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Biological monitoring: General guidelines for quality assurance

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These guidelines have been prepared by the ICES/OSPAR Steering Group on Quality Assurance of Biological Measurements in the Northeast Atlantic (SGQAE), as part of its role to encourage the production of biological data of consistent quality by member countries.[1]

The biological measures covered are: chlorophyll a, phytoplankton, macrozoobenthos, and macrophytobenthos, reflecting the initial remit of the Steering Group to address eutrophication-related studies according to the specifications of the OSPAR Joint Assessment and Monitoring Programme (JAMP). Tables of critical quality assurance (QA) factors and priority QA actions for these measures are presented. However, the guidelines for developing effective QA/AQC (analytical quality control) procedures governing field and laboratory work will be found to have a more general relevance to laboratories engaged in biological studies in the marine environment.

QA guidelines are presented across the full range of monitoring activities, i.e., from the objective-setting and sampling design stages of field surveys, to the generation, analysis, and archiving of data. Attention to all these activities is necessary in order to ensure the production of good quality information that continues to meet the purpose of scientific assessments.

In the preparation of these guidelines, every effort has been made to ensure compatibility with the recently revised ICES/HELCOM guidelines contained in the HELCOM Cooperative Monitoring in the Baltic Marine Environment (COMBINE) manual, and there has been free exchange of drafts between the respective QA Steering Groups.

Where possible, illustrative examples of good practice in relation to QA of biological measures are included, to aid in practical applications of the guidelines document, and to provide an indication of the likely direction of future QA developments for biological studies.

Key words: quality assurance, chlorophyll *a*, phytoplankton, macrozoobenthos, macrophytobenthos, sampling design, field surveys.

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[1] This document benefited from the contributions of several members of SGQAE since its creation in 1997, principally: Torgeir Bakke, Joe Breen, Franciscus Colijn, Einar Dahl, Jon Davies, Lars Edler, Karel Essink, Max Latuhihin, Kari Nygaard, Asger Olsen, Heye Rumohr, Wiebke Schwarzbach, Petra Schilling, Luis Valdez, and Sunhild Wilhelms.

1 INTRODUCTION

1.1 Need for Quality Assurance of Analytical Procedures in Marine Biological Monitoring

1.1.1 Background

As a consequence of the absence—or improper application—of measures to assure the quality of biological data, information about variations in the status of natural populations both in space and time is often uncertain or misleading, and the effects of political measures to improve the quality of the marine environment cannot be adequately assessed. Therefore, the acquisition of relevant and reliable data is an essential component of any research and monitoring programme associated with marine environmental protection. To obtain such data, the whole analytical process must proceed under a well-established Quality Assurance (QA) programme (see Section 7, below, for a definition of terms typically employed in QA activity).

In guiding such a development, the OSPAR Commission has formulated the following quality assurance policy:

1) Contracting Parties acknowledge that only reliable information can provide the basis for effective and economic environmental policy and management regarding the Convention area;

2) Contracting Parties acknowledge that environmental information is the product of a chain of activities, constituting programme design, execution, evaluation and reporting, and that each activity has to meet certain quality assurance requirements;

3) Contracting Parties agree that quality assurance requirements should be set for each of these activities;

4) Contracting Parties agree to make sure that suitable resources are available nationally (e.g., finances, ships, laboratories) in order to achieve this goal;

5) Contracting Parties fully commit themselves to following the guidelines adopted by the OSPAR Joint Assessment and Monitoring Programme (JAMP) and the Commission in accordance with this procedure of quality assurance.

Adherence to well-documented Quality Assurance/Quality Control (QA/QC) procedures is an established part of the activities of most chemical analytical laboratories, often occupying up to 25 % of staff time and, in recent years, much effort has been devoted within ICES to improving interlaboratory and inter-country data quality in national and international monitoring programmes. In contrast, much less effort has traditionally been devoted to QA/QC of biological measures employed in marine monitoring. This is largely due to the subsidiary role that many such measures have played in the past in coordinated international assessments of environmental quality, which have tended to concentrate on the distribution of chemical contaminants (with the notable exception of Baltic monitoring activity, see Section 1.1.2, below). Recently, there has been a shift in emphasis within ICES and OSPAR towards comparable holistic evaluations of the *biological* status of the marine environment in relation to man's activities. This shift, along with a quite separate development towards increased contracting out of biological analyses by resource-limited regulatory bodies to commercial consultancies (who are, as a result, under competitive pressure to deliver data of a consistent quality), has sharply highlighted the need for more effective and harmonized approaches to QA within and between member countries.

1.1.2 Rationale for SGQAE activity

The ICES/OSPAR Steering Group on Quality Assurance of Biological Measurements in the Northeast Atlantic (SGQAE) has developed the guidelines set out in this document with particular reference to the biological targets for eutrophication-related studies within its initial Terms of Reference, namely chlorophyll *a*, phytoplankton, macrozoobenthos, and macrophytobenthos. In doing so, SGQAE has freely adapted guidelines applicable to a much larger suite of variables under investigation in the HELCOM Coordinated Monitoring in the Baltic Marine Environment (COMBINE) Programme, Part B (see: http://www.helcom.fi/Monas/CombineManual2/CombineHome.htm). While the same general principles governing the development of QA programmes still apply, such adaptation was felt to be necessary as an acknowledgement of the very different organizational structures within which biological work may be conducted in OSPAR member countries. For example, at one extreme, local output may be vested in a single individual expert, where certification of that individual's expertise may clearly be more appropriate than a system of accreditation requiring a hierarchical QA management structure for its operation.

Biological studies at the community level (in this context, macrozoobenthos, macrophytobenthos, and phytoplankton) present particular challenges in QA, since each field-collected sample constitutes a unique *multivariate* entity, i.e., consisting of a combination of species and individuals peculiar to that sample. Of course, this is not to imply that all component species are unique in their occurrence, and some may be sufficiently widespread to be suitable for intercomparison exercises of identification proficiency among many countries. However, many species will be more localized in distribution, and competency in identification may, as a result, be more fairly tested at a regional level.

Proficiency in species identification is, of course, only one of many aspects of biological study that will determine the eventual quality of data sets. The present account covers the entire range of activities, from the initial setting of programme objectives and survey design, through to the collection of field samples, their processing in the laboratory leading to the generation of raw data and, finally, their compilation, analysis, and archiving. QA actions appropriate to each of these stages can be arrived at, in order to enhance consistency both within and between laboratories.

1.2 Strategy for Practical Implementation of QA Programmes for Biological Measures

Technical specifications related to the monitoring variables of interest, namely, chlorophyll *a*, phytoplankton, macrozoobenthos, and macrophytobenthos, can be found in the JAMP guidelines. (see http://www.ospar.org/)

For phytoplankton and chlorophyll *a*, the priority is likely to be for international-level QA assessment, at least at the level of sampling methodology, since the same (or similar) approaches will apply throughout the OSPAR area. It is also self-evident that the habitat, i.e., the water column, is dependably present at all locations, even if it is variable in terms of stratification, depth, and other characteristics. This is in contrast to some benthos studies, where gross variability in the physical habitat may result in entirely different species assemblages being encountered, which requires the adoption of markedly different sampling methods. Thus, not all countries will be involved in identical survey and sampling approaches. An example would be the presence or absence of a coastal rocky habitat.

Also, biogeographical factors affecting the species composition of phytoplankton, or the benthos of widely distributed habitats (such as soft bottoms), may, in practice, determine that intercomparisons of proficiency in species identification through "ring" tests should be

conducted at a regional rather than an OSPAR-wide level. For example, biogeographical provinces across the OSPAR area range from Arctic Boreal to Lusitanean. Such natural variability in biological systems determines that a tiered approach to QA initiatives, i.e., varying from the level of the laboratory to the national or international level, would be appropriate, depending upon the measure of interest.

It is also to be expected that there will be some examples of entrenched differences in sampling approaches between countries even for comparable habitats. For example, where evidence for the greater efficiency of one sampling device over another is unconvincing, personal preferences or historical precedents will be influential. There is no intrinsic reason why this should lead to significant problems with the quality of the resulting data, provided that acceptable documentation is available to demonstrate the comparability of data arising from different sampling methodologies. However, standard methods conforming to up-to-date guidelines should be adopted in any new monitoring programme.

SGQAE emphasizes the fundamental importance attached to agreement among participating countries on basic sampling issues such as mesh size, criteria for acceptance/rejection of samples, and consistency in timing of annual or more frequent surveys. Disparities here will nullify any benefits of sound QA, when it comes to intercomparisons of the results.

As part of this strategy, SGQAE identified a set of critical QA factors and priority QA actions for monitoring the relevant variables (chlorophyll *a*, phytoplankton, macrozoobenthos, and macrophytobenthos). These are given in Annex 1.

1.3 Objective of this Document

The objective of this document is to guide organizations (or individuals) towards the establishment of QA procedures, often for the first time, which will ensure that the data generated are suitable for contributing to international-level assessments of environmental quality. While some elements of any newly incorporated QA scheme must, from the outset, be considered mandatory, past experience suggests the need for a pragmatic view of how such a scheme will initially proceed. Thus, enhancements in performance may well be step-wise, in response to the adoption of new in-house working procedures, and as lessons are learned from intercomparison exercises, workshops, and other relevant activities. At this stage, a prevailing climate of encouragement will be the most helpful in facilitating such a progression.

2 THE QUALITY SYSTEM

2.1 General

"Quality system" is a term used to describe measures which ensure that a laboratory fulfils the requirements for its analytical tasks on a continuing basis. A laboratory should establish and operate a Quality System adequate for the range of activities, i.e., for the type and extent of investigations, for which it has been employed. The Quality System should refer to methodology, organization and staff, equipment and quality audit (see Annex 2).

The Quality System must be formalized in a Quality Manual that must be maintained and kept up-to-date. Some comments and explanations are given in this section.

2.2 Topics of Quality Assurance

In practice, **Quality Assurance** applies to all aspects of analytical investigation, and includes the following principal elements:

- A knowledge of the purpose of the investigation, which is essential to establish the required data quality.
- Provision and optimization of appropriate laboratory facilities and analytical equipment.
- Provision and regular updating of taxonomic keys and supporting literature for identification of biological specimens, including allowance for the possibility of the occurrence of introduced species.
- Selection and training of staff for the sampling and analytical task in question.
- Establishment of definitive instructions for appropriate collection, preservation, storage, and transport procedures to maintain the integrity of samples prior to analysis.
- Use of suitable pre-treatment procedures prior to the analysis of samples, to prevent cross-contamination and loss of the determinand in the samples.
- Validation of appropriate analytical methods to ensure that measurements are of the required quality to meet the needs of the investigations.
- Conduct of regular intralaboratory checks on the accuracy of routine measurements, by the analysis of appropriate reference materials, to assess whether the analytical methods are correctly employed and remain valid. Typically, control charts are used to evaluate the findings.
- Participation in interlaboratory quality assessments (proficiency testing schemes, ring tests, training courses) to provide an independent assessment of the laboratory's capability to produce reliable measurements.
- The preparation and use of written instructions, laboratory protocols, laboratory journals, etc., so that specific analytical data can be traced to the relevant samples and vice versa.
- The establishment of national/regional lists of all species likely to be encountered in surveys of marine communities, employing up-to-date nomenclature and recognized coding systems such as Species 2000 (see http://www.sp2000.org/), Encyclopaedia Taxonomica (see http://www.taxonomica.com/Taxonomica2/Introduction.asp) and the Integrated Taxonomic Information System (http://www.itis.usda.gov/).
- The management of the information in a suitable certified database/information system.

2.3 In-house Quality Manual

Every phase of a monitoring or assessment survey, even in small laboratories, must be enforced to ensure the quality of data acquisition, collection, handling and analysis, and subsequent data management and reporting. In-house Quality Manuals must be developed in accordance with appropriate national and international standards and followed rigorously.

The person responsible for authorization and compilation of the Quality Manual should be identified. A distribution list of the quality manual and identification of holders of controlled copies of the quality manual should be included.

The in-house quality manual should contain, as a minimum, the following items or their equivalent:

1) Scope;

2) References;

3) Definitions;

4) Statement of quality policy;

5) Organization and management;

6) Quality system audit and review;

7) A formal listing of the staff involved in the monitoring, analytical, and technical work as well as quality control management with respective training, professional qualification, and responsibilities within the laboratory;

8) Standard Operating Procedures (SOPs) (see Section 2.3.1, below);

9) Certificates and reports;

10) Sub-contracting of calibration or testing;

11) Outside support services and supplies;

12) Handling of complaints;

13) Contingency planning for the eventuality of problems arising (see also Section 4.1, below).

2.3.1 Standard Operating Procedures (SOPs)

An SOP may be defined as "a documented procedure which describes how to perform tests or activities *normally not specified in detail in study plans or test guidelines*" (Good Laboratory Practice, 1997). The italicized text helps to clarify some confusion that exists with regard to the role of an SOP. For example, in cases where international guidelines for a sampling or analytical procedure are written in sufficient detail, then these will perform the same function. However, guideline documents frequently cover large sea areas and a variety of habitats and cannot be expected to provide sufficient detail for the requirements of all local surveys. In these circumstances, an SOP bridges the gap between the activity of an individual laboratory and the wider need for harmonization of methodology. For example, a laboratory SOP might include a description of sample-processing equipment peculiar to that laboratory (though compatible with the performance needs of external guidelines), and perhaps its local source of manufacture.

A well-written SOP will help inexperienced members of staff in a laboratory to quickly develop expertise in a sampling or analytical area which is consistent with past practice at that laboratory, while being compatible with established approaches elsewhere. For those seeking laboratory accreditation, the production of SOPs will be essential as part of a wider QA package but, even for those who are not, they provide an important means to foster good practice internally. However, SOPs are clearly not, in themselves, guarantors of data quality.

SOPs should describe all steps performed in biological measurement. They should be established to cover the following areas of activity:

- Station selection and location, navigational accuracy;
- Handling, maintenance, and calibration of field and laboratory equipment;
- Handling and use of chemicals (i.e., fixatives, preservatives, reagents) used in marine environmental surveys;
- Collection of biological material;
- Storage of biological material including labelling, and checking of preservation status;
- Distribution of biological material to external contractors/taxonomic specialists;
- Analytical methods for biological material;
- Identification of biological material including taxonomic expertise of the personnel;
- Recording of biological and environmental data and subsequent data management;
- Analysis of biological and environmental data;
- QA of report writing and documentation including signed protocols in all steps of analysis.

SOPs should contain a description of operational procedures. An outline structure for an SOP (modified from ISO/IEC, 1999) is as follows:

- scope of procedure used;
- description of the study target;
- variable to be determined;
- equipment necessary, reference material (e.g., voucher specimens) and taxonomic literature used;
- specification of working conditions required for effective sampling;
- description of procedure/method with respect to the following aspects:
  - i) sampling and sample treatment, labelling, handling, transport and storage of samples, preparation for laboratory analysis,
  - ii) instrument control and calibration,
  - iii) recording of data,
  - iv) safety aspects;
- criteria to adopt or reject results/measurements;
- data to be recorded and methods for their analysis;
- assessment of uncertainty of measurements.

In considering "best practice", it is recommended that SOPs should:

- be structured logically by heading and sub-heading to cover the full sequence of activities in field sampling and laboratory analysis;
- carry an issue number, date, and name(s) of the individual(s) responsible for its drafting and updating. This anticipates a likely requirement for changes to SOPs in response to new equipment, guidelines, and so on;
- document in-house AQC procedures;

- account for the specific practices of the individual laboratory. At the same time, SOPs must of course reflect agreed guidelines applicable at national or international level, for example, relating to nomenclature and coding systems employed in documenting the outcome of the analysis of field-collected specimens;
- contain a full listing of taxonomic keys used for laboratory identification, and other useful reference works relating to procedures;
- be filed as paper copies in an accessible place, as well as being available on a computer network;
- be freely available to all interested parties (especially funding agencies);
- contain explicit instructions for the tracking of samples from the point of collection to the point of archiving of analysed material.

SOPs may usefully contain:

- diagrams depicting gear, especially where local modifications to equipment are made;
- a summary flow-chart as an accompaniment to a lengthy SOP, as an *aide memoire* for field and laboratory bench operators;
- details of local suppliers, manufacturers, etc., where relevant.

SOPs should not:

- contain vague generalizations;
- contain excessive detail: a sensible balance needs to be achieved which takes into account the basic level of training and common sense that a new operator will possess;
- cover too many activities: for example, it is logical to have separate SOPs for field and laboratory procedures. Different types of field activity such as intertidal core sampling and ship-board sampling are also sensibly treated separately.

Conclusion

The preparation of SOPs to cover field and laboratory analytical activities is one of the most important practical steps that a laboratory/institute can take in seeking to improve the quality and consistency of its scientific output and is, therefore, to be strongly recommended. This having been done, interlaboratory comparisons of SOPs may then provide a useful tool in identifying any remaining inconsistencies, and hence in promoting harmonization of methodology at a national and international level. Such periodic comparisons of SOPs are also to be strongly recommended (see, for example, Cooper and Rees, 2002).

2.4 Organization, Management, and Staff

2.4.1 Organization

The Quality System should provide general information on the identity and legal status of the laboratory and should include a statement of the technical role of the laboratory (e.g., employed in marine environmental monitoring).

The information must include general lines of responsibility within the laboratory (including the relationship between management, technical operations, quality control and support services, and any parent or sister organizations). In the case of smaller units, the organizational tasks must be allotted to fewer personnel or even one individual.

2.4.2 Management

Clear job descriptions, qualifications, training, and experience are necessary for all persons concerned with QA and QC. Job descriptions should include a brief summary of function, the pathways of reporting key tasks that the jobholder performs in the laboratory, and limits of authority and responsibility.

2.4.3 Staff

Minimum levels of qualification and experience necessary for the engagement of staff and their assignment to respective duties must be defined. Members of staff authorized to use particular items of equipment should be identified and the institution should ensure that all staff receive training adequate to the competent performance of the relevant methods and operation of equipment. A record should be maintained which provides evidence that individual members of staff have been adequately trained and that their competence to carry out specific methods, identifications or techniques has been assessed. Managers should be aware that a change of experienced and well-trained staff might jeopardize continuity in the production of data of consistent quality.

In the case of small units employing few staff or even single individuals responsible for the generation of data, a scheme for the certification of individual expertise (e.g., in aspects of species identification) may be a valid alternative to formal accreditation involving a hierarchy of quality managers, which may not be practicable.

2.5 Equipment

As part of its quality system, a laboratory is required to operate a programme for the necessary maintenance and calibration of equipment used in the field and in the laboratory to ensure against bias of results.

General service equipment should be maintained by appropriate cleaning and operational checks, where necessary. Calibrations will be necessary where the equipment can significantly affect the analytical result.

Performance checks and service should be carried out at specific intervals on microscopes, balances, and other instruments. The frequency of such performance checks will be determined by experience and based on the need, type, and previous performance of the equipment.

2.6 Documentation

All biological data produced by a laboratory should be completely documented ("meta-information") and should be traceable back to its origin. The necessary documentation should contain a description of sampling equipment and procedures, reference to SOPs for the sampling, sample handling and analytical procedures involved, and the names of persons responsible for Quality Control. In general, one signed protocol should accompany a sample through all steps of processing.

3 QA OF PROGRAMME PLANNING AND DESIGN

3.1 Introduction

The following account is an edited and amended version of text relating to this issue published by the Nordic Council of Ministers (1997b).

The planning process is critical to the production of sound environmental information. In order to design an effective environmental monitoring programme, the key issue for consideration is the final use of the data. The objectives of the planned programme should be clearly and precisely formulated by the lead scientist, mindful of the role of the outcome in environmental management, and should be put in writing.

In this formulation, precise sets of qualitative targets are essential in optimizing sampling, analysis, and data-handling programmes. If data are to be treated statistically, the number of samples, sampling frequency, sampling locations, and other quantitative aspects are of great importance. A statistician experienced in these types of problems should be consulted.

Available resources and monitoring costs influence the programme design. Clear specification of objectives in relation to costs will ensure that only necessary and relevant data are collected. Consideration should also be given to the possible risk of incorrect decisions based on insufficient data acquisition as a result of financial constraints. Again, the advice of a statistician may be useful in evaluating the effects of different levels of effort on the statistical power of monitoring programmes.

Information requirements in relation to available resources are the basic elements in the further planning process. The result of this planning process should be documented in the quality objectives plan.

Periodic evaluation of the information requirements should be based on monitoring results and changes in the requirements of the users. Stability and continuity are of great importance in the monitoring process that has an ongoing and iterative character. All changes should be documented and validated before being implemented.

3.2 Specification of Information Needs

Many different approaches indicating different information needs can be identified in the design of monitoring programmes. There are two broad categories:

1) compliance monitoring or the emission-based approach, including sampling and analysis according to national regulations;
2) ambient monitoring or the environmental quality approach, including sampling and analysis in order to establish baseline levels or trends, set from the original/desirable state of the environment.

These different approaches are interrelated and complement each other in many ways.

The information needs should be defined in detail:

- which questions are to be answered;
- which levels of overall reliability are to be attained;
- what are the intended uses of data/results.

The proper level of quality assurance can only be performed when the requirements of the information needed are made explicit.

In monitoring trends in the conditions of the environment, extreme care should be exercised that observed trends are not influenced or biased by changing methodology, change of laboratory, differences in sample stability, or time and frequency of sampling.

Reuse of monitoring information should always be kept in mind. In case of new and unforeseen environmental questions, thoroughly documented and accessible information may be re-evaluated in the far future, tackling quite new problems, and thus the reuse of data should be facilitated as far as possible.

The information needs, as the basis for further work, should be:

- detailed, and accompanied by written descriptions in order to avoid ambiguity;
- subject to review for conformity to legal, scientific, technical, and quality expectations;
- approved by top management and included in the quality management plan.

3.3 Strategy and Determinands

After defining the information needs (including considerations of spatial and temporal scales appropriate to meeting the survey objectives), a strategy for monitoring must be defined. This involves decisions about what information is to be produced by the monitoring system in order to translate the information needs to data-collecting activities.

The monitoring strategy will define what is to be determined and in which media, as well as its required quality. The strategy should also include information on the final use of data, including data analysis, compilations, statistical calculations, and evaluations.

In designing the monitoring strategy, the selection of relevant determinands is of the greatest importance. To obtain the most reliable and complete picture of the state of the environment, an integrated ecosystem-level monitoring approach should be adopted, involving coordinated chemical, physical, and biological sampling.

Spatial monitoring or mapping involves the coverage of chosen variables within selected areas. It can be made on one occasion or involve recurrent mapping. For certain environments, remote sensing (typically employing aerial photography or satellite imagery) can be an important tool in identifying features of interest for further study in field surveys at “ground” stations.

For some studies, model calculations may usefully complement the outcome of traditional sampling programmes and/or remote sensing. Models are based on the assumption that a dependent variable will continue to respond in the same manner as that established during the validation stage, in response to changes in one or more “control” variables. Models are used for calculating loads and concentrations and for making predictions. However, in practice, difficulties usually arise in simulating the complexities of biological interactions in the marine environment and models are invariably simplifications of reality. All models used in monitoring should be clearly described, documented, and validated. The quality of the output from a model depends not only on meeting the required levels of accuracy and precision for the measured variables at the data input stage, but also on the continued validity of the basic assumptions underlying its formulation. Defined action for continuous follow-up and corrections of the model should be included in the quality control plan. Modelling and environmental indicators are further discussed in Nordic Council of Ministers (1997b).

3.4 Data Quality Objectives

Liabastre *et al.* (1992) identified four stages in the quality assurance of environmental assessment activities, namely establishment of Data Quality Objectives (DQOs), design of the sampling and analytical plan, execution of the sampling and analytical plan, and data assessment. DQOs are defined as "interactive management tools used to interpret and communicate the data users' needs to the data supplier such that the supplier can develop the necessary objectives for QA and appropriate levels of quality control". The DQO process provides a logical and quantitative framework for establishing an appropriate balance between the time and resources that will be used to collect data, relative to the desired level of quality of the data needed to make a specified decision in an environmental management context. The quality level may be defined as the tolerable total measurement uncertainty in different sets of data in order to achieve an acceptable level of confidence in final decisions. The DQO process stresses the cooperation between the end users of the data and the scientific staff planning the monitoring programme.

The DQO process was developed by the U.S. Environmental Protection Agency. The process takes the form of seven steps:

1) state the problem;
2) identify the decision;
3) identify inputs to the decision;
4) define the study boundaries;
5) develop a decision rule;
6) specify limits on decision errors;
7) optimize the design.

These steps in the DQO process are fully discussed in the U.S. EPA Quality System Series documents (see: http://www.hanford.gov/dqo/index.html) and may profitably be applied to all projects where the intention is to collect environmental data and to make a specified decision. The seven-step DQO process provides a method for establishing decision performance requirements by considering the consequences of decision errors. A statistical sampling design satisfying the DQO can be generated. The introduction of the DQO process in the planning of monitoring programmes is to be recommended. A similar process has been termed *the graded approach*, where the level of quality is also determined from a consideration of the intended use of the data. In both cases, quality assurance encompasses the requirement to test, define, and document the quality level needed and to maintain this quality level in all subsequent steps.

3.5 Sampling Design

The previous parts of this section have emphasized the importance of precisely defining the objectives of the monitoring programme. The monitoring strategy considers what is to be measured, while the DQO process seeks to establish a proper balance between time, costs, resources, and the desired quality of the results. The sampling design concentrates on where and when: it specifies which determinands are to be measured at which location, at which time and frequency. In order to ensure that a sampling design is effective in generating data that will permit adequate description of a range of targeted habitats and allow statistical discrimination in space and time, programme designers should, ideally, have prior knowledge of the likely scales of temporal and spatial variability and other relevant knowledge of the system to be studied. If

not, pilot surveys may be required as a precursor to establishing a routine (see Rees *et al.* (1991) for procedural stages in the development of a benthic sampling programme).

In a representative sample, all relevant determinands have the same values as in the system at the point and time of collection. The validity of a sampling programme will be determined by the degree of accuracy with which it represents temporal and spatial variability in "environmental quality" for the duration of the monitoring programme.

Relevant factors in sampling design include the following:

- sampling location (degree of system homogeneity and hence the need for sample stratification, number of sampling locations, accessibility, and safety precautions);
- sampling time and frequency (system homogeneity over time, random and cyclic variations);
- estimated nature and magnitude of natural variation in the biological components to be measured;
- estimated nature and magnitude of the man-made impact under investigation;
- duration of sampling period—discrete or composite samples;
- economic and practical considerations;
- quality control.

After a complete sampling cycle, all results are to be evaluated and tested to meet the pre-set quality targets.

Expert assessment of the final results may identify weak points and inconsistencies that can be corrected to increase the quality of the programmes.

3.6 Specification of Sampling Procedures

Sampling is the starting point in the collection of information and a cornerstone in the monitoring process. Mistakes in sampling may invalidate the whole process and it is rarely possible, after the event, to correct any errors associated with this activity. By definition, environmental monitoring involves repeated sampling over time and, again, a missed field sampling opportunity as a result of inadequate planning can never be reproduced. Sampling procedures include sample collection, preservation, transport, and storage. All decisions relating to sampling strategy and sampling operations shall be thoroughly documented.

4 QA FOR FIELD WORK

The following account covers sampling activities for the determination of eutrophication-related changes in biological communities within the OSPAR area. General guidance on QA of field sampling is also contained in Nordic Council of Ministers (1997a).

The experience and competence of personnel are prime factors for consideration in relation to the aim of collecting high-quality data. QA covers issues relating to delegated responsibility and the authority of staff, as well as education, experience, and all aspects of special training.

4.1 Sea-going Procedures

The QA of sea-going procedures covers methods, instruments and equipment including their description, SOPs, applicability, limitations, calibration, and maintenance. Safety is also a critical consideration and will be an essential part of any QA programme. Guidelines on the conduct of surveys at sea are provided for the benthic macrofauna by Rumohr (1999), for phytoplankton by Sournia (1981), Tett (1987), and the HELCOM COMBINE manual, and for chlorophyll *a* by Aminot and Rey (2001).

The description of the measuring site (station, area, transect, etc.) covers not only its documented geographical location, typically employing a differential Global Positioning System (dGPS), but also the nature of the physical environment (depth, sediment type, etc.) and of the prevailing hydrographical and meteorological conditions (temperature, salinity, currents, wind direction and speed, cloud cover, etc.). There is an indispensable need for a comprehensive signed log of field activities that covers all aspects and steps of the sampling process including personnel, instruments and equipment, and recording activity including deviations and deficiencies. Accurate recording of time (as GMT) should be made, especially when the results from wide-scale surveys across time zones have to be combined.

The securing of results from instruments and data loggers is an indispensable and delicate step in QA that preferably should be safeguarded by keeping parallel hard copies of results.

The securing of samples and material is another important QA consideration; in particular, the use of durable and clear (internal and external) labels is essential for later tracking of archived samples. Parallel documentation by photo and video can increase reliability.

There is a need to anticipate, and plan for, the eventuality of deviations, malfunctions, and deficiencies in sampling equipment at sea (e.g., by taking duplicate items), and in cases of illness of personnel that makes them incapable of fulfilling their tasks.

Periodically, allowances must be made for the possibility of changes to sampling gear (e.g., as a result of design improvements) which may affect comparability with earlier surveys. Intercomparison of new and old equipment must be carried out before any change can be permitted. The recording and documentation of these results are very important.

The whole sea-going process (that ends when the samples, material, and documents are handed over to the analytical laboratory) must be accompanied by **quality control activities** such as:

- simultaneous recording by different observers, accompanied by evaluations of consistency;
- where necessary, parallel measurements with different instruments, accompanied by evaluations of consistency;
- test comparisons (intercomparisons);
- field blank samples (chlorophyll *a*);
- measurement of reference materials;
- securing the stability of measuring instruments in changing ambient conditions (temperature, humidity);
- checking for any interferences with other instruments or installations of the ship (this includes the need for a stable voltage supply).

4.2 Coastal and Land-based Procedures

Many of the above considerations apply equally to surveys employing divers and activities directly accessible from land, especially evaluations of intertidal habitats. Relevant guidelines for the conduct of such work include Baker and Wolff (1987), Holme and McIntyre, (1984), Davies *et al*. (2001), and Kroglund *et al*. (2002). Approaches to the QA of the main activities under this category are outlined below. It should be noted that surveys of macrobiota associated with hard substrata normally involve *in situ* identification and enumeration, where the only permanent record may be a photographic image. The quality of the data is directly dependent on the taxonomic skill of the surveyor. It is good practice to undertake pre-survey validation exercises with the intended field surveyors, possibly supported by a standard checklist of likely taxa, to quantify and then correct for any variation between surveyors.

4.2.1 Intertidal soft sediment surveys

QA of intertidal soft sediment surveys includes the following issues for attention:

Station positioning:

- To enable repeated sampling at the same stations, an accurate positioning system is necessary. For accurate relocation of sampling stations at successive surveys, dGPS is indispensable. Alternative methods of position finding can also be applied, for example, the use of well-defined permanent landmarks in the near vicinity. It should be noted that, for northern latitudes, experience has shown that staking out of sampling stations (along transects) will not be able to survive ice scour.

- The choice of sampling apparatus has to be adjusted to the burrowing depth of infaunal macrobenthos. Hand-operated corers of various diameters and lengths can be applied. Samples taken to 30 cm depth will take the majority of deep-burrowing macrofauna.

- It may be important to document the precise location of the sample stations in relation to micro-topographical features such as sediment waves (trough or peak) or creeks as these factors can influence the water retention within the sediment, and hence may affect the biological community composition.

Sample quality:

- The size of the sample (surface area and depth) as well as the number of replicates have to comply with the intended accuracy of determination of the density of macrobenthos and phytobenthos species. This may be different from species to species.

    - For phytobenthos, the counting of plants and collection of plant mass within randomly placed frames can be used.

    - For macrozoobenthos, the number of replicates to be taken is related to the desired relative standard error of the estimate of numerical density of species.

    - Macrozoobenthic core samples of insufficient depth should be replaced by a new sample.

- Prolonged and vigorous sieving of samples in the field has to be avoided in order to prevent unintended loss of small animals and damaging of delicate animals.

- In case of semi-quantitative measurements in the field (e.g., percentage cover in eelgrass), the personnel involved should be regularly trained in order to ensure the production of comparable results (in time as well as in space).

- Care should be taken to have an adequate volume ratio of sample material to neutralized fixative solution ensuring a final fixative concentration of at least, e.g., 4 % formaldehyde in sea water. Special care is required as to the final fixative concentration in samples containing large amounts of organic material. Large mollusc shells have to be opened to allow the fixative to penetrate the animal tissue.

Documentation of relevant background information:

- In intertidal sediment, the elevation of intertidal flats may change due to erosional and depositional processes. To obtain information on possible changes in elevation, regular echo-sounding is advised to be able to calculate possible changes in the period of tidal submersion;

- Description of the degree of sediment consolidation (on an arbitrary scale). This is important because in loose sediments some benthic animal species are not able to maintain their burrows;

- Presence of biogenic structures (e.g., position and extent of eelgrass beds, mussel beds);

- Signs of (recent) human activities (e.g., cockle fisheries).

4.2.2 Intertidal rocky habitat surveys

The following QA considerations were derived from the Norwegian standard for littoral and sublittoral rocky habitats (Kroglund *et al*., 2002) and the Marine Monitoring Handbook (Davies *et al*., 2001). A large variation of methods exists and there is a need for further harmonization in scientific approaches at an international level.

Topics to be covered in the development of a QA programme include:

- development of sampling programme;
- description of methods for data registration;
- species identification;
- storage of data and any collected material.

General information (see also Section 3.2, above)

Items of information which must be logged at the time of survey include the personnel involved, coordinates for sampling site including the geodetic parameters of the coordinate system employed (e.g., datum), season, time of sampling for each station, coastal type, substratum type, height above datum, horizontal extension (drawing, maps or photo), orientation, angle of gradient, wave exposure, weather conditions, visibility, exact position of mooring frames/quadrats, and the lower limit for macroalgal growth for the area and time of sampling. Useful supporting variables for measurement include salinity, temperature, nutrients, oxygen, tidal currents, air pressure, tidal phase, ice scouring, and reference to any other surveys of the area.

Sampling programme and survey design

Quality status assessments

These include spatial surveys for assessing general quality status and/or the effects of specific impacts arising from, for example, sewage discharges, aquaculture, industrial discharges, and oil spills. The species registration is either quantitative (percentage cover/counts/frequency) or semi-quantitative (abundance scale). The precise requirements for the number and locations of stations, including reference sites, will be site-specific, depending on the type and extent of pollution. However, minimum requirements must be met in order to ensure the generation of data of sound quality for management purposes (see Annex 3). It may be appropriate to determine the number of sampling stations in relation to the risk of making an error in the final assessment. The statistical technique of power analysis can be used for this purpose; sources of information on the use of power analysis are presented in Davies *et al*. (2001).

Trend monitoring

This activity involves repeated observations at fixed stations, for example, in order to determine long-term changes in populations of individual species or whole assemblages. The methods are generally the same as for quality status assessment, with some additions such as the number of stations required and the need for the precise relocation of a sampling station (see Annex 6). All sites are to be permanently marked in the field to ensure precise relocation (Annex 3).

Methods for data registration

The methods include:

1) Intertidal inventory: species composition/densities recorded in horizontal transects (typically involving a search area of 10 m^2) using abundance scales. The transect should be located within a single biological subzone. The method replaces quadrat surveys in areas where tidal variation is small (less than 50 cm) or where sampling at low water is not possible for other reasons. The source of the abundance scale used must be documented.

2) Intertidal quadrat survey: species composition/densities recorded in fixed or random quadrats using percentage cover/density of individuals. An alternative approach would be to use frames for frequency counts.

3) Intertidal algal density and size distribution in predefined areas.

4.2.3 Surveys of shallow coastal soft and hard substrata by means of diving

A European standard for scientific diving is being developed within the EU. Specifications concerning diver certification, including critical safety issues, are generally regulated at a national level. Certification should be a mandatory requirement for all those engaged in scientific diving activity.

Issues for attention covering QA of sampling programmes are, in many respects, similar to those under Sections 4.1, 4.2.1, and 4.2.2, above (see also Annex 3).

Reporting

Standard procedures must be established for the reporting of scientific surveys by divers, examples of which may be found in Kroglund *et al*. (2002) and Davies *et al*. (2001). A typical specification should include:

- project identification or project code;
- person or institute responsible for the recordings;
- person(s) that carried out the recordings;
- station code;
- date and time (start–stop);
- geographical coordinates for each station;
- methodology;
- substratum types—any sediment deposits and other loose material on hard substrate are noted;
- substratum slope (for diving surveys, a bottom profile is made using slope and depth);
- type of locality (for example, fjord, skerries, outer coast);
- station orientation;
- wave exposure. Subjective assessment (weak - moderate - strong) together with the number of open sectors of 10 degrees, with a radius of 7.5 km and the prevailing wind direction. In special circumstances, a more precise theoretical measure of wave exposure can be calculated;
- estimated recording conditions (percent cloud cover, wind, visibility in the water, light conditions);
- horizontal limits at the site (supra-littoral/littoral investigations) are described in words, diagrams or by photography;
- positioning of any fixed sampling grids in relation to the fixed reference point;
- positioning of stations and investigation areas for sub-littoral investigations, including the transect route, are described by giving the compass direction from a fixed reference point and by depth.

If depth below sea level is recorded, it may be corrected to datum using a tidal correction, although the source of the correction should be documented.

Methods for data registration

The methods include:

1) Subtidal inventory: species composition/densities recorded in vertical transects (typically 0–30 m) using abundance scales;

2) Subtidal quadrat survey: species composition/densities recorded in fixed quadrats using percentage cover/density of individuals, or frequency counts;

3) Subtidal photography: stereo photography at fixed positions.

5 QUALITY ASSESSMENT FOR LABORATORY ANALYSIS AND DATA HANDLING

The objective of a quality assurance programme is to identify the sources and magnitude of variability in the data, to reduce analytical errors to required limits, and to assure that the results have a high probability of being of acceptable quality.

5.1 Routine Quality Control at the Laboratory Analytical Level

Having developed an analytical system suitable for producing analytical results of the required accuracy, it is of extreme importance to establish a continuous control over the system and to show that all causes of errors remain the same in routine analyses (i.e., that the results are meaningful). In other words, continuous quantitative experimental evidence must be provided in order to demonstrate that the stated performance characteristics of the method chosen remain constant.

For marine environmental monitoring programmes, it is essential that the data provided by the laboratories involved are comparable. Therefore, activities such as participation in external quality assessment schemes, ring tests, and taxonomic workshops and the use of external specialists by the laboratories concerned should be considered indispensable.

While the use of a validated analytical method and routine quality control (see above) will ensure accurate results within a laboratory, participation in an external quality assessment or proficiency testing scheme provides an independent and continuous means of detecting and guarding against undiscovered sources of errors and acting as a demonstration that the analytical quality control of the laboratory is effective.

Most schemes are based on the distribution of samples or identical sub-samples (test materials) from a uniform bulk material to the participating laboratories. The test material must be homogeneous and stable for the duration of the testing period. Amounts of the material should be submitted that are sufficient for the respective determinations.

The samples are analysed by the different laboratories independently of one another, each under repeatable conditions. Participants are free to select the validated method of their choice. It is important that the test material is treated in an identical manner to the treatment of samples ordinarily analysed in the laboratory. In this way, the performance established by the proficiency testing results will reflect the actual performance of the laboratory.

Analytical results obtained in the respective laboratories are returned to the organizer where the data are collated, analysed statistically, and reports issued to the participants. In cases where laboratories are formally accredited, external quality audits are carried out in order to ensure that the policies and procedures, as formulated in the Quality Manual, are being followed. All data are computerized and back-up files can be mailed to the institute server or the forms containing data could be faxed to the institute to assure a paper copy.

The trend towards applications of internationally consistent AQC criteria to biological studies, especially of pelagic and benthic communities, is a relatively recent development. In practice, this determines that available procedures, a number of which are still subject to development or refinement, may fall some way short of the ideal. In Annexes 4–7, examples of best practice covering both field sampling and laboratory analysis are provided for the biological variables of interest, including imaging methods, as a supplement to the information on critical QA factors and priority QA actions identified in Annex 1.

5.2 Routine Quality Control at the Data Handling Stage

5.2.1 Data management

For the adequate management of the data obtained (especially when different laboratories are involved), an information management system is essential. The database should allow the storage/management of the full set of information relating to the data (including QA procedures,

and summaries of analytical methods). A proper reporting format or data entry system should allow the submission of the required information in order to describe fully, and if necessary to trace back, the data/samples.

Data checks performed by the (national) data manager should only be carried out on a data set that has already been subject to quality control procedures by the reporting institution. Therefore, information on QA/AQC procedures and outcomes has to accompany the data or, better, has to be regarded as part of the data submission (see below).

A central data management system should guarantee safe archiving (regular back-ups, computer virus checks, multiple storage, etc.) and access to the data.

Check routines performed by the data management system should look for:

- format compliance;
- completeness of data/information;
- compliance with the programme and guidelines;
- deviations from previous sampling/analysis procedures;
- plausibility (involving screening for outliers, e.g., arising from errors in position-fixing, or improbably high/low data values);
- conformity with agreed taxonomic nomenclature (parallel considerations include correct application of international coding systems such as Species 2000 or ITIS, taxonomic updates, and synonyms);
- species occurrences additional to those in standard lists which may include introduced species.

"Quick-look" visualization of the data/information (e.g., in the form of track plots or charts) should be provided by the data centre, as well as meta-information relating to the submission of the data, including its state of validation. The establishment of good communications between the data centre and the data originators is essential. Regular intercomparisons between the (national) data centre and ICES should be performed, and international standards for the management of the data should be met.

The qualifications of the data managers and programmers are of importance for the effective management of the data. A scientific background of the data manager is highly recommended, as well as training of both data managers and programmers in order to meet up-to-date standards. (See Annex 8 for a summary of draft guidelines for discrete water sample data, which provide useful information on approaches to the effective management of biological and chemical data.)

It is recognized that decisions regarding the overall acceptability of multivariate data arising from the analysis of biological communities can be difficult to arrive at, since elements of the submitted information may be unsuitable for some purposes, but nevertheless sufficient for others. For example, deficiencies in species identification may preclude the use of a submitted data set in "biodiversity" assessments, but the responsible laboratory may return biomass data of acceptable quality for the same samples, which may then be useful in assessments of ecosystem function. Criteria for determining the acceptability of data from surveys of biological communities to meet specified information needs at the international evaluation stage are still under development, and should be given high priority. However, systems for the "flagging" of data are already under development within certain countries (see Section 5.2.2, below) and this

experience may in due course find useful application for the quality control of the input to international databases. A useful practical approach to the screening and evaluation of data of variable quality can be found in ICES (2001), using as an example data on temporal trends in chemical contaminants.

5.2.2 Accreditation

In the aquatic sciences, formal accreditation schemes, typically governing the analytical practices of a laboratory as a whole or in part, exist both at national and international levels. The achievement and then maintenance of accredited status may be a necessary requirement for laboratories engaged in technically demanding approaches to the measurement of compliance against specified end-points (e.g., for Environmental Quality Standards: e.g., King, 1999). More generally, as a statement of conformity with established and sound practices, accreditation may enhance the reputation of a laboratory, and confer competitive advantage. However, it should be emphasized that formal accreditation *per se,* and the presumption of good practice that follows from it, are not absolute guarantors of data quality. All disciplines involving an element of routine may be amenable to a process of accreditation, but in the aquatic sciences, the activity is most commonly associated with analytical chemistry, microbiology, and toxicology.

In the context of evaluations of data quality, it may be considered appropriate for member states to adopt a data accreditation scheme for the purpose of assigning annual competence to laboratories engaged in the production of data of national/international importance. Through such a scheme, which may be overseen by a nationally appointed group of experts, national data are screened and "flagged" appropriately (e.g., acceptable, unacceptable, acceptable under certain conditions) prior to inclusion in a national database. These flags are assigned after a strict assessment of data against standards of performance, e.g., in relation to interlaboratory calibrations and external analytical quality control checks by an approved expert laboratory. It is envisaged that such national schemes would come under scrutiny by the relevant ICES/OSPAR/HELCOM QA Steering Groups, in order to ensure consistency of approaches and comparability of all data entering the ICES Environmental Databases. Further consideration needs to be given to the suitability of available schemes in relation to the conduct of biological community studies.

6 QUALITY ASSURANCE OF DATA ANALYSIS AND REPORTING

In the case of international evaluations of quality status, quality assurance of the outcome of analyses of the data following synthesis will be necessary. This task will be undertaken centrally by an organization responsible for database management, and therefore should be an inherently more straightforward exercise than will be the case for ensuring the quality and consistency of analytical outcomes from individual laboratories or countries. As there will usually be a requirement for both separate and combined analyses of environmental data sets to meet, respectively, national and international management needs, then issues of comparability in analytical outcomes and, ultimately, consistency in interpretations of these outcomes, are very important.

6.1 Data Analysis

Targets for AQC activity include:

- Avoidance of errors associated with inconsistent units for expressing results, such as area or volume sampled;

- The use of standard formulae for the calculation of derived measures such as diversity indices, and the avoidance of errors associated with different mathematical transformations (e.g., use of different log bases);

- Possible rounding errors associated with different computer software packages;

- Different outcomes associated with alternative versions of complex statistical procedures (e.g., multivariate analytical methods).

6.2 Reporting

The maintenance of consistent and objective standards in reporting survey outcomes is best addressed through systems of peer review. This will be especially important in the case of new or relatively inexperienced personnel, and a tiered approach should be adopted, depending upon the ultimate target audience, i.e., ranging from within-laboratory to between-country appraisals. As a general rule, every encouragement should be given to the publication of outcomes in the conventional peer-reviewed literature. However, recognizing that the level of detail required in the reporting of many monitoring outcomes (especially at the international level following syntheses of data from various sources) may preclude such conventional publication routes, then the use/establishment of expert groups to serve this need is to be recommended.

7 DEFINITIONS

Accreditation. The process of achieving competency and consistency in aspects of laboratory performance, in accordance with some recognized national or international standard.

Accuracy. Difference between the expected or true value and the actual value obtained. Generally accuracy represents the sum of random error and systematic error or bias.

Analytical method/process. The set of written instructions completely defining the procedure to be adopted by the analyst in order to obtain the required analytical result.

Analytical system. Such a system comprises all components involved in producing results from the analysis of samples, i.e., the sampling technique, the "method", the analyst, the laboratory facilities, the instrumental equipment, the nature (matrix, origin) of the sample, and the calibration procedure used.

Benthos. Fauna and flora living within, on, or in close association with, the bed of aquatic systems.

Calibration. The set of operations which establishes, under specified conditions, the relationship between values indicated by a measuring instrument or measuring system, or values represented by a material measure, and the corresponding known values.

External quality assessment. Evaluation of the effectiveness of QA/AQC procedures by outside expertise (see Section 5).

Intercalibration. Exercises involving the calibration of instruments or activities across laboratories.

Intercomparison. Comparative sampling, laboratory analysis, and evaluation with the aim of detecting systematic differences.

Macrophytobenthos. Macroscopic benthic flora.

Macrozoobenthos. Macroscopic benthic fauna, typically retained on a 1 mm or 0.5 mm mesh screen.

Matrix. The totality of all components of a material, including its chemical, physical, and biological properties.

Microzooplankton. Organisms typically defined as < 200 µm in length. For the sake of operational convenience, the microzooplankton include the pico- and nanozooplankton (0.2–2 µm and 2–20 µm, respectively). See also Zooplankton.

Performance characteristics. For an analytical method used under given experimental conditions, these are a set of quantitative and experimentally determined values for parameters of fundamental importance in assessing the suitability of the method for any given purpose (Wilson, 1970).

Phytoplankton. Free-living, drifting, mainly photosynthetic organisms in aquatic systems including cyanobacteria (Prokaryota) and algae (Protista).

Primary production. The uptake of inorganic carbon into particulate matter, typically expressed as mg carbon/m^3/day or, in the case of macrophytobenthos, as g carbon/m^2/day.

Precision. A measure of the variability of replicated analytical data due to coincidental sources of errors. Statistically, precision is typically expressed in terms of standard deviations or confidence intervals about the mean.

Proficiency testing. Determination of the performance of a laboratory in calibration or testing by means of interlaboratory comparisons.

Quality. Characteristic features and properties of an analytical method/analytical system in relation to their suitability to fulfill specific requirements.

Quality Assurance. Quality Assurance (QA) is the total management scheme required to ensure the consistent delivery of quality controlled information fit for a defined purpose. The QA scheme 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. The two principal components of QA are quality control and quality assessment.

Quality Assessment. The procedures which provide documented evidence that the quality control is being achieved.

Quality audits. Systematic reviews which are carried out in order to ensure that the policies and procedures of a laboratory, as formulated in the Quality Manual, are being followed.

Quality Control. The procedures which maintain the measurements within an acceptable level of accuracy and precision.

Quality Manager. The person responsible for QA (even in small laboratories).

Quality Manual. A document stating the quality policy and describing the quality system of an organization.

Quality policy. A statement of the overall quality objectives of a laboratory.

Quality system. A term used to describe measures which ensure that a laboratory fulfills the requirements for its analytical tasks on a continuing basis.

Ring test. A means for interlaboratory testing of performance which, for community-level studies, may involve the circulation of preserved specimens of individual species, whole samples collected in the field, or artificial composites.

Sample tracking. A procedure which is designed to ensure that results or data can be traced back to their origin.

Standard Operating Procedures. Detailed descriptions of sampling and analytical procedures in standardized format.

Technical Manager. The post-holder who has overall responsibility for the technical operation of the laboratory and for ensuring that the Quality System requirements are met.

Voucher specimens. Specimens from routine collections placed under museum curatorship to make later taxonomic checks possible.

Zooplankton. Organisms that drift in the open water, comprising most animal phyla and ranging in size from 0.2 µm (picozooplankton) to 1 m (megazooplankton); assemblages are typically composed of species living permanently in the pelagial (holoplankton) and species living for certain periods in the pelagial (meroplankton, including fish larvae and benthic larvae).

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ANNEX 1

CRITICAL QA FACTORS AND PRIORITY QA ACTIONS FOR MONITORING CHLOROPHYLL *A*, PHYTOPLANKTON, MACROZOOBENTHOS, AND MACROPHYTOBENTHOS

Table 1. Chlorophyll *a*

Steps	Method diversity	Critical QA factors	Priority QA actions
Sampling procedures	3–4 methods according to JAMP Guidelines - pump/hose - bottle sampler - *in situ* fluorescence Different QA procedure for chlorophyll *a* extracts	Variability in accuracy among methods (effectiveness of methods in coping with patchiness)	Intercomparisons (workshops) on sampling method performance: hose vs. bottle sampler vs. *in situ* fluorescence
Sample analysis	2 (3) principles recommended - spectrophotometer - fluorometer (-HPLC as clean-up option)	Accuracy and precision	Certified reference material International comparisons of analytical performance Calibration of *in situ* measurements (if *in situ* fluorometers are used, they should be calibrated with filtered water samples)
Data treatment	Low variety of statistical methods		Reporting of data should be followed by control charts

Footnote 1. Supplementary variables essential for the interpretation of chlorophyll results include: suspended particulate matter, particulate nitrogen and phosphorus, particulate organic carbon, temperature, salinity, and light penetration.

Footnote 2. HPLC is presently an optional method.

Table 2. Phytoplankton

Steps	Method diversity	Critical QA factors	Priority QA actions
Sampling procedures	High (4) - water bottles - hose - pumps - nets	Large variability in accuracy between methods, especially among nets	Intercomparison of methods
Treatment and storage of samples	High (4–6) - different fixatives - living samples	Algae may be impossible to identify as a result of group-specific fixation damage	Intercomparison of fixative effects
Concentration of samples	High (4) - sedimentation - centrifugation - filtration - no concentration	Large variability in accuracy between methods (species dependent)	Intercomparison of methods
Sample analysis	Use of light microscope offers different techniques such as: - brightfield - darkfield - phase-contrast - epifluorescence	Magnification Quality of optics (resolution)	Intercomparison exercises Control of optical quality
	Species identification	Taxonomic expertise	Training and intercomparison exercises Ring tests
		Change of species names (synonyms)	Common checklist including synonyms
Biomass transformation	Two main methods: - cell measurements - use of standard volumes	Large variability in size for the same species	Use of standard geometric cell shapes Establish lists of standard volumes
Data treatment	Use of “control charts” with relevant information accompanying the data	Simplicity and uniformity of control charts	Develop and maintain control charts

Footnote. Supplementary variables essential for the interpretation of phytoplankton results include: particulate and total organic carbon, particulate organic nitrogen, temperature, salinity, and light penetration.

Table 3. Macrophytobenthos

Steps	Method diversity	Critical QA factors	Priority QA actions
Sampling procedure	High. At least 3 different method principles recommended: - aerial surveillance, acompanied by ground-truth surveillance - shoreline and diving transects and frames - photography or video (either direct or from remote platforms)	Frame and transect work: representativity (accuracy) of stations	Guidelines on assessment of representativity of stations
		Taxonomic competence of field observers	Taxonomic intercomparison workshops
		Enumeration technique (semi-quantitative/quantitative; individual counts or area measurements)	Preparation of regional checklists of taxa
			Internal assessment of observer precision (repeated registrations)
		Operation of photographic and video equipment	Training courses
		Photo/video resolution and the deployment technique	Instrument intercalibration exercises
Sample analysis	Low for each of the above sampling procedures	Taxonomic competence	Taxonomic intercomparison workshops
			Preparation of regional checklists of taxa
		Precision in quantification of abundances/% cover from photo and video images and ground-truthing	Intercomparison workshop on image analysis procedures
Data treatment	Low in OSPAR recommendations	None	None

Footnote. Supplementary variables essential for the interpretation of macrophytobenthos results include: substrate type, depth in relation to sea level or standard datum, slope and bearing, presence of loose sediment, degree of wave exposure, tidal range, Secchi disk depth, and salinity.

Table 4. Macrozoobenthos: hard bottom

Steps	Method diversity	Critical QA factors	Priority QA actions
Sampling procedure	High. At least 3 different method principles recommended: - aerial surveillance - shoreline and diving transects and frames - photography or video (either direct or from remote platforms)	Frame and transect work: representativity (accuracy) of stations Taxonomic competence of field observers Enumeration technique (semi-quantitative/quantitative; individual counts or area measurements) Operation of photographic and video equipment Photo/video resolution and the deployment technique	Guidelines on assessment of representativity of stations Taxonomic intercomparison workshops Preparation of regional checklists of taxa Internal assessment of observer precision (repeated registrations) Training courses Instrument intercalibration exercises
Sample analysis	Low for each sampling procedure High diversity in quantification of abundance (abundance scales)	Taxonomic skill Precision of quantification of abundances from photo and video images	Taxonomic intercomparison workshops Standardized taxonomic lists Intercalibration workshops - image analysis procedures - abundance estimates
Data treatment	Variable principles with respect to inclusion/exclusion of species in community description Level in a taxonomic heirarchy used in analysis Numerous methods (and software packages) for univariate and multivariate analysis	Criteria for inclusion of epigrowth and colonial organisms Consensus on how to treat abundance of colony-forming species Inconsistency in handling uncertain identifications "Rounding" errors with different computer packages Mistakes in data compilation	Standard approaches to pooling/exclusions of species More specific guidelines Recommendations for best practice Intercomparisons of analytical output from a standard data set Standardized taxonomic lists

Footnote. Supplementary variables essential for the interpretation of hard-bottom fauna results include: substrate type, depth in relation to sea level or standard datum, slope and bearing, presence of loose sediment, degree of wave exposure, tidal range, dominating macroalgal cover, and salinity.

Table 5. Macrozoobenthos: soft bottom

Steps	Method diversity	Critical QA factors	Priority QA actions
Sampling procedure	Sample collection: Low: two main categories – grabbing and coring A wide variety of sampler designs is available within these categories Field processing: Low: the aim is invariably to extract fauna from sediments, and to preserve the material Approaches to processing can vary substantially in the details	Variability in sediment and faunal sampling efficiency according to sampler design and handling Winching speed, mesh design (round vs. square, plastic vs. metal), sieving procedures, especially hose pressure	Intercomparisons of sampling devices in the field Agreement on minimum acceptable sample volumes and sample quality Intercomparisons of methods for field sample processing Recommendations on "best practice"
Sample analysis	Low: manual counting, identifying and weighing of species Variability is encountered in: 1) means to extract fauna from residual sediment; 2) use of magnification during sorting; 3) access to up-to-date taxonomic keys; 4) biomass determinations	Extraction and sorting efficiency Proficiency of species identification Precision/accuracy of biomass estimates (method-determined)	Independent (in-house or external) checks on sorting and identification efficiency Workshops on species identification Access to up-to-date taxonomic keys Standardized taxonomic lists Ring tests (identification, counting, biomass) Compilation of biomass conversion factors
Data treatment	High: numerous methods (and software packages) for univariate and multivariate analysis	Inconsistency in handling of uncertain identifications Inconsistencies between different computer packages Mistakes in data compilation	Standard approaches to pooling/exclusions of species Intercomparisons of analytical output from a standard data set

Footnote 1. Supplementary variables essential to the interpretation of soft-bottom benthos data include: particle size analyses of sediment sub-samples; measurements of redox potential; concentrations of specified contaminants, e.g., heavy metals; organic matter content; chlorophyll *a*. QA procedures should already be established for many of these variables. However, for those not presently covered, advice is needed on the appropriate ICES/OSPAR groups to deal with them.

Footnote 2. Epifauna are sampled by a variety of means across both coarse and soft bottoms. QA procedures must also be developed for this group. A wide variety of sampling methods is currently employed (e.g., underwater photography, dredges/sledges, trawls) and, in most cases, the results are strongly method-dependent.

ANNEX 2

QUALITY AUDIT

Areas of particular importance to a chemistry laboratory (drafted by the WELAC/EURACHEM Working Group, 1992) but in most parts valid also for biology.

1 STAFF

- Staff are properly trained and up-to-date training records are being maintained.
- Tests are only carried out by authorized analysts.
- The performance of staff carrying out analyses is observed.

2 EQUIPMENT

- The equipment in use is suited to its purpose.
- Major instruments are correctly maintained and records of this maintenance are kept.
- Equipment, e.g., balances, thermometers, glassware, time pieces, pipettes, etc., is calibrated, and the appropriate calibration certificates demonstrating traceability to national or international standards are available.
- Calibrated equipment is appropriately labelled or otherwise identified.
- Instrument calibration procedures are documented and records of calibrations are satisfactorily maintained.
- Appropriate instructions for use of equipment are available.
- Instrument performance checks show that performance is within specifications.

3 METHODS AND PROCEDURES

- In-house methods are fully documented and appropriately validated.
- Alterations to methods are appropriately authorized.
- The most up-to-date version of the method is available to the analyst.
- Analyses are following the methods specified.

4 STANDARDS AND CERTIFIED REFERENCE MATERIALS

- The standards actually required for the tests are held.
- The standards are certified or are the “best” available.
- The preparation of working standards is documented.
- Standards and reference materials are properly labelled and correctly stored.
- New batches of standards are compared against old batches before use.
- The correct grade of materials is being used in the tests.
- Where reference materials are certified, copies of the certificate are available for inspection.

5 QUALITY CONTROL

- There is an appropriate degree of calibration for each test.
- Where control charts are used, performance has been maintained within acceptable criteria.
- QC check samples are being tested by the defined procedures, at the required frequency, and there is an up-to-date record of the results and actions taken where results have exceeded action limits.
- Results from the random re-analysis of samples show an acceptable measure of agreement with results from the original analyses.
- Where appropriate, performance in proficiency testing schemes and/or interlaboratory comparisons is satisfactory and has not highlighted any problems or potential problems. Where performance has been unsatisfactory, corrective action has been taken.

6 SAMPLE MANAGEMENT

- There is an effective documented system for receiving samples, identifying samples against requests for analysis, and showing progress of analysis and fate of sample.
- Samples are properly labelled and stored.

7 RECORDS

- Notebooks/worksheets include the date of test, analyst, analyte, sample details, test observations, all rough calculations, any relevant instrument traces, and relevant calibration data.
- Notebooks/worksheets are completed in ink, mistakes are crossed out and not erased, and the records are signed by the analysts.
- Where a mistake is corrected, the alteration is signed by the person making the correction.
- The laboratory's procedures for checking data transfers and calculations are being complied with.
- Vertical audits on random samples have not highlighted any problems (i.e., checks made on a sample, examining all procedures associated with its testing from receipt through to the issue of a report).
- Proof-reading of the final data report has been made.

8 REFERENCE

EURACHEM/WELAC (Cooperation for Analytical Chemistry in Europe/Western European Legal Metrology Cooperation). 1992. Information Sheet No. 1 (Draft): Guidance on the Interpretation of the EN 45000 series of Standards and ISO Guide 25. 27 pp.

ANNEX 3

MINIMUM REQUIREMENTS FOR HARD-BOTTOM SURVEYS BASED ON THE PURPOSE OF MONITORING AND REQUIRED PRECISION

The following example is taken from the Norwegian National Standard (Kroglund *et al.*, 2002). It suggests the minimum requirements for surveys of hard bottoms, according to the main aim of the survey. The number of stations is dependent on the size of the area to be monitored and the available substrate. pe = person equivalents. References to the methods (A.1–A.6) are for information only and full descriptions are presented in the standard.

Main aim	Minimum requirements for investigations in the littoral zone (shore)	Minimum requirements for investigations in the sub-littoral zone (maximum down to 30 m)
1. OVERVIEW SURVEYS	Inspection with listing of characteristic taxa/biotopes	Survey with listing of characteristic taxa/biotopes (diving, video, ROV, random stereo-photography)
2. DESCRIPTION OF ENVIRONMENTAL CONDITIONS		
2a. Smaller domestic effluent (< 5000 pe) or other lesser effluents (cooling water, industry, particle discharge, aquaculture, etc.)	Semi-quantitative investigations at 4–7 assumed affected stations, together with 3 reference stations (A.1)	No minimum requirements for investigations in the sub-littoral zone
2b. Larger domestic effluent (> 5000 pe) or other larger effluents (cooling water, industry, particle discharge, aquaculture, etc.); or physical constructions with expected effects	*Areas with mean tidal range < 0.5 m:* Semi-quantitative investigations at 7–10 assumed affected stations together with 3 reference stations (A.1) *Areas with mean tidal range > 0.5 m:* Quantitative grid analyses at 4–7 assumed affected stations together with 3 reference stations (A.3)	Semi-quantitative transect diving at minimum 2 stations (A.2). At least one station shall be placed outwith the assumed affected area of influence (reference station, see Section 4.5)
2c. Oil spill with minor pollution	Semi-quantitative investigations at 5 assumed affected stations, together with 5 reference stations (A.1). The first investigation shall be carried out within one week after the time of oil stranding. Thereafter, the investigation shall be repeated after 3 months, 6 months, and one year.	No minimum requirements for surveys in the sub-littoral zone
2d. Oil spill with major pollution (actual or potential)	*Areas with mean tidal range < 0.5 m:* Semi-quantitative investigations at fixed marked locations, at 5 assumed affected stations, together with 5 reference stations (A.1) *Areas with mean tidal range > 0.5 m:* Quantitative grid analyses at fixed locations, together with 5 reference stations (A.3)	Where dispersant agents are used, or in other conditions that can cause the oil to sink below the water surface, an overview of any effects in the sub-littoral zone shall be carried out

Main aim	Minimum requirements for investigations in the littoral zone (shore)	Minimum requirements for investigations in the sub-littoral zone (maximum down to 30 m)
2d (continued)	NOTE: The first investigation shall be carried out within one week after the time of oil stranding. Thereafter, the investigation is repeated after 3 months, 6 months, and one year. In the case of larger oil spills causing clear effects after one year, annual follow-up investigations may be required	
3. TREND MONITORING	*Areas with mean tidal range < 0.5 m:* Semi-quantitative investigations within fixed marked areas (A.1)	Semi-quantitative transect diving along a marked, fixed transect (A.2) plus one of the following:
	Areas with mean tidal range > 0.5 m: Quantitative grid analyses at fixed marked areas (A.3)	Quantitative grid analyses in minimum of one fixed depth. Only at depths < 10 m (A.4)
	NOTE: For trend monitoring, it should be aimed for quantitative analyses also in areas with low tidal range	Quantitative kelp forest analyses (A.5)
		Quantitative recording at fixed areas using stereo photography (A.6)

Reference

Kroglund, T., Oug, E., and Walday, M. 2002. Vannundersøkelse - Retningslinjer for marinbiologiske undersøkelser på litoral og sublitoral hardbunn/Water quality – Guidelines for marine biological investigations of littoral and sublittoral hard bottom. NS 9424.

ANNEX 4

GOOD PRACTICE IN THE SAMPLING AND ANALYSIS OF PHYTOPLANKTON AND CHLOROPHYLL *A*

Sampling

- Be sure that personnel responsible for sampling are well informed about sample location, type of sample, and sampling method.
- Register the information about sample location, type of sample (single or mixed sample), and sampling method (mesh size of the net, type of water sampler) in the protocol.
- Avoid contamination with sediment.
- Register date, time, and any other covariables such as water temperature, salinity, and extinction.
- Keep the samples cool and in the dark.
- Fixed (phytoplankton) samples: fixate immediately, avoid large air bubbles, do not shake the bottle.
- The samples should be counted as soon as possible; storage for more than one year is not recommended.
- Non-fixed (living) samples for qualitative analysis: keep the samples in the dark and at a temperature of 4 ± 2 °C. Deliver the samples *as soon as possible* (within 48 hours).
- For chlorophyll *a* it is recommended that the sample is filtered immediately after sampling or, at least, as soon as possible thereafter; avoid deposition of cells. If storage is unavoidable, the filters should be deep frozen (< −20 ^{o}C).

Phytoplankton analysis

- Take a sub-sample in case there is a need to count a non-concentrated sample.
- Make use of a determination protocol and fill in completely.
- Create and maintain an annotated species list that contains the Latin name, synonym, historic information, morphological description, measures and determination literature, supplemented with photo documentations and descriptions of rare species. The lists have to be adjusted to international codes with respect to the currently valid names of the species.
- Enumeration should be based on at least 50 cells/counting unit for a common/dominating species and the total count should exceed 500 (compare the error calculation in the HELCOM COMBINE Manual, C6–6).
- Be careful in preparation of the sedimentation chambers: the samples should be adapted to room temperature and the contents have to homogenize gently before filling the chambers.
- Avoid vibration and temperature changes during the settling time.
- Pay attention to the random distribution of the counting units after sedimentation.
- Perform on a regular basis a double check with a colleague, if possible.
- Keep track of unknown species, make a photo/video, and consult colleagues or experts, as appropriate.

- The laboratory personnel should regularly take part in taxonomic workshops and ring tests.

Spectrophotometric or fluorometric chlorophyll *a* analysis

- The analysis should follow ISO 10260; departure from this has to be documented, and evidence of comparability of the data provided.
- The samples/filters and the chlorophyll *a* extracts should be handled in subdued light.
- Avoid evaporation of the extraction solvent during extraction and measurement procedures.
- The measurements should be done immediately after clearing the extracts; the preference is for equipment for measuring the whole spectrum (800–350 nm) for easier checking of shifting of the chlorophyll peaks.
- Validate the spectrophotometer and the fluorometer at least once a year, or when changes of the equipment are required.
- Calibrate the equipment with a certified reference material, if possible; use control charts.
- The laboratory personnel should take part in ring tests regularly.

HPLC method:

- Validate the HPLC system (linearity, reproducibility, etc.). Validation is done at least once a year and when the system changes (new lamp, detector, etc.). When validation takes place, this should be logged.
- Reference sample: the amount of reference sample should be enough for two months or 40 days.
- Control chart: chlorophyll *a* content of the reference sample is registered on a control chart.

Performance criteria for HPLC analysis:

- Column pressure is allowed to vary within a certain range. Double check the peak shapes.
- Background signal detector: should be stable at a certain level.
- Retention time standard components chlorophyll *a*/*b* and phaeophytin *a*/*b*: check the location of the peaks and take action when there is a deviation of more then 10 %.
- Response factor standard components: should not deviate more then 10 % (compared with the last day).

References

HELCOM Manual for Marine Monitoring in the COMBINE Programme, Annexes C-4 and C-6. see http://www.helcom.fi/Monas/CombineManual2/CombineHome.htm.

ISO. 1992. ISO 10260: Water Quality – Measurement of biochemical parameters—Spectrometric determination of the chlorophyll-*a* concentration. International Standards Organisation, Paris.

ANNEX 5

GOOD PRACTICE IN THE SAMPLING AND ANALYSIS OF SOFT-BOTTOM MACROZOOBENTHOS

Sampling strategy: stations must be representative for the respective area. Representativeness should be checked by area sampling or video inspection. Area sampling schemes with random allocation of sampling effort could give better information on areas under investigation.

Position-fixing during sampling is required. Subtidal stations should be controlled by track plotting.

It is mandatory to follow method recommendations issued by OSPAR and ICES (e.g., Rumohr, 1999).

In subtidal sampling, winch operation should be standardized with a complete stop and slow lowering (< 0.5 m s^{-1}) the last few metres, before the grab touches the seabed.

All procedures during the sampling process must be documented in writing including the logging of events that may reduce the quality of samples. Sample volume should be measured. Criteria for the rejection of samples have to be developed (e.g., small sample volume, uneven bite, inclusion of drifting algae, stones or other material preventing jaw closure). All samples affected by spillage during sieving or transfer have to be regarded as non-quantitative.

Sampling devices (grabs, corers, etc.) must be used on a long-term basis. Gear changes have to be accompanied by intercalibration and a period of parallel sampling. There is no single standard gear for benthos investigations. The choice of an appropriate sampler depends on the average living depth of the fauna under investigation. It is always a compromise between specific sampling characteristics in different sediment regimes in the area to be sampled, good handling characteristics at sea under bad weather conditions, suitability for various ships, financial limitations, tradition, and scientific questions.

Dredging is not a quantitative sampling method in the subtidal zone, but can be useful for semi-quantitative sampling with a five-point scale of abundance. Standardized dredging should always be employed when grab samples are likely to be encountered with a very sparse (or absent) fauna. Special quantitative dredges (see, e.g., Bergman and Van Santbrink, 1994) can be used to collect rare mega-, in-, and epi-fauna.

Sieves should have a mesh opening of 1 mm (or 0.5 mm, if needed). The use of square or round sieve openings should be stated. Washing and sieving procedures should be sediment- and site-specific. Crumbling and gentle fragmentation of stiff clay material is explicitly allowed.

Samples should be fixed directly after sampling with 4 % formalin. All necessary measures should be taken in the field and in the laboratory to avoid health risks to individuals arising from the use of formalin. Fixed samples should not be sieved.

Sorting of samples should be done under a magnification aid (magnifier lamp/binocular), following removal of formalin in conditions conforming with accepted safety standards. Taxonomic identification should follow accepted identification aids. Voucher specimens should be kept in taxonomic reference collections to allow later taxonomic controls. The exchange of reference material between laboratories is encouraged. Laboratory reference collections should be validated by experts.

Laboratory personnel should regularly take part in taxonomic workshops and ring tests to improve and maintain personal professional skills.

References

Bergman, M.J.N., and Van Santbrink, J.W. 1994. A new benthos dredge ('Triple-D') for quantitative sampling of infauna species of low abundance. Netherlands Journal of Sea Research, 33: 129–133.

Rumohr, H. 1999. Soft bottom macrofauna: Collection, treatment, and quality assurance of samples. ICES Techniques in Marine Environmental Sciences, No. 27. 19 pp.

ANNEX 6

GOOD PRACTICE IN THE SAMPLING AND ANALYSIS OF HARD-BOTTOM MACROZOOBENTHOS AND MACROPHYTOBENTHOS

Background

Biological communities of rocky habitats exhibit a very high degree of variability in their taxonomic composition. In addition, biological interactions of predation, competition, and chance recruitment play their role in community structure. The effect of this is often to yield a very high variety of communities in an area, often changing markedly over a few metres. Such complexity in rocky habitats presents difficulties in both ecological monitoring and monitoring for man-induced change. On the shore and in the shallow kelp-dominated infra-littoral zone, one or several species may typically dominate (numerically or by space) community structure; however, in the deeper animal-dominated circa-littoral zone, communities tend to comprise a wide variety of species and present a much patchier community structure that can make effective monitoring difficult to establish.

General principles for sampling rocky habitats

The methods adopted need to be appropriate to the end requirements of the study. Consequently, the methods, equipment, and resources required will differ considerably between different types of monitoring studies. The scale of the study, be it local, national, or international, also has a marked effect on the techniques employed and the level of detail appropriate. The general strategy for sampling should be similar to both littoral and sub-littoral rocky habitats, although the specific methods adopted will differ according to the logistics of sampling in each zone.

Since rocky habitats by definition support epibiotic communities, they are visible by eye and sampling can typically be undertaken in a non-destructive *in situ* manner. For some studies, removal of samples (destructive sampling) may, however, be appropriate.

Quantitative sampling is difficult as many species are colonial in nature (and thus cannot be counted), are difficult to count (e.g., stands of filamentous algae), or adhere as a crust over the rock (and so cannot be collected or counted). More effective assessment of quantity for such species is given by estimates of percentage cover or frequency of occurrence in a grid. Semi-quantitative abundance scales may also be used, but the source of the scale must be clearly documented. An example of such a scale derived from integration of percentage cover estimates with a $\log_{10}$-based quantitative abundance scale for species that can be counted is provided in Hiscock (1996).

In the sub-littoral zone, use of SCUBA diving enables detailed recording and sampling to be made, which are particularly important in description of the community and monitoring. Remotely operated video (ROV) cameras confer some advantages in the maximum depth that can be surveyed, extending the time available underwater and providing a permanent record of the site. However, for species identification, remote video is only able to pick up the conspicuous species at a site, sometimes amounting to only about 50 % of the macrobenthic species present (Davies *et al*., 2001). Consequently, it is unsuitable where a detailed inventory of the species present in a habitat is required for monitoring purposes.

Monitoring for man-induced change requires previous knowledge of the nature of the community and its natural variability. Such basic information is lacking for the majority of rocky habitats, making the design of monitoring programmes critical to ensure that they effectively answer the aims of the study. Such monitoring should therefore include sufficient

study to establish natural variation at the site or parallel monitoring of a reference site of comparable nature.

Sampling strategy

All sampling of rocky habitats is subject to time constraints, either imposed by tidal movements on the shore or due to physiological factors whilst diving. Therefore, to reduce the travel time between stations, sampling strategies are generally based on a transect approach rather than the random placement of stations over an area. Safe diving practice dictates that the sampling should proceed from deep to shallow stations.

The time taken to record species composition and abundance at a sampling station (the sampling effort) should be consistent between surveys. This is especially important for trend monitoring studies.

The scale of sampling will determine the number of species recorded at a station and should be fixed at the outset. For example, recording the species living in kelp holdfasts or rock crevices will significantly increase the total number of species recorded from a station.

Adverse environmental conditions can have a serious effect on data quality; for example, rough sea conditions make recording in shallow water extremely difficult and maybe hazardous.

It is recommended that a permanent photographic record of sample frames is collected at the time of sampling. This allows for subsequent quality checks of recorded data. Photographs (or video) may be examined to make quantitative measurements of the species or community present (Lundälv, 1971).

Defining the position of sampling stations

Sampling stations for environmental descriptions and trend monitoring must be defined unambiguously, such that others can relocate them. Positions should be defined either using geographic coordinates with reference to the appropriate system for graticules (such as European Datum: ED-50, World Geodetic System: WGS-84), or using a grid system such as the UTM system. Positions should be defined according to appropriate national or international standards.

In addition to geographic coordinates, sampling stations can also be defined using characteristic landmarks and at least one fixed reference point or easily identifiable point immediately above the supra-littoral zone. For relocation of sub-littoral investigation areas, the depth and compass direction from the reference point should be recorded. The level on the shore can be given in relation to zone-forming organisms, but should also be measured according to a standard datum, if possible. In addition, station positioning should be documented by photography. Photographs should be kept in a central image library, preferably at the institute carrying out the survey or by the coordinating agency, and included in reports where the stations are first introduced.

For trend monitoring and in the case of larger impact investigations, permanent marking of stations will be necessary, for example, by fixing bolts in the rock. One bolt should be designated as a reference bolt and its position in relation to a known geographical position carefully documented. For example, the Norwegian monitoring programme uses the upper left bolt as the reference bolt and it is defined by its distance to a fixed reference point above the supra-littoral zone (Kroglund *et al.*, 2002). Bolts should be made of non-corrosive material and sufficient resources provided for their routine maintenance.

When permanent stations are not used, it is important to ensure that subsequent sample stations are located within the same biological subzone or biological community.

A graduated transect line or a ladder transect can be used where random stations are required. The position of stations along the transect are selected using random numbers (Davies *et al.*, 2001).

Requirements for diving

Diving surveys must be carried out in accordance with appropriate national rules and regulations. A guide to planning and carrying out scientific diving operations is given in Flemming and Max (1996).

Scientific requirements for personnel

The surveys must be carried out by appropriately qualified personnel (marine zoologists/marine botanists). They must be able to document competence within their specialist field, and participate in ring testing, when the appropriate routines are available. For investigations spanning several years, priority should be given to continuity in personnel carrying out the recordings. If changes of personnel occur, it is recommended that inter-personnel calibration exercises are completed.

It is good practice to undertake pre-survey validation exercises with the intended field surveyors, possibly supported by a standard checklist of likely taxa, to quantify and then correct for any variation between surveyors.

Timing of the investigation

Investigations on hard bottoms should be carried out during the summer season, mostly to ensure the full development of algal communities. For trend monitoring, the investigations must be carried out at the same time of year, each year. For trend monitoring that already has been established, continuity is given higher priority over the requirements for timing of the investigation.

Sample collection

Most rocky surveys use non-destructive methods, which make it possible to carry out repeated surveys of the same area. If sampling is required, it should be carried out with appropriate equipment to minimize destruction and damage to the biological communities. Taxa to be identified by microscopy should be collected and kept alive or fixed for analysis in the laboratory.

Image material should be archived with, at a minimum, the following information:

- project identification or project code;
- station code;
- date and time (start–stop);
- photograph.

Sampling equipment

The area of the sampling frame must be recorded and standardized for temporal trend monitoring. Ideally, the same equipment should be used throughout a monitoring study. Frames

that are designed for rapid assembly underwater should ensure that the individual components are securely held together with flexible cord to guard against the loss of an individual component. Such frames should also be designed to ensure that they cover the correct area each time they are assembled. Frames with three sides are easier to deploy in kelp forests although clear criteria should be established for "closing" the frame when enumerating the species present.

References

Davies, J., Baxter, J., Bradley, M., Connor, D., Khan, J., Murray, E., Sanderson, W., Turnbull, C., and Vincent, M. (eds.). 2001. Marine Monitoring Handbook. Joint Nature Conservation Committee, Peterborough, UK. 405 pp.

Flemming, N.C., and Max, M.D. (eds.). 1996. Scientific diving – a general code of practice. Best Publishing Company & UNESCO Publishing. ISBN (Best Publishing Company): 0–941332–51–9. 278 pp.

Hiscock, K. (ed.). 1996 Rationale and methods. Coasts and Seas in the United Kingdom. Marine Nature Conservation Series. ISBN 1 87 107 4107. 167 pp.

Kroglund, T., Oug, E., and Walday, M. 2002. Vannundersøkelse - Retningslinjer for marinbiologiske undersøkelser på litoral og sublitoral hardbunn/Water quality - Guidelines for marine biological investigations of littoral and sublittoral hard bottom. NS 9424.

Lundälv, T. 1971. Quantitative studies on rocky-bottom biocoenoses by underwater photogrammetry. Thalassia jugosl., 7: 201–208.

OSPAR. 1997. JAMP (Joint Assessment and Monitoring Programme). Eutrophication Monitoring Guidelines: Benthos. (version 9.6.97). OSPAR Commission, London. 12 pp.

ANNEX 7

GOOD PRACTICE IN THE USE OF IMAGING TECHNIQUES

General Comments

No binding procedures have so far been identified for QA/QC measures for imaging methods. Retrieval methods can be grouped as follows:

a) sea-going and land-based activities;

b) satellite and airborne imagery;

c) evaluation and processing of images, videos, and side-scan records;

d) storage and retrieval of image documents.

Important considerations in relation to diver-based retrieval include: parallel checks among several divers; photo and video documentation; double recording of profiles/transects; need for specific scientific diver training and certification; strict safety rules.

"Best Practice" Guidelines

a) Sea-going and land-based procedures should follow well-documented SOPs. In addition, a precise time log (i.e., TBC or data input on screen) is a prerequisite for proper evaluation and identification of photographic and video images. Marks on videos and photographs, and in the written log, can help in identifying and tracing back the image documents. One should note, however, that for certain purposes high quality pictures are needed for publication without the on-screen information.

All technical details of cameras, films, tapes, camera settings, angles, distances, lightings, and parallel measurements must be recorded in writing.

Films, tapes, and sonar records must be labelled and stored safely (in waterproof conditions).

Underwater pressure housings should be equipped with hydrophilic drying agents (silica-gel pellets in sacks) to provide proper functioning of cameras.

Greatest care should be given to O-ring sealings to avoid floating of pressure housings.

All safety instructions for diving, safety on board ships, and underwater electricity should be followed strictly.

b) For satellite and airborne images, the same rules apply in principle as for other imaging methods. In particular, it is important to fully document all parameters used in the registration and georectification to a coordinate system.

c) Images and recordings should be evaluated following well-documented and repeatable methods conforming to standards of the highest objectivity.

All steps involved in processing of the original image (e.g., colour enhancement) must be documented and stated in documents and figure legends. Geodetic parameters must be recorded.

d) Images and videos should be stored in suitable labelled magazines, to make later retrieval possible by other individuals.

Back-ups must be stored in other buildings as video copies, CD-ROMs of photograph collections, or stored on PC drives. Future storage media may include DVD drives.

Large collections of images should be stored in image data banks in digital form, to avoid mis-identifications and losses of images. The use of key words is strongly recommended, providing information about the subject, platform, format, position depth, remarks, etc.

Attention should be given to the possibility that videotapes may lose their magnetic information after > 15 years as well as CD-ROMs after > 10 years; thus, back-ups are advised every five years. This also applies to old film and photographic material that is of documentary and historical value.

ANNEX 8

SUMMARY OF DISCRETE WATER SAMPLE GUIDELINE

This summary document outlines the information contained in a data guideline prepared by the ICES Working Group on Marine Data Management (WGMDM). This guideline can be found at http://www.ices.dk/committe/occ/mdm/guidelines.

The discrete water sample guideline provides information relevant to data collected from discrete water samplers, including chlorophylls and other pigments. Here, discrete is used to characterize samples from instruments such as a single rosette bottle. Discrete does not include integrated samples from instruments such as pumping systems or combinations of rosette bottles. (See the above website for relevant guidelines). All guidelines follow the same structure as is given for discrete water samples.

The guideline is intended to provide the reader with information on what a data submission is expected to contain, the function of the data centre with regard to these data, and the service the data centre provides to clients requesting these data.

In the specific guideline for discrete water sample data, the reader is provided with an outline of required metadata. As well, the provider is reminded that all processing applied to the data set should be documented and provided to the data centre. This includes such things as flagging procedures, precision of methods, and handling of null values. Submission formats are also suggested, although data centres may accept submissions in a variety of formats. Cruise collection information is also itemized, which can serve as a metadata checklist to ensure that the collector acquires all required metadata at the source and time of initial sample collection.

The guideline also outlines the service provided by the data centre. The particular quality control procedures applied to the data set by the centre are outlined. During these procedures, any problems detected with the data set are brought to the attention of the provider and problem resolution between the centre and provider is sought. The data centre also maintains procedure and problem histories to provide value-added information to the data set. This value-added information is provided to other clients requesting the data.

The guideline also includes information on the general service of data delivery provided by the data centres. This service includes data descriptions and a full history of procedures and processing. Data quality flagging is described and any changes made to the data set are noted. In the event that a data centre cannot fulfil a client's need, the centre will provide a referral service to other data centres or experts.

Finally, a reference section includes links to other guidelines, quality control procedures, or data management techniques specific to that data type. This provides valuable information and acknowledgement of other related activities in the international community.

ICES Techniques in Marine Environmental Sciences

No. 1 Cadmium and lead: Determination in organic matrices with electrothermal furnace atomic absorption spectrophotometry
No. 2 Trace metals in sea water: Sampling and storage methods
No. 3 Cadmium in marine sediments: Determination by graphite furnace atomic absorption spectroscopy
No. 4 Lipophilic organic material: An apparatus for extracting solids used for their concentration from sea water
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