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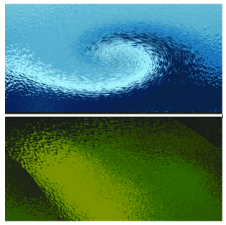

RESEARCH ARTICLE

Biovolume determination of phytoplankton guilds in transitional water ecosystems of Mediterranean Ecoregion

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Abstract

1 - Conceptually, morphometric measurements of phytoplankton guilds seem to have major advantages as descriptors of the ecological status of transitional water ecosystems (TW) with respect to classical taxonomic descriptors. However, at present, standardized or common methodologies for the use of morphometric descriptors do not exist.

2 - This paper aims to provide a starting point for the activation of standardized methods for the determination of morphometric descriptors of phytoplankton as a quality element in TW in accordance with the new directive of WFD 2000/60/EU.

3 - Phytoplankton biovolume is one of the most studied morphometric descriptors. It can be estimated by associating the algae with similar geometric forms and determining the volume of these by measuring the linear dimensions required for its calculation under the microscope. However, the lack of a standardized set of geometric forms and equations for calculating biovolume causes difficulties and produces data that are not comparable.

4 - A set of geometric models is suggested here for calculating the cell biovolumes of 201 phytoplankton genera found in transitional water ecosystems of Mediterranean Ecoregion. The equations were designed to minimize the effort of microscopic measurements. The main methodological problems, and the similarities and differences between our own and previously published proposals are discussed.

Keywords: morphometric descriptors, algal biovolume, geometric model, phytoplankton.

Introduction

Phytoplankton are listed in the Water Framework Directive (2000/60/EC) as a quality element for determining the ecological status of transitional water ecosystems. Nevertheless, at this time, only taxonomic parameters (diversity and abundance) are used as phytoplankton descriptors in the monitoring plans for water quality assessment. However, there are some disadvantages, in using these descriptors as indicators of the health status of transitional waters, because taxonomic parameters shown an high spatial and temporal variability, often insufficiently understood or documented, which is conceptually related to the heterogeneity, instability and structural complexity of transitional water ecosystems. Besides this, there are also methodological disadvantages, in that taxonomic identification is time-consuming and requires personnel who are highly experienced in taxonomy: since taxonomic structure from different transitional ecosystems are often not comparable (the phytoplankton taxonomic structure of a saltpan is very

different to that of a river delta). Since, a good descriptor of ecological status must have low internal variability, must respond unequivocally to internal selective pressures and external environmental forcing, and must be comparable between ecosystems; taxonomic structure and abundance of phytoplankton guilds simply do not meet these requirements. Based on these considerations, the search for innovative and more effective phytoplankton descriptors in transitional water ecosystems is being encouraged (Basset et al, 2004). Several studies seem to highlight the potential and the advantages of morphometric or body-size related descriptors in phytoplankton guilds in describing health status of aquatic ecosystems (Reynolds, 1997, Quinones et al., 2003 Sabetta et al., 2005).

Studies based on morphometric or body-size descriptors are concerned with variations in the size and shape of organisms as a result of ecological or evolutionary processes, including individual or population growth (Patapova and Snoeijs,1997), population interaction (Suttle et al., 1988), environmental forcing (Cottingham, 1999, Sin et al, 2000, Hashimoto and Shiomoto, 2002; Vadrucci et al., 2002, Perez-Ruzafa et al., 2002, Sabetta et al., 2005), ecological succession and bio-geographical distribution (Margalef, 1997; Smayda, 1978; Rohlf and Marcus, 1993). In phytoplankton guilds, morphometric descriptors can be defined at the following hierarchical levels: individual (biovolume, surface area, surface/volume ratio), population and guild (as body size-abundance distribution or body size-spectra; Sheldon et al., 1972, or as biomass size fractions of micro, nano and picophytoplankton, Sieburth, 1979).

The potential advantages of morphometric descriptors with respect to standard taxonomic descriptors are as follows:

- Body size is easy to measure
- Body size is easy to inter-calibrate
- Body size eliminates the difficulties of taxonomic identification
- Body size makes comparison between ecosystems easier, since it helps to resolve the difficulties resulting from the heterogeneity of taxonomic composition in different ecosystems.

Several theoretical and empirical studies have highlighted the role of body size in community organization. Moreover, there is much experimental evidence to support the influence of environmental forcing and pollution on the size structure of phytoplankton guilds in transitional water ecosystems and coastal marine areas (Cottingham, 1999, Vadrucci et al., 2002, Perez-Ruzafa et al., 2002, Ansotegui et al., 2003, Sabetta et al., 2005, Cermeño et al., 2005). However, body-size related descriptors require standardization of methodologies for their application.

Drawing up a standard protocol for the determination of phytoplankton morphometric descriptors requires several steps; one of the first concerns the definition of a standard procedure for the determination of the biovolume of phytoplankton cells and its subsequent conversion in biomass or carbon content. Biovolume and/or derived biomass are important parameters in studies of phytoplankton physiology, population dynamics, life cycles, (Papatova and Snoeijs, 1997), ecosystem energy flows (Menden-Deur and Lessard, 2000; Strathmann 1967, Montagnes et al., 1994, Reynolds, 1997) and even body size-abundance dynamics, since the body size-abundance distributions of phytoplankton guilds are constructed from biovolume or biomass data.

Biovolume can be estimated by several automated and semiautomatic methods. Some of these that can also be used in routine analysis include methods based on electronic particle counting (Boyd and Johnson, 1995), methods based on flow cytometry (Collier, 2000) and methods based on automatic microscopic image analysis. Nevertheless, these methods have several drawbacks that render them unsuitable in many research areas. For example, both electronic particle counting and flow cytometry yield a very low taxonomic resolution, limited to the level of easily discernible groups (e.g. algae of different size classes or pigment composition in flow cytometry). Moreover, coulter counters tend to underestimated cell volume and the magnitude is affected by cell size (Wheeler, 1999). Automated computer

mediated image analysis is used widely and successfully for the enumeration, biovolume estimation and classification of bacteria, but its application in phytoplankton guilds is less feasible because they are morphologically more variable than bacteria (Psenner, 1993, Sieracki et al., 1989). Other techniques, such as computer tomography of single cells or holographic scanning technology (Brown et al., 1989), despite being able to furnish an accurate estimate of biovolume, are not applicable for routine measurements because they require expensive equipment and long analysis times. For these reasons, at present, the most widely used method for calculating phytoplankton cell volume in routine analysis is based on the association of phytoplankton taxa with three-dimensional geometric forms (which are more similar to their real shape). This involves the direct measurement by light microscopy of the linear dimensions required for calculating the associated geometric volumes. The accuracy of the method depends on the set of selected geometric shapes. Indeed, one problem widely discussed among phytoplankton ecologists, is whether the phytoplankton should be assigned geometric forms that are complex (and thus require long analysis times) but similar to the cells' actual shape (Kovala and Larrance, 1966) or forms that are simple (and can thus be analysed rapidly) but less accurate (Edler,1979). Another more controversial aspect is related to the difficulty of comparing biovolume data from different aquatic ecosystems. Indeed, many different sets of geometric model are to be found in the literature but their application is frequently limited to the regional, ecosystem (marine or freshwater ecosystems) or guild level (phytoplankton or phyto-benthos) (Rott, 1981; Edler,1979). Some of these sets are strongly affected by the type of dominant species identified in the local plankton communities, and are often published in papers that are not widely available (Rott,1981, Kovala and Larrance,1966; Kononen et al., 1984), although in recent years, some papers (Hillebrand et al., 1999 and Sun and Liu, 2003) have been published in more visible journals. Although these studies are comprehensive and exhaustive, the proposed set of geometric forms are still not standardized, and their applicability needs to be expanded to include the phytoplankton of transitional waters, for which a set of geometric shapes has never been proposed. Also generally lacking in papers reporting sets of geometric models for calculating biovolumes is an explicit indication of the counting units used for each genus. In phytoplankton guilds, the counting unit is generally the single cell. Nevertheless, in some cases, when the single cell is not easy identifiable, the colony (as in the genera Phaeocystis, Snowella and Woronichinia), the coenobium (as iin Crucigenia, Pediastrum and Scenedesmus), group of cells (as in Chroococcus, Merismopedia), or fixed area (as in Microcystis) or the fixed filament (as in Anabaena, Nodularia, Oscillatoria) are used as the counting unit (Edler, 1979) .

This paper, based on the previous research by Edler, (Edler,1979), Hillebrand et al., (Hillebrand et al., 1999) and Sun and Liu (Sun and Liu, 2003), aims to provide a schematic protocol for calculating biovolumes of phytoplankton species, detectable with the Utermöhl method, in transitional ecosystems of the Mediterranean eco-region. Few papers have analyzed the methodological aspects of phytoplankton determination in transitional waters. This study, based on the consultation of scientific papers, was undertaken in collaboration with the most important research groups with experience in the analysis of phytoplankton in transitional water ecosystems and focuses on biovolume determination. Given that the protocol takes into account the morphometric characteristics of more than 600 species belonging to 8 groups and 201 genera listed in a range of publications concerning the phytoplankton of transitional water ecosystems, this paper will help to reduce the current fragmentation of the scientific knowledge of transitional water ecosystems in the Mediterranean Ecoregion, resulting in a common floristic list.

Moreover, it is also an attempt to integrate the most recent and widely-read papers (Hillebrand et al., 1999 and Sun and Liu, 2003) that propose sets of geometric forms for estimating

biovolumes, including specific sets for the phytoplankton of transitional water ecosystems.

Methods

Compilation of the unified floristic list.

We selected a set of geometric shapes for determining biovolume in phytoplankton guilds in transitional waters by consulting floristic lists available for a number of different types of transitional water ecosystems in the Mediterranean eco-region, including salt-pans, river deltas and coastal lagoons. These lists came from our own projects and from the literature (Vadrucci et al., 2004; Caroppo, 2000, Facca et al., 2001). In the case of our projects, the lists were drawn up by laboratories with experience in the field of phytoplankton analysis, such as the University of Lecce Laboratory of Ecology, the Department of Biological oceanography INOGS of Trieste, the University of Tirana Laboratory of Botany (Albania) and the Institute of Oceanology of the Bulgarian Academic of Sciences in Varna (Bulgaria).

The unified floristic list, obtained by combining the floristic lists from the various sources, included 643 species belonging to 201 genera. These genera are divided into 8 groups including one phytoplankton class or more classes as follows:

Group 1: Bacillariophyceae;
Group 2: Chlorophycea +.Prasinophyceae + +Prymnesiophyceae
Group 3: Chrysophyceae+ Dictyochophyceae+ +Haptophyceae
Group 4: Cryptophyceae+ Coanoflagellates+ +Kinetoplastides
Group 5: Cyanophyceae
Group 6:Dinophyceae
Group 7: Euglenophyceae
Group 8: Xantophyceae

The proportion of the genera accounted for by each group is reported in Fig. 1.

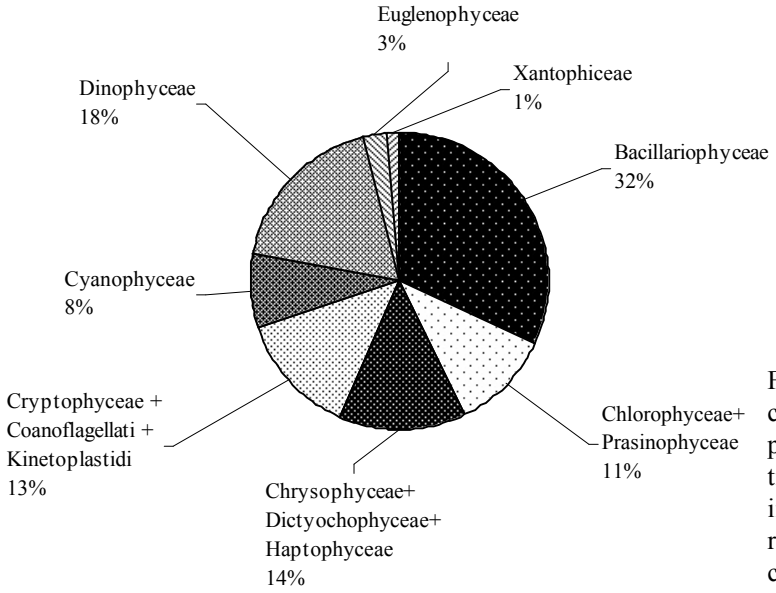

Figure 1. Relative contribution of the main phytoplankton groups in transitional water ecosystems in the Mediterranean eco-region (data from a number of credited lists of phytoplankton found in transitional ecosystems).

A proposed set of geometric shapes

We proposed a set of 23 geometric models to be used for the determination of microalgal biovolumes according to the principle of the most similar geometric shape. These shapes were applied at genus level. However, different geometric shapes were identified for species that shown significant deviation from typical

morphometric structure of the genus (as in the genera Protoperidinium, Ceratium, Navicula, Nitzschia ecc.). For these cases, the geometric shape was selected from those that had already been identified for the other genera. Moreover for species with evident elevations or extensions, such as apical and hypothecal horns, great capitate poles, conical apical elevations or very robust setae, biovolume was estimated adding them separately as cylinders or cones. Finally, for phytoplankton cell with unclear taxonomic position or cells classify with low taxonomic resolution biovolume was estimated associating to the cells the most similar geometric models selected from the set of proposed geometric models.

Application of geometric forms in different counting units

As a rule, these shapes should be applied to individual cells, even in coenobial, colonial, or filamentous species. However, when the single cell is not easy identifiable, the geometric form can be applied to an entire colony or fixed parts of filaments or parts of a colony (as in some Cyanophyceae genera).

Microscopic determination of linear dimensions.

The measurements should be carried out at high magnification (= 400 X) in order to minimize measurement errors. The methods for the concentration of the organisms should be non-destructive and free from vacuum or pressure forces. Utermohl's method, based on gentle sedimentation of phytoplankton in sedimentation chambers is recommended.

The linear dimensions can be measured manually using a micrometer during the quantification and identification of the phytoplankton cells. However, this is very time-consuming and requires great care, and therefore we suggest supporting microscopy with computerized image analysis systems. This favours the semi-automatic acquisition of the linear dimensions, cutting the time required.

Image analysis systems acquire in a short time an image of the field or part of the field of the plankton chambers. Using image processing software, it is then possible to determine, by outlining the contours of the single cells, morphometric information such as length, width, perimeter, etc. Some programmes are provided with applications for calculating cell volume directly, although this is limited almost exclusively to solids of rotation such as cylinders or spheres. In any case, even when it is supported by an image analysis system, microscopy limits the measurements per cell to two dimensions, and for some geometric forms the measurement of the third dimension (the thickness of the cell) is required. Measuring the third dimension of radially asymmetric cells is often a problem in microscopy. When possible, we suggest measuring this directly. Most good quality research microscopes are calibrated on the fine knob to indicate the distance travelled from high focal point on one side of the cells to the low focal point of the opposite, this distance is the thickness of the cell. Numerically abundant species are often seen from different viewpoints in microscope preparations so that every dimension is visible. In this case, the measured value or the average of a series of values can be used as the third dimension for each cell identified for a species. In contrast, rare species in natural samples may be seen from one angle only. In this case, the third dimension can be measured directly, going up and down with knob or after examination by light microscopy, by rolling the cell by gently tapping the coverslip with a pin-like object (Sun and Liu, 2003), or indirectly, if the aspect ratio of the species is known (Menden-Deur and Lessard, 2000). Finally, for species with maximum linear dimensions of less than 20 µm, we suggest following Verity et al. (Verity et al., 1992), for whom all cells can be associated with prolate spheroid forms in which depth equals width.

Size of sample required for calculation of biovolum.

We propose to estimate the biovolume of each counting unit included in the analysis. This approach requires great experimental effort but is necessary for an accurate estimate of the morphometric descriptors.

Results and Discussion

A check list for transitional waters ecosystems

Our first step in the determination of a set of geometric solids for calculating biovolumes was the identification of a list of phytoplankton found in transitional water ecosystems in the Mediterranean eco-region. This was in response to Smayda's suggestion (Smayda, 1978) that investigators should make representative sketches of each species to be measured before assigning it an appropriate geometric form. This helps to assess the appropriate shape and is useful subsequently as a guide to ensure that appropriate linear measurements are being made. Moreover, it was also useful in that it enabled us to draw up the list of phytoplankton species, combining information from various sources and reducing the current fragmentation of scientific knowledge on phytoplankton in transitional waters.

The proposed set of geometric shapes

We propose a set of 23 geometric forms, applied at genus level, for calculating biovolumes in phytoplankton guilds of transitional waters.

These forms were selected from the set of geometric models proposed in Hillebrand et al., (Hillebrand et al., 1999), Sun and Liu (Sun and Liu, 2003) and Edler (Edler, 1979). These sets of geometric forms were chosen because of their wider availability with respect to other papers reporting sets of geometric forms (such as Rott, 1981, Kovala and Larrance, 1966 etc.).

For each shape a schedule was drawn up (Annex I), which shows: the geometric shape, the formula for calculation, the number and the types of linear dimensions required, the number and the names of the genera to which the model was applied, some notes regarding the differences with respect to other proposed sets and potential difficulties in its application.

The selection of forms in this paper took account of the following: 1) the measurability of the dimensions, 2) the abundance and importance of the species, and 3) the need for useful descriptors.

Concerning the first point, we sought geometric shapes that were similar to the real shape of the organism but at the same time easy discernible and conveniently measurable with routine analysis. Simpler forms reduce the number of linear dimensions to be measured by light microscopy and consequently the time needed for each determination. Accordingly, from the sets of geometric forms analyzed, the simplest geometric forms proposed for transitional waters phytoplankton genera were selected. In our set, 13 are simple geometric solids, while 11 are the result of combining geometric solids. However, the latter were used in only 29% of the total genera identified (172 were associated with simple forms and 36 were associated with combined forms (Fig. 2A). Moreover, 8 of the combined forms were good for only 3 genera or less. Moreover, for 149 genera, measurement by light microscopy of linear dimensions in the X and Y viewing axes was sufficient, i.e. the additional measurement of cell thickness was not required (Fig. 2B).

On the second point, it is known that the degree of bias resulting from the use of an inappropriate model depends on the importance and the abundance of the species being analysed (Hillebrand et al., 1999). Accordingly, the geometric forms have been applied to the most important and abundant species of transitional ecosystems on the basis of the specific floristic list.

As for point three, most studies which have analyzed morphometric descriptors of phytoplankton guilds report group biovolume or biomass data into size classes. In these cases, the use of complex geometric forms to obtain a precise estimate of biomass is not necessary, provided that the use of simpler geometric forms would put the cells in the same or a strictly close size-class.

The counting units

Annex II shows the geometric model for each genus included in the phytoplankton list and the counting unit (CU) used for its calculation. This paper is one of the first to indicate explicitly the counting unit to which the geometric shape is applied. This is important in order to create less confusion and to allow more reliable comparison of data.

Accuracy of linear measurements by light microscopy

The measurement procedure will be the largest source of error in the estimate of biovolume if the samples are not handled in strict accordance with the standard protocol. The scale bar of the micrometers and of the image analysis system measurement module needs to be correctly calibrated at each magnification using a standard scale bar mounted on the microscopic objective.

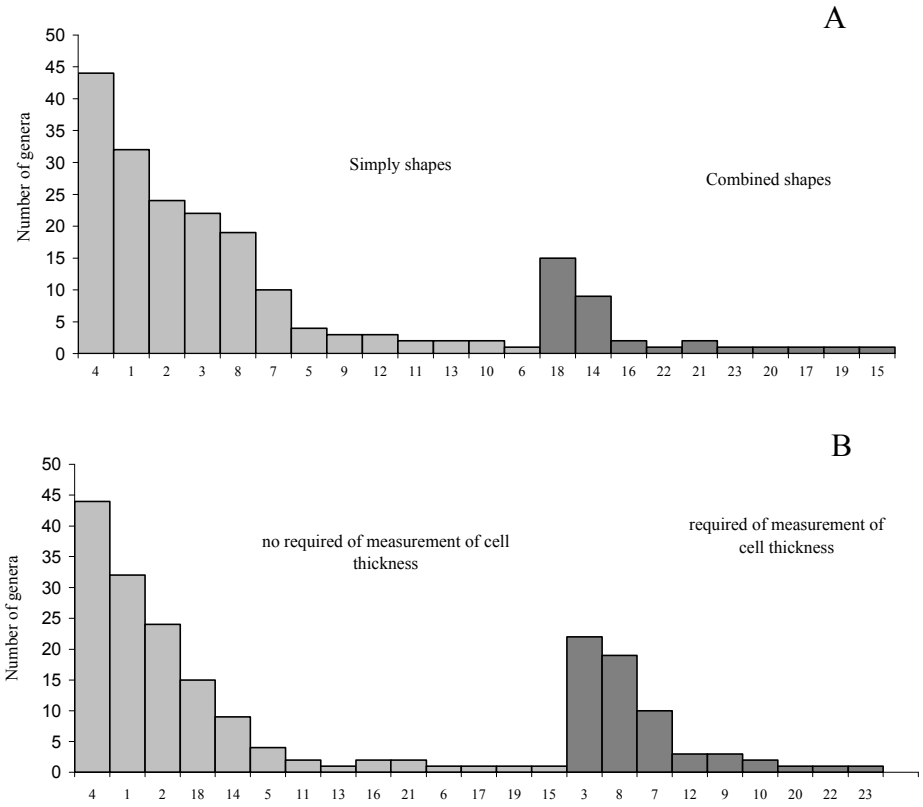

Figure 2. Number of genera associated with simple geometric forms (light grey) and combined geometric forms (dark grey). Number of genera that do not require (light grey) and that do require (dark grey) measurement of cell thickness (B). The numbers on the x-axis are taken from Annex I.

Light halos, which affect the measurements of the smallest cells, can be overcome by increasing the magnification of the microscope. In this protocol, we proposed to measure the linear dimensions using image analysis systems. Image Analysis systems include software that is able to measure semi-automatically a series of morphometric parameters of the phytoplankton cells. The system acquires a digital image of the microscopy field or part of the field, and the boundary of the objects (cells) to be measured is then traced on the digital image; the measurement module then provides measurements of morphometric features such as length, width, perimeter and circularity. In some cases, they provide measurements of biovolume directly, although this is usually limited to solids of rotation (spheres, cylinders, etc) (Sieracky, et. al., 1989). It is important to emphasize that the quality of the final image and the precision of the relative measurements is dependent on the quality of the original microscope image. No matter how good the digital or conventional camera is, it cannot produce outstanding images from a poorly configured microscope. At present the good quality of optical microscopes video cameras and image analysis systems and their relatively lower costs mean they are used in routine phytoplankton analysis more and more frequently. Moreover, the use of optical microscopes supported by image analysis

systems in the study of phytoplankton has a number of other advantages; digital images, for example, can be emailed for consultation and discussion, incorporated into other digital documents, or posted on a website, since they are easy to copy, store and archive. They can also be easily annotated with appropriate software for inclusion in presentations or archives. While photographic images can be scanned into a computer to produce digital images, digital image capture from the start saves time and effort. However, image analysis systems can improve the acquisition of the two linear dimensions of the visible plane of the cells but not of the "hidden dimension", that is, the thickness (or the "third dimension") of the cell, often required for the determination of the biovolume of some species. Various authors have suggested solutions, and in this protocol we have chosen to use some of these when it is not possible to measure the thickness of cells directly. This topic is currently the subject of much discussion, because none of the solutions proposed are able to provide more than an approximation of the third dimension. For example, the solution proposed by Verity et al. (Verity et al., 1992), which is to assimilate all phytoplankton cells to spheroids if their longest dimension is less than 20 μm, is valid for cells with spherical form, but it is no good when the cell height is significantly smaller than the width of the cell, as in some pennate diatoms. The aspect ratio, which takes into account the height/width, height/length and width/length ratios, is a good solution but unfortunately very few aspect ratios have been determined in phytoplankton cells or published in the literature. Thus, new solutions need to be tried out in order to achieve the most accurate possible estimate of the third dimension. A method proposed by McCartey and Loper (McCartey and Loper, 1989) involves adding a potentiometer to the adjustment knob of the light microscope in order to measure the distance from the focus on the bottom of the cells to the focus on the top. In addition, the new generation of inverted microscopes are supported with confocal microscopy and image analysis systems that are able to create 3D images and therefore to view and to measure phytoplankton cells in all dimensions. This is promising, but technological improvement is still needed.

Sample size

Given the variation of phytoplankton cell size in accordance with the season, life cycle, and physiological and environmental forcing, the application of "average" biovolume values for a certain species throughout the year and in different sites can produce significant inaccuracies. Biovolume needs to be calculated afresh for every experiment or set of samples. As regards the number of cells required to measure biovolume (i.e. the sample size), in our protocols we advise measuring the linear dimensions in all counting units. This contrasts with what has been proposed by other authors (Smayda, 1978, Hillebrand et al., 1999, Sun and Liu, 2003), where only a subset of cells is measured. For example Sun and Liu (Sun and Liu, 2003) advise measuring, for each phytoplankton sample, at least 10 randomly selected cells for each species; Smayda (Smayda, 1978) and Hillebrand et al. (Hillebrand et al., 1999) suggest 25 randomly selected cells for each species. However, we think that measuring the linear dimensions of a reduced number of cells renders the determination of morphometric descriptors less accurate, especially the body-size/abundance distributions, which are derived from biovolume or biomass data for each individual identified in the guild.

Conclusion

The use of morphometric parameters of phytoplankton for defining the health of transitional ecosystems status appears to be validated by the quantity of experimental evidence highlighting the sensitivity and symptomatic responses of these parameters to environmental forcing and pollution. However, a good descriptor needs to be not only sensitive and symptomatic of environmental risk but also comparable and reliable; this is possible only if there exists a standardize procedure for its

determination. Biovolume is one of the most important and basic morphometric descriptors of phytoplankton guilds. Accordingly, a standard protocol for its measurement is useful and necessary (Smayda, 1978; Hillebrand et al, 1999 Sun and Liu, 2003). However, biovolume determination is hampered by a series of methodological problems regarding various aspects, which have been widely discussed in the literature (from Kovala and Larrance, 1966 to Sun and Liu, 2003) and in this paper, and accurate measurement is still not always possible or practical. The most that can be achieved at present is an approximate estimate resulting from a compromise between the accuracy and the practicality of the determination. Practicality, understood as the necessity to minimize the effort of analytical determination above all in terms of the number of linear dimensions to measure by optical microscopy and thus the time required for each determination. Based on these considerations our protocol will be useful above all to overcome the problem of incomparable data resulting from the use of different sets of geometric shapes. At the same time, the implementation of new technologies must be encouraged in order to increase the accuracy of biovolume determination.

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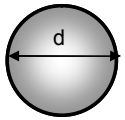
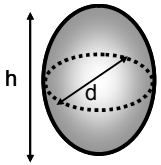
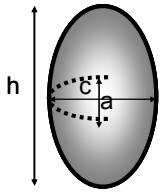
Annexes

Annex I - Schedule for geometric shapes showing: mathematical model, number and types of linear dimensions to measure, genera to which the shape is applied, notes on the

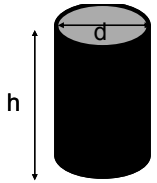
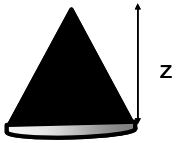
difference with respect to other sets of geometric shapes published and difficulties in application. (★ , very easy, ★★ easy, ★★★ difficult (often required the measure of the thickness of the cell,★★★★ very difficult (required measure of the thickness of the cell and an elevate number of linear dimensions).

Annex II – List of genera showing the counting units (C.U) for the application of geometric models. The table is sorted according to taxonomic group and lists the genera alphabetically. Species-specific deviations are also shown.

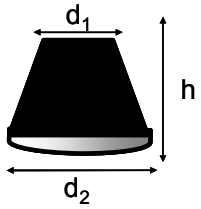
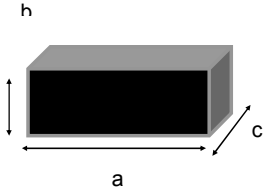
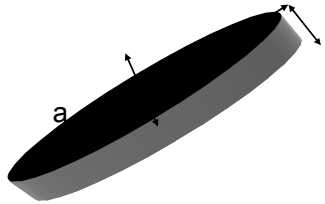
Annex I-

Geometric shape	**1) Sphere**	**2) Prolate spheroid**	**3) Ellipsoid**
Type of geometric shape	simple	Simple	simple
			
Formula for volume calculus	$V = \pi/6 \cdot d^3$	$V = \pi/6 \cdot d^2 \cdot h$	$V = \pi/6 \cdot a \cdot c \cdot h$
Number and type of linear dimension at light microscope	1: d= diameter	2 : d= diameter; h= height	3: a= length; c=width; h=height
Genera on which is applied	**Group 2***: Carteria, Halosphaera, Pterosperma, Trochiscia,* **Group 3:** *Braarudosphaera, Calyptrosphaera, Coccolithos, Coronosphaera, Dichtyocha, Ebria, Emiliana, Gephyrocapsa, Helladosphaera, Hermesinum, Marmiella, Parapedinella, Phaeocystis, Pontosphaera, Rhabdosphaera, Syracolithus, Syracosphaera.* **Group 4:** *Pseudobodo,* **Group 5:** *Chroococcus, Gloeocapsa, Microcystis, Synechoccocus, Woronichinia.* **Group 6:** *Goniodoma, Oblea, Porella, Protoceratium, Protoperidinium minutum.* **Group 8***: Meringosphaera.*	**Group 2***: Gonium, Oocystis, Pachysphaera, Pediastrium, Scenedesmus, Tetraselmis* **Group 3:** *Acanthoica, Apedinella, Chrysochromulina, Dinobryon, Halopappus, Monochrysis, Ophiaste, Padlova.* **Group 4***: Cryptomonas, Hilea.* **Group 5***: Coelosphaerium, Snowella.* **Group 6***: Cochlodinium, Oxyrrhis, Oxytoxum, Ptychodiscus, Torodinium, Warnowia, Amphidinium sphenoides, Dinophysis pulchella.*	**Group 1***: Amphora, Cymbella, Striatella, Surrirella gemma.* **Group 6:** *Alexandrium, Amphidinium, Blastodinium, Dinophysis, Exuviaella, Glenodinium, Gymnodinium, Gyrodinium, Lyngulodinium, Massartia, Nematodinium, Phalacroma, Pheopolykrikos, Polykrikos, Prorocentrum, Pyrophacus, Scrippsiella, Protoperidinium subinerme***. Group 7***:** *Astasia*
Number of genera and ratio (%) with total genera included in the check list	Number of genera: 32 + 1 specie Percentage: 14%	Number of genera: 24 + two species Percentage: 11.5%	Number of genera: 21 + two species Percentage: 10.1%
Note	The most simple form, requiring the measure of just one dimension. Calculated automatically by most image analysis software. The shape is also used in other sets of geometric forms for the above-mentioned genera. This form was applied above all to phytoflagellates groups of different taxonomic affiliation.	The shape is also used in other sets of geometric forms, in particular the set proposed by Hillebrand et al., (Hillebrand et al.,1999), except for the genus *Pediastrum*. For this genus, they proposed an elliptic prism applied to the whole colony. Previously Edler (Edler, 1979) had proposed a cylindrical form, also applied to the whole colony. We propose a prolate spheroid form applied to single cells. In this way, we overcome the problems related to the estimate of the third dimension required in both previous formulas; the width of each cell can be approximated to its thickness and to the thickness of the whole colony.	For some genera, there are some differences with respect to the geometric forms used in other sets. In particular, for *Cymbella* and *Amphora* the more recent papers have proposed a cymbelloid form, but some linear dimensions are very difficult to measure and are not practicable in routine analysis (two measures are in the transapical section of the cell). We argue that less bias results from using the more simple form of ellipsoid, although it can still overestimate biovolume by 35%. In dinophysis caudata appendix should added as cone.
Difficult for the application	★	★	★★★

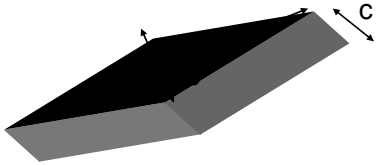
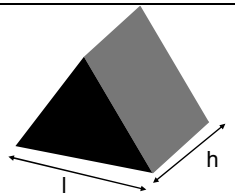
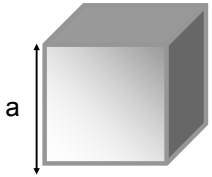
Annex I-continued

Geometric shape	**3.a) Ellipsoid -10%**	**4) Cylinder**	**5) Cone**
Type of geometric shape	simple	Simple	Simple
			
Formula for volume calculus	$V = (\pi/6 \cdot a \cdot c \cdot h)$-10%	$V = \pi/4 \cdot d^2 \cdot h$	$V = \pi/12 \cdot d^2 \cdot h$
Number and type of linear dimension at light microscope	3: a= length; c=width; h=height	2: d= diameter; h=height	2: d= diameter; z= height of cone
Genera on which is applied	**Group 1:** *Gyrosigma*	**Group 1***: Asterolampa, Asteromphalus, Bacteriastrum, Cerataulina, Chaetoceros, Coscinodiscus, Coscinosira, Cyclotella, Dactylosolen, Detonula, Ditylum, Ellerbeckia, Guinardia, Hemiaulus, Lauderia, Leptocylindrus, Lioloma, Melosira, Paralia, Planktoniella, Porosira, Proboscia, Rhizosolenia, Skeletonema, Stictocyclus, Surrirella, Thalassiosira, Thalassiothrix, Toxarium,* **Group 2***: Sticochoccus,* **Group 3***: Acanthosolenia, Calciosolenia, Ceratolithus,* **Group 4***: Bicosta,* **Group 5***: Anabaena, Anabaenopsis, Aphanizomenon, , Nodularia, Nostoc, Oscillatoria, Phormidium, Spirulina ,* **Group 6** *Amphisolenia.*	**Group 2***: Pyraminomonas, Calciopappus, Calycomonas.* **Group 6**: *Podolampas*
Number of genera and ratio (%) with total genera included in the check list	Number of genera: 1 Percentage: 0.5%	Number of genera: 43 Percentage: 21.0%	Number of genera: 4 Percentage: 1.9%
Note	This model was applied according to Edler's (1979) suggestion.	This model is easy to apply and is generally calculated automatically by most image analysis software. The shape is also used in other sets of geometric forms for the above-mentioned genera, with the exception of *Chaetoceros*. Hillebrand et al. (Hillenbrand et al., 1999) and Sun and Liu, (Sun and Liu 2003), proposed the elliptic prism form, whereas Edler (Edler, 1979) proposed the ellipsoid form. Here, we propose the cylindrical form, although it overestimates cell volume by as much as 40 % depending on the TA/AA ratio. Nevertheless, it is adequate for routine analysis (Montagnes and Franklin 2001).	This form was applied to just four genera, in accordance with Hillebrand et al. (Hillebrand et al., 1999).
Difficult for the application	★★ ★	★	★

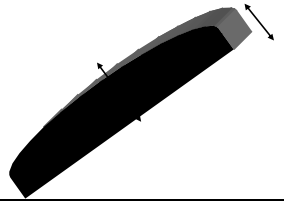
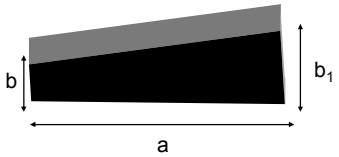
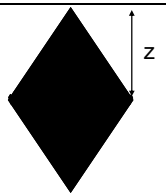
Annex I-continued

Geometric shape	**6) Truncated cone**	**7) Parallelepiped**	**8) Prism on elliptic base**
Type of geometric shape	simple	simple	simple
			
Formula for volume calculus	$V = \pi/12 \cdot h \cdot (d_1^2 + d_1 \cdot d_2 + d_2^2)$	$V = a \cdot b \cdot c$	$V = \pi/4 \ a \cdot b \cdot c$
Number and type of linear dimension at light microscope	3: d_1= minor diameter; d_2= minor diameter; h= height	3: a= length; b=width; c=thickness	3: a= length; b=width; c=thickness
Genera on which is applied	**Group 3:** *Pseudopedinella*	**Group 1:** *Asterionella, Bacillaria, Cymatopleura, Pinnularia, Rhabdonema, Synedra, Tabellaria, Thalassionema,* **Group 2:** *Pseudotetraedon, Tetraedon.*	**Group 1:** *Achnanthes, Amphiprora, Biddulphia, Campylodiscus, Cocconeis, Diatoma, Dimeregramma, Diploneis, Eucampia, Fragilaria, Fragilariopsis, Grammatophora, Lyrella, Mastogloia, Navicula, Stauroneis, Trachyneis* **Group 2:** *Closterium, Phacus.*
Number of genera and ratio (%) with total genera included in the check list	Number of genera: 1 Percentage: 0.5%	Number of genera: 10 Percentage: 4.7%	Number of genera: 19 Percentage: 8.61%
Note	This form was used just for one genus and was applied following the most similar geometric form in agreement with the most recent sets.	It is a very simple form that requires a limited number of linear measurements; however, its application can be difficult because it involves measuring the thickness of the cell. This was applied according to Hillebrand et al. (Hillebrand et al., 1999)	This form was introduced for the first time in Hillebrand et al.'s paper (Hillebrand et al., 1999) It provides a more accurate estimate of biovolume for the above-mentioned genera, because it is more similar to the real shape of the cell than the geometric form (parallelepiped) proposed by Edler (Edler, 1979). In this set, we are agree with Hillebrand et al.'s set of geometric forms, including the exceptions for *Navicula*. This genus is quite variable and therefore some species can require the use of a more appropriate geometric form according to their shape (such as a box or prism on a parallelogram base). The need to measure the third dimension can render the application of this model difficult.
Difficult for the application	★★	★★★	★★★

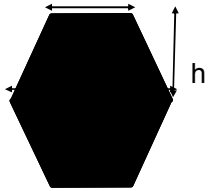
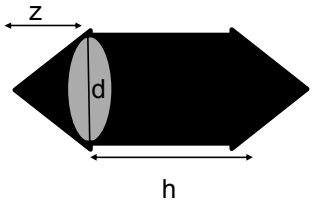
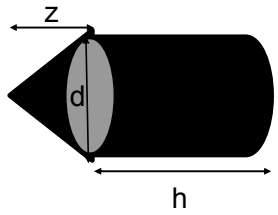
Annex I-continued

Geometric shape	**9) Prism on parallelogramm base**	**10) Prim on triangular base**	**11) Cube**
Type of geometric shape	simple	simple	Simple
			
Formula for volume calculus	$V = ½ a \cdot b \cdot c$	$V= ½ l \cdot m \cdot h$	$V = a^3$
Number and type of linear dimension at light microscope	3: a= length; b=width; c=thickness	3: m= height of a triangle; l=length of one side; h= heoght	1: a=length of one side
Genera on which is applied	**Group 1:** *Nitzschia, Pleurosigma, Pseudonitzschia*	**Group 1:** *Bellerochea, Triceratium*	**Group 2:** *Crucigenia;* **Group 5:** *Merismopedia*
Number of genera and ratio (%) with total genera included in the check list	Number of genera: 3 Percentage: 1.43%	Number of genera: 2 Percentage: 0.95%	Number of genera: 2 Percentage: 0.95%
Note	Like the previous form, it was introduced in Hillebrand et al's paper (Hillebrand et al., 1999). However, the genus *Nitzschia* includes species of different form. In this case, the most similar geometric forms should be used; accordingly, the sigmoid or rhombic species can be calculated as prisms on a parallelogram base, elliptic species as elliptic prisms, and linear species as boxes.	This form was introduced by Kononen et al., (Kononen et al. 1984). Before this paper, the biovolume of these genera was calculated using a cylindrical form. However, to associate a triangular diatom with a cylindrical form is inappropriate, since this leads to an overestimation of cell volume of about 80%. This form is also cited in the Hillebrand et al., (Hillebrand et al., 1999) and Sun and Liu (Sun and Liu, 2003) papers. The need to measure the third dimension can render its application difficult.	This model was applied to the same genera proposed in other sets of geometric forms.
Difficult for the application	★★★	★★★	★

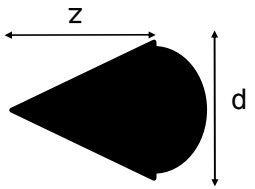
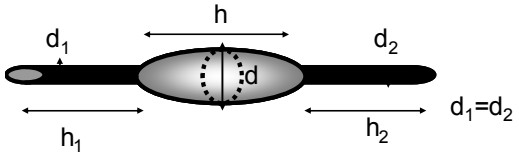
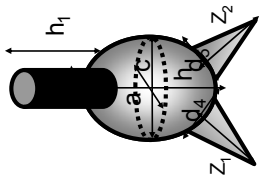
Annex I-continued

Geometric shape	**12) Half elliptic prism**	**13) Truncated pyramid**	**14) Two cone**
Type of geometric shape	simple	simple	Combined
			
Formula for volume calculus	$V = \pi/4 \ a \cdot b \cdot c$	$V= [(b_1+b \sqrt{\cdot} \ b_1 \cdot b) \cdot a]/3$	$V = \pi/6 \cdot d^2 \cdot h$
Number and type of linear dimension at light microscope	3: a= length; b=width; c=thickness	3: a= length; b=width minor base; b_1=width major base	2: d= diameter; z= height of cone
Genera on which is applied	**Group 1:** *Epithemia, Eunotia, Phaeodactylum*	**Group 1:** *Gomphonema, Licmophora*	**Group 2***: Chlorogonium, Monoraphidium, Schroderia,* **Group 6***: Heterocapsa, Oxyphysis, Peridinium, Protogonyaulax, Protoperidinium, Ceratium fusus, Gonyaulax spinifera, Gonyaulax scrippsiae, Oxitoxum viride.* **Group 7***: Lepocinclis*
Number of genera and ratio (%) with total genera included in the check list	Number of genera: 3 Percentage: 1.43 %	Number of genera: 2 Percentage: 0.95 %	Number of genera: 9 + 4 species Percentage: 4.30 %
Note	For this form, we agree with the proposal by Hillebrand et al. (Hillebrand et al., 1999) Edler (Edler, 1979) proposed ellipsoid forms for the first two genera, whereas Sun and Liu (Sun and Liu, 2003) proposed the sickle-shaped prism, but only for the Eunotia genus (the other two genera are not included in their list). We consider the half elliptic prism more suitable, because it has the same number of linear dimensions required but is more similar to the real shape of the cell.	The model differs by those proposed by other authors, e.g. Hillebrand et al. (Hillebrand et al., 1999), who proposed the gomphonemoid form. This form requires four linear dimensions, some of them very difficult to measure. For example, in his work, the linear measurement 'f' is the length of the transapically widest part of the head pole. Sun and Liu (Sun and Liu, 2003) proposed the same form but only for the *Gomphonema* genus; for the *Licmophora* genus, they proposed the sickle-shaped cylinder form. However, the volume of the sickle-shaped cylinder proposed by Sun and Liu for *Licmophora* can overestimate the volume, because it considers the two transapical views of the cell to be similar. We propose the truncated pyramid shape for both genera, because: the minor base, the major base and the height of a truncated pyramid with a square base is easier to measure and the lower accuracy of the shape is balanced by the greater replicability of the data.	This follows Hillebrand et al.'s (Hillebrand et al., 1999) set of geometric forms. Other sets of geometric forms reported only a few genera of those indicated. For example, Edler, (Edler, 1979), indicated the 2-cone form but reported just the first two genera in group 2, whereas Sun and Liu reported no genera for group 2. For ***Group 6*** they applied this form to the *Protoperdinium* genus but for *Peridium* they used the prolate spheroid form. The *Protoderidium* genus is highly variable in shape and for some species different geometric models can be required. Genarally in species with evident hypotecal horns, they add to be add as cones or cylinders Moreover, this model was also applied to 4 species of *Dinophyceae* that presented different shapes with respect to their genus. It is a combined solid of rotation, very easy to apply.
Difficult for the application	★★★	★★	★

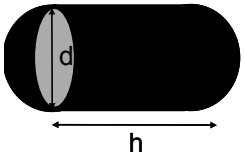
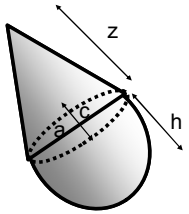
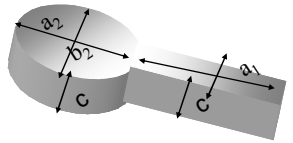
Annex I-continued

Geometric shape	**15) Two truncated cone**	**16) Cylinder+2 cones**	**17) Cylinder + cone**
Type of geometric shape	Combined	Combined	Combined
			
Formula for volume calculus	$V = \pi/6 \cdot h \cdot (d_1^2 + d_1 * d_2 + d_2^2)$	$V = (\pi/4 \cdot d^2 \cdot h) + (\pi/6 \cdot d^2 \cdot z)$	$V = (\pi/4 \cdot d^2 \cdot h) + (\pi/12 \cdot d^2 \cdot z)$
Number and type of linear dimension at light microscope	3: d_1= minor diameter; d_2= minor diameter; h= height	3: d= diameter; h=height; z= height of cone	3: d= diameter; h=height; z= height of cone
Genera on which is applied	**Group 2:** *Staurastrum*	**Group 2***: Actinastrum, Ankistrodesmus*	**Group 7***: Eutreptia, Eutreptiella*
Number of genera and ratio (%) with total genera included in the check list	Number of genera: 1 Percentage: 0.47 %	Number of genera: 2 Percentage: 0.95 %	Number of genera: 2 Percentage: 0.95%
Note	This follows other sets of geometric forms. It is a combined solid of rotation, very easy to apply.	This follows Hillebrand et al.'s set of geometric models 's (Hillebrand et al., 1999). We consider this form accurate enough. Edler (Edler, 1979) proposed the ellipsoid form for *Ankistrodesmus*, whereas Sun and Liu used two different geometric forms: the prolate spheroid for *Actinastrum* and the sickle-shaped cylinder for *Ankistrodesmus*. It is a combined solid of rotation, easy to apply, but some difficulties can arise when measuring the height of the cone	This follows Hillebrand et al.'s (Hillebrand et al., 1999) and Edler's (Edler, 1979) sets of geometric forms. It is a combined solid of rotation, easy to apply. We do not consider the suggestion of Sun and Liu (Sun and Liu, 2003) for *Eutreptia* (cylinder +cone +half sphere)**,** because some measurements are very difficult to make in routine analysis and in our opinion do not contribute to the precision of the biovolume estimate
Difficult for the application	★	★★	★★

Annex I-continued

Geometric shape	**18) Cone + half sphere**	**19) Spheroid + 2 cylinders**	**20) Ellipsoid + 2 cones + cylinder**
Type of geometric shape	Combined	Combined	Combined
			
Formula for volume calculus	$V = \pi/12 \cdot d^2 \cdot (z+d)$	$V= (\pi/6 \cdot d^2 \cdot h) + (\pi/2 \cdot d_{1/2}^2 \cdot h_{1/2})$	$V= (\pi/6 \cdot a \cdot c \cdot h) + (\pi/6 \cdot d_{4/5}^2 \cdot z_{1/2}) + (\pi/4 \cdot d_1^2 \cdot h_1)$
Number and type of linear dimension at light microscope	2: d= diameter; z= height of cone	6: $d_{1/2}$= average value of two cylinder diameters; $h_{1/2}$= average value of two cylinder heights; d=diameter of spheroid; h= height of spheroid	9: a= length; c=width;h=height of ellipsoid; d_1=diameter; h_1= height; $d_{4/5}$= average value of two cone diameters; $z_{1/2}$= average value of two cone heights
Genera on which is applied	**Group 3:** *Ochromonas, PrymneSunm.* **Group 4***: Chroomonas, Criptaulax, Leucocryptos, Micromonas, Plagioselmis, Rhinomonas, Rhodomonas, Spumella.* **Group 6:** *Diplopsalis, Goniaulax, Minuscula, Pachidinium, Pronoctiluca, Gyrodium lachrymae, Prorocentrum micans, Prorocentrum triestinum.*	**Group 1**: *Cylindrotheca*	**Group 6***: Ceratium*
Number of genera and ratio (%) with total genera included in the check list	Number of genera: 15 + 3 species Percentage: 7.65 %	Number of genera: 1 Percentage: 0.47 %	Number of genera: 1 Percentage: 0.47%
Note	This form was used for phytoflagellate and *Dinophyceae* genera. It was applied in accordance with other sets of geometric forms analyzed in this work and was also used for some *Dinophyceae* species that differ from the usual shape of their genera. It is a combined solid of rotation, very easy to apply.	This form was used only for the *Cylindrotheca* genus. It follows the form proposed by Hillebrand et al. (Hillebrand et al., 1999). Its application can be difficult due to the high number of linear dimensions to measure.	All authors agree that calculating biovolume for the genus *Ceratium* requires a series of complex combined forms. The ellipsoid+2 cones+cylinders is the most frequently used form, where the two cones are the hypothecal horns. However, the number of cones to add can vary in relation to the number of hypothecal horns present in the species. Some species showed a completely different shape from the genus; in this case other forms have to be used (for example, cone+cone for *Ceratium fusus*). This geometric solid is more difficult to apply, due to the high number of linear dimensions and the fact that it requires the measurement of the thickness of the cell.
Difficult for application	★	★★★★	★★★★

Annex I-continued

Geometric shape	**21) Cylinder + 2 half sphere**	**22) Half ellipsoid + cone on elliptic base**	**23) Parallelepiped + elliptic prism**
Type of geometric shape	Combined	Combined	Combined
			
Formula for volume calculus	$V = (\pi/4 \cdot d^2 \cdot h) + (\pi/6 \cdot d^3)$	$V = (\pi/12 \cdot a \cdot c) \cdot (h+z)$	$V= c\ (a_1 \cdot b_1 + \pi/4\ a_2 \cdot b_2)$
Number and type of linear dimension at light microscope	2: d=diameter; h= height	4: a= length; c=width;h=height; z= height of cone	5: a_1= length of parallelepiped; b_1=width of parallelepiped; c=thickness; a_2= length of prism; b_2=width of prism
Genera on which is applied	**Group 1:** *Corethron, Stephanodiscus*	**Group 7***: Euglena*	**Group 1:** *Climacosphenia*
Number of genera and ratio (%) with total genera included in the check list	Number of genera: 2 Percentage: 0.95 %	Number of genera: 1 Percentage: 0.47 %	Number of genera: 1 Percentage: 0.47 %
Note	Its application followed other sets of geometric formula, where the genera were reported. It is a combined solid of rotation, very easy to apply.	This shape was applied in accordance with Hillenbrand et al. (Hillebrand et al., 1999), but only to the *Euglena* genus. The *euglenoid* algae are variable in shape and cross section. Most *euglena* are not round, but flattened in cross section. Therefore, the obtuse pole is calculated as a half ellipsoid and the acute pole as a cone with an elliptic base. Edler (Edler, 1979) and Sicko-Goad et al. (Sicko-Goad at al., 1977) propose a similar shape with a cylinder instead of a cone. Finally, Sun and Liu (Sun and Liu, 2003) proposed the cylinder+half sphere+cone, but we do not agree with this geometric shape because it often does not coincide with the real shape of the cell.	It was in accordance with Hillebrand et al.'s (Hillebrand et al., 1999) set of geometric shapes.
Difficult for the application		★★★	★★★★

Annex II

Genus	shape	C.U.	Genus	shape	C.U.
Group 1			Striatella	3	colony
Achnanthes	8	cell	Surrirella	4	cell
Amphiprora	8	cell		3 [1]	cell
Amphora	3	cell	Synedra	7	cell
Asterionella	7	cell	Tabellaria	7	cell
Asterolampa	4	cell	Thalassionema	7	cell
Asteromphalus	4	cell	Thalassiosira	4	cell
Bacillaria	7	cell	Thalassiothrix	4	cell
Bacteriastrum	4	cell	Toxarium	4	cell
Bellerochea	10	cell	Trachyneis	8	cell
Biddulphia	8	cell	Triceratium	10	cell
Campylodiscus	8	cell	**Group 2**		
Cerataulina	4	cell	Actinastrum	16	cell
Chaetoceros	4	cell	Ankistrodesmus	16	cell
Climacosphenia	23	cell	Carteria	1	cell
Cocconeis	3	cell	Chlorogonium	14	cell
Corethron	21	cell	Closterium	8	cell
Coscinodiscus	4	cell	Crucigenia	11	cell
Coscinosira	4	cell	Gonium	2	cell
Cyclotella	4	cell	Halosphaera	1	cell
Cylindrotheca	19	cell	Monoraphidium	14	cell
Cymatopleura	7	cell	Oocystis	2	cell
Cymbella	3	cell	Pachysphaera	2	cell
Dactylosolen	4	cell	Pavlova	2	cell
Detonula	4	cell	Pediastrium	2	colony
Diatoma	8	cell	Pseudotetraedon	7	cell
Dimerogramma	8	cell	Pterosperma	1	cell
Diploneis	8	cell	Pyramimonas	5	cell
Ditylum	4	cell	Pyraminomonas	5	cell
Ellerbeckia	4	cell	Scenedesmus	2	cell
Epithemia	12	cell	Schroderia	14	cell
Eucampia	8	cell	Staurastrum	15	cell
Eunotia	12	cell	Sticochoccus	4	cell
Fragilaria	8	cell	Tetraedon	7	cell
Fragilariopsis	8	cell	Tetraselmis	2	cell
Gomphonema	13	cell	Trochiscia	1	cell
Grammatophora	8	cell	**Group 3**		
Guinardia	4	cell	Acanthoica	2	cell
Gyrosigma	3a	cell	Acanthosolenia	4	cell
Hemiaulus	4	cell	Apedinella	2	cell
Lauderia	4	cell	Braarudosphaera	1	cell
Leptocylindrus	4	cell	Calciopappus	5	cell
Licmophora	13	cell	Calciosolenia	4	cell
Lioloma	4	cell	Calycomonas	5	cell
Lyrella	8	cell	Calyptrosphaera	1	cell
Mastogloia	8	cell	Ceratolithus	4	cell
Melosira	4	cell	Chrysochromulina	2	cell
Navicula	8	cell	Coccolithos	1	cell
Nitzschia	9	cell	Coronosphaera	1	cell
Paralia	4	cell	Dichtyocha	1	cell
Phaeodactylum	12	cell	Dinobryon	2	cell
Pinnularia	7	cell	Ebria	1	cell
Planktoniella	4	cell	Emiliana	1	cell
Pleurosigma	9	cell	Gephyrocapsa	1	cell
Porosira	4	cell	Halopappus	2	cell
Proboscia	4	cell	Helladosphaera	1	cell
Pseudonitzschia	9	cell	Hermesinum	1	cell
Rhabdonema	7	cell	Mamiella	1	cell
Rhizosolenia	4	cell	Monochrysis	2	cell
Skeletonema	4	cell	Ochromonas	18	cell
Stauroneis	8	cell	Ophiaster	2	cell
Stephanodiscus	21	cell	Parapedinella	1	cell
Stictocyclus	4	cell	Phaeocystis	1	cell

Genus	shape	U.C	Genus	shape	U.C.
Pontosphaera	1	cell	Lyngulodinium	3	cell
Prymnesium	18	cell	Massartia	3	cell
Pseudopedinella	6	cell	Minuscula	18	cell
Rhabdosphaera	1	cell	Nematodinium	3	cell
Syracolithus	1	cell	Oblea	1	cell
Syracosphaera	1	cell	Oxyphysis	14	cell
Group 4			Oxyrrhis	2	cell
Bicosta	4	cell	Oxytoxum	2	cell
Chroomonas	18	cell		14 [8]	cell
Criptaulax	18	cell	Pachidinium	18	cell
Criptoficee undet.	4 or 1	cell	Peridinium	14	cell
Cryptomonas	2	cell	Phalacroma	3	cell
Hilea	2	cell	Pheopolykrikos	3	cell
Leucocryptos	18	cell	Podolampas	5	cell
Micromonas	18	cell	Polykrikos	3	cell
Plagioselmis	18	cell	Porella	1	cell
Pseudobodo	1	cell	Pronoctiluca	18	cell
Rhinomonas	18	cell	Prorocentrum	3	cell
Rhodomonas	18	cell		18 [9]	cell
Spumella	18	cell	Protoceratium	1	cell
Group 5			Protogonyaulax	14	cell
Anabaena	4	filament	Protoperidinium	14	cell
Anabaenopsis	4	filament		14 [10]	cell
Aphanizomenon	4	filament		14 [11]	cell
Aphanizonium	4	filament		3 [12]	cell
Chroococcus	1	cell		1 [13]	cell
Coelosphaerium	2	colony	Ptychodiscus	2	cell
Gloeocapsa	1	single, colony	Pyrophacus	3	cell
Merismopedia	11	cell	Scrippsiella	3	cell
Microcystis	1 [10]	part of colony	Torodinium	2	cell
Nodularia	4	filament	Warnowia	2	cell
Nostoc	4	cell	**Group 7**		
Oscillatoria	4	filament	Astasia	3	cell
Phormidium	4	filament	Euglena	22	cell
Snowella	2	colony	Eutreptia	17	cell
Spirulina	4	filament	Eutreptiella	17	cell
Synechoccocus	1	cell	Lepocinclis	14	cell
Tetrapedia	1	cell	Phacus	8	cell
Woronichinia	1	colony	**Group 8**		
Group 6			Meringosphaera	1	cell
Alexandrium	3	cell	**Exceptions:**		
Amphidinium	3	cell	[1] Exception: *Surrirella gemma*		
	2 [2]	cell	[2] Exception: *Amphidinium sphenoides*		
Amphisolenia	4	cell	[3] Exception: Ceratium fusus cone+cone. The genus *Ceratium* is quite variable in shape. We poposed according to Hillebrand et al., 1999 to calculate biovolume of central body as ellipsoid, the hypotecal horns as cones and the apical horn as clinders.		
Blastodinium	3	cell			
Ceratium	20	cell			
	14 [3]	cell			
Cochlodinium	2	cell	[4] Exception: *Dinophysis caudata* add horn as a cone		
Dinophysis	3	cell	[5] Exception: *Dinophysis pulchella*		
	3 [4]	cell	[6] Exception: *Gonyaulax spinifera , Gonyaulax scrippsiae*		
	2 [5]	cell	[7] Exception: *Gyrodium lachrymae*		
Diplopsalis	18	cell	[8] Exception: *Oxytoxum viride*		
Exuviaella	3	cell	[9] Exception: *Prorocentrum micans, Prorocentrum triestinum*		
Glenodinium	3	cell	[10] Exception: *Prototeridinium brevipes, Protoperidinium divergens* add horns as cones		
Goniaulax	18	cell			
	14 [6]	cell	[11] Exception: *Protoperidinium elegans* : two hypotechal horns as cones, central body as cone and apical horn as cylinder		
Goniodoma	1	cell			
Gymnodinium	3	cell	[12] Exception: *Protoperidinium subinerme*		
Gyrodinium	3	cell	[13] Exception: *Protoperidinium minutum*		
	18 [7]	cell	[14] Exception: *species that form non–uniform colonies (as in the Microcystis genus), the biovolume of the whole colony would be measured as the sum of the biovolumes of smaller, spherical areas.*		
Heterocapsa	14	cell			
Katodinium	17	cell			