samples collected by grabs are given by Rees et al. (1991), ICES (1994) and Rumohr (2009). Measurements of redox potential and shear-strength should be made on samples collected by a box corer rather than a grab sampler because grab samplers are likely to distort the sample.

2. Sampling equipment

Sampling equipment appropriate for soft-bottom macrozoobenthos is described in detail by Rumohr (2009) and Eleftheriou and McIntyre (2005). Coarse sediments which cannot be sampled using normal procedures may be sampled using either a Hamon grab or appropriate dredges (e.g. an anchor dredge). Sediment structure and bioturbation depth may be checked with sediment profile imagery (see below). A hand-operated corer should be used for Wadden Sea sediments (TMAP, 2000). It should be noted that more sophisticated gear, such as epibenthic sledges, might be required for sampling hyperbenthic or bentho-pelagic species. Such gear is particularly valuable for studies of species (especially crustaceans) which constitute an important component of the diet of fish. Epibenthic and hyperbenthic sledges (Rothlisberg and Pearcy, 1977), dredges (see also Brattegard and Fosså, 1991) and the Sorbe sledge (Sorbe, 1983) 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 (e.g. 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).

Photographic and video records are recommended as a complement to traditional sampling methods (Rumohr, 1995; Smith & Rumohr 2005). Sediment profile imaging (see Rhoads and Germano, 1982; Solan et al., 2003) may provide a useful means for rapid surveys and classification of soft sediment areas (Nilsson and Rosenberg, 1997a and 1997b). 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. These records should be ‘ground-truthed’ by underwater video recording and/or grab sampling of sediments.

3. Storage and pre-treatment of samples

Procedures for the storage and pre-treatment of soft-bottom macrozoobenthos samples are described at sections 3.1-3.2 of Rumohr (2009).

4. Analytical procedures

Procedures for the sorting and biomass determination of soft-bottom macrozoobenthos samples are described at sections 3.4 and 3.5 of Rumohr (2009).

The following taxa groups do not belong to macrozoobenthos and therefore they should not be determined and counted:

- fishes;
- Harpaticoide Copepoda;
- Nematoda.

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