Baltic Marine Environment Protection Commission

Working Group on the State of the Environment and Nature Conservation

Tallinn, Estonia, 7-11 November, 2016

STATE & CONSERVATION 5-2016

Document title	Monitoring of Chlorophyll-a – draft monitoring guidelines
Code	2MA-21
Category	CMNT
Agenda Item	2MA – Revision of HELCOM Monitoring
Submission date	17.10.2016
Submitted by	Finland

Background

This document contains the draft monitoring guidelines for Chlorophyll-a submitted by Lead Country Finland.

The draft has been reviewed by representatives from Finland, Germany and Sweden.

Action requested

The Meeting is invited to:

- comment and amend the draft guidelines as needed.

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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, however, 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 a taxonomy-based indicator.

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 measurements or earth observation (EO).

1.2 Purpose and aims

The purpose for Chl *a* monitoring is to describe spatiotemporal trends in phytoplankton biomass that is a sensitive indicator of anthropogenic pressures laid on the ecosystem. Here, Chl *a* is used as an index of eutrophication. Chl *a* is thus an element of eutrophication monitoring, and its monitoring collects input data for the HELCOM core indicator “Chlorophyll-a”.

2. Monitoring methods

2.1 Monitoring features

2.2 Time and area

Station-based determination of Chl *a* using wet analytics is a mandatory element of COMBINE monitoring 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.

EE, FI, and SE 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.

#this part lacks information from the contracting parties# FI employs Earth Observation (EO) data for the Chl *a* mapping using a 300-m ground resolution for the entire Baltic Sea - both the open sea and coastal areas - excluding the westernmost parts.

2.3 Monitoring procedure

2.3.1 Monitoring strategy

The *in vivo* fluorescence yield, recorded at 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).

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To fulfill the status 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

Sampling strategies for determining Chl *a* are as follows (HELCOM 2015a):

- Ship-based monitoring
    - 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
- Ferrybox samples are collected at 3–5 m depth
- EO at visible band collects information from the upper part of the euphotic water, depending on the transparency of the water.

Sampling equipment

- Discrete sampling bottles
- CTD-rosette bottles
- ISCO-sampler for Ferrybox

2.3.3 Sample handling and analysis

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). Samples for Chl *a* measurement are filtrated through GF/F filters, and extracted. The following amendments are suggested to the current methodology HELCOM (2015a):

	Current guideline	Recommended	Reference	Suggesting party
Drying of filters	Filters should be dried in darkness at room temperature before extraction	Filters need not to be dried before extraction	Seppälä et al., unpubl.	FI
Storing of filters	Damp filters can be stored frozen up to one month before extraction	Filters should not be stored dry, but extracted as soon as possible The filters should be stored in an deep freezer (< -20 °C)	Wasmund et al. (2006)	SE
Storing of extracts	Extracts cannot be stored longer than 24 hours in room temperature	The extracts can be stored in an ultracold freezer (-80°C), or otherwise at -20°C, up to three months	Wasmund et al. (2006)	SE

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#suggestion: the method descriptions could have their own guidelines#

2.4 Data analysis

2.4.1. Ferryboxes

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 such; 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. 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.

2.4.2. EO

#this part lacks information from the contracting parties# EO-based Chl *a* concentration is determined by modelling pigment absorption at visible wavelengths. The model to derive Chl *a* from emergent flux varies according to the satellite instrument. For FI, ENVISAT / MERIS Chl *a* products were determined with FUB processor (Schroeder et al. 2007) using processing steps for by Attila et al. (2013, 2016) for calculating Chl *a*. For GE, EO data is processed with the FUB processor applied to the MERIS employing algorithms suited best for the German waters in the Baltic Sea.

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 r-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 intercalibrations.

#this part lacks information from the contracting parties# For FI, An EO estimation of Chl *a* is carried out via automated processing, complemented with manual cloud mask processing, that undergoes quality check before archiving and distribution (Attila et al. 2016).

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

5.3 Additional literature