Guidelines for monitoring of chlorophyll *a*

1. Background

1.1 Introduction

Increase in phytoplankton biomass is a direct consequence of advancing eutrophication. For monitoring purposes, phytoplankton biomass is estimated by chlorophyll a (Chl *a*) concentration.

The amount of Chl *a* is not a direct proxy for phytoplankton biomass because of a highly variable ratio of cellular carbon to Chl *a* in phytoplankton (Geider 1987). Phytoplankton biomass, except for picoplankton, is more accurately assessed by quantitative taxonomical analysis. It is, however, laborious and thus provides with a smaller amount of data than the Chl *a* method, which lowers the status confidence of any taxonomy-based indicator. Regardless of its shortcomings, the Chl *a* method – being easy to sample and fast to analyze – is the method of choice for environmental studies.

The scope of this guideline is the determination of Chl *a* concentration; measured from water samples using wet analytics as well as estimated from *in vivo* Chl *a* fluorescence recordings.

1.2 Purpose and aims

Monitoring of Chl *a* provides information that is used for assessing direct effects of eutrophication. The aim is to provide spatiotemporal information for detection of short-term status and long-term trends and to ensure that the data is compatible for the HELCOM core indicator 'Chlorophyll a'. The indicator description, including its monitoring requirements, is given in the HELCOM core indicator web site: [http://helcom.fi/baltic-sea-trends/indicators/chlorophyll-a].

2. Monitoring methods

2.1 Monitoring features

Chl *a* is an optically active pigment, and hence, its concentration within a sample can be determined optically by spectrophotometry (light absorption), fluorometry (fluorescence emission), and high-performance liquid chromatography (HPLC).

2.2 Time and area

2.2.1 Station-based determination of Chl-a

Station-based determination of Chl *a* using wet analytics should be carried out during the summer months (June–September), keeping in mind that the vernal peak in the northern parts of the Baltic Sea may be prolonged to June. Chl *a* concentration varies substantially both in space and time. For this reason, sampling is advised to cover the entire growth season. This leads to the possibility of assessing mean values for the spring / autumn season, and for the entire growth season.

Chl *a* monitoring is carried out by all HELCOM Contracting Parties, and the monitored area covers the entire Baltic Sea area, both the open sea and coastal areas.

2.2.2. SOOP

Estonia, Finland and Sweden probe Chl *a* also in the Ship-of-opportunity (SOOP) approach. Chl *a* is estimated along the routine operating merchant routes using both wet analytics and *in vivo* Chl *a* fluorescence emission recorded by Ferryboxes.

2.3 Monitoring procedure

2.3.1 Monitoring strategy

The *in vivo* fluorescence yield, recorded at red Chl *a* fluorescence band, is considerably lower for cyanobacteria than for eykaryotic groups, and hence, the traditional *in vivo* Chl *a* fluorescence method (Lorenzen et al. 1966) does not reliably detect cyanobacterial biomasses (Seppälä et al. 2007).

To reach sufficiently high confidence criteria of the core indicator, the joint monitoring should produce annually at least 15 measurements in June–September for each assessment unit, i.e., for open sea sub-basins. The measurements should be as evenly spatially distributed as possible.

2.3.2 Sampling method(s) and equipment

2.3.2.1 Ship based monitoring

Sampling method

Chl *a* samples are collected in the ship-based monitoring as follows (HELCOM 2015a):

- For the open sea, the sampling depths follow the HELCOM protocol down to 20 m depth. Additional sample(s) should be obtained from Chl *a* maxima present at other depths
- In coastal monitoring, the sample at 1 m or an integrated sample (1 to 10 m) can be collected
- Additionally, Chl *a* should be analyzed from the sample used for phytoplankton taxonomy or primary production analyses

Sampling equipment

- Discrete sampling bottles
- CTD-rosette bottles

2.3.2.2. SOOP

Ferrybox samples are collected underway at 3–5 m depth, and stored by an autonomous sampler / refridgerator.

2.3.3 Sample handling

2.3.3.1 Ship based monitoring

The sample water for Chl *a* measurement should be filtered immediately after sampling through GF/F filters, and extracted with 10 cm^3 of ≥ 96 % ethanol without any drying period (Seppälä et al., unpubl.).

Filters should not be stored dry, but extracted as soon as possible (Wasmund et al. 2006); only a short time storage (up to 1 month) for filters at –80°C is allowed. Extracts can be stored in an ultracold freezer (–80°C), or otherwise at –20°C, up to three months until the measurement (Wasmund et al. 2006).

2.3.3.2. SOOP

The samples cannot be filtered immediately after sampling in the SOOP approach, so the sample bottles should be shaken well prior to filtration.

2.3.4. Sample analysis

2.3.4.1. Spectrophotometric method

The Chl *a* concentration is measured from individual extracts using light absorption at 663–665 nm with 2 nm band-width (absorption at 750 nm subtracted). Centrifugation for 10–20 minutes at about 10,000 m/s^2 is necessary in order to reduce the blank reading to < 0.005 for a 1-cm cuvette. The method is based on ISO 10260, and Arvola (1981), except that here the Chl *a* determination is not corrected by phaeopigment content operated by an acidification step.

Calculation:

$$Chl\ a\ (mg/m^3) = 1000 \times e \times A/(83 \times V \times I)$$

where e = volume of ethanol (cm^3), A = absorbance at 665 nm minus that at 750 nm after correction by the cell-to-cell blank, 83 = absorption coefficient in 96% ethanol, V = water volume filtered (dm^3), and I = length of cuvette (cm).

The sample volume, ethanol volume and the length of cuvette must be chosen so as to deliver absorbance at a range of 0.1–0.8 at 663–665 nm, i.e., within the optimum range of the instrument.

2.3.4.2. Fluorometric method

The Chl *a* concentration is measured from individual extracts against the instrument calibration curve based on known concentrations of pure Chl *a* as determined with spectrophotometry. The wavelength combination for the determination is 425–430 nm / 663–672 nm (excitation / fluorescence emission, depends on the instrument). The method is based on Holm-Hansen et al. (1965), Lorenzen (1966), and Jespersen and Christoffersen (1987).

Calculation:

$$Chl\ a\ (mg/m^3) = R \times f \times s \times e/V$$

where R = fluorescence reading, f = calibration factor, s = slit correction, e = ethanol volume (cm^3), and V = sample volume (dm^3).

For the measurement of phaeopigments, an additional fluorescence reading is recorded after the acidification of the sample. Phaeopigments are determined as:

$$Phaeopigment\ (mg/m^3) = f_a \times \left((r \times R_a) - R\right) \times s \times e/V$$

where R_a = fluorescence reading after acidification, r = ratio of R to R_a obtained from an extract free from phaeopigments, and f_a = calibration factor.

2.3.4.3. HPLC

HPLC techniques allow simultaneous determination of the concentrations of a wide range of carotenoids, chlorophylls and their degradation products. For pigment separation, most of HPLC methods employ reversed-phase conditions and columns packed with stationary phases having an aliphatic chain length of C_8, C_{18} or C_{30} (Bidigare et al. 2005, Carrido et al. 2011). Separated pigments peaks are identified by a comparison of retention times with those of standards. Quantification of pigments is typically based on their absorption or fluorescence signals.

2.4 Data analysis: SOOP

For high-frequency Chl *a* mapping with Ferryboxes, flow-through based *in vivo* Chl *a* fluorescence data is converted to Chl *a* concentration by validating a fluorescence emission – recorded at the time of sampling – with a parallel wet Chl *a* measurement from the water sample. A statistical relation is derived from the collected transect data and the resulting conversion factor is extrapolated with certain reliability to flow-through data between the sampling points. This conversion factor varies regionally and seasonally due to variations in the phytoplankton physiology and community structure, and has to be treated as transient; the factor should be re-estimated every time Chl *a* data is being collected.

This basic modelling provides adequate results during the times of season when algal biomasses are high or eukaryotic groups dominate. The relation weakens when the algal biomasses are low or cyanobacteria comprise a significant part of the phytoplankton community. In that case, auxiliary parameters, such as phycocyanin fluorescence (indicating the presence of cyanobacteria) and turbidity correct the relation substantially (Seppälä et al. 2007).

Ferrybox system probes the phytoplankton inhabiting the upper layers of the water column. There, phytoplankton exhibit non-photochemical fluorescence quenching under excessive light levels, which lowers *in vivo* Chl *a* fluorescence emission relative to Chl *a* concentration. This diel variation in *in vivo* Chl *a*

fluorescence needs to be taken into account when using samples collected in the daytime to convert fluorescence data recorded in the nighttime, and vice versa (Babin 2008). Therefore, the calibration factors should be determined for day and night separately.

3. Data reporting and storage

The wet analytics data is included in the station data along with depth-dependent variables, stored by the contracting parties, and reported annually to the COMBINE database hosted by ICES.

4. Quality control

4.1 Quality control of methods

Laboratories carrying out Chl *a* analyses should have established a quality management system according to EN ISO/IEC 17025 standard.

Replicate samples, producing data for X-bar charts, are included in the analysis in order to clarify the magnitude of random error for Chl *a* results introduced by handling of the samples.

In order to clarify the magnitude of systematic error in the analytical chain, laboratories should participate in ring-tests and inter-calibrations.

Contracting parties should follow the HELCOM monitoring guideline but minor deviations from this are acceptable if the method achieves comparable results. Validation of the adopted method needs to be performed on the relevant matrix and concentration range e.g. by taking part regularly at inter-comparison studies or proficiency testing schemes.

4.2 Quality control of data and reporting

Measurement uncertainty should be estimated using ISO 11352 standard. Estimation should be based on within-laboratory reproducibility, data from proficiency testings, and internal / commercial reference material.

Data must be flagged if normal QA routines or recommended storage conditions cannot be followed.

See HELCOM (2015b).

5. Contacts and references

5.1 Contact persons

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5.2 References

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* For undated references, the latest edition of the referenced document (including any amendments) applies