OSPAR COMMISSION

Protecting and conserving the North-East Atlantic and its resources

CEMP Guidelines: Phytoplankton monitoring[1]

(OSPAR Agreement 2016-06)

About this document

This document describes phytoplankton species composition monitoring guidelines for the OSPAR area. "Phytoplankton" is here used throughout as a simple umbrella term to encompass prokaryotes as well as eukaryotes, but is limited to protists, whatever their size and trophic mode (i.e. auto-mixo or heterotroph). Micrometazoa <200 um and also larger metazoans are thus excluded from this definition. The scope of the guidelines includes eutrophication, biodiversity, invasive species, harmful algal blooms and climate change.

This document replaces the document 'JAMP Eutrophication Monitoring Guidelines: Phytoplankton Species Composition', adopted 1997 in Brussels. (Ref. No.: Agreement 1997-05).

About the draft version from 14 January 2016

This version include changes suggested by Abigail McQuatters-Gollop (OSPAR-COBAM Pelagic Habitats), Veronique Creach, Paul Tett, Eileen Bresnan and Mike Best, all from the United Kingdom. Also suggestions by Felipe Artigas, France are included. The changes were incorporated in the document by Bengt Karlson, Sweden. Some of the suggested changes were only used in part and/or rephrased.

About the draft version from 11 December 2015

The draft was produced by Bengt Karlson and Marie Johansen from Sweden together with Hans Ruiter from the Netherlands. This version of the document incorporates many changes proposed by ICES in a review published on 16 June 2015. Also some changes proposed by Germany and the United Kingdom have been included. In a separate document *Response to advice and comments on draft JAMP phytoplankton monitoring guidelines.docx* the motivation for some of the choices made are described.

About the draft version from 25 February 2015

The draft was produced by Bengt Karlson and Marie Johansen from Sweden together with Hans Ruiter from the Netherlands. The work was partly done during a video conference and partly through correspondence. Comments on the version from 7 January by OSPAR-EUT, which discussed the document during a meeting in London 26-28 January, 2015, have largely been taken into account. Annika Grage submitted comments by Germany and Suzanne Painting submitted comments by the United Kingdom. Additional comments from Philip Axe, Swedish Agency for Marine and Water Management and Abigail McQuatters-Gollop, Sir Alistair Hardy Foundation for the Ocean Science, have also been received. The authors of the guidelines would like to thank everyone for their comments and suggestions. Many, but not all, have been accepted.

[1] This Agreement replaces Agreement 1997-05.
CEMP Guidelines were previously referred to as JAMP Guidelines. Many of the existing JAMP Guidelines are due for review; until this review is complete they continue to be referred to as JAMP Guidelines.

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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) Changes in plankton lifeform pairs
- D1 PelHab 2 Plankton biomass and/or abundance
- D1 PelHab 3 Plankton diversity index

Phytoplankton monitoring guidelines are relevant for several other indicators in development. The CEMP guideline aims 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 these data, assuming that the species can be classified to functional groups, e.g. 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. It aims at characterising maritime areas with regard to their eutrophication status. Depending on the status of the areas, monitoring of phytoplankton is relevant. Two types of area-specific phytoplankton indicator species can be distinguished: nuisance species, forming dense "blooms", and toxic species. Nuisance species and potentially toxic species (e.g. some dinoflagellates) are direct effect assessment parameters. The nuisance species show increased "bloom" levels (cell concentrations) and increased duration of "blooms" compared with previous years. It should be noted that there is scientific uncertainty in the use of toxic phytoplankton species as indicators of direct eutrophication effects. Shifts in species composition from diatoms to flagellates (some of which are toxic) could indicate eutrophication.

Long-term monitoring is essential for tracking change in marine ecosystems. In order to design a suitable sampling protocol, a number of decisions about resolution in time and space are required. The use of frequent sampling and consistent and quality- assured methods and analytical procedures ensures the production of comparable data. If new methods are proposed (for instance to save costs, or to improve precision), it is important to understand fully how the results from them relate to those from the existing methods. New parameters may be added as methods and knowledge improve and as they fit with long term monitoring aspects. In this version of the guidelines, options to include autotrophic picoplankton, and novel ways of estimating biomass of phytoplankton and monitoring of some rare phytoplankton species have been added. All zooplankton are outside the scope of this guideline.

Definitions

These guidelines concerns phytoplankton, i.e. cyanobacteria, unicellular eukaryotic algae, ciliates and related groups. The unicellular eukarotic organisms are often called protists. "Phytoplankton" is here used throughout as a simple umbrella term to encompass prokaryotes as well as eukaryotes, but is limited to protists, whatever their size and trophic mode). Micrometazoa <200 um metazoans are thus excluded from this definition. Phototrophic, mixotropohic and heterotrophic organisms are included. The terms pico-, nano-, micro-, and mesoplankton 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, microplankton (20–200 µm) also include phytoplankton, but can also include some large phytoplankton.

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 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.

Sampling

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 phytoplankton communities and within the sampling variability. The European standard EN-15972 states that 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.

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, biomass and biodiversity of the plankton community.

Sampling equipment for quantitative sampling

Sampling equipment is described in Section 5.1 of the European standard EN-15972. 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 phytoplankton. Tube sampling must be carried out with care to avoid damaging ciliates.

Sampling depth

- The minimum requirements include sampling near surface waters, i.e. either at 1 m below 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, by using a sampling tube at 0–10 m or another type of depth-integrating sampling device. It is necessary to use the same volumes of water from each depth when pooling. This sampling strategy will miss any sub-surface phytoplankton maxima deeper than 10 m. In addition to the required near surface sampling it is recommended to carry out sampling at other depths according to local conditions. Depth profiles of in situ chlorophyll fluorescence can be used for guidance when sampling thin layers of phytoplankton. This should be considered especially when sampling stratified waters in both coastal and open-water areas. Surface water samples and integrated samples are not directly comparable

Supporting parameters

For the best interpretation of data on phytoplankton, several supporting parameters are recommended to be included at each sampling event:

- chlorophyll a
- meso-zooplankton diversity, abundance and biomass
- inorganic nutrients (dissolved inorganic nitrogen [DIN], dissolved inorganic phosphorus [DIP], and Si)
- light penetration (Secchi depth, photosynthetically active radiation [PAR])
- CTD profiles that include depth, chlorophyll fluorescence, turbidity, oxygen, temperature, and salinity.

Other relevant parameters to include:

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

Preservation and storage of samples

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. 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.

Storage

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

Analytical procedures

Abundance and identification (the Utermöhl method)

These OSPAR guidelines recommend the use of the Utermöhl method (using an inverted microscope) described in the European standard EN-15204 for the determination of the abundance and species identification (see section 7, Annex D and and Annex F). 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. Using the Utermöhl method it is often difficult to quantify the pico- and nanoplankton. To include all phytoplankton in the analysis it is recommended to use the methods described in sections 8.1-8.4 in addition to the Utermöhl method. It is foreseen that molecular methods described in section 8.5 will complement the other methods in the near future.

Using the Utermöhl method a relatively small volume of water, usually 10-50 mL, is analysed. This means that the detection limit is about 20 cells L^{-1} if the whole bottom of a 50 mL chamber is analysed. In practice the detection limit is higher due to stochastic reasons. Many large phytoplankton occur in abundances near this detection limit, this is also true for many harmful species. This means that they will often not be part of the results (see also Rodriguez-Ramos et al. 2014). To include also the rare organisms it is recommended to also analyse a larger volume, e.g. 1 L, concentrated by sedimentation.

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, wet weight and/or carbon content of the organisms. Standard methods for estimating biovolume are described in the European standard EN-16695. In the HELCOM area a standard procedure to estimate biovolumes is well established (HELCOM-COMBINE 2015 and Olenina et al. 2006). The HELCOM phytoplankton biovolume list is updated yearly and available at www.ices.dk. Carbon content is a metric that is very useful in the foodweb context. It is part of Annex C in the European standard EN 16695. The calculations of carbon content are based on cell volume. It is recommended to follow the equations in Menden-Deuer and Lessard (2000).

Trophic type

Phytoplankton 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. Epifluorescence microscopy is a useful tool for determining if organisms contain chloroplasts.

The data collected in the entire OSPAR area should be cohesive and comparable to be applicable for comparable assessments between countries. A species list of trophic types for the OSPAR area would need to be developed to ensure that 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. A joint OSPAR-HELCOM list may be based on the HELCOM phytoplankton biovolume list that includes information on trophic type.

Quality assurance

Accredited laboratories

Laboratories carrying out analyses of phytoplankton have to establish a quality management system according to the international standard EN ISO/IEC 17025. An accreditation by a recognised

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 comparisons and proficiency testing to provide external verification of laboratory performance.

Regular participation in quality assurance/quality control (QA/QC) schemes is very important to ensure data quality. One good example is the BEQUALM phytoplankton ring test, run under the auspices of the National Marine Biological Analytical Quality Control (NMBAQC) scheme. Organisations can also acquire certification through national, European, or international accreditation schemes.

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.

Standardised lists

New plankton organisms are continuously being described, and changes in the naming and categorisation of organisms is common. Changes should be based on internationally accepted rules which have been established in nomenclatural codes (e.g. ICN International Coode of Nomenclature for algae, fungi and plants). It is essential to keep standardised lists, which are updated in a systematic way. Therefore for consistent reporting purposes the following lists should be used:

- The naming of species should follow the World Register of Marine Species (WoRMS) www.marinespecies.org. For algae WoRMS is based on AlgaeBase www.algaebase.org.
- Lists of cell shapes and equations for calculating cell volumes should follow recommendations by EN-16695 or the HELCOM system (Olenina et al. 2006) with yearly updates at www.ices.dk.
- The IOC–UNESCO Taxonomic Reference List of Harmful Micro Algae http://www.marinespecies.org/hab/ is used to designate species as harmful (toxic/nuisance). It should be noted that many taxa can only be identified to the genus level if light microscopy is used; it is 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. The HELCOM phytoplankton biovolume list provides information on trophic types for species included in the list.

Reporting requirements

Reporting data on the abundance, biomass, biodiversity and distribution

Each Contracting Party to OSPAR should report data annually as required and specified by OSPAR. Data will be freely available and accessible following the requirements of the EU INSPIRE directive. Data on indicator species should be included in the reporting, e.g. the common indicator *Phaeocystis*.

Reporting of invasive/non-indigenous/cryptogenic species

Invasive and non-indigenous species are part of Descriptor 2 of the MSFD. The EU regulation on indigenous-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.

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/.

Additional possible monitoring techniques

Autotrophic picoplankton

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 food web together with nanoplankton. They constitute part of the microbial foodweb and can form a large part of the plankton biomass.

Sampling and analysis

Sampling is identical to the sampling for nano- and microplankton, but the preservation method is different. 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, or fixed in 0.5% to 1% glutardialdehyde (HPLC-grade), and stored at –80°C. In this case, the analysis should take place in the next 3 months.

Qualitative sampling

As a supplement to the quantitative sampling of phytoplankton it is useful to carry out a vertical net tow using a 10 or 20 µm plankton net. Sampling using nets with this mesh size 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. Observations carried out on living samples are recommended. Sampling equipment for qualitative sampling is described in Section 5.2 of the European standard EN-15972.

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 difficult to enumerate and identify coccolithophorids. There are at least three alternative methods available: (1) electron microscopy, (2) polarised light microscopy (Frada et al., 2010, and references therein), and (3) molecular methods. Beside the Utermöhl method it is recommended that at least one of the methods 1, 2, and 3 is used in the monitoring of

coccolithophorids. Another alternative for enumerating coccolithophorids and also other nanoplankton is flow cytometry.

Automated flow cytometry

Automated flow cytometry is a technique useful for automated enumeration and identification of plankton organisms. There are three main types of flow cytometry useful for plankton studies. Non-imaging flow cytometry is based on measuring fluorescent and scattering properties of the organisms and is mainly suitable for pico-and nanoplankton. Imaging flow cytometry uses automated image analysis to identify organisms based on their size and morphology in addition to parameters measured in non-imaging flow cytometry. Pulse shape recording flow cytometry use the basic parameters in non-imaging flow cytometry and also records the pulse shape which is related to morphology. Flow cytometry may be considered the state of the art for quantitative enumeration of picoplankton and in general automated flow cytometry is more accurate than the Utermöhl-mehod for nanoplankton. For microplankton it is also very useful. 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. It may be possible to deploy imaging and/or pulse shape-recording flow cytometers *in situ* for autonomous phytoplankton sampling, enumeration, and identification to some extent. Before the results of automated flow cytometry can be used alongside those of microscope–based methods, a comprehensive comparison of the two techniques is required. In addition, inter-comparisons between different types of automated flow cytometers would be needed.

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 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. For molecular analysis samples there is evidence that Lugol's, formaldehyde and gluteraldehyde are not suitable fixatives. Commonly, samples should be preserved in 95% molecular-grade ethanol after removing seawater. Failing that, samples should be frozen as soon as possible below minus 20 degrees Celsius.

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 of cell abundance and biomass, 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.

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.).

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.

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, automated flow cytometers, 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. Automated molecular dvices could also be deployed on buoys and fixed platforms.

Continuous Plankton Recorder

The Continuous Plankton Recorder (CPR) is a device that is towed behind ships to sample the upper layer mixed by the propellor. 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. For phytoplankton it may be semi-quantitative while for mesozooplankton it may be quantitative. 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 CPR- data exist. It is recommended that the data from the CPR surveys are used to complement other plankton data.

Satellite remote sensing

During cloud-free conditions, satellite remote sensing can provide data on ocean colour. These 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 analytical methods. Remote sensing algorithms are being developed further to quantify the relative contribution of broad sizes or taxonomic classes of phytoplankton. In particular, information on the distribution and frequency of blooms of coccolithophorids can also be obtained (Shutler et al., 2010). The data must be used together with the results from water sampling as it is necessary to interpret and validate the satellite data. Information on in situ conditions such as the turbidity of the water, the content of coloured dissolved organic matter, the composition of the plankton community etc. will influence the ocean colour.

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