1.6.6.2 OSPAR request on review of draft OSPAR JAMP eutrophication guidelines on phytoplankton species composition

Advice summary

ICES provides a revised version of the OSPAR JAMP phytoplankton monitoring guidelines. The motivation for the suggested changes is summarized below.

ICES advises that:

- there are no reliable widespread "indicator" plankton species yet agreed by the scientific community, whether for ocean acidification or for eutrophication;
- standard lists are still required for trophic type/functional groups and non-indigenous/cryptogenic species, and a further process to keep these and other lists up to date is also required;
- if OSPAR wishes to collectively store, use, analyse, or assess phytoplankton data (including for biodiversity) then a common data reporting, handling, and storage system is required, as well as agreed analysis and assessment systems;
- some choices by OSPAR may therefore be needed in relation to phytoplankton data;
- OSPAR encourages its Contracting Parties to invest in both the equipment and personnel necessary to monitor picoplankton.

Request

ICES is requested to advise OSPAR on the revision of the OSPAR JAMP Eutrophication Guidelines which will be revised by experts from Germany, The Netherlands and Sweden.

ICG-EUT 2014 concluded, and HASEC 2014 endorsed, that these guidelines were in need of a review. The guidelines should be revised to reflect new knowledge about phytoplankton and needs within (directives such as) the EU Marine Strategy Framework Directive (MSFD) and the Water Framework Directive (WFD).

It is the intention of the revision that the existing aims described in the guidelines will be supplemented with the following:

- *to identify harmful algae species and blooms in line with MSFD Descriptor 5.*
- *to identify invasive (non-indigenous) species in line with MSFD Descriptor 2.*
- *to monitor effects of ocean acidification as e.g. on coccolithophorids (e.g.* Emiliania huxleyi*) in line with Descriptor 1 in MSFD.*

The revised guidelines should incorporate coming monitoring and measurement techniques such as (but not limited to) spectrofluorometry, flow cytometry and qualitative observations of foam production, and should make use of existing standards, such as EN 15972 and EN 15204 and reflect developments within the OSPAR ICG–COBAM which is working on biodiversity monitoring and assessment. Data handling issues, such as the format required for reporting to ICES, should also be addressed.

Elaboration on the advice

The primary advisory work carried out by ICES was a full review of a draft of the revised JAMP phytoplankton monitoring guidelines dated 25 February 2015. A revised set of guidelines, incorporating changes advised by ICES is attached as the Annex to this advice. Some explanation of the reasoning for the changes is given in the Basis for the advice section below.

Indicator species

ICES advises that there are no reliable widespread "indicator" plankton species yet agreed by the scientific community, whether for ocean acidification or for eutrophication. While such indicators may exist locally, further studies are required if widespread indicators are required.

Indicators of ocean acidification

ICES and OSPAR have been evaluating possible indicator organisms that are potentially suitable for ocean acidification (OA) monitoring. Among these organisms are the coccolithophores, principally *Emiliania huxleyi*, but also other species. ICES has concluded that *E. huxleyi* is not a suitable candidate indicator for the following reasons. *E. huxleyi* is a species that consists of a wide range of strains, each with a different genetic signature. Research on the responses of this species suggest that its sensitivity to OA is highly dependent on the strain involved with only some strains showing sensitivity to OA, as indicated by reduced calcification under elevated pCO_2. Determination of the particular strain requires genetic sequencing, which is impractical to implement as part of any routine monitoring programme. Moreover, recent research indicates that even strains of *E. huxleyi* that show a negative response to OA appear capable of rapidly evolving tolerance. For example, after 500 generations, populations showed increased growth and partially restored calcification rates under high CO_2, which suggests that this species possesses the ability to adapt to OA.

Other coccolithophore species (e.g. *Gephyrocapsa oceanica,* strain PC7/1; *Coccolithus pelagicus,* strain AC400) have shown an increase in the proportion of malformed coccoliths under elevated pCO_2, though the responses may also be strain-specific and therefore subject to the same caveats as for *E. huxleyi*. Moreover, the distributions of these species may not extend throughout the OSPAR maritime area, which would mean that monitoring would need to focus on different species in different parts of the area.

Eutrophication indicators

ICES advises that there is at present no scientific agreement on using indicator species/ratios as an eutrophication index, although different indexes – such as harmful algal bloom (HAB) species, diatom:dinoflagellate ratio, or *Phaeocystis* sp. – may be used locally.

For many coastal regions, attempts to relate trends in the occurrence of HABs to nutrient enrichment are confounded by increased monitoring effort and reporting of HABs, the effects of climate change, and the introduction and transfer of HAB species. Thus the occurrence and abundance of HAB species and HABs should not be used to diagnose eutrophication unless a link to anthropogenic nutrient enrichment can be demonstrated for a specific area.

There are similar problems with *Phaeocystis*. Only in the Netherlands has the occurrence of *Phaeocystis* blooms been linked to nutrient enrichment, while *Phaeocystis* blooms can occur in other regions in the OSPAR area without being associated with nutrient enrichment.

The diatom:dinoflagellate ratio is also not linked conclusively to any one driver of change, but may be used as a general indicator of change in a planktonic community.

Standard lists

ICES advises that standard lists are still required for trophic type/functional groups and non-indigenous/cryptogenic species, and a further process to keep these and other lists up to date is also required. Without such work there is a risk that there will not be coherence in phytoplankton monitoring in the OSPAR area.

Data reporting, handling, and storage

ICES advises that if OSPAR wishes to collectively store, use, analyse, or assess phytoplankton data (including for biodiversity), a common data reporting, handling, and storage system is required, as well as agreed analysis and assessment systems.

ICES advises that some choices by OSPAR may therefore be needed in relation to phytoplankton data. OSPAR's position in relation to phytoplankton data is at present unclear to ICES. The original version of the OSPAR JAMP eutrophication

guidelines on phytoplankton species composition has a section in square brackets stating: "*[As a component of the 1997 ICES Work Programme, the Oslo and Paris Commissions have formally requested ICES to establish a databank for phytoplankton species. The work will include the development of a reporting format and a species code list. The reporting procedures should include a national report containing information on methods used and any other comments or information relevant to an ultimate assessment of the data. In order to establish the acceptability of the data, they should be reported together with the dates and results of participation in intercalibration exercises.]"*

ICES developed the database and has provided a reporting format, both of which have been subsequently updated (the same database is used for contaminant and biological community data, as well as for HELCOM). The draft revised version of the OSPAR JAMP eutrophication guidelines dated 25 February 2015 states "*Each partner country in OSPAR is required to report data yearly to ICES*", but this sentence does not seem to be based on an official decision by any part of the OSPAR community. There are also suggestions within this draft of the guidelines for the database to be further developed – fortuitously most of these suggested features have either been incorporated already or would be easy to incorporate.

Picoplankton
ICES advises OSPAR to encourage its Contracting Parties to invest in both the equipment and personnel necessary to monitor picoplankton. These plankton are very important components of marine foodwebs and the current low availability of analytical equipment and trained personnel has caused ICES to conclude that these plankton could not be included in the guidelines at present.

Suggestions

Foam production
ICES was requested to include qualitative observations of foam production within the review of the OSPAR JAMP eutrophication guidelines. However, there are few (if any) scientific articles on monitoring of phytoplankton foam production. A standard monitoring technique would need to be developed and published if the surface scums of, e.g. *Phaeocystis* were to be included in monitoring. Observations of foam production should, therefore, not be included in the OSPAR JAMP eutrophication guidelines at present.

Satellite observations
Satellite observations have been examined by others as way of monitoring chlorophyll. Further development is needed before satellite observations can reliably be included in the OSPAR JAMP eutrophication guidelines.

Basis of the advice

Motivation for the substantive changes in the draft OSPAR JAMP guidelines on phytoplankton from the version dated 25 February 2015

All recommended changes are designed to make these guidelines more user-friendly and logical, as well as updating where relevant.

Section 1 – Introduction
ICES considered it helpful to address the overall aims of this monitoring, especially as related to the Marine Strategy Framework Directive and to provide more detail on the indicators that OSPAR Contracting Parties have agreed to use. These aims have been described. ICES noted that a secondary objective is to attempt to distinguish between the various drivers of change in phytoplankton communities. ICES rearranged the introduction to provide a logical flow. ICES is not aware of existing OSPAR guidelines for monitoring mesozooplankton; if these are under development or not yet adopted, then a full reference is required.

Section 2 – Objectives
ICES found some overlaps in the objectives listed – they have been reformatted to two main purposes: (i) improving the understanding of phytoplankton blooms, and (ii) establishing long-term trends in phytoplankton community composition. The results of the second objective can be used for a number of purposes.

Section 3 – Sampling
This section combines the contents of three previous sections (3, 4, and 5), all of which covered various aspects of sampling. ICES removed some duplication of text and has clarified other parts.

Section 4 – Preservation and storage of samples
This section relates primarily to existing European standards. ICES has made recommendations for some aspects that go beyond or clarify the use of these standards.

Section 5 – Analytical procedures
This section also relates to existing European standards; again, ICES has made recommendations for some aspects that go beyond or clarify the use of these standards. In other parts of the section European standards have not been defined; in these cases ICES makes recommendations based on common current best practice.

Section 6 – Quality assurance
A paragraph recommending a good quality assurance process has been added, together with a catalogue of standard lists that should be used.

Section 7 – Reporting requirements
ICES has expanded the section on reporting requirements slightly on the assumption that OSPAR will agree to the recommendation in the draft that plankton data should be reported to ICES (this is not currently the case, except for those Contracting Parties that are also Party to HELCOM). ICES also notes that there is no scientific agreement that there are consistent species that indicate eutrophication, or that coccolithophorids are susceptible to ocean acidification. ICES therefore does not recommend treating any species as "indicators" of these pressures.

Section 8 – Additional optional monitoring techniques
An additional technique for analysing picoplankton has been included. This sub-section is a strong candidate for inclusion in the main guidelines as these plankton are very important components of the marine foodweb. The section has not been promoted due to the current low availability of analytical equipment and trained personnel for monitoring purposes.

The sections in the previous draft guidance on rare species and microzooplankton have been incorporated into the main parts of this draft of the guidance. Some additional text has been added in places and clarified elsewhere. ICES was unable to determine if reliable standard methods exist for converting satellite colour observation to an index of chlorophyll *a* for the OSPAR area.

Section 9 – References
This section has been supplemented, tidied up, and standardized.

Appendix
The Appendix that was attached previously has been deleted as it was non-standard, and the technique described was close to the preferred methods for concentrating samples described in the European standard EN-15972 (2011).

Additional information

ICES notes that the European standards referenced in the OSPAR Eutrophication Guidelines are relatively expensive. This cost may inhibit their use, but ICES nevertheless supports their use. OSPAR Contracting Parties should ensure these standards are available for use in relevant laboratories.

Sources and references

EN-15972. 2011. Water quality – Guidance on quantitative and qualitative investigations of marine phytoplankton. ISO 15972:2011.

ICES. 2014. Report of the Joint OSPAR/ICES Ocean Acidification Study Group (SGOA), 6–9 October 2014, Copenhagen, Denmark. ICES CM 2014/ACOM:33. 33 pp.

ICES. 2015a. Report of the Working Group on Introductions and Transfers of Marine Organisms (WGITMO), 18–20 March 2015, Bergen, Norway. ICES CM 2015/SSGEPI:10. 195 pp.

ICES. 2015b. Report of the ICES/IOC/IMO Working Group on Ballast and Other Ship Vectors (WGBOSV), 16–18 March 2015, Bergen, Norway. ICES CM 2015/SSGEPI:09. 102 pp.

ICES. 2015c. Report of the Working Group on Phytoplankton and Microbial Ecology (WGPME), 10–12 March 2015, Gothenburg, Sweden. ICES CM 2015/SSGEPD:06. 16 pp.

ICES. 2015d. Interim Report of the ICES–IOC Working Group on Harmful Algal Bloom Dynamics (WGHABD), 13–18 April 2015, Lisbon, Portugal. ICES CM 2015/SSGEPD:17. 77 pp.

Annex 1

1 Introduction

As part of its North-East Atlantic Environment Strategy, OSPAR aims eventually to have a regional set of indicators that are used by all relevant Contracting Parties to address the requirements of relevant EU directives, e.g. the Marine Strategy Framework Directive (MSFD). Some of these relate to plankton. These "common indicators" for plankton may be relevant to Descriptors 1 (biodiversity), 2 (non-indigenous (including some invasive) species), 4 (foodwebs), and 5 (eutrophication, including harmful algal blooms). Existing OSPAR common indicators are:

- D1 PelHab 1 (D4 Foodweb 5) Plankton lifeform index;
- D1 PelHab 2 Plankton biomass and/or abundance;
- D1 PelHab 3 Plankton diversity index.

Further indicators under development are described as "candidate indicators", some of which are prioritized. The candidate indicator that is most relevant for the OSPAR JAMP phytoplankton monitoring guildlines is:

- D4 FoodWeb 2 Production of phytoplankton.

Phytoplankton are relevant for other candidate indicators, including: D4 FoodWeb 7 Biomass and abundance of functional groups; D4 FoodWeb 8 Changes in the distribution of biomass and species over trophic levels or body size; and D4 FoodWeb 9 Ecological network analysis indicator (e.g. trophic efficiency, flow diversity). Both the common and the candidate indicators may apply only to certain regions within the OSPAR area.

The JAMP guidelines aim to ensure the delivery of consistent, high-quality phytoplankton data that can be used to evaluate the state of each of the indicators and then ultimately be used for OSPAR status assessments. Sampling under these guidelines should also help assist in producing assessments that distinguish between the various drivers of change in the phytoplankton community, e.g. eutrophication and climate change effects.

The basic data needed from any phytoplankton sample are therefore species identity, abundance, and biomass. Information for any of the above indicators can be derived from this data, assuming that the species can be classified to functional groups, i.e. to nuisance, toxic, or non-indigenous/cryptogenic species.

A further purpose of monitoring phytoplankton is under the OSPAR Common Procedure (COMP). The Common Procedure is a means of establishing eutrophication status of OSPAR seas on a common basis. Two types of area-specific phytoplankton species groups have been distinguished: nuisance species (that form dense "blooms"), and toxic species (that are toxic also at low concentrations). It has been suggested (HELCOM–OSPAR, 2014) that shifts in species composition from diatoms to flagellates (some of which are toxic) could indicate eutrophication. However, it should be noted that there is considerable scientific uncertainty in the use of phytoplankton species as indicators of eutrophication.

In order to design a suitable sampling protocol, a number of decisions about resolution in time and space are required. Long time-series are essential for tracking change in marine ecosystems. Frequent and consistent sampling and analysis is important to maintain such long time-series. If new methods are proposed (for instance to save costs, or to improve precision), it is important to understand fully how the results from the new methods relate to those from the existing methods. New parameters may be added as methods and knowledge improve. In this version of the JAMP guidelines, options to include autotrophic picoplankton, microzooplankton, and novel ways of estimating biomass of phytoplankton and monitoring of some rare phytoplankton species have been added.

The guidelines on phytoplankton also include microzooplankton, as this would be cost-effective and scientifically sound. The methods used for phyto- and microzooplankton monitoring are the same or similar.

1.1 Definitions

The terms pico-, nano-, micro-, and meso-plankton are used in this document. These terms are widely used and reflect size groups: picoplankton (0.2–2 µm) include heterotrophic bacteria and the smallest phytoplankton; nanoplankton (2–20 µm) include phytoplankton and unicellular zooplankton; microplankton (20–200 µm) also include phytoplankton and unicellular zooplankton (microzooplankton); and mesoplankton (200 µm to 2 mm) mainly include multicellular zooplankton, e.g. copepods, but can also include some large phytoplankton. In the context of this document, "microzooplankton" only includes ciliates and heterotrophic dinoflagellates. Copepod nauplii and other metazoans < 200 µm will not be sampled or preserved adequately with the proposed methods.

2 Objectives

Information from monitoring phytoplankton can be used to:

- establish the composition, spatial distribution, and frequency of phytoplankton blooms;
- establish long term temporal and spatial trends in phytoplankton and micro-zooplankton species composition and their relative abundance, in order to detect

    - changes in length of growing season, timing of blooming, etc.,
    - changes that may be caused by eutrophication, warming, ocean acidification, etc.,
    - changes in frequency and magnitude of harmful algal blooms,
    - occurrence of non-indigenous/cryptogenic species,
    - changes in the foodweb,
    - changes in diversity indices.

3 Sampling

3.1 General considerations

The aim of the monitoring is to sample all the regions within the OSPAR area at an adequate temporal and spatial scale sufficient to detect any signals of change within the natural variability of the phyto- and microzooplankton communities and within the sampling variability. The sampling frequency, period, and spatial scale should be adjusted to meet the aims of the monitoring. A commonly used approach is to sample selected localities frequently (weekly–fortnightly) in addition to carrying out wide-scale (monthly) surveys with sampling at many locations. It is recommended to include coastal and offshore localities as well as both problem areas and non-problem areas (OSPAR, 2005) in the sampling design. Harbours and ports are the most likely areas to find non-indigenous/cryptogenic species and sampling in these locations is recommended for schemes aimed at detecting these species.

An understanding of the complexity of the hydrography of estuarine or coastal seas is necessary before starting to survey or sample the phytoplankton. Thus, there is a need for routine hydrographic observations at the same time as the surveys/sampling. Apart from the influence of water column structure on phytoplankton dynamics there is a need to consider horizontal (spatial) and temporal variability in order to establish the frequency and location of sampling. Sample sites should be further apart than the horizontal tidal amplitude, but sufficiently close to resolve the presence of gradients. Similarly, the timing of sampling should consider the state of the tide at each location. It would be preferable that sampling be conducted at the same state of the tide on each sampling occasion. For instance, in estuarine or coastal locations it might

be preferable to sample at high water (±1 hr) to ensure that marine phytoplankton are sampled as consistently as possible. Sampling frequency should take account of seasonal variability in the abundance of the species of interest.

3.2 Sampling equipment for quantitative sampling

Sampling equipment is described in Section 5.1 of the European standard EN-15972 (2011). Water sampling shall be carried out using suitable water sampling bottles or tubes. The design of sampling bottles and tubes must allow free water flow when lowered through the water column. Materials should be non-toxic for phyto- and microzooplankton. Tube sampling must be carried out with care to avoid damaging ciliates.

3.3 Sampling depth

The minimum requirements include sampling near surface waters, i.e. either at the surface or a depth-integrated sample at 0–10 m. This can be accomplished by pooling samples (from bottles) from depths of 0, 2.5, 5, 7.5, and 10 m, or by using a sampling tube at 0–10 m. It is necessary to use the same volumes of water from each depth when pooling. This sampling strategy will miss any sub-surface chlorophyll maxima deeper than 10 m; however, these may be sampled separately if detected by fluorescence profiles in CTD casts. This should be considered especially when sampling stratified waters in both coastal and open-water areas.

3.4 Supporting parameters

For the best interpretation of data on phytoplankton and microzooplankton, several supporting parameters are required at each sampling location: total chlorophyll*,* inorganic nutrients (dissolved inorganic nitrogen [DIN], dissolved inorganic phosphorus [DIP], and Si), light penetration (Secchi depth, photosynthetically active radiation [PAR]), and CTD profiles that include depth, oxygen, temperature, and salinity.

Other relevant parameters include specific photosynthetic pigments (high-performance liquid chromatography [HPLC]-analysis), coloured dissolved organic matter (CDOM, used to correct ocean colour data), organic carbon, total and particulate phosphorous and nitrogen, and zooplankton. Parameters relevant for ocean acidification are: pH, pCO_2, total alkalinity, and DIC (dissolved inorganic carbon).

4 Preservation and storage of samples

4.1 Preservation for analysis of nano- and microplankton

Sample fixation is described in Sections 5.4 (including Annex D) and 6.5 of the European standard EN-15972 (2011). If (calcareous) coccolithophorid abundance is to be examined, then a separate sample from that used for other phytoplankton will be needed due to differing (non-acidic) preservation methods. Recommended concentrations of preservatives for microzooplankton are not specified in the European standard EN-15972; these should be similar to those used for phytoplankton blooms (see Section 6.5 of the European standard EN-15972 [2011]).

4.2 Storage

Storage is described in Section 6.8 in the European standard EN-15972 (2011). Ideally, analysis should be carried out as soon as possible after collection as some species, e.g. *Pseudo-nitzschia* can deform rapidly.

5 Analytical procedures

5.1 The Utermöhl method

Species identification and sample processing is described in Section 7 and Annex F in the European standard EN-15972 (2011), although it is not recommended to use biovolumes to calculate chlorophyll content. These OSPAR guidelines recommend the use of the Utermöhl method described in the European standard EN-15204 (2006). It should be noted that

all organisms observed in a sample should be identified to the lowest taxonomic level possible. This includes also heterotrophic unicellular eukaryotic organisms, e.g. some microzooplankton. For recording rare and/or non-indigenous/cryptogenic species, a full search of the counting chamber is required (in contrast to the sub-sampling suggested in the European standard EN-15972 [2011]). If further concentration or sedimentation is required, follow the procedures detailed in Section B4 of the European standard EN-15204 (2006).

5.2 Biovolume and carbon content

Phytoplankton differ in size from ~0.8 µm to >500 µm. To correct for differences in size of phytoplankton it is preferable to estimate the cell volume and estimate the wet weight and/or carbon content of the organisms. Standard methods for estimating biovolume are under development for the European standard EN-16695 (in prep.). At present laboratories in the OSPAR area are using Hillebrand *et al*. (1999) or the HELCOM system (www.helcom.fi/helcom-at-work/projects/phytoplankton) to estimate biovolumes. Carbon content is a metric that is very useful in the foodweb context, but there are at present no standards for calculating this. Calculations of carbon content based on cell volume shall where possible follow equations in Menden-Deuer and Lessard (2000). There is no current standard for calculating carbon content in microzooplankton, so it is important that the methods used to calculate this are detailed when reporting.

5.3 Trophic type

Microzooplankton include organisms that are mixotrophic, i.e. they feed on other organisms as well as using photosynthesis. To interpret data from phytoplankton analysis it is important to specify their trophic type. Four types have been designated: (1) autotrophic, (2) heterotrophic, (3) mixotrophic, and (4) not known/specified.

The data collected in the entire OSPAR area should be cohesive and comparable to be applicable for assessments between countries. A species list of trophic types for the OSPAR area would need to be developed to ensure this occurs. Such a list would contain many unknowns; it is therefore expected that the list would improve over time as more observations are made.

6 Quality assurance

6.1 Accredited laboratories

Laboratories carrying out analyses of phytoplankton should establish a quality management system according to the international standard EN ISO/IEC 17025 (2005). An accreditation by a recognized accreditation authority is recommended. The quality assurance programme should ensure that the data are fit for the purpose for which they have been collected, i.e. that detection limits are adequate and accuracy is compatible with the objectives of the monitoring programme. The quality assurance procedures must cover all steps of the determinations, including sampling, storage of samples, analytical procedures, maintenance and handling of the equipment, training of the personnel, as well as an audit trail. The laboratory should take part in intercalibration exercises between countries and proficiency testing to provide external verification of laboratory performance.

Participation in quality assurance/quality control (QA/QC) schemes such as the annual BEQUALM phytoplankton ring test, run under the auspices of the National Marine Biological Analytical Quality Control (NMBAQC) scheme, is a useful and widely used way to help ensure data quality. Organizations can also acquire certification through national, European, or international accreditation schemes, e.g. Good Laboratory Practice (GLP) and the United Kingdom Accreditation Service (UKAS).

Uncertainty in results should be estimated by analysing replicate samples on a regular basis in order to understand the statistical power of the programme to detect change. Inter-laboratory reproducibility should be evaluated regularly as described in Section 8.4 of the European standard EN 15204 (2006).

6.2 Standardized lists

New plankton organisms are continuously being described, and changes in the naming and categorization of organisms is common. It is essential to keep standardized lists, including standard size categories for unidentified organisms. The lists should be updated in a systematic way that includes coordination with accepted international standard lists. These lists are:

- Taxonomic nomenclature: The naming of species (and any updates) should follow the World Register of Marine Species (WoRMS) http://www.marinespecies.org/. For algae WoRMS is based on AlgaeBase http://www.algaebase.org/. Laboratories may wish to consider the inclusion of standard agreed images of taxa in WoRMS and use these to validate taxonomic lists in order to facilitate intercomparisons.
- Lists of cell shapes and equations for calculating cell volumes will follow recommendations by EN-16695 (in prep.), Hillebrand *et al*. (1999) or the HELCOM system should be used.
- The IOC–UNESCO Taxonomic Reference List of Harmful (toxic/nuisance) Micro Algae http://www.marinespecies.org/hab/ is used to designate species as harmful**.** It should be noted that many taxa can only be identified to the genus level if light microscopy is used; it is therefore not always possible to distinguish between toxic and non-toxic species and strains.
- A verified database of non-indigenous/cryptogenic species can be found on the AquaNIS website http://www.corpi.ku.lt/databases/index.php/aquanis/.
- Standard lists are still required for trophic type/functional groups and non-indigenous/cryptogenic species.

Any standard lists may contain mistakes and source literature may need to be consulted.

7 Reporting requirements

7.1 Reporting data on the biodiversity and the distribution of organisms

Each Contracting Party to OSPAR should report data annually to ICES using a standard format (ERF 3.2) that includes metadata. Data will be freely available and accessible following the requirements of the EU INSPIRE directive. The reporting procedures should include a national report containing information on methods used and any other comments or information relevant to an ultimate assessment of the data. The following ICES links can be used:

- Data submissions and inquiries: accessions@ices.dk
- Download reporting format ERF3.2: http://www.ices.dk/marine-data/data-portals/Pages/DOME.aspx
- Pre-submission data checks: http://dome.ices.dk/datsu/
- Help on format requirements and checks: http://dome.ices.dk/datsu/selRep.aspx?Dataset=73
- Overview and status of existing submissions: http://www.ices.dk/marine-data/tools/Pages/Submission%20status.aspx

7.2 Reporting of non-indigenous/cryptogenic species

Non-indigenous species are part of Descriptor 2 of the MSFD. The EU regulation on invasive alien species (EU/1143/2014; EU, 2014) requires recording, monitoring, and assessment of invasive alien species. Observations of non-indigenous/cryptogenic species are reported annually.

7.3 Reporting of harmful algal blooms

Harmful algal blooms are part of Descriptor 5 of the MSFD. Observations of harmful algal bloom species should be reported annually as part of the reporting of quantitative plankton data. Harmful algal events should be reported to the Harmful Algae Events Database http://haedat.iode.org/.

8 Additional, complementary monitoring techniques

8.1 Autotrophic picoplankton

8.1.1 Introduction

Autotrophic picoplankton, e.g. *Prochlorococcus*, *Synechococcus*, and small eukaryotic organisms are the dominant primary producers under oligotrophic conditions in many seas. *Synechococcus* is probably the most abundant phytoplankton in European coastal waters in summer. Until the late 1970s these organisms were unknown, but scientific results published since then have shown their important role in the marine foodweb. They constitute part of the microbial foodweb and can form a large part of the plankton biomass.

8.1.2 Sampling and analysis

Sampling is identical to the sampling for nano- and microplankton, but the preservation method is different. Pre-filtering using a 45 µm nylon mesh can be carried out for samples that will be analysed using fluorescence microscopy or flow cytometry. For fluorescent microscopy (e.g. MacIsaac and Stockner, 1993), samples should be preserved using glutaraldehyde (HPLC-grade) or paraformaldehyde and should be analysed as soon as possible (within a week) to avoid degradation of fluorescent pigments. Final concentration should be 0.5% (or 0.2% for paraformaldehyde). Both of these chemicals need to be handled according to their safety sheets. It is recommended that samples should be stored in the dark at 4°C. For flow cytometry (e.g. Campbell, 2001), analysis should ideally be carried out immediately; if this is not possible, samples should be preserved, snap-frozen in liquid nitrogen, and stored at –80°C.

8.2 Qualitative sampling

Sampling using nets is not quantitative; however, important information on the presence of robust and/or rare taxa may be obtained. To aid the identification of species observed in the quantitative samples, net samples are useful to obtain more individuals for observation. As a supplement to the quantitative sampling it is useful to carry out a vertical net tow using a 10 or 20 µm plankton net. Sampling equipment for qualitative sampling is described in Section 5.2 of the European standard EN-15972 (2011).

8.3 Methods for coccolithophorid enumeration and identification

Coccolithophorids are phytoplankton with calcium carbonate scales (coccoliths). They are identified as being one of the groups that is potentially most susceptible to ocean acidification, but this is controversial. As they can form extensive blooms covering very large areas, these could be recorded in a systematic way. Data from satellite remote sensing and automated measurements from ships of opportunity or buoys (see below) may be included.

When using the Utermöhl method it is often difficult to enumerate and identify coccolithophorids. There are at least three alternative methods available: (1) electron microscopy, (2) polarized light microscopy, and (3) molecular methods. Method (1) requires a costly instrument and personnel specialized in electron microscopy methods. The use of methods (2) and (3) are recommended for routine work. The polarized microscopy method (Frada *et al*., 2010, and references therein) is relatively low cost and has been used extensively in investigations of coccolithophorids in micro-paleontological research, but also in studies of present day coccolithophorids. Method (3) is described briefly below. It is recommended that at least one of the methods 1, 2, and 3 is used in the monitoring of coccolithophorids.

8.4 Imaging flow cytometry

Imaging flow cytometry shows promise as a technique useful for automated enumeration and identification of plankton organisms. This technique also allows cell volumes of individual organisms to be estimated. The algorithms for automated identification of plankton need to be carefully designed and assessed by a trained phytoplankton specialist (González *et al.*, 2013; Álvarez *et al.*, 2012, 2014). An advantage to this technique is that less manpower would be needed for analyses of samples once a system is calibrated for local phytoplankton. There are currently (Dec. 2014) at least three different imaging flow cytometers commercially available. It may be possible to deploy imaging flow cytometers *in situ* for autonomous phytoplankton sampling, enumeration, and identification. It should be noted that each cytometer samples a relatively small volume.

Before the results of imaging flow cytometry can be used alongside those of microscope–based methods, a comprehensive comparison of the two techniques is required. In addition, intercomparisons between different types of imaging flow cytometers would be needed.

8.5 Molecular methods

Molecular methods for identifying plankton organisms such as sequencing of part of genomes (e.g. rDNA or rRNA), sometimes called barcoding, and Real Time PCR, have evolved significantly the last decades. An advantage to these methods is that they produce more objective results, from an analytical point of view, compared to methods where identification of an organism is dependent on the skill of a person. The molecular methods are now established in the research community but not yet in the marine monitoring community. The cost of sequencing a large number of samples is not high; however, the analyses of the resulting data is time consuming. The results from most molecular methods are in general not directly comparable to results from cell counts using a microscope as there are issues with quantification, but these methods yield other information on biodiversity, especially for organisms < 5 µm, the organisms with the highest cell numbers in plankton samples.

Before the results of molecular methods can be used alongside those of microscope-based methods, a comprehensive comparison of the two techniques is required. Molecular techniques may generate large quantities of data; the handling and analysis of such data needs to be considered when a decision to use these techniques is taken.

8.6 Sampling platforms

Research vessels constitute the main sampling platforms. In addition, to increase temporal and spatial resolution sampling may also be carried out from e.g. ships of opportunity (FerryBox systems) and other platforms (buoys, piles, autonomous underwater vehicles, etc.). This increased temporal and spatial resolution may generate large quantities of data; the handling and analysis of such data needs to be considered when a decision to use these systems is taken.

8.6.1 FerryBox systems

Research vessels, ferries, and cargo vessels may be fitted with automated water sampling devices and instruments for automated measurements of bio-optical properties of seawater or the organisms in the water. This facilitates frequent sampling of near-surface waters. It is recommended that phytoplankton sampling occurs prior to measuring the bio-optical properties in the water sampling systems. It may also be possible to deploy imaging flow cytometers as part of FerryBox systems for autonomous phytoplankton sampling, enumeration, and identification.

8.6.2 Oceanographic buoys and other platforms

Oceanographic buoys, other fixed platforms such as piles and bridges, and autonomous underwater vehicles such as gliders may be fitted with automated water sampling devices and/or instruments for automated measurements of bio-optical properties. This facilitates sampling at several depths. It is recommended that the water sampling systems are used for phytoplankton sampling and that the bio-optical data is used to supplement other data.

8.6.3 Continuous Plankton Recorder

The Continuous Plankton Recorder (CPR) is a device that is towed behind ships. Plankton organisms are collected on a silk mesh (270 µm) which is later analysed using microscopy. The method is selective for relatively large and robust organisms. After sampling, the colour (greenness) of the silk is used as an index of phytoplankton biomass. An advantage to this method is that CPR sampling covers large sea areas. Long time-series of semi-quantitative data exist. It is recommended that the semi-quantitative data from the CPR surveys are used to complement the quantitative plankton data.

8.7 Satellite remote sensing

During cloud-free conditions, satellite remote sensing can provide data on ocean colour. This data can be used to estimate near-surface chlorophyll *a* concentrations, a proxy for phytoplankton biomass. Converting satellite colour observation to chlorophyll *a* concentrations in the OSPAR area requires reliable standard methods [*note to editors: this needs further editing if such work has occurred already*]. The method used to carry out this conversion needs to be recorded along with other metadata.

Information on the distribution and frequency of blooms of coccolithophorids can also be obtained, using robust automated techniques applied to a long time-series of ocean colour data (Shutler *et al.*, 2010). The data can be used together with the information from water sampling and *in situ* optical measurements to describe the frequency of algal blooms and the horizontal distribution of blooms.

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