

1.5.5.4 Review and update of JAMP Eutrophication monitoring guidelines

Request

Review and update of JAMP Eutrophication monitoring guidelines (OSPAR 6-2009)

Given that the JAMP Monitoring Eutrophication Monitoring Guidelines for nutrients, oxygen, benthos, phytoplankton and chlorophyll are now over 10 years old, there is a need to review, and where required update, the guidelines to reflect technical developments, best practice and to ensure that the guidance remains fit-for-purpose. The purpose is to support the monitoring of these parameters for the assessment of eutrophication under the Comprehensive Procedure and, more generally, for WFD and MSFD monitoring.

The request to ICES has two aims:

- a) add more specifications in the current guidelines which includes not only listing different possibilities on analysis but also expressing the most commonly used method if it comes to a choice between different methods and prioritise recommended methods and illustrating best practice so it should be more clear which option to go for as a priority.
- b) add standards and protocols to be used for developing techniques that have not been used as a standard parameter but have recognised added value to support assessments from a more general validation perspective to complement ship-borne measurements.

For each parameter further clarification in the guidelines is needed on the aspects set out below:

Inorganic/organic nitrogen

- a) advice on the period and frequency of sampling to have an accurate idea on winter nutrient concentrations
- b) a more detailed explanation of contamination risks during sampling and analysis and appropriate temperatures and duration of preservation
- c) standards and protocols for moored instrumentation
- d) standards and protocols for satellite assessments to complement ship-based measurements

Biomass of phytoplankton: chlorophyll a

- a) advice on the kind of analysis to be performed on chlorophyll a (advantages and disadvantages of acidification procedure)
- b) advice on the type of chlorophyll a most suitable to report on (total, active, Phaeophytin)
- c) required frequency of sampling for accurate estimate of mean and 90th percentiles during growing season (study of Sweden)

Oxygen

- a) a more detailed explanation of contamination risks during sampling and analysis and appropriate temperatures and duration of preservation needs to be included
- b) advantages of developing sampling methodology and analysis needs to be included
- c) recommendations on accurate analysis of trends (decreased concentration, increased frequency of low O₂ concentration, increased consumption rate)

Benthic community structure

- a) advice on monitoring of sufficient surface should be included (advantages of sampling with different devices: Van Veen grab, Reineck boxcore, others)
- b) advice on fixation should be added: different fixation mechanisms are in place, like fixation before and after sieving the samples, including advice on staining
- c) monitoring of zoobenthos should be done in accordance to ISO 16665 at accredited laboratories or laboratories that can show to perform on this basis
- d) advice on calculating biomass of benthos.

Source of information

ICES 2009a. Report of the Benthos Ecology Work Group (BEWG 2009)
ICES 2009b. Report of the Marine Chemistry Working Group (MCWG 2009)
ICES 2009c. Report of the Working Group on Harmful Algal Bloom Dynamics (WG HABD 2009)

Summary

Revised versions of the JAMP Eutrophication Monitoring Guidelines: Chlorophyll in Water and the JAMP Eutrophication Monitoring Guidelines: Benthos are attached as Annexes. Advice is also provided on parts of the JAMP Eutrophication Monitoring Guidelines: Nutrients but ICES considers it premature to revise the document at this time. Eutrophication is one of the 11 descriptors of good environmental status that is addressed under the European Union's Marine Strategy Framework Directive. A Task Group has been convened to provide recommendations to the European Commission on criteria and methodological standards necessary to assess progress in achieving good environmental status with respect to eutrophication. Therefore ICES recommends that OSPAR await the outcome of that activity in early 2010 before seeking further advice on this subject.

ICES Response

In the past advice on Quality Assurance and Control for Biological Measurements for the JAMP has been provided by a joint OSPAR/ICES Expert Group however for various reasons this group has been disbanded. This advice is collated from the work of a number of ICES Expert Groups. OSPAR was unable to clarify some issues related to the request prior to the ICES scientific meetings and in addition the ICES expert groups identified several critical questions during their considerations of this request. Furthermore the European Commission is developing criteria and methodological standards for the implementation of the MSFD. This includes an assessment of the achievement of good environmental status with respect to eutrophication; specifically the stated goal is that "human-induced eutrophication is minimised, especially adverse effects thereof, such as losses in biodiversity, ecosystem degradation, harmful algae blooms and oxygen deficiency in bottom waters." ICES recommends that OSPAR await the outcome of that activity in early 2010 before seeking further advice on this subject.

Background information

Inorganic/organic nitrogen

a. advice on the period and frequency of sampling to have an accurate idea on winter nutrient concentrations

A report on *Temporal and spatial monitoring of eutrophication variables in CEMP* prepared by SMHI (Axe *et al.*, 2008) was considered by ICES. This report concluded *inter alia* that the optimum sampling programme to observe rapid events is likely to be a combination of ferrybox systems, which appear to be reliable and give both good spatial and temporal coverage, and moored buoy observations. Periodic and discrete sampling from vessels will not be enough to determine accurate winter nutrient concentrations. In addition, to ensure that the data is of sufficient quality, data must be verified with conventional research vessel observations. Research vessels still have a role in seasonal mapping, and in providing data of sufficient quality for trend analysis from a large area.

Under the JAMP, nutrients are monitored in order to determine their spatial distribution, determine temporal trends, and to determine the degree of nutrient enrichment in the OSPAR areas. Monitoring for these purposes should take place at the time of lowest algal activity, which is usually the winter. This is because surface waters become progressively depleted in inorganic nutrients during spring, summer and autumn due to their removal by phytoplankton. Therefore, for the Maritime Area as a whole, the sampling period and the sampling frequency cannot be specified in terms of months or dates; the period is dependent on regional and inter-annual differences. Although it would be desirable to define sampling periods and frequencies for different parts of the Convention area this may be difficult because of spatial differences in the hydrographical conditions. Moreover, there may be inter-annual differences and winter blooms of phytoplankton consuming nutrients may occur at times when it is normally a phytoplankton minimum or nutrient maximum.

b. a more detailed explanation of contamination risks during sampling and analysis and appropriate temperatures and duration of preservation

Sampling

Sampling activities always include the risk of contamination, which may have various sources depending on specific sampling situations. Care should be taken to ensure good laboratory practice during sampling procedure (e.g. avoidance of contamination from ship, cleaning of instrumentation and bottles, etc.). It is recommended that

laboratories performing measurements check contamination risks and document how to minimize and control potential contamination during sampling. Among the common nutrients ammonia is usually the most challenging to determine due to airborne contamination. This can be more challenging at sea as it is more difficult to control the environment in which the analysis occurs. It is also important to avoid contact with cigarette smoke, both in the air and on workers' fingers.

Storage

Nutrient determinations should be carried out as soon as possible after sampling. Ammonia must be determined immediately after sampling, while nitrate, phosphate and silicate should be determined within a few hours after sampling with samples protected from light and stored in a refrigerator between sampling and analysis.

If immediate analysis is not possible samples must be preserved. Commonly used preservation methods are freezing the samples or adding a preservative, e.g. HgCl₂. If the sample contains amounts of particulate matter which may compromise the analysis, it should be filtered to remove the particles before freezing. Care must be taken to ensure that filters are not a source of contamination. Samples for the determination of silicate, which have been frozen, should be defrosted for sufficient time for de-polymerisation to occur. This is particularly important when there are high silicate concentrations in the water.

Since no preservation method for nutrients can, at present, be recommended for general use, each laboratory must validate, and document, its storage methods for each nutrient before they are used routinely. The validation should be done over the whole seasonal cycle to investigate varying conditions e.g. during high and low nutrient concentrations and during high and low primary productivity. The QUASH (Quality Assurance of Sampling and Sample Handling) project (1996–2000) carried out an inter-comparison of sample handling and preservation methods for nutrients in seawater for a number of laboratories. The outcome demonstrated the need for laboratories to validate and document their procedures and highlighted the particular challenges of preserving samples for subsequent ammonia analysis. (QUASH, 2000)

c. standards and protocols for moored instrumentation

ICES is unable to provide advice regarding standards and protocols for moored instrumentation at this time. In order to develop advice for this request ICES recommends that a joint OSPAR/ICES workshop should be held involving scientists and technicians using this technology.

d. standards and protocols for satellite assessments to complement ship-based measurements

ICES is not aware of any use of satellite imagery for the determination of nutrients in seawater.

Biomass of phytoplankton: chlorophyll a

Recommendations for revisions to the “JAMP Eutrophication Monitoring Guidelines: Chlorophyll a in Water” addressing the specific issues under a, b, and c of this part of the request are provided in Annex I.

a. advice on the kind of analysis to be performed on chlorophyll a (advantages and disadvantage of acidification procedure)

b. advice on the type of chlorophyll a most suitable to report on (total, active, Phaeophytin)

ICES recommends that the fluorometric method with an acidification step to distinguish phaeopigments be used and be reported as chlorophyll and phaeopigment. Furthermore ICES advises that where possible all contracting parties should use the same analytical method. This may pose problems for the evaluation of long time series when laboratories change methods. The spectrophotometric method is still used by a number of laboratories but the fluorescence method which is more sensitive is preferred. If the method is changed a long period with parallel use of the new and old method will be necessary.

c. required frequency of sampling for accurate estimate of mean and 90th percentiles during growing season (study of Sweden)

ICES cannot provide any additional guidance regarding sampling frequency more than the existing text in the guidelines. Considering the short generation time of phytoplankton a very high sampling frequency is needed to cover the succession and development of the phytoplankton communities. Knowing the area to be monitored will help optimize the sampling frequency. Sampling frequency must be often enough to resolve bloom events at minimum, sampling should be weekly or biweekly but this may need to be more frequent depending on local

conditions and the species being observed. In order to assess trends it is essential that all of the transient high and low chlorophyll values are resolved.

In this context it is also important to highlight the paragraph in Section 5 of the JAMP Guidelines, Sampling Strategy, dealing with additional microscopic quantitative and qualitative analysis of phytoplankton; note the revised text in Section 5 of Annex 1.

The “study of Sweden” was not available however this is believed to refer to establishing a ‘background’ summer concentration for an area and then as long as 90 % of the measured concentrations are below this no ‘accelerated growth’ is deemed to have occurred. To establish an accurate 90%ile however, it is critical to have an appropriate historical data set based on reference condition waters. The database must be carefully evaluated to ensure that natural events such as increases in chlorophyll due to occasional upwelling of nutrients, or oligotrophic waters do not skew the determination.

Oxygen

- a. *a more detailed explanation of contamination risks during sampling and analysis and appropriate temperatures and duration of preservation needs to be included*

Sampling and Storage and pre-treatment of samples

Many different water samplers may be used to collect discrete samples for oxygen determination. It is essential however, that the water sampler used completely isolates the sample from the surroundings so that no leakage or exchange occurs. In particular circumstances it may be necessary to use a special bottom water sampler.

Immediately after taking the water sample, an aliquot has to be transferred into a calibrated Winkler bottle. Care must be taken to minimize contact between the water sample and atmosphere, especially in samples with low oxygen concentrations. This includes the process of transferring the water from the sample bottle into the Winkler bottle as well as by introducing air into the sample bottle due to leakage. As this transfer of the sample is one of the steps in the whole determination procedure which is responsible for the greatest error, only well trained personnel should be allowed to take the samples.

Oxygen may also be determined using sensors. These sensors may be used attached to a CTD system, as part of an autonomous system on moored platforms or installed on ships for continuous measurements. The advantage of sensor measurements is the provision of high resolution data in space or time, depending on the instrumentation used. Sensors can be particularly useful, compared with conventional discrete sampling techniques, for determining temporal and spatial variability and for capturing short term oxygen deficiency or supersaturation events. On a commercial basis Clark type and Optode type sensors are widely available (Moore *et al.*, 2009).

As all sensors have limitations in their performance, no type of sensor can be generally recommended. These limitations may include the sensitivity, the precision of measurement, low response time, instability of measured results, instability due to varying environmental conditions, poisoning in anoxic waters, etc. Therefore it is necessary to test different sensors and select the one most suitable for measurements in the area to be observed.

Apart from a proper selection of a sensor, the calibration and handling of any sensor has to be validated. Furthermore, regular control, using the Winkler method as reference, is essential. Intervals of calibration, control of measurements and maintenance depend on the type of sensor and the environmental conditions in which the sensor is used. These intervals have to be evaluated and controlled as one element in the validation process of the sensor. The validation process also includes a description of the handling of the sensor in order to obtain the specified precision of the sensor.

Oxygen in discrete samples must be fixed immediately after collection to bind the oxygen in the sample. The precautions mentioned above must be maintained. After fixation, samples have to be kept in a dark place at a constant temperature - if possible the same as the *in situ* temperature - for at least one hour. The fixed sample should be titrated within 24 hours of collection. In some cases longer storage of the fixed sample may be necessary. Although not recommended, longer storage is possible, provided that storage conditions and handling procedures are validated and clearly documented. Zhang *et al.* (2002) noted that storage under seawater is advisable in such circumstances. Sensors for oxygen determination are designed for *in situ* measurements and should not be used for analysis of discrete samples.

b advantages of developing sampling methodology and analysis needs to be included

ICES notes that there are no new methods that need to be included, but of note there are several technical developments that improve oxygen measurements or allowing high frequency sampling. Due to specific requirements of different sampling sites, no general recommendation for specific developments can be made.

c recommendations on accurate analysis of trends (decreased concentration, increased frequency of low O2 concentration, increased consumption rate)

ICES is unable to respond to this request at this time due to the unavailability of statistical expertise.

Benthic community structure

The revised guidelines are included as Annex 2 and these revisions address the specific following requests (a) through (d).

- a. advice on monitoring of sufficient surface should be included (advantages of sampling with different devices: Van Veen grab, Reineck boxcore, others)*
- b. advice on fixation should be added: different fixation mechanisms are in place, like fixation before and after sieving the samples, including advice on staining*
- c. monitoring of zoobenthos should be done in accordance to ISO 16665 at accredited laboratories or laboratories that can show to perform on this basis*

ICES notes that the size and scope of the ISO and JAMP documents are quite different. It must be remembered that the JAMP document is not a manual, per se, whereas the ISO document, by its nature, has to be considerably more prescriptive. The guidelines have been revised to make certain that they are consistent with the ISO specifications and refer the user to the ISO guidelines where more detail is required.

d. advice on calculating biomass of benthos.



JAMP Eutrophication Monitoring Guidelines:
Chlorophyll in Water

JAMP Eutrophication Monitoring Guidelines: Chlorophyll in Water 7

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JAMP Eutrophication Monitoring Guidelines: Chlorophyll in Water

About this document

This document is an updated version of a document with reference number 1997-4 *OSPAR Guideline on Eutrophication Chlorophyll a*. The modified text is highlighted.

Title: Monitoring changes in phytoplankton chlorophyll

1. Introduction

Nutrient enrichment of coastal waters can give rise to increased primary production and phytoplankton biomass which is part of the process of eutrophication. The human driven process of coastal eutrophication is considered to be undesirable and there is a requirement for an integrated monitoring programme to characterise the status and changes in coastal waters with respect to eutrophication. This will include: the loadings of dissolved nutrients to a given coastal area and winter concentrations of dissolved inorganic nutrients; an estimate of phytoplankton biomass and evidence of accelerated growth (an increase in biomass); undesirable disturbance to the balance of organisms and to the quality of the water. Chlorophyll is used as an estimate of phytoplankton biomass and to provide evidence of accelerated growth.

2. Purposes

The measurement of chlorophyll concentrations in microplankton cells (either in vivo, following extraction of pigments from cells or in vivo, within the cells) is carried out for, *inter alia*, the following purposes:

1. to establish temporal trends, in phytoplankton biomass
2. to compare the measured level of phytoplankton biomass against a predetermined background threshold to provide evidence for or against accelerated growth.

3. About chlorophyll a

Phytoplankton biomass is regularly reported as chlorophyll or chlorophyll a and has been for some 40 years. However, the use of chlorophyll is not without difficulties (the chlorophyll content of cells can vary, as does the cellular carbon to chlorophyll ratio) and there are methodological issues. For long term studies, which is the main purpose of JAMP, it is essential to keep methods unchanged if possible. If the method is changed a long period with parallel use of the new and old method is necessary.

Chlorophyll a is a light harvesting pigment that occur in most microalgae including cyanobacteria (blue green algae). Related pigments found in microalgae include chlorophylls b, c₁, c₂ divinyl chlorophyll a and divinyl chlorophyll b. To specifically measure concentrations of chlorophyll a, the pigment must first be separated from the other chlorophylls and breakdown products using a technique such as HPLC (High Performance Liquid Chromatography) (e.g. Wright, 1991). The standard fluorometric methods for determining chlorophyll recommended by JAMP do not completely separate the different chlorophylls or distinguish between chlorophyll a and chlorophyllide a. The term "*chlorophyll*" or "*Chl*" should therefore be used when reporting results from these methods. For data on Chlorophyll a obtained using HPLC the term "*chlorophyll a*" or "*Chl. A*" should be used.

Pigment extracts will also contain variable amounts of the chlorophyll precursor pigments/ breakdown products chlorophyllide a, phaeophorbide a and phaeophytin a. It is possible to discriminate the two phaeopigments from chlorophyll a by making measurements of fluorescence on the pigment extract before and after the addition of acid; the so called 'acidification method'. This method relies on the changes in fluorescence when chlorophyll is converted to phaeophytin following acidification of a pigment extract.

The non acidified fluorescence method gives an estimate of pigment concentration which is a combination of chlorophyll a + chlorophyllide a + phaeophorbide a + phaeophytin a. This should therefore be reported as chlorophyll pigment.

The fluorescence method with an acidification step gives chlorophyll a + chlorophyllide a and phaeophorbide a + phaeophytin a (together with some chlorophyll b, c₁ and c₂) and should be reported as chlorophyll and phaeopigment.

Terms, acronyms and units in summary

Chlorophyll (Chl. $\mu\text{g l}^{-1}$) is measured using extracted pigments in a filter fluorometer

Chlorophyll and phaeopigment (Chl.+Phaeo., $\mu\text{g l}^{-1}$) is measured before after acidification in a filter fluorometer.

Chlorophyll a (Chl. a $\mu\text{g l}^{-1}$) is measured following HPLC separation of pigments.

4. Quantitative objectives

The quantitative objective must be to unambiguously link an increase in chlorophyll to nutrient enrichment. To achieve this, the monitoring programme must resolve the natural seasonal and inter-annual variability in chlorophyll and include reference sampling stations outside the region of enriched water which can be used to resolve the effects of other pressures such as climate change.

It is intended that the region-specific temporal trend monitoring programme should have the power (90%) to detect a change in concentration (50%) over a selected period (10 years). To clarify the situation and to help define objectives Contracting Parties should undertake statistical analyses of their existing data sets. This would help to determine the representativeness of the monitoring stations and thus the selection of suitable sampling stations and sampling frequencies.

The monitoring programme should enable Contracting Parties to determine the representativeness of their monitoring stations with regard to the spatial and temporal variability in chlorophyll concentrations. This would include a definition of the extent of the monitoring area and selection of representative monitoring stations.

5. Sampling strategy

In many coastal waters there is a pronounced seasonal cycle of phytoplankton growth and both the timing and magnitude of seasonal events such as the spring bloom exhibit interannual variability. As such sampling needs to cover the entire growing season and resolve both intra and inter-annual variability.

Since chlorophyll is only a proxy for phytoplankton biomass the correlation with phytoplankton biomass estimated using microscopic phytoplankton community analysis with cell counts and cell volume measurements (Olenina *et al.*, 2006) should be established in a given sea area. Data on phytoplankton composition would also provide information on whether the abundance of particular harmful species is increasing and on changes in the structure of the phytoplankton community. To do this a subset of samples covering the whole growing season should be analysed using both techniques every year.

Suspended particulate matter (SPM) (see e.g. Yeats and Brüggemann, 1990), temperature, salinity and light penetration measured as PAR (Photosynthetically Active Radiation) or Secchi depth or derived from measurements of SPM should also be measured, as supporting/interpretation variables.

6. Sampling equipment

A suitable water sampler should be used to collect samples for chlorophyll measurement. A non transparent sampling device is recommended and because chlorophyll is photolabile (is broken down to colourless compounds in the light) extraction procedures and measurements should be carried out in low light. In particular circumstances it may be necessary to use a profiling chlorophyll fluorometer/CTD to detect and to sample chlorophyll maxima. The problem of uneven chlorophyll distribution (patchiness) can be alleviated by using a hose-type device (*cf.* ICES, 1996) but it should be borne in mind that this gives an integrated sample.

Automated measurement systems and sampling equipment on research vessels and ships of opportunity (FerryBox systems), instrumented continuous plankton recorders (CPRs) and undulating oceanographic recorders can provide useful supporting information. Instrumented moorings can also be used to provide high frequency measurements of chlorophyll to resolve short term events but are single point measurements. Anti fouling devices must be used on moored instruments and service intervals must be high enough to make sure that bio fouling does not influence results.

When using in situ chlorophyll fluorometers the effect of photoquenching (i.e. reduced chlorophyll fluorescence signal at high light intensities) should be taken into account. One means of doing this is to measure light. For moored instrument systems photoquenching can be avoided by only using data recorded during the hours of darkness.

7. Storage and pre-treatment of samples

Chlorophyll samples should be filtered immediately after sampling and filtering should be carried out under green or low light conditions. Filters should be extracted immediately, and the extract should be kept deep-frozen. If it is not possible to follow this procedure the filters should be kept frozen at $<-20^{\circ}\text{C}$ for no longer than 21 days. If stored longer a temperature of $<-80^{\circ}\text{C}$ should be maintained to avoid degradation of chlorophyll.

8. Analytical procedures

Standard procedures for the determination of chlorophyll are given in Strickland and Parsons (1968), UNESCO (1994) and HELCOM (1988). If HPLC is used for chlorophyll a analysis the method by Wright *et al.* (1991) should be used. This HPLC method has been commonly used, and it is accepted that it does not distinguish between Chl-a and Divinyl Chl-a forms. If that level of differentiation is required an intercomparison of different HPLC methods can be consulted (eg. Claustre *et al.*, 2004). It is important to state the method used and that the same method of measuring chlorophyll concentration is used and the same procedure (sample collection, filtration, extraction and storage) is followed.

If in-situ chlorophyll fluorometers are used, they should be calibrated with local natural water samples with a range of chlorophyll concentrations. All measuring instruments should be calibrated with filtered water samples and standard chlorophyll a. The recommended detection limit for chlorophyll is 0,1 µg l⁻¹ with a precision of 6 %.

9. Analytical quality assurance¹

The quality assurance programme should ensure that the data are fit for the purpose for which they have been collected, *i.e.* that they satisfy the detection limits and levels of accuracy compatible with the objectives of the monitoring programme.

A Certified Reference Material standard (CRM) chlorophyll should be used for calibration purposes. Internal methods should be properly calibrated. Results should be subject to international calibration exercises.

Laboratories carrying out analyses are recommended to be accredited for chlorophyll analysis by a recognized accreditation authority.

10. Reporting requirements

Data reporting should be in accordance with the requirements for National Comments and with the latest ICES reporting formats, together with information on methods used, detection limits, reference values and any other comments or information relevant to an ultimate assessment of the data. In order to establish the acceptability of the data, they should be reported together with the dates and results of participation in intercalibration exercises and summary information from recent control charts, including dates, sample sizes, means and standard deviations.

11. References

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¹ A joint ICES/OSPAR Steering Group on Quality Assurance of Biological Measurements related to eutrophication parameters was established in 1997 in order to coordinate the development of quality assurance procedures, the implementation of quality assurance activities (*e.g.*, the conduct of workshops and intercomparison exercises) and the preparation of appropriate taxonomic lists of species. This work will cover chlorophyll a and is a fairly long-term programme of about five years. Good cooperation will be ensured with the ICES/HELCOM steering group on Quality Assurance of Biological Measurements in the Baltic Sea.

Annex 2 Revised JAMP Eutrophication Monitoring Guidelines: Benthos

JAMP Eutrophication Monitoring Guidelines: Benthos

Reviewed and amended by ICES April 2008 and 2009

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JAMP Eutrophication Monitoring Guidelines: Benthos

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 focuses 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 conduct 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 ISO 16665 International Standard guidelines on quantitative sampling and sample processing of marine soft-bottom macrofauna. (ISO 2005). These ISO guidelines should be consulted when detailed questions on sampling and sample processing are to be cleared.

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.

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

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 three primary objectives of benthic monitoring are as follows:

- a. to test the hypothesis that eutrophication is responsible for changes in community composition and function, biomass and community structure;
- b. to test the hypothesis that eutrophication is responsible for an increase in the abundance of ephemeral/annual algae such as *Cladophora*, *Enteromorpha* and *Ectocarpus* and a decrease in perennial algae such as *Laminaria* and *Fucus* and the angiosperm *Zostera marina* (eelgrass);
- c. to test the hypothesis that changes in eutrophication levels are responsible for a decreased depth distribution of the macrophytes (*e.g.* due to increased turbidity).

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

Sampling strategy

Sample sites should be representative of the whole monitoring area and so 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. 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 control/reference sites (preferably at least two) 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.

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 samples must be formulated and followed strictly. All steps in the sampling and analytical procedure must be documented in written form.

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.

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.

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 will require storage 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).

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 together with weight loss on ignition (500°C–520°C), total organic carbon and pigments (recommended). Other more advanced methods such as Laser diffraction, sedimentation columns *etc.* may also be used. To measure nutrients (particulate N) in sediments samples should be dried at 60°C until constant weight (12-24h), treated with HCl, held for 24h in a desiccator, dried again at 60°C and analysed in a CHN analyser.

Analytical quality assurance

Effectively the quality assurance (QA) programme should ensure that the data are fit for the purpose for which they have been collected (see Rees, 2004). Appropriate QA schemes should be established before the onset of survey work. 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, a calibration of their cover estimates must be performed. This can be done 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. 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 intercalibration exercises, ring tests and associated taxonomic workshops. Voucher specimens should be deposited regularly at museums to make later taxonomic checks possible.

Reporting requirements

Reporting formats need to be developed which will allow the exchange and evaluation both of the raw data and of all relevant ancillary information. Such formats must be readily usable by both the data centres and the originators of the data. 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. Reporting formats will develop with the programme. As a component of the 1997 ICES Work Programme, the Oslo and Paris Commissions have formally requested ICES to establish a databank for phytobenthos and zoobenthos.

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Technical Annex 1

Hard-bottom macrophytobenthos, soft-bottom macrophytobenthos and hard-bottom macrozoobenthos

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) and Rees (2009). 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

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 calibrated by means of quantitative field inspections at selected locations (*cf.* 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.

Diving transects

Diving transects are used to provide a description of the depth distribution and abundance of the dominant plant and animal communities. The 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 (Braun-Blanquet) 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. Transects 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 in prep., Krause-Jensen *et al.*, 1994; Karlsson, 1995; Pedersen *et al.*, 1995.). 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;
- photographic and/or video documentation (video/photographic profiles of the transect, panoramic views and, at fixed marked sites if possible, stereo photographs);
- the degree of wave exposure, Secchi disk depth (*i.e.* light transmission) and salinity (if possible).

- The use of satellite image based software e.g GOOGLE Earth. is suggested to visualize the exact location of a diving transect.

Quantitative sampling

Depending on the time spent on the transect, direct observations by divers may overemphasise the importance of particular eye-catching species. Quantitative sampling 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 (*cf.* Elliott, 1983). For example small but randomly 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) Dybem *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 organisms of each species;
- e) the biomass of plant species and animal species;
- f) the size structure of some animals (mainly molluscs).

Biological material could also be collected as reference specimens for herbaria *etc.* and for algal toxins (in conjunction with other monitoring programmes).

Sampling equipment

Submarine video in combination with GPS is useful for choosing 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 the most cost-effective techniques.

Surveys estimating abundance should sample within a large area containing the same biotope in order to reduce edge-effects or effects resulting from irregular species distribution. Quadrangular 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).

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.

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 quadrangular 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. Biomass should be expressed as either “g dry weight” samples should be dried at 60°C until constant weight (this can be up to one week depending on volume of sample) or as “g ash-free weight per m²” (samples should be dried at 500°C until constant weight (at least 6h)). Biomass expressed as volume (*e.g.* using water displacement) should be measured in the field whenever possible.

The degree of accuracy required for taxonomic sorting depends on the purpose of the monitoring programme. For the present programme it should be sufficient to identify organisms, whose taxonomic specification is difficult or time-consuming, to the generic level rather than to the specific level. (e.g. *Cladophora* spp., *Enteromorpha* spp.). Rare species should be determined to higher taxonomic levels. Functional groups should be kept intact as far as possible.

Biological material could also be collected as reference specimens for herbaria *etc.* and for algal toxins (in conjunction with other monitoring programmes). The use of fixation and preservation media has to be cleared the receiving institutes.

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Technical Annex 2

Soft-bottom macrozoobenthos

Sampling strategy

An initial spatially extensive “baseline” survey will facilitate the selection of representative stations within and 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.

Large-scale sampling of the macrozoobenthos community in offshore subtidal soft-bottoms should comprise many stations but with adequate replication 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 representativity 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 with a view to detecting the possible effects of nutrient enrichment (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 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;
- the time of day;
- the weather conditions during sampling and sea state;
- a description of the sediment, including:
 - surface colour and colour change with depth (as a possible indicator of redox state);
 - smell (H₂S);
 - a description of sediment type, including important notes such as the occurrence of concretions, loose algae;
- the type and specification of the sampler (weight and sampled area);
- mesh size of the sieve.

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.

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 benthopelagic 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, P. C. and Pearcy, W. C., 1977 dredge; see also Brattegard and Fosså, 1991; Sorbe sledge (Sorbe, 1983))

are useful for the small mobile crustaceans and boundary fauna. If automatic closing mechanisms and dredge distance recorders are added, then 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).

(see also Rees, 2009., for guidance on epibenthic sampling)

Photographic and video records are recommended as a complement to traditional sampling methods (Rumohr, 1995; Smith and Rumohr, 2005). Sediment profile imaging (cf. Rhoads and Germano, 1982; Solan *et al.*, 2003) may provide a useful means for rapid surveys and classification of soft sediment areas. 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.

Storage and pre-treatment of samples

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

Analytical procedures

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

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