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Water quality — Guidance on the estimation of phytoplankton biovolume

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**Water quality - Guidance on the estimation of
phytoplankton biovolume**

Qualité de l'eau - Lignes directrices pour l'estimation
du biovolume des microalgues

Wasserbeschaffenheit - Anleitung zur Abschätzung des
Phytoplankton-Biovolumens

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European foreword

This document (EN 16695:2015) has been prepared by Technical Committee CEN/TC 230 “Water analysis”, the secretariat of which is held by DIN.

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by March 2016 and conflicting national standards shall be withdrawn at the latest by March 2016.

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Introduction

The abundance or number of counting units of individual phytoplankton taxa does not necessarily reflect the real ratio of single taxa to the complete biomass of a phytoplankton community. Few big cells/counting units can contribute far more biomass to the system than many small ones. Hence, abundance data alone is often not an ideal measurement of population size. Biomass estimations give very important information for ecological studies, classification schemes and ecosystem modelling. Therefore, it is necessary to determine the biomass of phytoplankton taxa, particularly because phytoplankton delivers energy in the form of carbon, to other trophic levels of food webs. It is not possible to directly analyse the carbon content on the taxonomic level in natural phytoplankton samples, therefore the biovolume of the phytoplankton taxa is a suitable measure to determine the biomass of an ecosystem according to the taxonomic composition. Neither particle size analysis using laser analysis, nor flow cytometry, nor Coulter Counters, nor chemical analyses of chlorophyll-a concentration as well as total carbon allow statements on the taxon level. An estimation of the carbon content is possible using conversion factors (see Annex C).

Further, the biovolume is a quantitative basis for assessing hazards from those algae and cyanobacteria, which (can) contain noxious or toxic metabolites, and is used in combination with cell numbers or chlorophyll-a concentration within WHO guidelines and national regulations for risk assessments.

Up to now, various guidelines for estimating the biovolume of microalgae have been used in different national and international monitoring programs (e.g. [1], [2], [3], [4]). The main objective of this document is the standardization of the procedure for determining the phytoplankton biovolume in order to achieve comparability of data. For this reason, the estimation of the biovolume in phytoplankton samples in sedimentation chambers (according to Utermöhl) using an inverted microscope will be described in detail.

This European Standard is also applicable for image analysis of pictures derived from microscope and flow cytometry cameras. The use of a standard catalogue containing basic and some composed geometrical shapes is recommended. Of course, such a standard list will not reflect the variety of all naturally existing shapes and will not match the exact biovolume values of each taxon. It will always be a compromise between accuracy and efficiency. However, the usage of agreed geometrical shapes and the application of the relevant formulae will improve the comparability of phytoplankton data and will be an important step forward for the implementation of quality assurance measures in phytoplankton analysis.

1 Scope

This European Standard specifies a procedure for the estimation of biovolume of marine and freshwater phytoplankton taxa using inverted microscopy (Utermöhl technique according to EN 15204), in consideration of some heterotrophic protists ($< 100 \mu\text{m}$) that are not considered in routine zooplankton analysis and benthic microalgae, which can be found in pelagic water samples.

This European Standard describes the necessary methods for measuring cell dimensions and for the calculation of cell or counting unit volumes to estimate the biovolume in phytoplankton samples. This shall be done using harmonized assignments of geometrical shapes to avoid errors.

2 Normative references

The following documents, in whole or in part, are normatively referenced in this document and are indispensable for its application. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

EN 15204, *Water quality - Guidance standard on the enumeration of phytoplankton using inverted microscopy (Utermöhl technique)*

EN 15972, *Water quality - Guidance on quantitative and qualitative investigations of marine phytoplankton*

EN 16698, *Water quality - Guidance on quantitative and qualitative sampling of phytoplankton from inland waters*

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

3.1

biomass

total mass of living organic matter within a system or taxon

3.2

biovolume

total volume of (living) organisms within a system or taxon

Note 1 to entry: The biovolume is usually expressed in cubic millimetres per litre (mm^3/l).

3.3

cell volume

counting unit volume

total volume of a single cell or one counting unit

Note 1 to entry: The cell volume or counting unit volume includes the cell wall (if existing) but excludes lorica and/or mucilaginous envelopes and cell surface structures such as spines, bristles and scales.

Note 2 to entry: The cell volume or counting unit volume is usually expressed in cubic micrometres (μm^3).

4 Principle

Generally, the estimation of the total or taxon specific biovolume in phytoplankton samples of natural communities or cultures is based on measurements of a representative number of individuals. By

multiplying the average or median cell or counting unit volume with the abundance, the total biovolume of each taxon in the sample is determined.

Three approaches are feasible:

- 1) Estimation by representative measurement:** A representative number of individuals (in most cases single cells) or counting units of all recorded or dominating taxa is measured in each sample or a specified number of samples within a comparable series. These data are used to calculate the average or median cell or counting unit volume of each taxon using the applied geometrical formulae.
- 2) Estimation using size classes based on representative measurements:** For taxa with a high variability in cell size (e.g. several diatoms, different stages in life cycle) reasonable size classes can be determined first, and then the individuals are assigned to both the relevant taxon and size class. Basis for the definition of the size classes are measurements in the same manner as described in (1).
- 3) Estimation using standard volumes based on representative measurements:** A reasonable general standard cell or counting unit volume is defined for each taxon once. These standard values are determined by representative measurements and calculated by the formula of the assigned geometrical shapes as described in (1).

A geometrical shape shall be assigned to each taxon in all approaches to calculate the cell or counting unit volume. Thus, to harmonize these approaches the geometrical shapes are pre-assigned to all taxa (see Annex D). These shapes have been chosen to reflect the corresponding taxa shapes as accurately as possible, and to allow effective taxa measurement with little effort (i.e. with as few dimensions as possible; usually only two are necessary). Seventeen different geometrical shapes are utilized (for the catalogue of geometrical shapes see Annex A). If it is impossible to describe the actual shape of a taxon with a simple basic geometrical shape, composite shapes (e.g. cone with half sphere) are used. If the actual geometry of taxa does not fit exactly to the assigned shape, a “geometry correction factor” is used for the final cell or counting unit volume calculation.

Taxon lists describing the preferred geometrical shapes have been published before (see e.g. [1], [3], [4]), based on specific taxonomical levels or for particular areas. This guidance document provides harmonized geometrical shapes for phytoplankton organisms spread across European marine, brackish, and freshwater systems. Annex D contains an alphabetical list of genera with the assigned geometrical shapes. If there are divergent forms on species, subspecies, form, or variety level within a genus they are listed as well.

5 Equipment and preservatives

The following equipment is required for biovolume analysis of phytoplankton samples.

5.1 Inverted microscope equipped with a condenser featuring a numeric aperture (NA) of at least 0,5 and plan objectives with a NA of 0,9 or more allowing for total magnification between 63× and 400× at a minimum. The microscope should have binocular, bright field (additional phase contrast is useful), 10× or 12,5× eyepieces.

Though inverted microscopy is the recommended method for analysing of phytoplankton, conventional (non-inverted) compound light microscopes may also be used for measuring phytoplankton under some conditions.

5.2 Calibrated object micrometre.

5.3 Eyepiece (ocular) micrometre.

5.4 Counting-graticule.

5.5 Sedimentation chambers according to EN 15204.

5.6 Image analysis software, if available.

5.7 Sampling bottles according to EN 15204.

5.8 Preservatives, acidic Lugol's iodine and/or alkaline Lugol's iodine according to EN 15204.

6 Procedure

6.1 Sampling and sample preparation

The sampling and determination of phytoplankton abundance and composition is a precondition for the calculation of the biovolume of a phytoplankton sample. Sampling shall be carried out according to EN 16698 for freshwater samples and EN 15972 for marine samples. For counting and species determination, see EN 15204.

The dimensions needed for the biovolume estimation of the relevant phytoplankton taxa are analysed in sedimentation chambers, which are prepared in the same manner as for counting and species determination (see EN 15204), using an inverted microscope and an eyepiece micrometre or image analysis software.

For specific scientific purposes, measurements can be carried out also with a conventional (non-inverted) compound light microscope.

6.2 Calibration of the eyepiece micrometre, counting-graticule and image analysis software

The required dimensions for estimation of the cell or counting unit volume shall be measured using an eyepiece (ocular) micrometre or an image analysis software. For the application of size classes a calibrated counting-graticule can also be used.

Prior to measurement, all systems shall be calibrated with a calibrated object micrometre for every microscope and all objectives and eyepieces used.

The scale of commercially available calibrated object micrometres has a length of 1 mm (or 2 mm) where the millimetre is divided into 100 equal parts. The distance between the graduation lines is 10 µm. By aligning the scale of the eyepiece micrometre with the scale of the object micrometre or the grid boxes of the counting-graticule, the scale value (S) or conversion factor of the eyepiece micrometre can be determined for each magnification as follows:

$$S = \frac{n_{\text{obj}} \times 10}{n_{\text{eye}}} \quad (1)$$

where

S is the scale value (conversion factor) for the eyepiece micrometre in micrometres (µm);

n_{obj} is the number of graduation lines of the object micrometre;

n_{eye} is the number of graduation lines of the eyepiece micrometre or the number of grid boxes of the counting-graticule.

The conversion factor should be specified with up to two decimal places. The intervals between the graduation lines of the scale shall be separately determined with the calibrated object micrometre for every objective used.

If image analysis software is used, this equipment shall be calibrated with the calibrated object micrometre separately for every level of magnification, following the instructions in the operating manual of the software.

6.3 Statistical requirements for determination

The required dimensions of the relevant geometrical shape shall be measured for each taxon of interest. At least 20 individuals per taxon should be measured to ensure that the standard error of cell or counting unit volume will be generally < 10 %.

For taxa, which are very variable in size, the number of measured cells/counting units should be increased until the standard error is < 10 %, to a maximum of 50 individuals ([3], [5]).

Where the size variability of a taxon is small, the number of measured cells may be minimized to only 5 to 10 individuals. In all cases, it is advisable to check that the standard error of cell or counting unit volume is low. The standard deviation and standard error can be calculated according to Formulae (2) and (3) as follows:

$$s = \sqrt{\frac{\sum_{i=1}^n (x_i - \bar{x})^2}{n-1}} \quad (2)$$

where

- s is the taxon volume standard deviation;
- x_i is the volume of a single cell/counting unit of the taxon;
- $\bar{x}$ is the mean volume of all measurements of the taxon;
- n is the number of the respective measured cells of the taxon.

From the standard deviation s , the standard error of the mean $\sigma_{\bar{x}}$ can be calculated using Formula (3):

$$\sigma_{\bar{x}} = \frac{s}{\sqrt{n}} \quad (3)$$

To obtain the percentage range σ_{rel} of the standard error, divide the standard error of the mean $\sigma_{\bar{x}}$ by the mean value $\bar{x}$ of the samples using Formula (4):

$$\sigma_{rel} = \frac{\sigma_{\bar{x}}}{\bar{x}} \times 100 \quad (4)$$

From the results, the 95 % confidence limits can be determined using Formulae (5) and (6):

$$\bar{x}_u = \bar{x} + (\sigma_{\bar{x}} \times 1,96) \quad (5)$$

$$\bar{x}_l = \bar{x} - (\sigma_{\bar{x}} \times 1,96) \quad (6)$$

where

$\bar{x}_u$ is the upper 95 % confidence limit;

$\bar{x}_l$ is the lower 95 % confidence limit.

Since the required number of individuals to be measured for any taxon is dependent upon the size variability of that taxon, it is helpful to calculate the cell or counting unit volume after each measurement. This will allow continuous statistical analysis and check of precision, thus minimizing the number of measurements required. Ideally, the confidence limits should be set at 95 % as a measure for precision. By ensuring that only as many cells as necessary for achieving this limit will be counted, the amount of laboratory work will be minimized.

If not enough cells/counting units of a taxon are present in the sample in order to achieve the minimum statistical requirements described, all of the (few) cells of this taxon shall be measured or a mean standard biovolume may be used (see below).

Measuring a high number of cells for every taxon in every sample is a time consuming procedure. For routine monitoring programmes mean cell or counting unit volumes calculated from own measurements, for a particular project and area, may be used. These mean volumes shall be checked regularly by measuring actual cell dimensions (see 6.4) and calculating actual cell and counting unit volume (see 6.5). For taxa with a high variability in cell size and representing more than 50 % of the total biovolume, these checks are strongly advised.

6.4 Measurement

6.4.1 General

Measurement of the cells/counting units can be carried out in a separate step or parallel to the counting process. Depending on the cell size of the taxa, the determination of the required dimensions (e.g. diameter, height, length, width, etc.) should be carried out at magnifications between 63× and 1 000×, in order to obtain corresponding precision.

The so-called empty magnification, which does not reveal any new detail, should be avoided. Therefore, the total magnification of the microscope should preferably be higher than 500× but smaller than 1 000× of the NA of the used objectives to work in the area of useful magnification.

It is important that the cells to be measured are chosen randomly to avoid a discrimination of special size classes. This can be achieved by selecting the cells from randomly distributed visual boxes all over the sedimentation chamber.

If single cells can clearly be distinguished in chain-building or filamentous species or other colony forming taxa, only one cell per chain, filament or colony should be measured. Filamentous algae often form lumps and are distributed very unevenly in the chamber. Also in these cases, it shall be ensured that cells from different lumps are measured.

By rotating the eyepiece micrometre and moving the sedimentation chamber with the microscope stage, the scale of the eyepiece micrometre is placed over the required dimension of the cell/counting unit to be measured. The number of covered graduation lines is read, and with the application of the conversion factor (see 6.2), the length of the dimension is calculated by multiplication.

The measurement results should be reported in micrometres, with up to two decimal places. If the end of the cell dimension to be measured is between two graduation lines of the ocular scale, the share is to be estimated to a maximum of one decimal place.

When using the size classes approach based on representative measurements, the calibrated counting-graticule may also be used for the assignment of the individuals to the corresponding size classes.

When image analysis software is used, the corresponding details of the operating manual shall be followed.

6.4.2 Using size classes based on representative measurements

The appropriate number of size classes depends on the size variation of each taxon. The size classes, for example, can be derived from cluster analyses applied to a representative dataset of measured cells of the taxon (see 6.4.1).

For each size class the average cell or counting unit volume is calculated using the formula of the taxon specific geometrical shape with mean dimension lengths. By multiplication with the respective abundance and addition of all size classes of a taxon, the total taxon specific biovolume in the sample is determined. In routine monitoring programmes, standard size classes should be used (e.g. [4]). If regional lists of size classes are available, they should be used instead [4]. It is recommended to create a new size class, when the biovolume of individuals significantly exceeds the biovolume of existing size classes.

6.4.3 How to deal with hidden dimensions

For some particular taxa, it is often not possible to measure all dimensions needed to calculate the cell volume ("hidden dimensions", e.g. height of prismatic cells of different shapes or small diameter of elliptical cells) during routine analysis. Then it is necessary to estimate the length of missing dimensions as a proportion of one of the visible dimensions using a calculated species-specific factor. The visible to hidden size relationship can be obtained from measurements of other samples from the same sample series or from the same area if the position of the cells allows this. Measurements should be used preferentially over estimates. The taxon list in Annex D gives suggestions for the dimension relations for most of the taxa, which usually have a "hidden dimension". For those taxa, where no suggestion for the "hidden dimension" is given, corresponding nominal values shall be taken from the literature if available. If the "hidden dimension" shall be determined exactly for a special problem, the cells can be turned around in paraffin oil. With special microscopes, it is also possible to measure the distance between focus of the upper and lower end of the "hidden dimension".

6.4.4 Measurement of filamentous taxa

With some filamentous taxa (e.g. cyanobacteria), it is often difficult to distinguish individual cells within a filament, especially when the cells are directly connected without any gaps. In such cases, filament pieces of a fixed length, e.g. 100 µm or 10 µm, can be counted and measured (100 µm or 10 µm length and diameter), and multiplied by the total enumeration of this counting unit in the sample.

Alternatively, mean dimensions of filaments can be measured to calculate the volume of one filament, a value that is then multiplied by the number of filaments in the sample.

A third method, which is more precise for filamentous forms, particularly those, which have no distinct boundaries between cells, is as follows: Instead of counting individual filaments, the total length of the fraction of each filament that is within the boundaries of a counting grid shall be measured, ignoring the fraction outside of the grid boundaries (see Figure 1). The sum of the total length of all fractions of filaments within the grid shall be calculated after counting of the transect is completed. Afterwards, the diameter of at least 20 filaments shall be measured and the mean filament diameter is calculated. To obtain the biovolume of the respective taxon, the sum of total filament lengths (h) shall be multiplied with the square of the median filament diameter and the factor of $\pi/4$, because the filament is a cylinder with the volume:

$$V = \frac{1}{4} \times \pi \times d^2 \times h \quad (7)$$

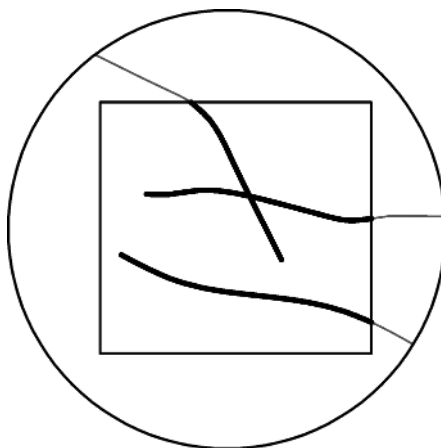


Figure 1 — Determining biovolume for filaments without distinct cell boundaries using a counting grid

NOTE The lengths of all fractions within the counting grid (black rectangle) are measured, excluding fractions outside of the counting grid boundaries [6].

6.4.5 Measurement and counting of colony- and coenobium-forming taxa

In most cases, the assignment of a geometrical shape should be based on the shape of an individual cell. For some colony- and coenobium-forming species where individual cells are difficult to distinguish or have very complex contours, it can be expedient to assign a geometrical shape based on the shape of the whole colony or coenobium or to use ultrasonic treated samples (where the association of cells have become disintegrated into individual cells) for counting and measuring the cells.

For example in some species, small individual cells are aggregated into compact spatial colonies. The individual cells are usually indistinguishable, and the number may be very difficult to assess in these colonies or coenobia. In such cases, the estimation of the volume can be based on the geometry of the entire colony or coenobium, in particular during the analysis of routine monitoring samples. For some taxa an additional geometrical shape correction factor shall be taken into account for the volume calculation. The same applies for colony- or coenobium-forming taxa with very complex contours of individual cells. Table 1 lists examples. In Annex D, it is specified for the respective taxa if the entire colony or coenobium with its geometry should be measured.

Table 1 — Geometrical shapes and correction factors for some colony- and coenobium-forming taxa.

Taxon name	Geometry based on colony	Geometrical shape correction factor	Hidden dimension factor
<i>Woronichinia</i>	sphere	0,2	-
<i>Coelosphaerium</i>	sphere	0,2	-
<i>Snowella</i>	sphere	0,75	-
<i>Botryococcus</i>	spheroid	-	-
<i>Eudorina</i>	sphere	0,25	-
<i>Pandorina</i>	sphere	-	-
<i>Coelastrum</i>	sphere	-	-
<i>Pediastrum</i>	cylinder	species dependend	height of colony = height of single cell (factor see Annex D)
<i>Crucigenia</i>	cuboid	species dependend	third edge length (height) = $0,5 \times$ second edge length (width)

A more precise method to achieve the biovolume of such colonial forms, especially the cyanobacterial genera *Microcystis*, *Aphanothece*, *Aphanocapsa*, and the *Dolichospermum* species forming “ball of yarn” colonies, is to measure cell dimensions and the greatest axial linear dimension of the colony in the sedimented sample, and then separate the colonies by ultrasonic treatment, and count the individual cells in the treated sample [7].

6.4.6 Measurement of complex geometrical shapes

Some taxa show very complex cell outlines requiring a composition of multiple geometrical shapes and thus, the application of complicated formulae for biovolume calculations. In order to ease that work, some simplified combined forms have been assigned to these taxa that can be easily measured and will require only minimum effort for the estimation. However, depending on the required precision, more complex and thus more precise geometrical subdivisions shall be applied to those taxa. Another possibility is to resort to pre-determined cell volumes from literature (mean standard cell or counting unit volume), bearing in mind that the size of the cells depends on a number of environmental factors and thus, may vary widely. On the other hand, cell volume estimation for some species is easier on half-cell basis (for example in some desmid genera like *Cosmarium* and *Staurostrum*).

In any case, everything shall be documented in the protocol. As an example, Annex E shows how to measure the needed dimensions for four taxa from the conducted interlaboratory comparison as well as for two additional taxa, which are not easy to measure.

6.5 Calculation of biovolume

The cell or counting unit volume is calculated on the basis of the taxon-specific geometrical shape and the linear dimensions determined for the individual cell or counting unit (e.g. diameter, height, length, width, etc.; see Annex A). For some taxa, the associated geometrical form will not fit exactly to the actual cell shape, for example, if a cuboid is assigned to a pennate diatom with rounded apical cell ends. For such cases, the cell volume shall be multiplied by a correction factor, which is given in Annex D for relevant taxa.

The average cell or counting unit volumes of the various taxa shall be generally calculated as the median of all individual cell or counting unit volumes.

NOTE If the individual volumes per taxon are normally distributed (according to Chi-squared test or Kolmogorov-Smirnov test), the arithmetic mean can be used for the calculation of the average volume instead of the median. For answering specific questions in ecology, it can be necessary to use also the arithmetic mean for non-normally distributed values.

These calculations shall be carried out for every phytoplankton taxon recorded in all or selected samples using the “representative measurement” approach (see Clause 4) or basically once for characteristic samples using the “standard factor” approach (see Clause 4). If applying the “size class” approach (see Clause 4 and 6.4.2) the average cell or counting unit volume for each size class is calculated using the mean of the upper and lower dimensions of size class borders.

The biovolume per taxon in a sample is calculated by multiplying the number of cells/l (or cells/ml) or counting units (e.g. number of 100 µm filament pieces per liter) with the median (or mean) of the determined taxon-specific cell or counting unit volumes (µm³) as measured by one of the three methods listed above:

$$V_{\text{bio}, i} = \frac{n_i \times \tilde{V}_i}{10^9} \quad (8)$$

where

$V_{\text{bio}, i}$ is the biovolume of taxon or size class i in cubic millimetres per litre (mm³/l);

n_i is the number of cells (or number of counting units) of taxon or size class i per litre (l⁻¹);

$\tilde{V}_i$ is the median (or mean) of the cell or counting unit volumes of taxon or size class i in cubic micrometres (μm^3).

The total biovolume to be determined for each sample results from the sum of the biovolume determined for each phytoplankton taxon or size class.

Statistical performance data from the conducted European wide interlaboratory comparison for validation are listed in Annex B.

6.6 Biovolume biomass relations

In phytoplankton ecology, the biomass is usually expressed as chlorophyll-a concentration ($\mu\text{g/l}$), biovolume (mm^3/l) or carbon content ($\mu\text{g/l}$). Assuming the density of organisms being equal to the density of water ($1,0 \text{ g/cm}^3$, [8]), the biomass as wet weight may be estimated as follows:

$$1 \text{ mm}^3/\text{l} (\text{biovolume}) = 1 \text{ cm}^3/\text{m}^3 (\text{biovolume}) = 1 \text{ mg/l} (\text{wet weight});$$

$$1 \text{ mm}^3/\text{m}^3 (\text{biovolume}) = 10^6 \mu\text{m}^3/\text{l} (\text{biovolume}) = 1 \mu\text{g/l} (\text{wet weight}).$$

NOTE For the estimation of the carbon content, see Annex C.

6.7 Reporting

The specific cell or counting unit volumes are expressed in cubic micrometres (μm^3) without decimal places or for picoplankton with two significant places (e.g. $4,2 \mu\text{m}^3$). The biovolume of a single taxon and the complete sample is given in cubic millimetres per litre (mm^3/l or mm^3/ml) with three significant digits or places (e.g. $12,5 \text{ mm}^3/\text{l}$, $3,75 \text{ mm}^3/\text{l}$, $0,138 \text{ mm}^3/\text{l}$, $0,004 \text{ mm}^3/\text{l}$).

As an example, the taxon specific part of a sample report can contain information as shown in Table 2.

Table 2 — Example report for taxon specific sample part.

Taxon name	Abundance	Geometry	Average cell volume	Total biovolume	Carbon content (according to ...)
	n/l		μm^3	mm^3/l	$\mu\text{g/l}$
<i>Thalassiosira nordenskiöldii</i>	12 109	cylinder	9 817	0,119	6,53
<i>Odontella aurita</i>	265 725	elliptic cylinder	14 091	3,74	188,5
....					
Sum of sample 5	765 180			4,43	231,2

Some taxa (e.g. flagellates without solid cell wall) are inclined to shrink during the fixation process. Shrinkage depends on many influencing factors (preservative, life stage, physiological status, species, etc., even diatoms can shrink). Often it is difficult to find good correction factors for this process in the literature. As a consequence, applying a general correction factor to preserved material can lead either to overestimation or to underestimation of the biovolume. For monitoring activities with the objective of detecting trends, the exact compensation by preservation correction factors is not of the highest priority. In other cases where it might be mandatory to determine the exact biomass, correction factors for influence of fixation should be derived from comparisons between fixated and living organisms. This shall be noted in the protocol.

7 Quality Assurance

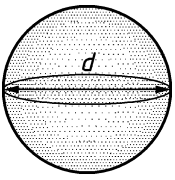
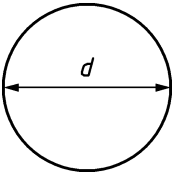
The quality assurance associated with this European Standard should be in accordance with EN 14996.

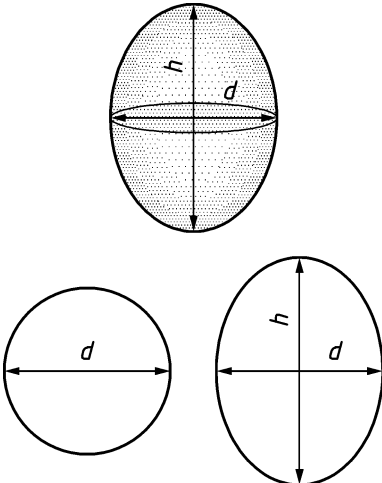
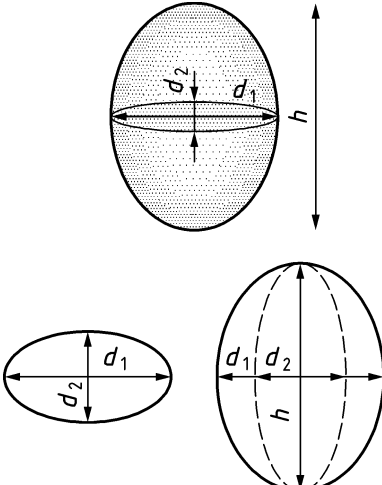
Annex A (informative)

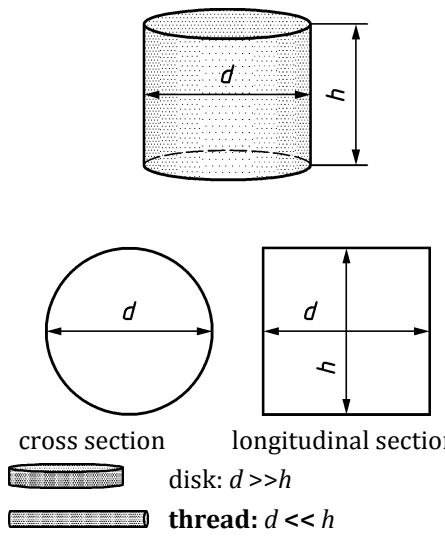
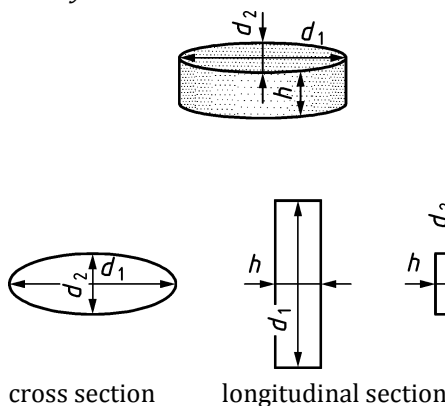
List of geometrical shapes

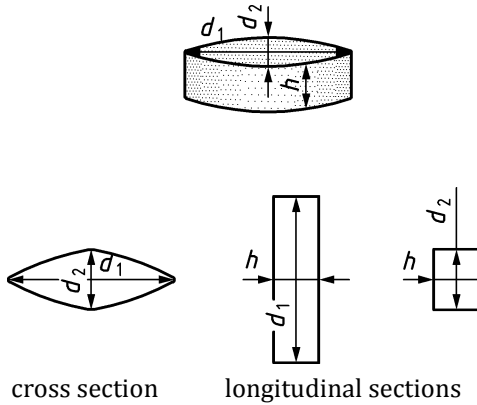
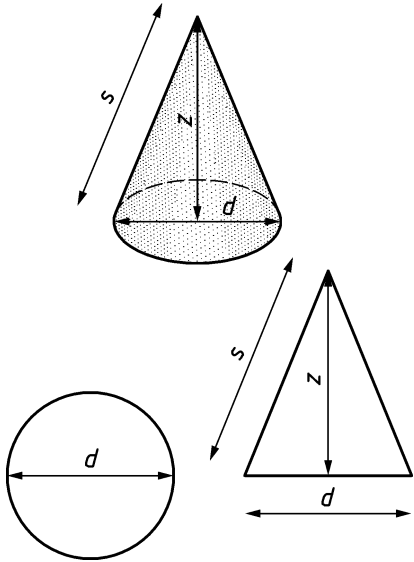
Table A.1 provides information about 17 different geometrical shapes used for the biovolume estimation of phytoplankton. The first column simply contains a consecutive number (ID-number) for each shape. Listed in the second column is the official geometrical name of the form alongside any other synonymous names. The images of each shape show a 3D-view, a cross-section, and a longitudinal-section in which the dimensions are designated to be used for biovolume calculation. The third column shows some general information about each shape, a description about the needed dimensions as well as some taxon examples. Firstly, it is listed if it is a basic shape or a composed form. If the latter is the case it is also indicated from which basic shapes the form is composed. The volume calculation precision distinguishes between exact calculation, if there is a clearly defined formula for volume, and approximate calculation, if the formula is derived by approximate integration. The dimensions necessary for calculation of biovolume are listed with full name and abbreviation as well as with information about measurability of these dimensions in phytoplankton organisms using an inverted microscope. “m” means that this dimension is measurable, “hd” means that this is a hidden dimension that cannot be measured in a sedimentation chamber. If both specifications are listed (m; hd), this indicates that cells can have different positions, the orientation of which can affect if the dimension is measurable or not. Finally, some typical taxon examples for each shape are listed. In the last column, the formulae needed for biovolume calculation are specified, with the deduced form based on diameter that can clearly be measured in the cells, and with the original geometrical form based on radius.

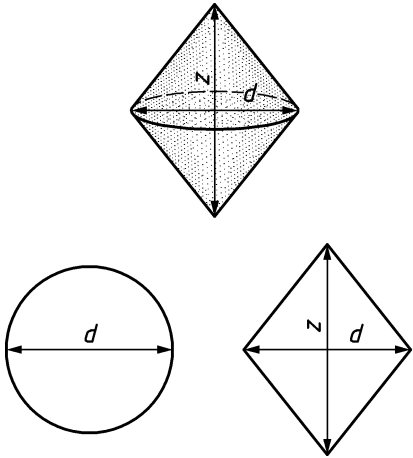
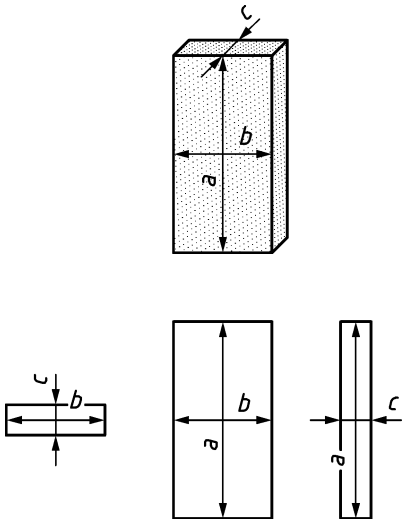
Table A.1 — Geometrical shapes

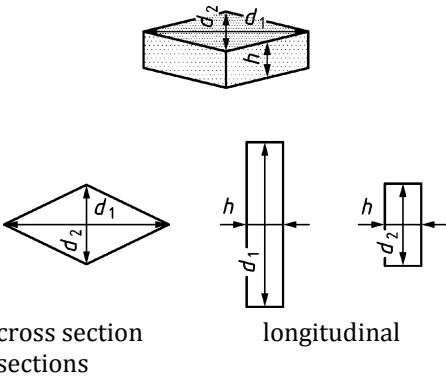
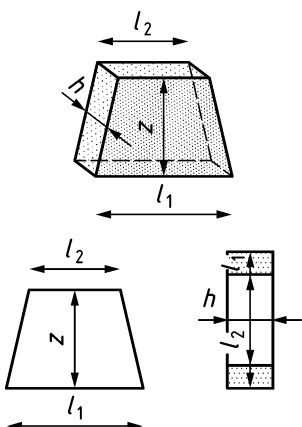
ID	Geometrical shape (<i>Synonyms</i>)	Information	Volume formula
1	Sphere   cross section	<i>basic shape</i> <i>Volume calculation precision:</i> <i>exact</i> Dimensions: <i>diameter (d) - m</i> Examples: <i>Chroococcus, Coelastrum,</i> <i>Gloeocapsa, Microcystis, Oblea,</i> <i>Pterosperma</i>	$V = \frac{1}{6} \times \pi \times d^3$ For radius $r = \frac{d}{2}$: $V = \frac{4}{3} \times \pi \times r^3$

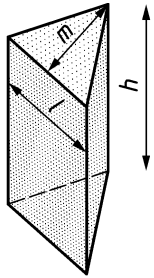
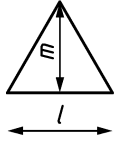
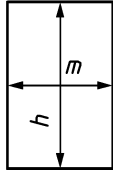
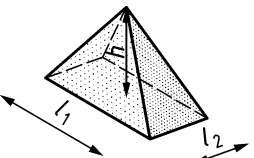
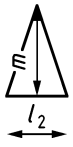
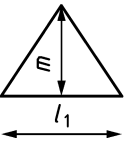
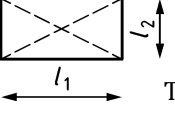
ID	Geometrical shape (<i>Synonyms</i>)	Information	Volume formula
2	Spheroid <i>rotational ellipsoid</i> <i>ellipsoid of revolution</i>  cross section longitudinal section	<i>basic shape</i> <i>Volume calculation precision: exact</i> Dimensions: <i>diameter (d) - m</i> <i>height (h) - m</i> Examples: <i>Aphanothece, Chlamydomonas, Desmodesmus, Trachelomonas, Woronichinia</i>	$V = \frac{1}{6} \times \pi \times d^2 \times h$ For radius $r = \frac{d}{2}$: $V = \frac{4}{3} \times \pi \times r^2 \times \frac{1}{2} \times h$
3	Ellipsoid <i>tri-axial ellipsoid,</i> <i>flattened ellipsoid</i>  cross section longitudinal section	<i>basic shape</i> <i>Volume calculation precision: exact</i> Dimensions: <i>large diameter (d₁) - m</i> <i>small diameter (d₂) - m</i> <i>height (h) - m</i> Examples: <i>Amphidinium, Cosmarium, Dinophysis, Gymnodinium, Mallomonas</i>	$V = \frac{1}{6} \times \pi \times d_1 \times d_2 \times h$ For radius r : $V = \frac{4}{3} \times \pi \times r_1 \times r_2 \times r_3$ with $r_1 = \frac{d_1}{2}; \quad r_2 = \frac{d_2}{2}; \quad r_3 = \frac{h}{2}$

ID	Geometrical shape (<i>Synonyms</i>)	Information	Volume formula
4	Cylinder <i>circle based cylinder</i>  cross section longitudinal section disk: $d \gg h$ thread: $d \ll h$	<i>basic shape</i> <i>Volume calculation precision: exact</i> Dimensions: <i>diameter (d) - m</i> <i>height (h) - m; hd</i> Examples: <i>Aphanizomenon, Aulacoseira, Coscinodiscus, Phormidium, Rhizosolenia, Thalassiosira</i>	$V = \frac{1}{4} \times \pi \times d^2 \times h$ For radius $r = \frac{d}{2}$: $V = \pi \times r^2 \times h$
5	Elliptic cylinder <i>prism on elliptic base</i> <i>oval cylinder</i>  cross section longitudinal sections	<i>basic shape</i> <i>Volume calculation precision: exact</i> Dimensions: <i>large diameter (d₁) - m</i> <i>small diameter (d₂) - m; hd</i> <i>height of cylinder (h) - hd; m</i> Examples: <i>Achnanthes, Chaetoceros, Fragilaria, Odontella, Surirella</i>	$V = \frac{1}{4} \times \pi \times d_1 \times d_2 \times h$ For radius r: $V = \pi \times r_1 \times r_2 \times h$ with $r_1 = \frac{d_1}{2}; \quad r_2 = \frac{d_2}{2}$

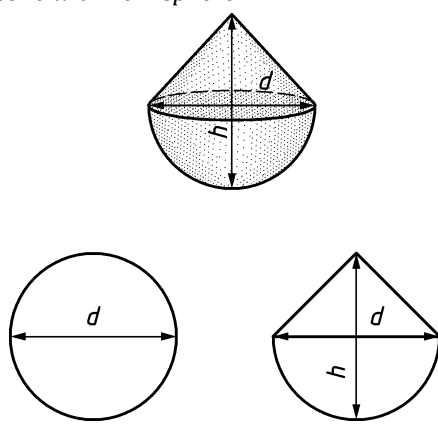
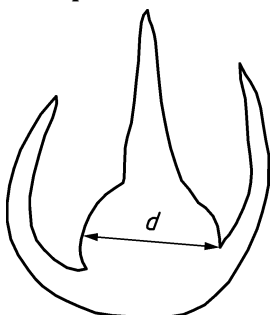
ID	Geometrical shape (<i>Synonyms</i>)	Information	Volume formula
6	Lanceolate cylinder <i>naviculoid</i>  cross section longitudinal sections	<i>basic shape</i> <i>Volume calculation precision:</i> <i>approximate</i> Dimensions: <i>large diameter (d₁) - m</i> <i>small diameter (d₂) - m; hd</i> <i>height of cylinder (h) - hd; m</i> Examples: <i>Haslea, Navicula</i>	$V = \frac{2}{\pi} \times d_1 \times d_2 \times h$ For radius <i>r</i>: $V = \frac{8}{\pi} \times r_1 \times r_2 \times h$ with $r_1 = \frac{d_1}{2}; \quad r_2 = \frac{d_2}{2}$
7	Cone  cross section longitudinal section	<i>basic shape</i> <i>Volume calculation precision:</i> <i>exact</i> Dimensions: <i>diameter (d) - m</i> <i>slant height (s) - m</i> <i>height of cone (z) - hd</i> NOTE With an inverted microscope, the slant height can exactly be measured. The cone height is a hidden dimension because of the oblique projection. Examples: <i>Calciopappus, Minuscula, Pyramimonas longicauda</i>	$V = \frac{1}{12} \times \pi \times d^2 \times \sqrt{s^2 - \frac{1}{4} \times d^2}$ For radius $r = \frac{d}{2}$ and height $z = \sqrt{s^2 - r^2}$: $V = \frac{1}{3} \times \pi \times r^2 \times z$

ID	Geometrical shape (<i>Synonyms</i>)	Information	Volume formula
8	Double cone <i>two cones</i>  cross section longitudinal section	<i>composed shape</i> <i>basic shape: cone</i> <i>Volume calculation precision: exact</i> Dimensions: <i>diameter (d) - m</i> <i>height of double cone (z) - m</i> NOTE Due to the position in the sedimentation chamber, the total height can only be measured approximately because of the oblique projection. Examples: <i>Gyrodinium spirale,</i> <i>Heterocapsa triquetra,</i> <i>Protoperidinium crassipes</i>	$V = \frac{1}{12} \times \pi \times d^2 \times z$ For radius $r = \frac{d}{2}$: $V = 2 \times \frac{1}{3} \times \pi \times r^2 \times \frac{1}{2} \times z$
9	Cuboid <i>rectangular box</i> <i>rectangular parallelepiped</i>  cross section longitudinal sections	<i>basic shape</i> <i>Volume calculation precision: exact</i> Dimensions: <i>first edge length (a) - m</i> <i>second edge length (b) - m; hd</i> <i>third edge length (c) - hd; m</i> Examples: <i>Bacillaria, Hantzschia,</i> <i>Pinnularia, Tabellaria</i>	$V = a \times b \times c$

ID	Geometrical shape (<i>Synonyms</i>)	Information	Volume formula
10	Rhombic prism <i>parallelepiped prism on parallelogram base</i>  cross section sections longitudinal sections	<i>basic shape</i> <i>Volume calculation precision: exact</i> Dimensions: <i>large diagonal (d_1) - m</i> <i>small diagonal (d_2) - m; hd</i> <i>height of prism (h) - hd; m</i> Examples: <i>Brachysira, Nitzschia, Pseudonitzschia</i>	$V = \frac{1}{2} \times d_1 \times d_2 \times h$
11	Trapezoid prism  cross section longitudinal section	<i>basic shape</i> <i>Volume calculation precision: exact</i> Dimensions: <i>length of large trapezoid side (l_1) - m</i> <i>length of small trapezoid side (l_2) - m</i> <i>height of trapezoid (z) - m; hd</i> <i>height of prism (h) - hd; m</i> Examples: <i>Crucigenia fenestrata</i>	$V = \frac{1}{2} \times (l_1 + l_2) \times z \times h$

ID	Geometrical shape (<i>Synonyms</i>)	Information	Volume formula
12	Triangular prism <i>prism on triangle base</i>   cross section  longitudinal section	<i>basic shape</i> <i>Volume calculation precision: exact</i> Dimensions: <i>length of triangle side (l) - m</i> <i>height of triangle (m) - m; hd</i> <i>height of prism (h) - hd; m</i> NOTE For a prism on basis of an equilateral triangle, only the triangle side is needed. The triangle height can be expressed by this side. Examples: <i>Bellerochea, Ditylum, Goniochloris, Lithodesmium, Triceratium</i>	$V = \frac{1}{2} \times l \times m \times h$ or $V = \frac{1}{4} \times \sqrt{3} \times l^2 \times h$ for a prism on basis of an equilateral triangle with $m = \frac{1}{2} \times \sqrt{3} \times l$
13	Pyramid Cross sections     Top view	<i>basic shape</i> <i>Volume calculation precision: exact</i> Dimensions: <i>length of first basic side (l₁) - m</i> <i>length of second basic side (l₂) - m; hd</i> <i>height of pyramid (h) - hd; m</i> Examples: <i>Gomphonema, Licmophora</i>	$V = \frac{1}{3} \times l_1 \times l_2 \times h$

ID	Geometrical shape (<i>Synonyms</i>)	Information	Volume formula
14	Tetrahedron lateral view top view	<i>basic shape</i> <i>Volume calculation precision: exact</i> Dimensions: <i>edge length (a) - m</i> <i>height of tetrahedron (h) - hd</i> Examples: <i>Staurostrum, Staurodesmus, Tetraedriella, Tetraedron</i>	$V = \frac{1}{12} \times \sqrt{3} \times a^2 \times h$ or $V = \frac{1}{12} \times \sqrt{2} \times a^3$ for a regular tetrahedron with $h = \frac{1}{3} \times \sqrt{6} \times a;$
15	Spindle cross section longitudinal section	<i>basic shape</i> <i>Volume calculation precision: approximate</i> Dimensions: <i>diameter (d) - m</i> <i>height (h) - m</i> Examples: <i>Ankistrodesmus, Closterium, Monoraphidium</i>	$V = \frac{2}{15} \times \pi \times d^2 \times h$ For radius $r = \frac{d}{2}$: $V = \frac{8}{15} \times \pi \times r^2 \times h$

ID	Geometrical shape <i>(Synonyms)</i>	Information	Volume formula
16	Cone with half sphere <i>cone with hemisphere</i>  cross section longitudinal section	<i>composed shape</i> <i>basic shapes: cone, sphere</i> <i>Volume calculation precision: exact</i> Dimensions: <i>diameter (d) - m</i> <i>total height (h) - m</i> NOTE Due to the position in the sedimentation chamber, the total height can only be measured approximately because of the oblique projection. Examples: <i>Gonyaulax, Hemiselmis, Katodinium, Teleaulax</i>	$V = \frac{1}{12} \times \pi \times d^2 \times \left(h + \frac{1}{2} \times d \right)$ For radius $r = \frac{d}{2}$: $V = \frac{1}{3} \times \pi \times r^2 \times (h - r) + \frac{1}{2} \times \frac{4}{3} \times \pi \times r^3$
17	Ceratium-shape  lateral view	<i>Complicated composed shape</i> <i>Volume calculation precision: approximate</i> Dimensions: <i>girdle diameter (d) - m</i> Examples: <i>Ceratium</i>	$V = x \times d^y$ x and y are factors derived from measurements [4], [9] <i>Ceratium</i> x: 0,32359 y: 2,9953 <i>C, furca</i> x: 2,3038 y: 2,532 <i>C, fusus</i> x: 35,198 y: 1,9156 <i>C, hirundinella</i> x: 2,3038 y: 2,532 <i>C, horridum</i> x: 0,32437 y: 3,0474 <i>C, lineatum</i> x: 1,2375 y: 2,5989 <i>C, longipes</i> x: 0,32437 y: 3,0474 <i>C, macroceros</i> x: 0,32437 y: 3,0474 <i>C, tripos</i> x: 0,32359 y: 2,9953

Annex B (informative)

Performance data

The performance data given in Table B.1 to Table B.4 were determined in a European interlaboratory comparison for validation carried out in March 2014 on two mixed marine and two freshwater samples taken from the North Sea (Helgoland, Germany), the Baltic Sea (Stralsund, Germany) and different lakes around Oldenburg (Germany) and Bad Saarow (Germany). 52 participants from the following European countries have provided results: Denmark (4), Finland (3), France (1), Germany (20), Ireland (1), Italy (3), Lithuania (3), Norway (1), Poland (2), Portugal (3), Spain (2), Sweden (3) and United Kingdom (6).

All results have been calculated according to the German standard document DIN 38402-45:2014-06.

Table B.1 — Performance data for measurements of first dimension in biovolume analysis of phytoplankton species in natural samples - Abbreviations for dimensions according to Annex A.

Taxon name	l	n	X μm	X_{med} μm	X_{mean} μm	X_{HS} μm	S_R μm	$C_{V,R}$ %	S_r μm	$C_{V,r}$ %
<i>Dactyliosolen fragilissimus</i> (d)	45	1 225	11,74	11,93	12,06	12,04	2,01	16,7	1,80	14,9
<i>Dinophysis acuminata</i> (d_1)	42	961	33,48	33,90	34,07	34,07	4,24	12,5	3,23	9,5
<i>Ceratium tripos</i> (d_1)	44	998	66,79	65,93	66,74	66,66	6,12	9,2	4,56	6,8
<i>Pseudo-nitzschia cf. pungens</i> (d_1)	44	1 054	111,87	111,69	111,12	111,19	12,43	11,2	11,18	10,1
<i>Thalassionema nitzschioides</i> (d_1)	42	1 013	32,95	32,82	33,15	33,15	4,29	13,0	4,25	12,8
<i>Rhizosolenia imbricata</i> (d)	42	1 154	9,68	10,19	10,16	10,19	2,49	24,4	2,14	21,0
<i>Ditylum brightwellii</i> (l)	40	1 114	26,76	23,83	23,64	23,69	4,89	20,7	4,18	17,6
<i>Stephanopyxis turris</i> (d)	37	964	43,15	40,35	39,96	39,96	7,60	19,0	7,07	17,7
<i>Odontella sinensis</i> (d_1)	40	1 106	167,28	163,87	175,38	174,21	40,62	23,3	33,02	19,0
<i>Chaetoceros debilis</i> (d_1)	39	982	13,39	14,25	13,65	13,65	3,84	28,2	2,77	20,3
<i>Fragilaria crotonensis</i> (d_1)	53	1 391	76,94	76,58	78,15	77,47	12,51	16,2	14,27	18,4
<i>Tabellaria fenestrata</i> (a)	47	1 052	51,62	50,91	50,77	50,77	4,77	9,4	4,20	8,3
<i>Aulacoseira granulata</i> (d)	50	1 183	5,48	5,88	5,89	5,90	1,02	17,3	0,84	14,2
<i>Cryptomonas erosa</i> (d_1)	50	1 133	12,63	12,83	12,96	12,96	1,58	12,2	1,32	10,2
<i>Rhodomonas lacustris</i> (d)	49	1 249	5,20	5,27	5,28	5,28	0,66	12,5	0,71	13,5
<i>Planktothrix agardhii</i> (d)	47	1 260	3,32	3,34	3,44	3,42	0,72	21,0	0,15	4,3
<i>Monoraphidium arcuatum</i> (d)	42	1 085	1,73	1,69	1,83	1,78	0,54	30,3	0,32	17,8
<i>Woronichinia naegeliana</i> (d)	44	1 184	2,92	3,27	3,26	3,26	0,60	18,3	0,39	12,0
<i>Cosmarium ocellatum</i> (d_1)	43	977	28,60	26,21	26,44	26,46	2,54	9,6	1,85	7,0
<i>Trachelomonas hispida</i> (d)	45	1 068	21,15	20,96	20,86	20,87	2,08	10,0	1,75	8,4
Explanation of symbols										
l	number of laboratories with $ z_u\text{-score} < 3$									
n	number of total individual test results of all laboratories with $ z_u\text{-score} < 3$									
X	assigned reference value based on 7 replicates with each having a minimum of 30 single measurements									
X_{med}	interlaboratory ^a median value									
X_{mean}	interlaboratory ^a arithmetic mean value									
X_{HS}	interlaboratory ^a robust mean value (Hampel Schaetzer)									
S_R	reproducibility standard deviation									
$C_{V,R}$	relative reproducibility standard deviation									
S_r	repeatability standard deviation									
$C_{V,r}$	relative repeatability standard deviation									
^a	Based on arithmetic means of individual laboratory measurements; only laboratories with $ z_u\text{-score} < 3$ included.									

Table B.2 — Performance data for measurements of second dimension in biovolume analysis of phytoplankton species in natural samples - Abbreviations for dimensions according to Annex A.

Taxon name	<i>l</i>	<i>n</i>	<i>X</i> µm	<i>X</i> _{med} µm	<i>X</i> _{mean} µm	<i>X</i> _{HS} µm	<i>S</i> _R µm	<i>C</i> _{V,R} %	<i>s</i> _r µm	<i>C</i> _{V,r} %
<i>Dactyliosolen fragilissimus (h)</i>	45	1 225	60,61	59,69	59,32	59,32	12,01	20,3	11,86	20,0
<i>Dinophysis acuminata (d₂)</i>	43	994	20,09	19,45	20,58	20,32	5,58	27,5	1,90	9,4
<i>Ceratium tripos (d₂)</i>	45	1 018	43,41	38,00	38,53	38,09	11,82	31,0	2,12	5,6
<i>Pseudo-nitzschia cf.pungens (d₂)</i>	43	1 024	4,81	4,51	4,59	4,58	1,01	22,0	0,84	18,4
<i>Thalassionema nitzschioides (d₂)</i>	42	1 013	3,99	4,35	4,26	4,23	0,99	23,4	0,77	18,2
<i>Rhizosolenia imbricata (h)</i>	42	1 154	188,20	201,34	200,16	200,46	45,13	22,5	41,59	20,7
<i>Ditylum brightwellii (m)</i>	40	1 114	23,17	22,99	22,87	22,96	4,47	19,5	4,51	19,7
<i>Stephanopyxis turris (h)</i>	37	964	66,83	59,20	58,30	58,30	10,36	17,8	9,62	16,5
<i>Odontella sinensis (d₂)</i>	40	1 106	66,91	64,27	64,17	63,63	20,62	32,4	11,50	18,1
<i>Chaetoceros debilis (d₂)</i>	38	959	10,04	9,31	9,53	9,40	2,59	27,6	1,59	16,9
<i>Fragilaria crotonensis (d₂)</i>	47	1 171	4,40	3,96	3,99	3,98	0,96	24,2	0,23	5,9
<i>Tabellaria fenestrata (b)</i>	47	1 052	5,16	7,57	7,39	7,41	2,33	31,4	1,07	14,4
<i>Aulacoseira granulata (h)</i>	43	966	27,33	26,04	26,11	26,11	3,16	12,1	2,68	10,3
<i>Cryptomonas erosa (d₂)</i>	49	1 113	10,73	10,13	9,95	9,99	2,10	21,1	0,95	9,5
<i>Rhodomonas lacustris (h)</i>	49	1 249	11,43	10,53	10,61	10,61	1,85	17,4	1,67	15,7
<i>Planktothrix agardhii</i>	-	-	-	-	-	-	-	-	-	-
<i>Monoraphidium arcuatum (h)</i>	41	1 065	49,31	42,00	41,25	41,25	7,92	19,2	4,98	12,1
<i>Woronichinia naegeliana (h)</i>	43	1 077	5,35	5,38	5,33	5,36	0,69	12,9	0,49	9,2
<i>Cosmarium ocellatum (d₂)</i>	43	956	14,30	13,39	13,52	12,19	2,52	20,7	0,87	7,2
<i>Trachelomonas hispida (h)</i>	45	1 068	27,12	27,13	26,83	26,80	3,01	11,2	2,35	8,8
For explanation of symbols, see Table B.1.										

Table B.3 — Performance data for measurements of third dimension in biovolume analysis of phytoplankton species in natural samples - Abbreviations for dimensions according to Annex A

Taxon name	l	n	X μm	x_{med} μm	x_{mean} μm	x_{HS} μm	s_R μm	$C_{V,R}$ %	s_r μm	$C_{V,r}$ %
<i>Dactyliosolen fragilissimus</i>	-	-	-	-	-	-	-	-	-	-
<i>Dinophysis acuminata (h)</i>	42	961	46,69	48,71	48,88	48,77	5,38	11,0	4,58	9,4
<i>Ceratium tripos (h₁)</i>	42	938	40,33	42,67	41,39	41,44	6,27	15,1	3,34	8,1
<i>Pseudo-nitzschia cf.pungens (h)</i>	44	1 054	4,81	4,06	4,14	4,12	1,51	36,7	0,66	16,1
<i>Thalassionema nitzschioides (h)</i>	42	1 013	4,59	4,69	4,77	4,76	1,00	21,0	0,78	16,4
<i>Rhizosolenia imbricata</i>	-	-	-	-	-	-	-	-	-	-
<i>Ditylum brightwellii (h)</i>	40	1 114	74,17	70,10	71,00	70,88	17,30	24,4	17,01	24,0
<i>Stephanopyxis turris</i>	-	-	-	-	-	-	-	-	-	-
<i>Odontella sinensis (h)</i>	40	1 106	195,30	180,70	175,04	175,34	47,20	26,9	42,85	24,4
<i>Chaetoceros debilis (h)</i>	38	954	9,14	8,83	8,99	8,91	2,10	23,6	1,57	17,6
<i>Fragilaria crotonensis (h)</i>	49	1 291	4,40	3,76	3,48	3,48	0,98	28,2	0,30	8,6
<i>Tabellaria fenestrata (c)</i>	41	929	7,82	4,88	5,47	5,40	2,06	38,2	0,70	13,0
<i>Aulacoseira granulata</i>	-	-	-	-	-	-	-	-	-	-
<i>Cryptomonas erosa (h)</i>	49	1 113	25,85	25,75	25,91	25,91	3,64	14,1	3,53	13,6
<i>Rhodomonas lacustris</i>	-	-	-	-	-	-	-	-	-	-
<i>Planktothrix agardhii</i>	-	-	-	-	-	-	-	-	-	-
<i>Monoraphidium arcuatum</i>	-	-	-	-	-	-	-	-	-	-
<i>Woronichinia naegeliana</i>	-	-	-	-	-	-	-	-	-	-
<i>Cosmarium ocellatum (h)</i>	46	1 014	26,82	26,86	27,04	27,04	2,32	8,6	2,12	7,8
<i>Trachelomonas hispida</i>	-	-	-	-	-	-	-	-	-	-
For explanation of symbols, see Table B.1.										

Table B.4 — Performance data for biovolume analysis of phytoplankton species in natural samples

Taxon name	<i>l</i>	<i>n</i>	<i>X</i> µm ³	<i>X</i> _{med} µm ³	<i>X</i> _{mean} µm ³	<i>X</i> _{HS} µm ³	<i>S</i> _R µm ³	<i>C</i> _{V,R} %	<i>S</i> _r µm ³	<i>C</i> _{V,r} %
<i>Dactyliosolen fragilissimus</i>	45	1 225	6 824	6 911	7 045	7 033	2 566	36,5	2 497	35,5
<i>Dinophysis acuminata</i>	43	992	16 718	16 334	17 241	17 190	6 906	40,2	3 940	22,9
<i>Ceratium tripos</i>	44	998	109 160	85 983	95 007	88 516	37 516	42,4	13 216	14,9
<i>Pseudo-nitzschia cf. pungens</i>	44	1 054	1 355	1 058	1 167	1 135	541	47,7	348	30,7
<i>Thalassionema nitzschioides</i>	41	1 006	490	555	536	535	218	40,7	161	30,0
<i>Rhizosolenia imbricata</i>	42	1 154	13 980	16 089	17 031	16 988	8 001	47,1	6 982	41,1
<i>Ditylum brightwellii</i>	40	1 114	24 055	18 753	20 080	20 080	8 571	42,7	7 689	38,3
<i>Stephanopyxis turris</i>	37	964	99 642	80 331	77 611	77 611	34 081	43,9	27 694	35,7
<i>Odontella sinensis</i>	40	1 106	1 785 462	1 560 584	1 624 194	1 586 715	785 689	49,5	723 632	45,6
<i>Chaetoceros debilis</i>	39	982	1 022	854	938	937	468	49,9	328	35,0
<i>Fragilaria crotonensis</i>	50	1 311	688	526	503	498	247	49,7	85	17,1
<i>Tabellaria fenestrata</i>	45	1 009	2 111	1 648	1 863	1 758	880	50,1	463	26,3
<i>Aulacoseira granulata</i>	45	1 032	680	753	761	760	272	35,9	216	28,4
<i>Cryptomonas erosa</i>	50	1 133	1 914	1 725	1 809	1 809	655	36,2	545	30,2
<i>Rhodomonas lacustris</i>	49	1 249	102	100	103	102	33	32,4	31	30,8
<i>Planktothrix agardhii</i>	47	1 260	866	884	969	941	362	38,4	68	7,2
<i>Monoraphidium arcuatum</i>	47	1 246	64	51	57	55	27	49,5	19	35,1
<i>Woronichinia naegeliana</i>	45	1 151	24	30	31	31	12	39,7	8	25,3
<i>Cosmarium ocellatum</i>	47	1 057	5 820	5 083	5 071	5 070	1 624	32,0	987	19,5
<i>Trachelomonas hispida</i>	45	1 068	6 410	6 414	6 302	6 287	1 716	27,3	1 309	20,8
For explanation of symbols, see Table B.1.										

Annex C (informative)

Carbon content calculation

Organic carbon is a main constituent of organisms. It represents the energy source transported within food webs and therefore is an important basis for ecological modelling. The carbon content of phytoplankton in natural samples cannot be determined directly (e.g. with a CN-Analyser), because chemical methods are not suitable to discriminate between the C-contents of different organism groups such as phytoplankton, zooplankton, bacteria, detritus, etc. Additionally, it will be impossible to distinguish phytoplankton groups on the taxonomic level. Therefore, a common method for estimating carbon biomass is the calculation from the microscopically determined biovolume using conversion factors. Mainly due to the correlation between total cell volume and vacuole size as mentioned before, the estimation of the carbon content with an exponential function applied on the cell volume should be preferred to the use of fixed conversion factors per volume:

$$c(C)_i = a \cdot (V_{\text{cell},i})^b \quad (\text{C.1})$$

where

$c(C)_i$ is the carbon content of taxon i in picograms per cell (pg/cell);

a and b are constant factors, see Table C.1;

$V_{\text{cell},i}$ is the average cell volume of taxon i in cubic micrometres per cell ($\mu\text{m}^3/\text{cell}$).

Because the carbon content of phytoplankton cells varies depending on different physiological and environmental factors, there are no universal values for the factors a and b . Some values used in the literature are shown in Table B.1. Due to the fact that the ratio of total cell volume to vacuole size may significantly differ from one species to another, especially for diatoms and other taxonomic groups under the same conditions, different conversion factors are used.

Table C.1 — Conversion factors for converting cell volume to carbon content from different sources

Group	Function $c(C)_i = a \cdot (V_{\text{cell},i})^b$		Source
	a	b	
pelagic diatoms	0,485	0,712	Strathmann (1967) [10]
	0,263	0,740	Tagushi (1976) [11]
	0,357	0,814	Vidal (1978) [12]
	0,513	0,757	Eppley et al. (1970) [13]
	0,131	0,890	Pavlovskaya & Kondratyeva (1981) [14]
	1,094	0,859	Blasco et al. (1982) [15]
	0,288	0,811	Menden-Deuer & Lessard (2000) [16]
marine dinoflagellates	0,387	0,900	Pavlovskaya & Kondratyeva (1981) [14]
phytoplankton without diatoms	0,347	0,866	Strathmann (1967) [10]
	0,251	0,940	Eppley et al. (1970) [13]
	0,216	0,939	Menden-Deuer & Lessard (2000) [16]
total phytoplankton	0,436	0,829	Moal et al. (1987) [17]
	0,109	0,991	Montagnes et al. (1994) [18]

The calculation of the carbon content is not mandatory, but if this procedure is carried out, then it is recommended to use the factors by Menden-Deuer & Lessard (2000) [16] or Eppley et al. (1970) [13]. Reference to what carbon factors have been used should always be included in the data report. Nevertheless, more extensive studies for different environmental conditions are needed to standardize the estimation of the carbon content of phytoplankton organisms via the biovolume.

Sometimes colony forming taxa shall be counted in cell aggregates. In these cases, it is necessary to distinguish between counting of cell packages (e.g. 100 cells of *Microcystis* sp.) and filaments (e.g. 100 µm pieces of *Nodularia* sp.). For these two special cases, the carbon content shall be calculated by using the following formulae (see also [2]):

For multi-cell colonies as

$$c(C)_i = a \cdot CPU \cdot \left(\frac{VCU}{CPU} \right)^b \quad (C.2)$$

and for filaments as

$$c(C)_i = a \cdot \frac{LCU}{CL} \cdot \left(\frac{VCU \cdot CL}{LCU} \right)^b \quad (C.3)$$

where

VCU is the volume of the counting unit in cubic micrometres (µm³);

CPU is the number of cells per counting unit;

CL is the cell length;

LCU is the length of the counting unit in micrometres (µm), mostly 100 µm.

Especially for small cells (e.g. cyanobacterial picoplankton) the carbon content can be calculated by multiplying the average cell volume with a fixed factor using a simple linear dependency (e.g. [19]):

$$c(C)_i = x \cdot V_{\text{cell},i} \quad (C.4)$$

where

$c(C)_i$ is the carbon content of taxon *i* in picograms per cell (pg/cell);

x is a constant factor (pg/µm³);

$V_{\text{cell},i}$ is the average cell volume of taxon *i* in cubic micrometres per cell (µm³/cell).

Annex D (informative)

Alphabetical list of recommended geometrical shapes and hidden dimension factors

Table D.1 contains recommended geometrical shapes and additional information, which should be used for biovolume estimation. This information is generally assigned on genus level. If some species, subspecies, forms, or varieties within a genus are different, they are listed additionally. The taxon list also contains genera of typical benthic microalgae as well as some heterotrophic flagellates or other protists. Both groups often are determined in the routine monitoring in parallel to the real phytoplankton organisms (in the strict ecological sense), either because they are stirred up from the sediment into the pelagial or because they belong to taxonomical groups also containing autotrophic species (e.g. Euglenophyceae, Dinophyceae or Cryptophyceae).

Basis for the used taxonomical names is the current valid status provided by the international databases “World Register of Marine Species” (WoRMS) [20] and AlgaeBase [21]. These taxa list can not be a complete list of all phytoplankton taxa occurring in European waters but is intended as a recommendation for the most important taxa. If some taxa seem to be missing, possibly due to using synonyms, the validity of the used name has to be checked in the above mentioned databases to find the assigned geometrical shape.

For the listed taxa the geometrical shape relating to Annex A is given. Additionally it is listed to which unit (cell, filament, coenobium, etc.) the geometry has been assigned. Sometimes the allocated geometrical shape does not correctly cover the effective volume. In these cases, the result has to be multiplied by a “geometrical shape correction factor” to estimate the effective volume more precisely. Some of these factors have been taken from the Helsinki Commission (HELCOM) monitoring program [4]. All others are estimated suggestions based on the results of the mandated CEN project.

For some particular taxa, it is often not possible to measure all dimensions needed for the calculation of the cell volume during routine analysis. In the last column of Table D.1 recommendations are given for relations to measurable dimensions for most of the taxa, which usually have such a “hidden dimension” depending on the position in the microscopic view. Some of these indicated factors have also been taken from the HELCOM monitoring program [4] or derived from their size class dimensions. Others have been calculated from measurements or from several pictures from different taxonomical databases or literature.

Various taxa are quite variable in appearance. Thus, few species sometimes can be rather spherical, in other cases rather elliptical. Moreover, different stages within the life cycle (e.g. cysts) may have various forms. In addition, amoeboid taxa exist. In the following taxon-list, the assignments of a geometry have been made according to the common appearance of the vegetative form. If a corresponding taxon in the sample to be analysed shows a different shape deviating from the list, an appropriate geometrical shape will have to be selected according to Annex A.

If taxa are analysed on a higher taxonomical level than genus (e.g. family, order, class, etc.) a well-fitting shape according to Annex A will have to be assigned.