Monitoring of phytoplankton species composition, abundance and biomass

1. Background

1.1 Introduction

Phytoplankton primary producers constitute the basis of the pelagic food web and phytoplankton community composition directly affects the nutrition, growth, reproduction and survival of different organisms (see Hällfors & Uusitalo 2013 and references therein) as well as the biogeochemical cycles of the Baltic Sea (Tamelander & Heiskanen 2004, Spilling & Lindström 2008).

In addition to providing data on the food web, phytoplankton monitoring provides essential information on the consequences of eutrophication (Suikkanen et al. 2007, 2013, Hällfors et al. 2013a). In the Baltic Sea, eutrophication has resulted in increases in summer phytoplankton abundance and biomass (Carstensen & Heiskanen 2007, Fleming-Lehtinen et al. 2008, Jaanus et al. 2011) as well as more frequent and intense blooms (Finni et al. 2001, Carstensen et al. 2007). Also the phytoplankton species composition has been observed to change with different nutrient levels and ratios (Gasiunaite et al. 2005, Carstensen & Heiskanen 2007, Suikkanen et al. 2007, Jurgensone et al. 2011).

Long-term monitoring has enabled determination of the annual phytoplankton succession and facilitates the recognizing of aberrant phenomena and their progression in the phytoplankton community (e.g. Hajdu et al. 2006, Fleming & Kaitala 2006, Klais et al. 2011, Majaneva et al. 2012, Olli et al. 2013). Phytoplankton monitoring also provides data on the biodiversity of phytoplankton communities (Uusitalo et al. 2013, Hällfors 2013, Olli et al. 2014), on harmful taxa (Leppänen et al. 1995, Wasmund 2002), and makes possible the detection of invasive alien species (Olenina et al. 2010).

Phytoplankton species composition, abundance and biomass are monitored by counting phytoplankton from preserved water samples using the Utermöhl inverted light microscopical method (Utermöhl 1958), by the relevant authorities.

1.2 Purpose and aims

In short, analysis of phytoplankton species composition, abundance and biomass is carried out for the following purposes:

- to describe temporal trends in phytoplankton species composition, phytoplankton abundance, biomass as well as the intensity and occurrence of blooms
- to describe the spatial distribution of phytoplankton species
- to identify key phytoplankton species (e.g. dominating, harmful, potential non-indigenous and/or invasive species, as well as indicator species)
- to provide basic data for complex ecosystem analyses, food web studies, modelling as well as political and social requirements such as indicators in the frame of the Marine Strategy Framework Directive of the European Union (MSFD; European Union 2008) and the EU Water Framework Directive (WFD; European Union 2000).

2. Monitoring methods

2.1 Monitoring features

Monitoring methods have to be conservative over a long time-period to facilitate the detection of changes and trends. The used monitoring methods should allow comparability of results within a monitoring program.

The method using the inverted light microscope is a universal method for phytoplankton identification and has been applied on a world-wide scale for decades. For quantification of phytoplankton, the Utermöhl method (Utermöhl 1958) has become the commonly used method. Monitoring guidance should include detailed information concerning the counting procedure, species identification, biovolume estimation and biomass calculation (as well as conversion into carbon units, if required). Concerning species identification, an equal level of knowledge among the persons contributing to the monitoring program is necessary.

2.2 Time and area

See HELCOM Map and Data service.

2.3 Monitoring procedure

2.3.1 Monitoring strategy

Phytoplankton species composition, abundance, and biomass in the euphotic zone form the basis for the determination of temporal trends of phytoplankton. The phytoplankton community is very dynamic, reacting quickly to changes in its environment and making the community structure spatially and temporally variable (Dybern & Hansen 1989). High-frequency sampling at a number of stations covering all basins in the Baltic Sea area is needed to reveal reliable trends. Since phytoplankton shows a substantial seasonal variation (e.g. Hällfors et al. 1981, Wasmund & Siegel 2008, Jaanus 2011), sampling needs to cover the entire growth season, which in parts of the Baltic Sea extends over the entire year. Microscopic determination is the only method by which it is possible to acquire information on the whole species composition of phytoplankton samples. However, in addition to the sampling at fixed sampling stations, ships-of-opportunity transects, satellite image interpretations and aerial surveillance could help to identify variability in the temporal and spatial extent of phytoplankton (e.g. Kanoshina et al. 2003; Lips et al. 2014). Such synoptic surveys are necessary for the study of the extent of the annually recurring phytoplankton blooms.

2.3.2 Sampling method(s) and equipment

For the purpose of quantitative studies in the open sea, the minimum requirement is to take an integrated sample from 0 – 10 m depth using a hose (Lindahl 1986) or by pooling equal amounts of water collected from fixed depths between 0 and 10 m using a water sampler (see Majaneva et al. 2009 for examples). The recommended sampling depths are 0 – 1 m, 2.5 m, 5 m, 7.5 m and 10 m. The integrated sample should be thoroughly mixed in a bucket or similar container. One subsample of 200 cm^3 is drawn from the well-mixed sample for quantitative phytoplankton counts. The same integrated sample should be used for chlorophyll determination and, if desired, primary production. An additional sample, 10 – 20 m, is recommended. If a subsurface chlorophyll *a* maximum is observed, additional phytoplankton and chlorophyll *a* samples may be taken at this depth.

When utilising automated flow-through sampling onboard ships-of-opportunity a single sample from the mixed surface layer can be taken (Rantajärvi et al. 1998, Kononen et al. 1999, Majaneva et al. 2009). A single surface layer sample can also be collected from a helicopter.

In coastal areas, sampling is more dependent of water depth and local environmental conditions and should be modified accordingly; e.g. a sample from 0 – 1 m or an integrated sample (0 – 10 m) could be collected.

It is recommended to take additional net samples from the water column (e.g. from 0 – 20 m) in order to obtain concentrated phytoplankton samples. These samples serve as a support for species identification of especially large-sized sparsely occurring species. Observation of unpreserved and living material facilitates identification of taxa which are deformed or even destroyed by preservatives (see e.g. Hällfors et al. 1979 and references therein) or which get heavily stained by the preservative. A plankton net with a 10 µm mesh-size is recommended. In case of higher phytoplankton concentration it is advisable to use a net with 25 µm mesh-size.

2.3.3 Sample handling and analysis

2.3.3.1 Preservation and storage of samples

Net samples to be studied alive can be kept fresh for a few hours in an open container in a refrigerator. All other samples have to be immediately preserved to prevent samples from decaying before analysis and also to immobilize flagellates to facilitate their sedimentation.

Acid Lugol's solution is the most suitable preservative (fixative) for Baltic Sea phytoplankton (Hällfors et al. 1979). However, if coccolithophorids need to be preserved with the coccoliths intact, a parallel subsample should be fixed with alkaline Lugol's solution, since acid Lugol's solution dissolves the coccoliths. If the thecal plate pattern of dinoflagellates needs to be investigated, a parallel subsample could be fixed with neutral Lugol's solution, to facilitate subsequent dyeing and distinguishing of the tabulation. Neutralized formaldehyde gives incomparable results to Lugol's solution and should not be used, except at a few coastal stations where long time series are already established using formaldehyde.

For preservation of water samples, 0.25 – 0.5 cm^3 of acid Lugol's solution per 100 cm^3 sample has to be added immediately after sampling. The parallel subsamples for investigating coccolithophorids or dinoflagellates should be fixed with the same amount, 0.25 – 0.5 cm^3, of alkaline or neutral Lugol's solution, respectively, per 100 cm^3 sample. If the cells are too strongly stained by iodine for comfortable identification, surplus iodine can be chemically reduced to iodide by dissolving a small amount of sodium thiosulphate ($Na_2S_20_3$. 5 H_20) in the aliquot to be sedimented.

Clear, colourless iodine-proof (i.e. glass) bottles with tightly fitting screw caps should be used for iodine-preserved material. With clear bottles it is easy to see when the iodine becomes depleted and more preservative needs to be added. Samples should be stored in dark and cool conditions and counted as soon as possible, at least within a year. With samples stored for more than one year there is a risk of the species composition being distorted due to unequal preservation and deterioration of different taxa.

Acid Lugol's solution (Willén 1962):

200 cm^3	distilled or deionized water
20 g	potassium iodide (KI)
10 g	resublimated iodine (I_2)
20 cm^3	glacial acetic acid (conc. CH_3COOH)

Mix the ingredients in the order listed. Make sure the previous ingredient has dissolved completely before adding the next. Store in a tightly sealed glass bottle cooled and in the dark.

Alkaline Lugol's solution (modified after Utermöhl 1958):

Replace the acetic acid of the acid solution by 50 g sodium acetate (CH_3COONa). Use a small part of the water to dissolve the acetate.

Neutral Lugol's solution (from Andersen & Throndsen 2003):

Prepare as acid Lugol's solution, but without the glacial acetic acid.

2.3.3.2 Sample settling procedure

The recommendation is based on the counting technique using an inverted microscope as described by Utermöhl (1958). A detailed account of the method is given by Edler and Elbrächter (2010).

Before settling (sedimentation) the sample should be adapted to room temperature to avoid excessive formation of gas bubbles in the sedimentation chambers. Gas bubbles will adversely affect sedimentation, the distribution of cells in the bottom-plate chamber, and microscopy.

Immediately before the sample is poured into the sedimentation chamber, the bottles should be shaken firmly but gently in irregular jerks to homogenize the contents. Too violent shaking will produce a lot of small bubbles, which may be difficult to eliminate. A rule of thumb is to gently turn the bottle upside-down at least 50 times. If the sample must be shaken vigorously in order to disperse tenacious clumps, this should not be done later than one hour before starting sedimentation.

The chambers should be placed on a horizontal surface and should not be exposed to temperature changes, draught or direct sunlight. For the cells to settle evenly, it is essential that the supporting surface is level and vibration free, since vibrations will cause the cells to collect in ridges. Covering the settling chamber(s) with an overturned plastic box will provide a fairly safe and uniform environment for sedimentation. Including moistened tissue paper or e.g. a small flask of water under the hood considerably reduces problems caused by evaporation.

In order to achieve a reasonable accuracy in counting, the sedimented sample should first be examined for general distribution of cells on the chamber bottom, as well as the abundance and size distribution of the organisms. The settled sample should be discarded if the distribution is visibly uneven, one-sided or in ridges, indicating convection, a sloping surface, or vibration, respectively. If this occurs consistently, measures should be taken to eliminate the sources of disturbance.

Settling time is dependent on the height of the chamber and the preservative used (e.g. Hasle 1978, Rott 1981). The times given in Table 1 are recommended as minimum times. If vibration is a problem, the minimum time should not be significantly exceeded. Otherwise it is recommended that counting be performed within four days. Sedimented samples not counted within a week should be discarded. Separated bottom chambers not counted immediately should be kept in an atmosphere saturated with humidity.

Table 1. Settling time for phytoplankton samples preserved with Lugol's solution for sedimentation chambers of different volumes.

Volume of chamber (cm3)	Height of chamber (cm)	Settling time (h)
2 – 3	0.5 – 1	3
10	2	8
25	5	18
50	10	24

Sedimentation chambers of 100 cm^3 (height 20 cm and settling time 48h for Lugol's solution) should be used with caution since convection currents are reported to interfere with the settling of plankton in chambers taller than five times their diameter (Nauwerck 1963, Hasle 1978). Such chambers can be used

only when phytoplankton is very sparse, as in late autumn and winter. For such samples it is recommended that phytoplankton is counted from the whole chamber bottom.

2.3.3.3 Cleaning of the sedimentation chambers

After use no part of the combined sedimentation chamber should be allowed to dry out before it is carefully cleaned. Dried phytoplankton or formalin preservative may be quite difficult to remove. The separate parts are first rinsed under running tap water, and then soaked for a few minutes in lukewarm water with some nonabrasive detergent added, thereafter cleaned with a soft brush or soft tissue paper, and rinsed with tap water. The sedimentation chamber may also be cleaned with 95% ethanol. Finally, they are rinsed with deionised or distilled water, and are put away to dry. Special care should be taken not to scratch either end of the top cylinder and the entire upper surface of the bottom plate. Storage of chamber plate should be horizontal in order to avoid bending of the plate.

2.3.3.4 Quantitative determinations (phytoplankton counting procedure)

After having examined and approved the general distribution of cells in the chamber bottom, counting begins at the lowest magnification, followed by analysis at successively higher magnifications (or the other way around, starting with the highest magnification). For the sake of adequate comparison between samples, regions and seasons, it is important to always strive to count the specific species at the same magnification. In special situations, such as bloom conditions, however, this may not be feasible. However, as far as possible it is preferable to always keep the same magnification and instead decrease the volume settled or the area counted if a species is very abundant. Large, easily identifiable species (e.g. *Ceratium* spp.), which are usually also relatively sparse are counted at the lowest magnification and preferably over the entire chamber bottom. Smaller species are counted at higher magnification and possibly on only a part of the chamber bottom.

Small microplankton species can preferably be counted together with the nanoplankton when they occur in abundance, or they can be counted using an objective with intermediate magnification, 20 – 25x. A grid of 5 x 5 (or 10 x 10) squares in one of the oculars is very helpful when counting dense fields of small cells. The recommended magnifications for phytoplankton of different sizes are listed in Table 2.

Table 2. Recommended magnifications for counting of different size classes of phytoplankton.

Size class	Magnification
0.2 – 2 µm (picoplankton)*	1000x
2 – 20 µm (nanoplankton)	200 – 630x
>20 µm (microplankton)	100 – 250x

* picoplankton cannot be properly analysed using the Utermöhl method

Counting the whole chamber bottom is performed by traversing back and forth (or up and down) across the chamber bottom. The parallel eyepiece threads delimit the transect, where the phytoplankton are counted (Fig. 1.). Phytoplankton cells crossing the upper thread are counted, but not those crossing the lower thread.

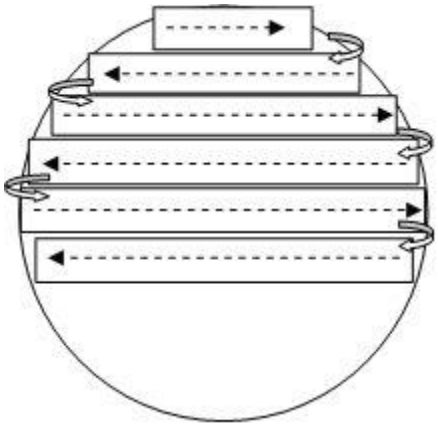

Figure 1. Traversing the whole chamber bottom with the parallel eyepiece threads to indicate the counted area (from Edler & Elbrächter 2010).

Counting part of the chamber bottom can be done in different ways. If half the chamber bottom is to be analysed, every second transect of the whole chamber counting method is counted. If a smaller part is to be analysed, one, two, three or more diameter transects are counted. After each transect is counted the chamber is rotated 30-45°. Also a number of fields-of-view, or ocular grids of 10 x 10 squares, can be counted.

If ocular squares (grids) are used in counting, single cells crossing two sides of the square (e.g. the bottom and the right sides of the square) should be taken into account, and cells crossing the other two sides (e.g. the left and the upper sides of the square) should be ignored (Fig. 2.). In the case of filamentous and colonial species, those cells of the filaments and colonies that occur inside the square should be taken into count, whereas the cells of the same filaments and colonies occurring outside the square should not be counted. Thus, all parts of the filaments and colonies inside the square should be taken into account, irrespective of which side of the square the filament or colony crosses (Fig. 3.).

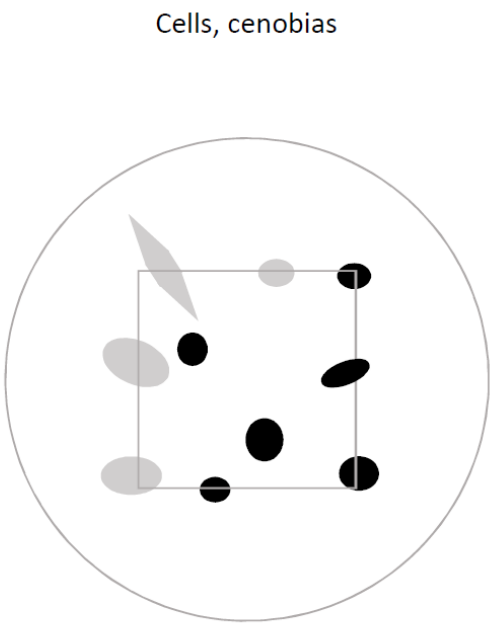

Figure 2. How to count single cells and cenobias.

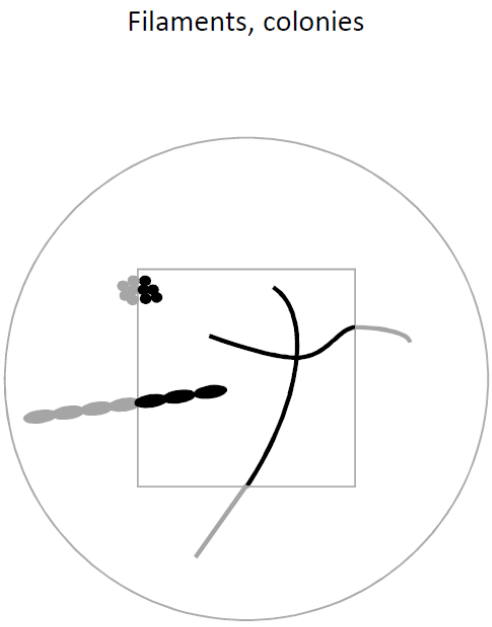

Figure 3. How to count filaments and colonies.

How much of the chamber area should be counted and which magnification to be used is dependent on the size of the organisms and their abundance, and on the kind of counting units used. The common counting unit is the cell. This applies also to colonies with irregular numbers of cells. Estimation of cell numbers in small-celled and densely-packed colonies may be realized by visual dividing of the colony into sub-areas, counting cell numbers in one sub-area and multiplying with the number of sub-areas. When estimating the total cell number of a colony it is important to take into account its potential three-dimensionality, and whether the colony is hollow or filled with cells.

Colonial phytoplankton which occur regularly as groups of four cells or a multiple are most conveniently counted and reported as colonies, e.g.:

Acutodesmus

Choricystis

Crucigenia

Crucigeniella

Desmodesmus

Dictyosphaerium

Elakatothrix

Gonium

Lacunastrum

Merismopedia

Monactinus

Pandorina

Pediastrum

Pseudopediastrum

Scenedesmus

Tetrastrum

Willea

Filamentous cyanobacteria are to be counted in lengths of 100 µm. Numbers of 100 µm pieces per volume of sea water are reported. Diatoms with any plasma inside the cell should be counted as a living cell.

When counting phytoplankton in a sedimentation chamber, it is suitable to count also protozooplankton (e.g. ciliates and colourless flagellates). This recommendation is also valid for these forms. However, it must be stressed that the protozooplankton are a separate group and must not be mixed with the phytoplankton (and that the sample volume analysed is not always enough for reliable counts of microplankton-sized protozooplankton). Thus, they must not be included in abundance or biomass values of phytoplankton. The exceptions are the mixotrophic ciliates *Mesodinium rubrum* and *Laboea* spp. that should be counted and included in abundance and biomass values of phytoplankton.

While colony forming pico-celled cyanobacteria should be analysed, the picoplankton fraction cannot be properly analysed using the Utermöhl method. Reliable quantitative counting of the picoplankton fraction requires fluorescence microscopy or flow cytometry (see e.g. OSPAR 2016 and references therein).

While counting, the species (individuals) have to be allocated to size classes and trophic type (autotrophic, i.e. phototrophic, i.e. chlorophyll-bearing; heterotrophic; or mixotrophic) according to the scheme of Olenina et al. (2006) and the latest update of its appendix (the latest update should always be used). This information is important for a reliable biovolume calculation; however it must be borne in mind that light microscopical analysis of Lugol's preserved samples does not give fully reliable results regarding trophic type since the presence or absence of chloroplasts is difficult to distinguish.

At least 50 counting units of each dominating taxon should be counted, and the total count should exceed 500 units. All cells encountered in the area examined should be counted and reported even if fewer counted units progressively will decrease the precision of the count and increase the statistical error of the population estimate. The approximate 95% confidence limits of a selected number of counted units are given in Table 3. They have been calculated according to the formula:

$$95\%\ \text{C.L.} = n \pm 2 \times (100/\sqrt{n})\%$$

where n is the number of units counted. Actually the error is not symmetrical, but increasingly asymmetrical with lower counts. Thus, for four units counted the theoretical limits are -73 to +156% (Lund et al. 1958, Kozova & Melnik 1978).

Table 3. The approximate 95% confidence limits of a selected number of counted units.

Count	95 % C.L. (%)
4	100
5	89
7	76
10	63
15	52
20	45
25	40
40	32
50	28
75	23
100	20
200	14
400	10
500	8.9
700	7.6
1000	6.3
2000	4.5
5000	2.8
10000	2

It should be recognized that these are not maximum errors. The statistics assume perfectly random-distribution of cells on the bottom of the sedimentation chamber, a condition which is probably never realized. The several subsampling steps involved also tend to increase the variance (cf. Venrick 1978a-b).

With species for which the counting unit is smaller than the individual, e.g. some colonial forms, chain forming diatoms, and filamentous species with the average filament length in excess of 100 µm, the distribution of the counting units will be aggregated even in perfectly sedimented samples. The variance will be higher, and the precision accordingly lower. If it is necessary to keep the error within the same limits as for "randomly" distributed units, the number of counted units should be increased in the ratio average size of individual/size of counting unit.

The number of counting units per volume (dm^3) of sea water is calculated by multiplying the number of units counted with the coefficient C, which is obtained from the following formulas:

$C(dm^{-3}) = A*1000 / (N*a_1*V)$ or $C(dm^{-3}) = A*1000 / (a_2*V)$

where:

A = cross-section area of the top cylinder of the combined sedimentation chamber; the usual inner diameter is 25.0 mm, giving A = 491 mm^2 (the inner diameter of the bottom-plate being irrelevant)

N = number of counted fields or transects

a_1 = area of single field or transect

a_2 = total counted area

V = volume (cm^3) of sedimented aliquot

2.3.3.4.1 Biomass determinations

Biomass data are a much better descriptor of phytoplankton than abundance, especially because the latter is strongly influenced by the highly abundant picoplankton and nanoplankton, which can be analysed only with limited certainty. It should be taken into account that abundance results are given as counting units per volume of sea water, not cells per volume of sea water. Thus, abundance results as such are not directly comparable, since certain taxa can be counted as single cells or as different sized colonies (e.g. many cyanobacteria). Hence, some recalculation of units into cells is necessary, if the results are to be given as cells per volume of sea water. However, biomass (wet weight and carbon content) results can be used directly as final results.

All in all, biomass data are preferred for characterizing spatial and temporal phytoplankton patterns and for modelling. Depending on the purpose of the investigation, phytoplankton biomass can be expressed as cell volume (or weight) or carbon. The transformations to cell volume are based on measurements of the size of the species and the adaptation of the shapes to geometrical shapes. The mandatory geometric formulas, size groups and the resulting biovolumes per counting unit are compiled in the paper of Olenina et al. (2006) and its updated appendix.

2.3.3.4.2 Biovolume calculation

As specified above, during the counting process the species (individuals) have to be allocated to size classes according to the scheme of Olenina et al. (2006) and its updated appendix (available at ICES website: http://www.ices.dk/marine-data/Documents/ENV/PEG_BVOL.zip). The individual biovolumes of the different counting units have to be multiplied with their abundance to get the biovolume per dm^3.

Biovolume $_{taxon}$ [$mm^3\ dm^{-3}$] = abundance [dm^{-3}] x VCU x 10^{-9}

VCU = volume of counting unit (in μm^3)

From the biovolume data, the biomass (wet weight) is simply derived by a rough assumption of a plasma density of 1 g cm^{-3}, as follows (CEN 2015):

1 mm^3 l^{-1} (biovolume) = 1 cm^3 m^{-3} (biovolume) = 1 mg l^{-1} (wet weight):

1 mm^3 m^{-3} (biovolume) = 10^6 μm^3 l^{-1} (biovolume) = 1 µg l^{-1} (wet weight)

2.3.3.4.3 Carbon content calculation

In a further step, the carbon content can be calculated, because organic carbon is the universal component of organisms and is the energy source transported along the food chain. The calculation of the carbon content is non-obligatory, but if executed it has to be done according to the below formulas.

In early guidelines (HELCOM 1988) it was recommended to calculate the carbon content from the plasma volume by a constant factor. Since the calculation of the plasma volume of diatoms bears a lot of uncertainties and, moreover, the conversion factor is not constant in reality, the calculation of carbon was suspended for some years. Formulas by Menden-Deuer and Lessard (2000) take into account the decrease in specific carbon content with cell size and calculate the carbon content of diatoms directly from the cellular biovolume without plasmavolume calculation. The carbon formulas are used according to the conclusions section in Menden-Deuer and Lessard (2000).

For **phytoplankton** in general (**including cyanobacteria and dinoflagellates**):

$$\text{Carbon [pg C cell}^{-1}\text{]} = 0.216 \times CV^{0.939}$$

For diatoms:

$$\text{Carbon [pg C cell}^{-1}\text{]} = 0.288 \times CV^{0.811}$$

CV = cell volume

The above formulas are for carbon content in single cells. If cell aggregates are the counting unit (CU), their carbon content has to be calculated via the carbon content of the cells according to the formulas below. It has to be differentiated between counting of multi-cell colonies (e.g. 100 cells of *Microcystis* as a CU) and filaments (e.g. 100 µm of *Nodularia* as a CU). In filaments, the cell length has to be known.

The formula for **multi-cell colonies**:

$$\text{Carbon [pg C CU}^{-1}\text{]} = 0.216 \times CPU \times (VCU/CPU)^{0.939}$$

The formula for **filaments**:

$$\text{Carbon [pg C CU}^{-1}\text{]} = 0.216 \times LCU/CL \times (VCU*CL/LCU)^{0.939}$$

CU = counting unit

VCU = volume of counting unit (in μm^3)

CPU = number of cells per counting unit

CL = cell length (in µm)

LCU = length of counting unit (mostly 100 µm)

2.3.3.5 Semi-quantitative analysis of phytoplankton samples

Microscopic determination is the only method by which it is possible to acquire information on the whole species composition of phytoplankton samples. This information is needed in order to reveal changes in the phytoplankton communities in time and space and e.g. to estimate the potential toxicity of a bloom. The quantitative analysis (i.e. counting of actual cell numbers) is time-consuming, and in some cases, a semi-quantitative counting method can be used instead. In this method, all taxa are identified and listed, but their abundance is estimated using a semi-quantitative ranking (Leppänen et al. 1995); the allocation of taxa to different size-classes is optional and depends on the level of information strived for.

Although quantitative phytoplankton analysis is the more commonly used method there are several benefits of using semi-quantitative abundance estimations, as discussed by Hällfors (2013). First, the semi-quantitative method is less time-consuming and makes possible the analysis of a larger number of samples. Second, the semi-quantitative method better takes into account even the smallest phytoplankton cells, which are often belittled when expressing abundance in units of biomass. Third, multivariate analysis of the phytoplankton community does not require quantitative data; unbiased qualitative data, in which the species abundances are in realistic proportions to each other (e.g. on scales of 0–5 or 0–10), are sufficient (Sarvala 1984). Indeed, if the data consist of cell counts or biomasses, it is often necessary to use transformations that result in a roughly equivalent scale anyway (Sarvala 1984).

2.3.3.5.1 Counting procedure

For the semi-quantitative analysis, the inverted microscope technique is used. At least half of the chamber bottom (preferably the whole) should be analysed using a small magnification (10x objective) and two bottom diameter transects with a larger magnification (40x objective). All taxa found should be listed; if using the HELCOM counting software the net sampling option should be chosen. A semi-quantitative 5-level abundance scale ranking should be used (Table 4). Several species can and do get the same ranking, even the highest one. Provided that the same sample volume is always sedimented and examined, the samples are comparable; see Hällfors 2013 and references therein. The rearrangement of taxa and size classes, required in most cases when analysing phytoplankton species data, necessitates the recalculation of taxon semi-quantitative abundances. For this a formula has been developed (see Hällfors et al. 2013b).

Table 4. 5-level semi-quantitative abundance scale used for estimating taxon abundances.

1. ***very sparse***, one or a few (less than five of the >20 µm fraction) cells or units in the analysed area, i.e. in the sedimented sample
2. ***sparse***, slightly more cells or units in the analysed area
3. ***scattered***, irrespective of the magnification several cells or units in many fields of view
4. ***abundant***, irrespective of the magnification several cells or units in most fields of view
5. ***dominant ****, irrespective of the magnification many cells or units in every field of view

* in terms of abundance, not biomass. Large sized taxa may be dominant in terms of biomass even if not dominant in terms of abundance.

If information on the accurate abundance of a species (e.g. a potentially toxic one) is needed in addition to the semi-quantitative abundances, at least 20 fields (with 40x objective), or one transect (with 10x objective) should be counted using the quantitative method.

2.3.3.6 Qualitative determinations

Net samples can be studied with either an inverted or a standard research microscope. The advantages of using a standard research microscope include a potentially higher resolution, thinner preparations and the possibility to turn the cells around by tapping the cover glass; this is not possible if the net sample has been pipetted onto a chamber bottom or the slide has been turned upside down (as is necessary when using an inverted microscope). Tapping the cover glass to turn over cells or to crush them is especially helpful when examining the plate structure of dinoflagellates. Dinoflagellate plates are also well studied using the epifluorescence method with Calcofluor (Andersen & Throndsen 2003).

3. Data reporting and storage

At least raw data, but preferably also calculated data and metadata are to be reported to and stored in national environmental databases. The national databases report the phytoplankton data further to the ICES database.

4. Quality control

4.1 Quality control of methods

Extensive knowledge of the taxonomy, identification and counting procedures of phytoplankton is essential in order to produce high-quality data. To achieve and maintain such knowledge, persons performing phytoplankton analysis should regularly participate in training courses, intercalibrations and proficiency tests. The most recent version of the biovolume file should be used and this file needs to be updated regularly based on cell measurements and expert judgement. (The biovolume file is updated yearly by the HELCOM Phytoplankton Expert Group).

In order to check the precision of the method and analyst it is recommended to count one dominating species using a low and one using a high magnification in a new subsample in every 20th sample.

4.2 Quality control of data and reporting

Immediately after having finished counting the sample, the analyst should go through the results to check that no errors have slipped in (i.e. checking that the correct taxa have been recorded and that the abundances/biovolumes/carbon values are reasonable) before saving the data in the national data base.

5. Contacts and references

5.1 Contact persons

Chairperson of the HELCOM Phytoplankton Expert Group; see: http://www.helcom.fi/helcom-at-work/projects/phytoplankton/

5.2 References

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Additionally valuable taxonomic information can be found in various scientific papers, Baltic Sea Phytoplankton Identification Sheets (Ann. Bot. Fennici) and ICES Identification Leaflets for Plankton.

For phytoplankton images validated by the HELCOM Phytoplankton Expert Group see the HELCOM PEG Gallery at www.nordicmicroalgae.org: http://nordicmicroalgae.org/galleries/helcom-peg