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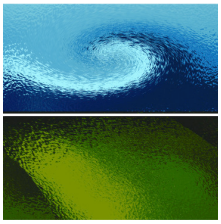

RESEARCH ARTICLE

Cell biovolume and surface area in phytoplankton of Mediterranean transitional water ecosystems: methodological aspects

M. R. Vadrucci[1*], C. Mazziotti[2], A. Fiocca[3]

[1]ARPA Puglia-Regional Agency for the Environmental Prevention and Protection, Department of Lecce, Via Miglietta 2, 73100 Lecce, Italy.
[2]ARPA Emilia-Romagna - Regional Agency for the Environmental Prevention Viale Vespucci, 2, 47042 Cesenatico, Italy.
[3]Department of Biological and Environmental Sciences and Technologies, University of Salento Via Provinciale Lecce-Monteroni, 73100 Lecce, Italy.

*Corresponding author: E-mail address: m.vadrucci@arpa.puglia.it

Abstract

1 - A series of experimental researches highlighted the role of morphometric parameters of phytoplankton guilds as descriptors of the ecological status of transitional water ecosystems (TWs).

2 - However, at present, standardized or common methodologies for their use do not exist. In this work, we develop a procedure for the determination of biovolume and surface area in phytoplankton guilds of Mediterranean TWs.

3 - Phytoplankton biovolume and surface area are included among the most studied morphometric descriptors. They 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 light microscope.

4 - Here, a set of geometric models is suggested for calculating the cell biovolumes and surface area of 235 phytoplankton genera, deriving from the analysis of 869 phytoplankton species, found in transitional water ecosystems of the Mediterranean Ecoregion. The equations were designed to minimize the effort of microscopic measurements.

5 - The similarities and differences between the geometric models here proposed and previously published are discussed.

Keywords: morphometric descriptors, cell biovolume, surface area, phytoplankton, transitional water ecosystems.

Introduction

The Water Framework Directive (2000/60/EC) sees phytoplankton as one of the biological elements for evaluating the ecological status of transitional aquatic ecosystems. However, this regulation provides only general indications on the relative descriptors to be included in the monitoring programs. Descriptors are measurable variables of a quality elements that are able to respond at environmental pressures. In the WFD the measurable variables indicated as phytoplankton descriptors are taxonomic composition, number of species, numerical abundance including abundance of harmful algae species and biomass. These descriptors are directly associated to taxonomic identification of phytoplankton species.

However, for the phytoplankton, there is currently much debate among scientists over the suitability of descriptors and indices based on the taxonomic recognition of the species, indicated as "taxonomic descriptors", for which the regulations also provide classification criteria, rather than descriptors relating to functional trait of the species, also indicated as "non taxonomic descriptors" such as body–size related descriptors. They concerned with aspects relating to the morphological and dimensional characteristics of the phytoplankton organisms such as: biovolume, surface area, surface area/volume ratio, body size abundance spectra (Sheldon *et al.*, 1972), fractionated biomass (Sieburth,1979), morphological functional groups (Reynolds, 1997).

Individual size affects most aspects of a phytoplankton cell, since allometric relationships link cell size to processes such as nutrient uptake (Munk and Riley, 1952), light affinity (Ruiz *et al.*, 1996; Cermeno *et al.*, 2005), photosynthesis and respiration (Banse and Mosher, 1980), settling rates, physical transport (Semina, 1968; Jackson, 1989), and plant-herbivore interactions (McCauley and Downing, 1985; Sommer *et al.*, 2000). Size dependency of both metabolic rates and cell density regulation has important implications for species coexistence relationships. Consistently, at the phytoplankton guild level, common patterns of population abundance (Duarte *et al.*, 1987) and biomass (Rodriguez and Mullin, 1986; Cavender-Bares *et al.*, 2001) with individual cell size have been observed. Various structural factors in the abiotic environment, including water dynamics (Rodriguez *et al.*, 2001; Serra *et al.*, 2003), depth of photic zone (Gaedke, 1992), trophic state and nutrient concentration (Sprules and Munawar, 1986), have also been found to explain the patterns of variation of phytoplankton size structure on spatial and temporal scales.

There are much experimental evidences showing the sensitivity of dimensional structures to environmental forcing and human-generated pressures. Recent works, carried out in marine coastal areas, have demonstrated the existence of significant variations in the size fractions as a function of the trophic state of the ecosystems as in phytoplankton as in macrobenthos communities (Basset *et al.*, 2004; Glover *et al.*, 1985; Ponti *et al.*, 2009; Vadrucci *et al.*, 2002; Watson and Kalff, 1980). Other studies have shown how the size-abundance distribution of the phytoplankton communities varies significantly in relation to human-generated or environmental forcing (Suttle *et al.*, 1988; Echevarrìa *et al.*, 1990; Gaedke, 1992; Rojo and Rodriguez, 1994; Cottingham, 1999; Sin *et al.*, 2000; Perez-Ruzafa *et al.*, 2002; Quinones *et al.*, 2003; Sabetta *et al.*, 2005, Cermeño *et al.*, 2005). Concerning their use as descriptors of ecological status of transitional aquatic ecosystems, morphometric and body-size related descriptors seem to have certain advantages (related to their determination, inter-calibration, to make habitat comparisons), as already demonstrated for other biological quality elements (Basset *et al.*, 2012) which make them potentially suitable for environmental monitoring programs.

However, currently lack sufficient data and methodologies comparable to those existing for taxonomic studies, for which in contrast, there are standardized or at least common methodological procedures. On the procedural and methodological level the development of morphometric and body size related descriptors depends on the capacity to support biometric and ecological knowledge with a technologically adequate detection tools. Drawing up a protocol for the determination of phytoplankton morphometric and body size related descriptors requires several steps; one of the first concerns with the

definition of a common procedure for the determination of the biovolume and surface area of the phytoplankton cells. An accurate biovolume determination is fundamental for the estimation of phytoplankton biomass and other derived body size related descriptors, such as functional groups, size spectrum. On the other hand, phytoplankton biomass, expresses directly by biovolume or in term of carbon units, is included in the list of descriptors for phytoplankton in the WFD for inland waters. Biovolume can be estimated by various automatic or semiautomatic methods, including those based on electronic particle counting (Boyd and Johnson, 1995), flow cytometry (Collier, 2000) and automatic microscopic image analysis. Nevertheless, these methods have several drawbacks that restrict their application to specific topics. For example, both electronic particle counting and flow cytometry yield a very low taxonomic resolution, limited to the analysis of phytoplankton size classes or pigment composition. Moreover, coulter counters tend to increasingly underestimate cell volume with increasing 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 communities is less feasible because they are morphologically more variable than bacteria (Sieracki *et al.*, 1989; Psenner, 1993). Other techniques, such as computer tomography of single cells, holographic scanning technology or electronic microscopy 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 and surface area in routine analysis is based on the association of phytoplankton taxa with three-dimensional geometric forms. This involves the direct measurement by light microscopy of the linear dimensions required for calculating the associated geometric volumes and areas. 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 complex geometric forms that are similar to the actual shape but require long analysis, (Kovala and Larrance, 1966), or simple geometric forms that are less accurate but can be analysed rapidly (Edler, 1979; Olenina *et al.*, 2006). Many different sets of geometric shapes can be found in the literature regarding regional areas (Edler, 1979; Olenina *et al.*, 2006), ecosystem types (marine or freshwater ecosystems) or communities (phytoplankton or phyto-benthos) (Kovala and Larrance, 1966; Edler, 1979; Rott, 1981; Kononen *et al.*, 1984). Recent studies also propose an extended set of geometric shapes for phytoplankton of different aquatic ecosystems (Hillebrand *et al.*, 1999; Sun and Liu, 2003). However, since WFD divided aquatic ecosystems into 6 categories: running waters, lakes, transitional waters, coastal waters and ground waters, the availability of guidelines and procedure specific for every aquatic ecosystem categories is becoming increasingly important.

The general aim of the this work is to propose a protocol for calculating algal cell biovolume and surface area of phytoplankton species in transitional aquatic ecosystems of the Mediterranean Ecoregion. The specific objectives are:

- To propose a specific set of geometric equations to estimate cell biovolume and surface area of phytoplankton species;
- To identify the counting unit for the application of the geometric shapes;
- To establish the modality to make the linear measurements with the inverted microscope;
- To quantify the number of cells to measure for each species.

Material and Methods

Compilation of the unified floristic list

The compilation of the unified floristic list at species level was draw up on the basis of a series of floristic lists coming from a number of different types of transitional water ecosystems of the Mediterranean Ecoregion, including salt-pans, river deltas, lagoons and coastal lakes. These lists came from our own projects (in particular from the 18 transitional ecosystems analyzed in fall 2004 and spring 2005 during the TWReferenceNET project) and from the literature (see Appendix 2). In the case of our projects, the lists were drawn up by laboratories with experience in the field of phytoplankton analysis. The quality assurance of the data set was guaranteed or trough the development of a rigid working protocol for the sampling and sampling analysis, supported by workshop and training sessions for standardize sampling, methods and level of expertise, thus minimizing laboratory-specific biases in the data-set, or using lists coming from papers published only on peer-reviewed journals The taxonomic position was checked on the basis of the AlgaeBase web-site (Guiry and Guiry, 2007) and the most recent literature supporting it.

Geometric shapes

We propose a set of 22 geometric shapes to be used for the determination of phytoplankton cell biovolume and surface area. Geometric shapes were applied at the genus level, even though different shapes were selected for species that showed a significant deviation from the typical morphometric structure of the genus (e.g., *Protoperidinium, Ceratium, Navicula, Nitzschia* etc.). For species with apical and hypothecal horns, large capitate poles, conical apical elevations or very robust setae, cell biovolume was estimated by adding them separately as cylinders or cones.

Geometric shapes are applied to solitary cells, even in coenobial, colonial, or filamentous species. However, when the single cell is not easy identifiable, the geometric shape can be applied to the entire colony or fixed parts of the colony as in some genera of the Cyanophyceae. Geometric shapes and equations proposed in this work are taking into account the works of Edler (1979), Hillebrand *et al.* (1999), Sun and Liu (2003) and Vadrucci *et al.* (2007).

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

Cell measurements should be made under an inverted microscope, with a specific magnification in relation to cell size, operating in phase contrast optics following Utermöhl, 1958.

Linear dimensions can be measured manually with an eyepiece micrometer during the identification and enumeration of the phytoplankton cells. However, this procedure is very time consuming and therefore, we suggest using a computerized image analysis system to support the acquisition of morphometric data.

Microscopy limits the measurements of the counting unit to two visible dimensions, even though the measure of biovolume or surface area of some species, requires the measurement of a third dimension (the thickness of the cell or hidden dimension-HD). Measuring a third dimension of radial asymmetric cells is often a problem in microscopy. When possible, we suggest measuring it directly. Most good quality research microscopes are calibrated to indicate the distance travelled from high

focal point on one side of the cell to the low focal point of the opposite. The thickness is given from the distance of the high and low focus position on the cell. Besides, the third dimension can be measured, after counting, by turning the cell by gently tapping the coverslip with a pin-like object (Sun and Liu, 2003). Finally, numerically abundant species can be often seen from different sides. In this case, the value of the median of a series of measured values can be used for estimating the third dimension for each cell identified for a species. The third dimensions can be estimated also indirectly from bibliographic values related to the aspect ratio (Olenina *et al.*, 2006) of species (Menden-Deuer and Lessard, 2000). Finally, for species with maximum linear dimensions less than 20 µm, we advise following Verity *et al.* (1992), suggesting that all cells can be associated with prolate spheroid forms in which depth equals width.

How many cells to measure

To determine the minimum number of cells to be measured for each taxon in order to have an accurate estimate of the biovolume and surface area of phytoplankton cells, we have consider phytoplankton data collected in 18 transitional water ecosystems of Mediterranean Ecoregion. A total of 321 sampling stations were samples and four water sample replicates were collected for each sampling station for phytoplankton analysis. It included the taxonomic recognized of taxa as well as the measurements of linear dimensions and the estimate of biovolume and surface area. For each replicates a total of 400 cells (±10% of accuracy; Lund, 1958) were identified and measured for a total of 1600 cells for sampling stations. Linear dimensions were measured with a video-interactive image analysis system (L.U.C.I.A. Version 4.8 Laboratory Imaging s.r.o.) connected to an inverted microscope (T300E NIKON Instruments). The variation of the linear dimensions with the increase of cell numbers was evaluated on five taxa identified at species level, (*Chaetoceros whigamii* Brightwell; *Navicula transitans* Cleve; *Cylindrotheca closterium* (Ehrenberg) Lewin and Reimann; *Prorocentrum minimum* (Pavillard) J. Schiller; *Prorocentrum micans*, Ehrenberg) for which more than 50 cells in the same sampling point were identified and measured. These species were selected on the basis of cell size (small *vs* large species: biovolume average range from 51.39 ± 21.86 μm^3, for *C. whigamii* to 8,262.67 ± 619.72 μm^3 for *P. micans*) and of ratio between cell length (L) and cell width (W). Accordingly, species with different average L/W ratio were selected: L/W ratio ranged from 1.27±0.28, for *P. micans*, to 99.20±38.53 for *C. closterium*. Moreover, we have also evaluated the variation of average biovolume of phytoplankton guilds to increase of cells numbers measured in a samples. Using a randomisation test (PopTools, Excel-routine re-sample), subsamples of 25, 50, 100, 200, 300, 400, 500, 600, 700, 800, 1000 of phytoplankton cell were re-sampling from a population of 1600 individuals and the average biovolume for each sub-sample were calculated. Using a Montecarlo simulation routine the iterations were repeated for 100 time for each subsample.

Results

A check list for transitional water ecosystems

Our first step was to fill a list of phytoplankton species found in transitional water ecosystems in the Mediterranean Ecoregion. The floristic list included phytoplankton species from 30 transitional water ecosystems located in 7 European countries: Italy, Albania, Greece, Bulgaria, Romania, Spain and France. Different types of transitional water ecosystems (estuaries, coastal lakes, lagoons, salt pans) were considered to take into account the largest taxonomic heterogeneity of phytoplankton. Altogether, the floristic list

included 869 species belonging to 235 genera, grouped in 12 classes: Bacillariophyceae or Diatoms (BAC), Chlorophyceae (CHL), Chrysophyceae (CHR), Cryptophyceae (CRY), Cyanophyceae (CYA), Dictyochophyceae (DIC), Dinophyceae (DIN), Euglenophyceae (EUG), Prasinophyceae (PRA), Prymnesiophyceae (PRY), Xanthophyceae (XAN), Zygnematophyceae (ZYG). The proportion of the genera accounted for each group is reported in figure 1.

Geometric shapes

We propose a set of 22 geometric shapes for calculating biovolumes and surface area of phytoplankton species in transitional waters. These shapes take into account some set of geometric models proposed by others authors, (Edler, 1979; Hillebrand *et al.*, 1999; Sun and Liu, 2003), which were selected because of their wider availability with respect to other papers reporting set of geometric shapes (Kovala and Larrance, 1966; Rott, 1981). In addition, our list included also 6 genera (*Trochiscia*, *Bicosta*, *Strombomonas*, *Aulacomonas*, *Woronichinia*, *Lohmannosphaera*) that were not included in the above mentioned set of geometric shapes. A schedule for each geometric shape shows the formula for calculation of biovolume and surface area, the number and types of linear dimensions required, the names of the genera to which the model was applied, some notes regarding differences respect to exiting set of geometric formula and species-specific deviation (Appendix 1).

The selection of geometric shapes was based mainly on the degree of difficulty to measure linear dimensions. Specifically we chose geometric shapes that were similar to the real shape of the organism but at the same time easy discernible and conveniently measurable in routine analysis. Simple geometric shapes reduce the number of linear dimensions to be measured in light microscopy and consequently the time needed for each measurement. Accordingly, from the sets of geometric forms taken into account, the simplest geometric shapes, were selected. In our set, 13 are simple geometric solids, while 9 are the result of combining different geometric solids. However, the

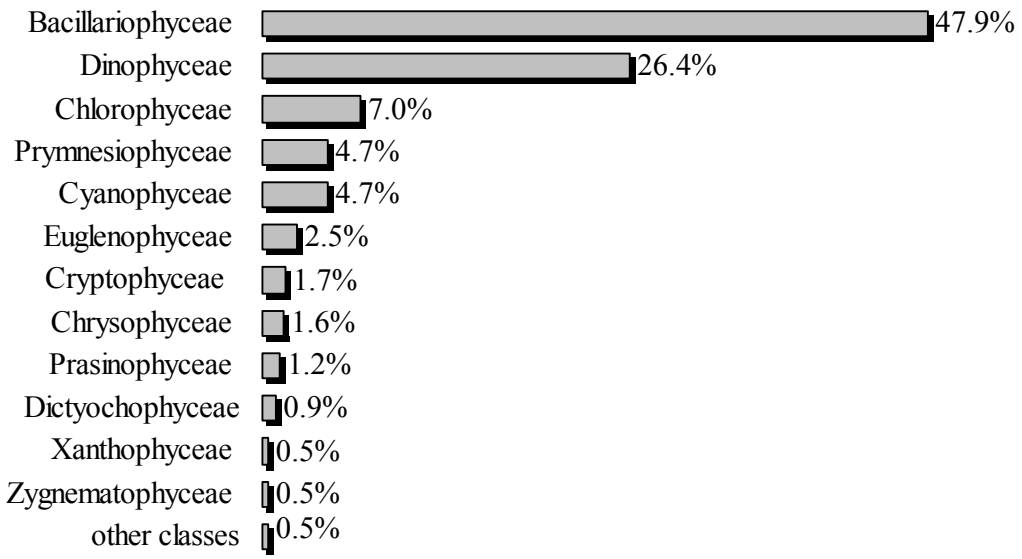

Figure 1. Relative contribution of the main phytoplankton classes in transitional water ecosystems in the Mediterranean Ecoregion (data from a number of published lists of phytoplankton in TWs).

latter were used in only 16% of the total genera identified (36 genera on 235 genera). Moreover, for 152 genera (66 % of the total), the measurement of linear dimensions in x and y viewing axes was sufficient, i.e. the additional measurement of cell thickness was not required.

This is in accordance with other sets of geometric shapes. Simple models were used in the 89% and in the 90% of total genera analyzed by Hillebrand *et al.* (1999) and Sun and Liu (2003) respectively. The third dimensions were not necessary in 61% of the genera analyzed by Hillebrand *et al.*, (1999), but only in 40% of the genera analyzed by Sun and Liu (2003). The appendix II shows the geometric shape and the counting unit to apply it. The counting unit (CU) can be referred to single cell, colony or part of colony. This paper is one of the first to indicate explicitly the counting unit to which the geometric shape is applied. In the case of species that form non–uniform colonies (e.g. Microcystis), the biovolume of the whole colony would be measured as the sum of the biovolumes of smaller areas.

Accuracy of linear measurements by light microscopy

Measurements are the main source of error in calculating cells biovolume. The scale bar of the eyepiece or of the image analysis system measurement modules need to be correctly calibrated at each magnification using a standard scale (micrometer). Light halos, which affect the measurements especially of the smallest cells, can be overcome by increasing the magnification or using phase contrast microscopy that can increase cell contrast. In this protocol, we proposed to measure the linear dimensions using image analysis systems with a semi-automatic method. We propose to use image analysis system to determine cell linear dimensions, because it is more precise (precision ±0.1 µm) than the eyepiece micrometer (precision ±0.5 µm), and because image analysis system allows an easier estimate of the cross sectional area of the cell, rather than its use reduce the time of the analysis (Fig. 2).

Nevertheless, image analysis systems can improve the acquisition of the two linear dimensions of the visible plane of the cells but not the thickness ("hidden dimension", "third dimension") of the cell, required for the determination of the biovolume and surface area of some species. Various authors have suggested solutions, but the 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.* (1992), 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 inaccurate when the cell thickness is significantly smaller than the width of the cell, as in some pennate diatoms. The aspect ratio (thickness/width, thickness/length) based on the regression models between thickness measurements and width or length measurements can be a good solution but unfortunately very few studies have been made and published in the literature. Finally, methods based on the measurement of the distance between the high and the low focal point can be used, but there is no a method for standardizing that and the error increases with the decrease of cell thickness. Thus, new solutions need to be tried in order to achieve the most accurate estimate of the third dimension and in general of all dimensions of phytoplankton cells. A good direction can be given by the new generation of inverted microscopes supported by motorized focus and image analysis systems with software able to create 3D images or DIC microscopy (differential interference contrast). In particular, due to the absence of halo effects, DIC microscopy gives visually clear images of cells with well

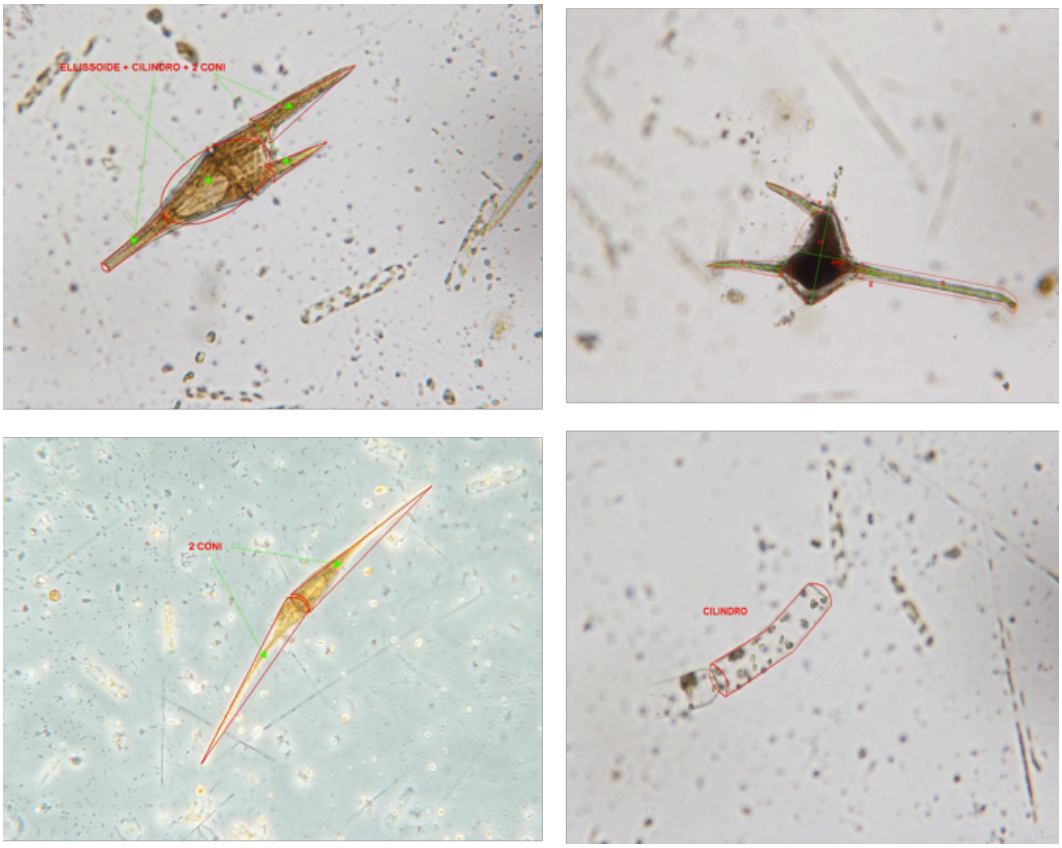

Figure 2. Examples of phytoplankton cell measurements using image analysis system Lucia Ltd–Nikon Instruments SpA.

defined edges, and offers the best prospect for cell recognition. This is promising and open perspectives versus automatic imagining analysis systems for counting and measuring phytoplankton cells, even if technological improvement is still needed (Gray *et al.*, 2002).

Number of cells to measure for cell biovolume and surface area estimation and average biovolume

Given the variation of phytoplankton cell size with the season, life cycle, and physiological and environmental forcing, the use of "average" biovolume values applied to species level throughout the year and in different sites can produce significant inaccuracies (Wetzel and Likens, 1991).

Biovolume needs to be calculated afresh for every experiment or set of samples. In our study we observed a large intra-specific biovolume variability, above all in species characterized by a high length/width ratio and small size where the standard error was high. In *Cylindrotheca closterium* and *Navicula transitans* for which high variability of apical length is well documented the standard error was higher than 17% and 25%, respectively after the measurement of 50 cells (Fig. 3). This aspect was supported also by statistical analysis carried out on biovolume variation at community level. In particular, the variation of average biovolume of randomic selected phytoplankton guilds with increasing numerical abundance of cells measured (from 25 to 1000 cells within a population of 1600

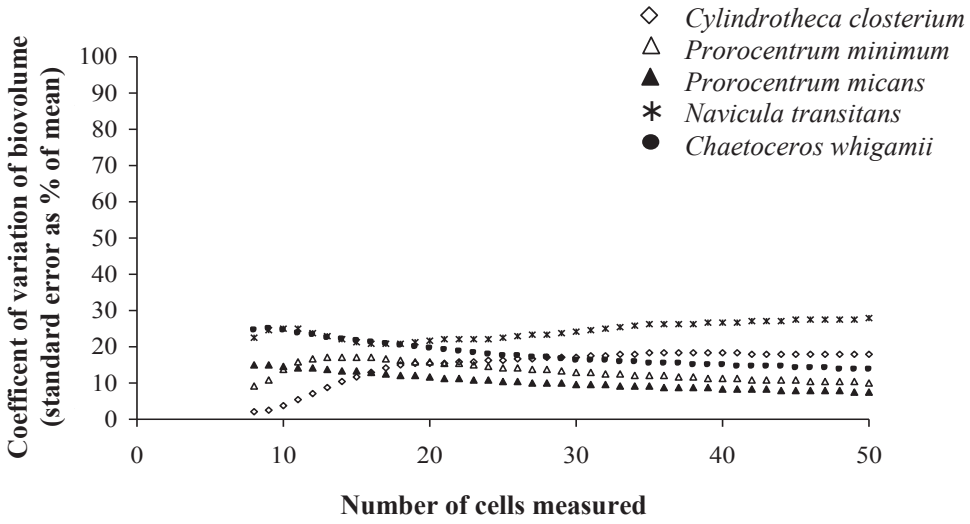

Figure 3. Microscopical measurements of linear dimensions. In the graph is reported the coefficient of variation in relation to the number of cells measured.

individuals) shown the highest variability when the number of cells measured is low; the variability decrease with the increase of number of cell measured (Fig. 4). This is probably due to reduction of the errors due to the inter and intra-specific variability of phytoplankton cells include in the determination. Average biovolume range for each subset of randomized phytoplankton guild is represented in figure 5. On the basis of this results, in our protocols we advise measuring the linear dimensions in all

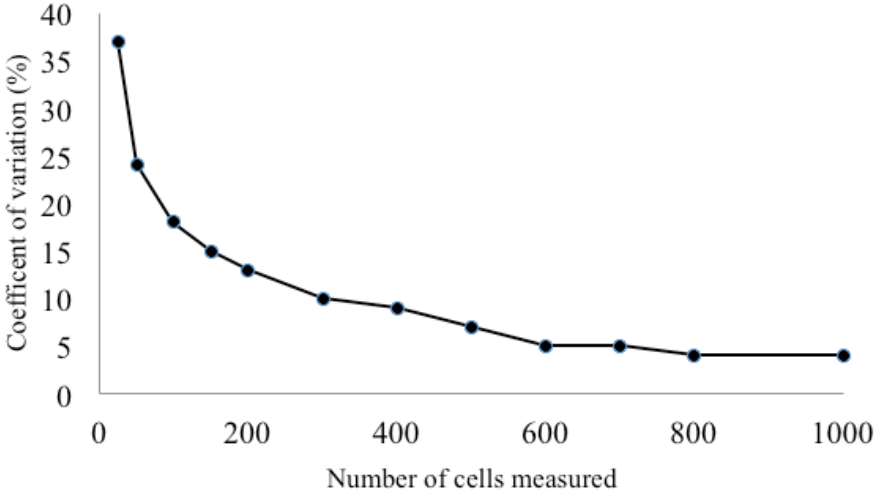

Figure 4. Coefficient of variation of average biovolume of random subset of phytoplankton guilds with increasing numerical abundance of cells measured.

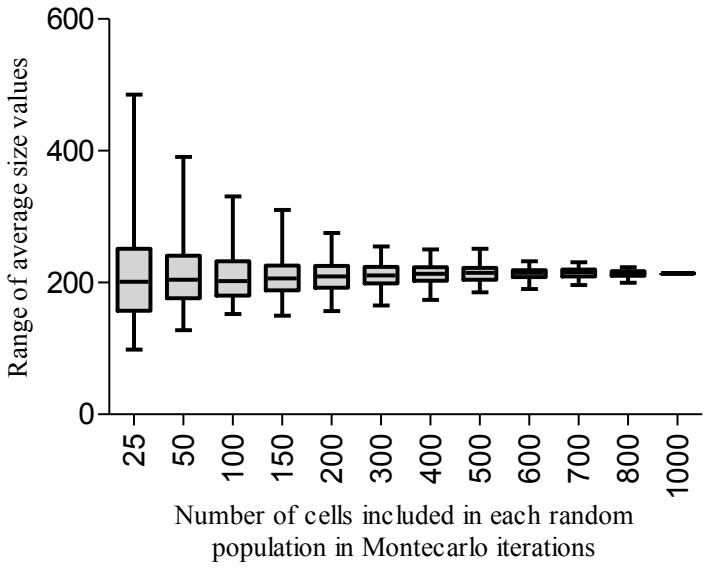

Figure 5. Range of phytoplankton average biovolume (μm^3) with the increasing of the number of cell measured. The bars represented the minimum and maximum, the box represented the 25 and 75 percentile and the average biovolume values.

phytoplankton cells or counting units of the most abundant species counted in a sample, in relation to the level of accuracy required. On the other hand, in the figure 4, the coefficient of variation of randomized subset of phytoplankton guilds shows values under the 10% when almost 300 phytoplankton cells were measured. Therefore, this is the number of cells recommended to be measured in our protocol.

These results are in contrast with other authors (Smayda, 1978; Hillebrand *et al.*, 1999; Sun and Liu, 2003) who found sufficient to measure a subset of cells of the most abundant species and to estimate biovolume from the mean or median of the measured linear dimensions. Nevertheless, the use of mean or median linear dimensions for biovolume or surface area calculus results in loss of information relatively to intra-specific biovolume variability (Potapova and Snoeijs, 1997; Olenina *et al.*, 2006). Specifically, the size structure of populations of phytoplankton species respond to ecological forcing as phytoplankton communities as a whole, but with constrain due to structural characteristic of the species. For this we think that in studies having the aim to evaluated how phytoplankton size structure respond to environmental or anthropogenic forcing, it is necessary to include variability at population level. This was also supported by other studies specifically focusing on the variability of phytoplankton size structure, in which all counting unit, included in the analysis, were measured (Quinones *et al.*, 2003; Reul *et al.*, 2005).

Discussion

This work proposed a protocol for estimating biovolume and surface area of phytoplankton species of transitional water ecosystems of Mediterranean Ecoregion. It is an implementation of a previous work carried out on a reduced lists of phytoplankton taxa (Vadrucci *et al.*, 2007). This is the first step

in a dynamic process, aimed to the realization of standardized methods for determination of body-size related descriptors: cell biovolume and surface area are the basic morphometric descriptors of phytoplankton communities, by which other morphometric and body–size related descriptors can be obtained (i.e. biomass, surface to volume ratio, length to width ratio, size spectrum or morphological functional groups).

A series of experimental evidences (Reynolds, 1997; Tokeshi, 1999; Quinones *et al.*, 2003; Weithoff, 2003; Reul *et al.*, 2005) highlight that they can furnish additional information which can not be supply directly by taxonomic parameters. For this morphometric descriptors would be included in monitoring plans for the evaluation of health status of transitional waters ecosystems, as well taxonomic descriptors. Moreover, using biovolume when presenting phytoplankton results has became more and more important, when realizing that only cell numbers are often inadequate. In many European countries, the health status classification of water bodies based on phytoplankton quality elements carried out within EU Water Framework Directives implementation taken into account of biovolume and other size-related parameters.

However, it is necessary to create common procedure for their determination in order to make body-size descriptors comparable and reliable. Accordingly, the checklist of more than 800 species of phytoplankton recorded in 30 transitional water ecosystems and the related list of geometric shapes constitutes one of few available on phytoplankton guilds of transitional water ecosystems in the Mediterranean Ecoregion.

On the other hand, cell biovolume and surface area determination are hampered by a series of methodological problems regarding various aspects, which have been widely discussed in the literature and in this paper, and for this reason an accurate measurement of these parameters is still not always possible or practical. Currently, the most that can be achieved is an approximate estimate resulting from a compromise between the accuracy and the practicality of the determination. Practicality is understood as the necessity to minimize the effort of analytical determination as in terms of the number of linear dimensions to measure by optical microscopy as in the time required for each determination.

Based on these considerations our protocol will be useful to overcome the problem of the incomparability of data, resulting from the use of different sets of geometric shapes, in study regarding phytoplankton guilds in transitional aquatic ecosystems. Moreover, according to WFD (2000/60 EC) and Italian regulation (Italian decree 152/2006), it respond at the necessity to have common and specific guidelines and procedures to determine descriptors of phytoplankton quality element for each water body category. At the same time, the implementation of new technologies must be encouraged in order to increase the accuracy of biovolume and surface area determination.

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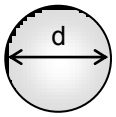
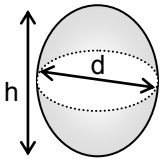
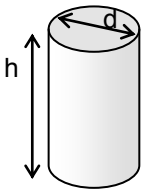
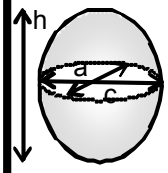
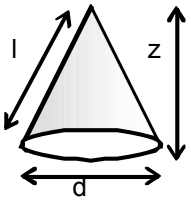
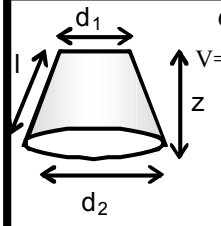
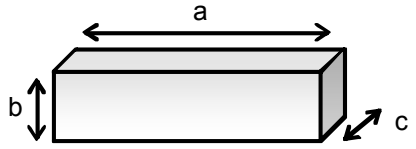
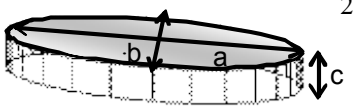
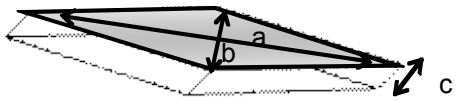
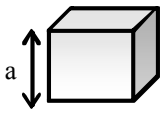
Appendix 1 - Schedule for geometric shapes showing: mathematical equation, number and types of linear dimensions to measure, genera to which the shape is applied, the difference with respect to other sets of geometric shapes published and notes for species deviation.

Geometric shape and formulas	***Genera on which is applied:***
 1) Sphere $V = \pi/6 \cdot d^3$ $A = \pi \cdot d^2$ *Number and type of linear dimensions to measure* 1: d = diameter	***BAC*****:** *Hyalodiscus (1),* ***CHL****: Carteria,Chlamydomonas, Coelastrum,Micractinium,Tetrape-dia, Tetrastrum ,****CHR****: Parapedinella,****CRY****: Pseudobodo,****CYA****: Chroococcus ,Coccoid cyanobacteria, Gloeocapsa, Gomphosphaeria (2), Microcystis, Synechoccocus, Trochiscia, Woronichinia,****DIC****: Dictyocha* ***DIN****: Goniodoma, Oblea, Protoceratium* ***PRY****: Alisphaera, Braarudosphaera, Calyptrosphaera, Ceratolithus, Coccolithus, Coronosphaera, Emiliana, Gephyrocapsa, Helladosphaera, Lohmannosphaera, Pavlova , Pontosphaera, Rhabdosphaera, Syracolithus, Syracosphaera* ***PRA****: Halosphaera, Pterosperma* ***XAN****: Meringosphaeria,* ***OC****: Ebria, Hermesimum, Rhizochloris* **Notes:** (1) For more flattened forms elliptic prism can fit better (2) species with elongated forms should be calculated as cylinder or prolate spheroid
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. This form was applied above all to phytoflagellate groups of different taxonomic affiliation.	
 2) Prolate spheroid $V = \pi/6 \cdot d^2 \cdot h$ $A = \frac{\pi \cdot d}{2}\left(d + \frac{h^2}{\sqrt{h^2 - d^2}} \cdot \sin^{-1} \cdot \frac{\sqrt{h^2 - d^2}}{h}\right)$ *Number and type of linear dimensions to measure* 2: d = diameter; h = height	***CHL****: Gonium, Oocystis, Pediastrum, Scenedesmus,* ***CHR****: Chrysococcus, Dinobryon, Mallonomas, Monochrys* ***CRY****: Cryptomonas, Hillea,* ***CYA****: Coelosphaerium, Snowella,* ***DIC****: Apedinella,****DIN****: Cochlodinium, Oxytoxum (1), Oxyrrhis, Pyrocystis, Torodinium,Warnowia,****PRA****: Aulacomonas, Mamiella, Micromonas, Pachysphaera, Tetraselmis,* ***PRY****: Acanthoica, Chrysochromulina, Halopappus,Ophiaster* **Notes:** (1) *O. viride*: cone+cone
The shape is also used in other sets of geometric forms, in particular the set proposed by Hillebrand et al., 1999, except for the genus *Pediastrum*. For this genus, Hillebrand proposed an elliptic prism applied to the whole colony. Previously 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. This also resolves the problems of volume overestimation of the colony, which both previous sets had put at roughly twice the actual volume.	
 3) Cylinder $V = \pi/4 \cdot d^2 \cdot h$ $A = \pi \cdot d \cdot (d/2 + h)$ *Number and type of linear dimensions to measure* 2 : d = diameter; h = height	***BAC****: Actinoptycus, Asterolampra, Asteromphalus, Bacteriastrum, Cerataulina, Coscinodiscus, Coscinosira, Cyclotella, Dactylosolen, Detonula, Ellerbeckia, Guinardia, Hemiaulus, Lauderia, Leptocylindrus, Lioloma, Melosira, Paralia, Planktoniella, Porosira, Proboscia, Rhizosolenia, Skeletonema, Stictocyclus, Thalassiosira, Toxarium,****CHL****: Planktonema, Sticochoccus,* ***CYA****: Anabaenopsis, Ana-baena, Aphanizomenon, Filamentous cyanobacteria, Lyngbya, Nodularia, Nostoc,Oscillatoria, Phormidium, Spirulina* ***DIN****: Amphisolenia,* ***PRY****: Acanthosolenia, Calciosolenia ,****ZYG****: Cosmarium, Mougeoutia* ***OC****: Bicosta* *Notes:*
This model is easy to apply and is generally calculated automatically by most image analysis software.	

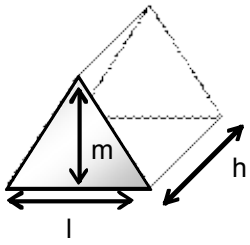
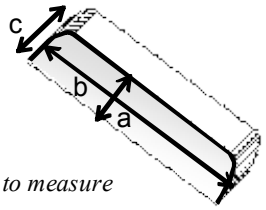
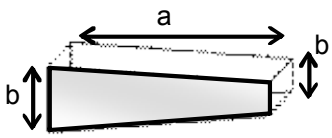
Appendix 1 - Continued.

Geometric shape and formulas	***Genera on which is applied :***
4) Ellipsoid $V = \pi/6 \cdot a \cdot c \cdot h$  $A = \approx \frac{\pi}{4} \cdot (a+c) \cdot \left[\left(\frac{a+c}{2}\right) + \frac{2h^2}{\sqrt{4h^2-(a+c)^2}} \sin^{-1} \frac{\sqrt{4h^2-(a+c)^2}}{2h^2}\right]$ *Number and type of linear dimensions to measure* 3: a = length; c = width; h = height	***BAC**: Amphora,,Cymbella, Gyrosigma (1) Surrirella* ***CHL**: Lagerheimia,**DIN**: Akashiwo, Alexandrium,Amphidinium (2), Blastodinium,Dinophysis(3), Diplopelta, Glenodinium, Gymnodinium (4), Gyrodinium (5), Lingulodinium , Nematodinium ,Peridinium, Phalacroma,Pheopolykrikos, Polykrikos,Prorocentrum (6), Ptychodiscus, Pyrophacus, Scrippsiella,**EUG**: Astasia, trombomonas, Trachellomonas* **Notes:** (1) Ellipsoid -10%, (2) *A. sphenoides*: prolate spheroid (3) *D. pulchella*: prolate spheroid; *D. caudata*, *D. tripos* horns as to be added as cones, (4) *G. rhomboides*: cone+cone (5) *G. lachymae*: cone+half sphere, (6) *P. arcuatum*, *P. dentatum, P. micans, P. triestinum*: cone+half sphere
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%.	
 5) Cone $V = \pi /12 \cdot d^2 \cdot z$ $A = \pi \cdot \frac{d}{2} \cdot \left(\frac{d}{2} + l\right)$ *Number and type of linear dimension to measure* 2: d = diameter; h = height ; for area l = side of cone	***CHR**: Calycomonas, Paulinella **CHL**: Treubaria (1) ,**DIN**: Podolampas ,**PRA**: Pyramimonas **PRY**: Calciopappus* **Notes:** (1) The biovolume is calculated as sum of the number of cones. This form was applied to six genera, in accordance with Hillebrand et al., 1999
 6) Truncated cone $V = (\frac{\pi}{12} \cdot h \cdot (d_1^2 + d_1 \cdot d_2 + d_2^2)) \cdot 2$ $A = \frac{\pi}{4} \cdot (d_2^2 + d_1^2 + 2l[d_2 + d_1])$ *Number and type of linear dimensions to measure* 3: d_1 = minor diameter; d_2 = major diameter; z= height; for area l = side of truncated cone	***ZYG:** Cosmarium* This form was used just for one genus and was applied following the most similar geometric form in agreement with the most recent sets. Some species can be considered as prolate spheroid
7) Parallelepiped $V = a \cdot b \cdot c$ $A = 2 \cdot (a \cdot b) + 2 \cdot (b \cdot c) + 2 \cdot (a \cdot c)$  *Number and type of linear dimensions to measure* 3: a = length; b = width; c = thickness	***BAC**: Asterionella, Bacillaria, Cymatopleura, Hantzschia, Pinnularia, Rhabdonema, Synedra(1), Tabellaria (2), Thalassionema, Thalassiothrix **CHL**: Tetraedon* **Notes:** (1) Species with elliptic valves should be calculated as prism on elliptic base (2) Transapical axis (width) is measured as the mean of the minimum and the maximum
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.	

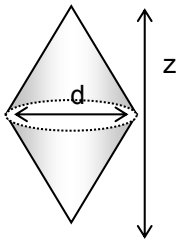
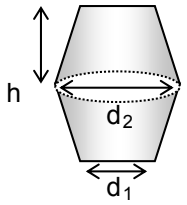
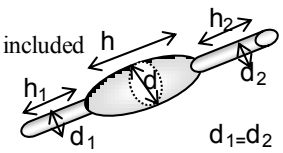
Appendix 1 - Continued.

Geometric shape and formulas	***Genera on which is applied***
8) Elliptic prism $V = \pi/4 \cdot a \cdot b \cdot c$ $A = \frac{\pi}{2} \cdot (a \cdot b + [a + b] \cdot c)$  *Number and type of linear dimensions to measure* 3: a = length; b = width; c = thickness	***BAC**: Achananthes, Amphiprora, Berkeleya, Biddulphia (1), Campylodiscus, Chaetoceros, Cocconeis, Diatoma, Dimerogramma, Diploneis (2) , Eucampia, Fragilaria, Fragilariopsis, Grammatophora (3), Haslea, Lyrella, Mastogloia, Navicula (4), Stauroneis (5), Striatella, Trachyneis, Tropidoneis **CHL**: Pediastrum (6)* ***DIN**: Mesoporos **EUG**: Phacus* **Notes:** (1) Elevations or extensions should be added separately as cylinders or cones; (2) Transapical axis (width) is measured as the mean of the minimum and the maximum (width) (3) Species with linear valves should be calculated as box , 4) Species with rhombic valves should be calculated as prism on parallelogramm base, species with linear valves as box, (5) Species with linear valves should be calculated as box (6) The conting unit is the colony , as thckness of the colony is considered the diameter of a single cell.
This form was introduced for the first time by Edler (1979) with the named of ellipsoid, but it was reported in Hillebrand as elliptic prism . We are agree with Hillebrand's shapes-genera associations, 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. The need to measure the third dimension can render the application of this model difficult.	
9) Prism on parallelogramm base $V = \frac{1}{2} a \cdot b \cdot c$ $A = a \cdot b + \frac{\sqrt{a^2 + b^2}}{4} \cdot c$  *Number and type of linear dimensions to measure* 3: a = length; b = width; c = thickness	***BAC:** Nitzschia (1), Pleurosigma, Psammo-dictyon, Pseudonitzschia (2),* *Pseudosolenia, Rhaphoneis* **Notes:** (1) Sigmoid or rhombic species can be calculated as prisms on a parallelogram base, elliptic species as elliptic prisms, and linear species as boxes. (2) Species with linear valves should be calculated as box
This form was introduced for the first time in Hillebrand's paper. However, the genus *Nitzschia* includes species of different form. In this case, the most similar geometric forms should be used.	
 10) Cube $V = a^3$ $A = 6a^2$ *Number and type of linear dimensions to measure* 1 :a = length of one side	***CHL**: Crucigenia (1)* ***CYA**: Merismopedia* **Notes:** (1*) C.quadrata* = sphere x 4;
This model was applied to the same genera proposed in other sets of geometric forms.	

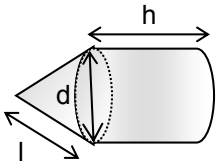
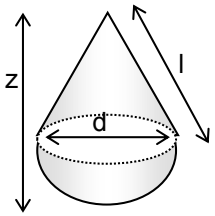
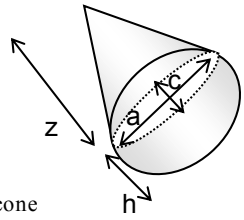
Appendix 1 - Continued.

Geometric shape and formulas	***Genera on which is applied:***
11) Prism on triangular base $V = \frac{1}{2} \cdot l \cdot m \cdot h$ $A = l \cdot m + (3 \cdot h \cdot l)$  *Number and type of linear dimensions to measure* 3: m = height of a triangle; l = length of one side; h = height	***BAC**: Asterionellopsis, Bellarochea, Ditylum Triceratium*
This form is also cited in the Hillebrand and Sun and Liu and Olenina et al., papers. The need to measure the third dimension can render its application difficult.	
12) Half elliptic prism V = $\pi/4 \cdot a \cdot b \cdot c$ A = $\pi/4 \cdot (a \cdot b + a \cdot c + \cdot b \cdot c) + a \cdot c$  *Number and type of linear dimensions to measure* 3: a = length; b= width; c = thickness	***BAC:** Epithemia, Eunotia, Phaeodactylum*
For this form, we agree with the proposal by Hillebrand et al 1999. Edler (1979) proposed ellipsoid forms for the first two genera, whereas 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	
 13) Truncated pyramid V= $[(b_1 + b\sqrt{\cdot b_1 \cdot b}) \cdot a]/3$ *Number and type of linear dimensions to measure* 3 a = length; b = width minor base; b_1= width major base	***BAC**: Gomphonema, Licmophora*
The model differs by those proposed by other authors, e.g. Hillebrand et al. 1999, who proposed the gomphonemoid form. This form requires four linear dimensions, some of them very difficult to measure. In his work, the linear measurement 'f' is the length of the transapically widest part of the head pole. 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. 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.	

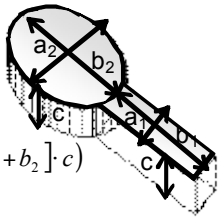
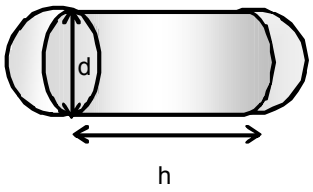
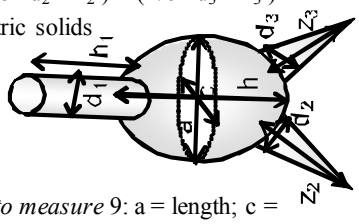
Appendix 1 - Continued.

Geometric shape and formulas	***Genera on which is applied***
14) Two cones $V = \pi/12\ d^2 \cdot z$ $A = \pi/2 \cdot d \cdot \sqrt{z^2 \cdot d^2}$  *Number and type of linear dimensions to measure* 2: d = diameter; z = height of cone. For area : l = side of cone	***CHL**: Chlorogonium, Kirchneriella, Monoraphidium, Schroderia , Actinastrum, Ankistrodesmus* ***DIN**: Gonyaulax (1), Heterocapsa, Heterodinium, Oxyphysis, Protoperidinium (2)* ***EUG**: Lepocinclis(3), **PRY**: Anoplosolenia* **Notes** (1) *G. spinifera*, *G. scrippsae*: cone + half sphere (2) *P. elegans*, cilinder + 3 cones; *P. brevipes*, *P. thorianum*, *P. pallidum*: cone+half ellipsoid; *P. minutum*: sphere; *P. divergens,* cone+half ellipsoid but prominent hypotecal horns should be added as cones, P. bipes**,** cone+half sphere (3) Species apically elongated should be calculated as prolate spheroid
It is a combined solid of rotation, very easy to apply. This follows Sun's set of geometric forms. For Group DIN *(2),* this form was applied to the *Protoperdinium*. The *Protoperidium* genus (2) is highly variable in shape and for some species different geometric models can be required.	
15) Two truncated cones $V = \pi/6 \cdot h \cdot (d_1^2 + d_1 \cdot d_2 + d_2^2)$ $A = \frac{\pi}{2} \cdot \left(d_2^2 + d_1^2 + 2l\left[d_2 + d_1\right]\right)$  *Number and type of linear dimensions to measure* 3: d_1= minor diameter; d_2= major diameter; h = height length; b = width; c = thickness	***ZYG:** Staurastrum* This follows other sets of geometric forms. It is a combined solid of rotation, very easy to apply. However, cones can be add in relation the species shape
16) Prolate spheroid + two cylinders $V = (\pi/6 \cdot d^2 \cdot h) + (\pi/2 \cdot d_{1/2}^2 \cdot h_{1/2})$ A = as sum of the three geometric solid included  *Number and type of linear dimensions to measure* 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	***BAC**: Cylindrotheca* This form was used only for the *Cylindrotheca* genus. It follows the form proposed by Hillebrand et al 1999. Its application can be difficult due to the high number of linear dimensions to measure. Two cones can be also used for biovolume estimation

Appendix 1 - Continued.

Geometric shape and formulas	***Genera on which is applied***
17) Cylinder + cone $V = (\pi/4 \cdot d^2 \cdot h) + (\pi/12 \cdot d^2 \cdot z)$ $A = \frac{\pi}{2} \cdot d \cdot \left(\frac{d}{2} + l + 2h\right)$  *Number and type of linear dimensions to measure* 3: d = diameter; h = height; z = height of cone. For area : l = side of cone	***DIN:*** *Katodinium,* ***EUG:*** *Eutreptia, Eutreptiella*
This follows Hillebrand's and Edler's sets of geometric forms. It is a combined solid of rotation, easy to apply. We do not consider the suggestion of 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	
18) Cone + half sphere $V = \pi/4 \cdot d^2 \ z$ $A = \frac{\pi}{2} \cdot d \cdot (l + d)$  *Number and type of linear dimensions to measure* 2: d = diameter; z = height of cone. For area : l = side of cone	***CHR****: Ochromona, Spumella,* ***CRY:*** *Chroomonas, Leucocryptos, Plagioselmis, Rhinomonas, Rhodomonas* ***DIN****: Diplopsalis, Pachidinium, Pronoctiluca* ***PRY****: Prymnesiunm*
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.	
19) Half ellipsoid + cone on elliptic base $V = (\pi/12 \cdot a \cdot c) \cdot (h + z)$ *A = as half ellipsoid + area of cone*  *Number and type of linear dimensions to measure* 4: a = length; c = width; h = height; z = height of cone	***EUG****: Euglena* ***PRY****: Phaeocystis*
This shape was applied in accordance with Hillenbrand et al 1999, but only to the Euglena genus. The euglenoid algae are variable in shape and cross section. Most euglena species 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,1979 and Sicko-Goad at al., 1977 propose a similar shape with a cylinder instead of a cone. Finally, 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.	

Appendix 1 - Continued.

Geometric shape and formulas	***Genera on which is applied***
20) elliptic prism+parallelepiped V= $c\,(a_1 \cdot b_1 + \pi/4\ a_2 \cdot b_2)$ $A = 2(a_1 \cdot b_1) + 2c(a_1 + b_1) + \frac{\pi}{2}(a_2 \cdot b_2 + [a_2 + b_2] \cdot c)$  *Number and type of linear dimensions to measure* 5: a_1= length of parallelepiped; b_1 = width of parallelepiped; c = thickness; a_2= length of prism; b_2 = width of prism	***BAC****: Climacosphaenia* It was in accordance with Hillebrand et al.'s (Hillebrand et al., 1999) set of geometric shapes
21) Cylinder + 2 half spheres V = $(\pi/4 \cdot d^2 \cdot h) + (\pi/6 \cdot d3)$ A = $\pi \cdot d \cdot (h+d)$  *Number and type of linear dimensions to measure* 2: d=diameter; h= height	***BAC:*** *Corethron,* Its application followed other sets of geometric formula, where the genera were reported. It is a combined solid of rotation, very easy to apply.
22) Ellipsoid + 2 cones + cylinder V = $(\pi/6 \cdot a \cdot c \cdot h) + (\pi/4 \cdot d_1^2 \cdot h_1) + (\pi/6 \cdot d_2^2 \cdot z_2) + (\pi/6 \cdot d_3^2 \cdot z_3)$ A = as sum of areas of the three geometric solids  *Number and type of linear dimensions to measure* 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	***DIN****: Ceratium (1)* **Notes** (1) *C. fusus*: cone+cone; *C. trichoceros*, *C. macroceros* all horns calculated as cylinders; *C. tripos* central body as cone
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 dimension. Moreover,it is very time consuming and thererefore not very likely to be used in the monitoring programs. G. Hansen 1992 proposed to use a correlation between calculated volume and the width of the cingulum. This is also used in Olenina et al., 2006 and it makes the measurements far more easy.	

Appendix 2 - 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. The list was based on the analysis of 869 species and was drawn up on the basis of the floristic list published in Caroppo and Cardellicchio 1995,; Caroppo, 2000, Gilabert 2001 Facca et al., 2002, Perez et al., 2002, Nuccio et al., 2003, Vadrucci et al., 2004; Hendwood, pers comm. and for data collected during the TW Reference NET project. Shape code are referred to Appendix 1.

genus	shape	C.U.	genus	shape	C.U.
Bacillariophyceae					
Achnanthes	8	single cell	Leptocylindrus	3	single cell
Actinoptychus	3	single cell	Licmophora	13	single cell
Amphiprora	8	single cell	Lioloma	3	single cell
Amphora	3	single cell	Lyrella	8	single cell
Asterionella	7	single cell	Mastogloia	8	single cell
Asterolampra	3	single cell	Melosira	3	single cell
Asteromphalus	3	single cell	Navicula	8	single cell
Asterionellopsis	11	single cell	Nitzschia	9	single cell
Bacillaria	7	single cell	Paralia	3	single cell
Bacteriastrum	4	single cell	Phaeodactylum	12	single cell
Bellarochea	11	single cell	Pinnularia	7	single cell
Berkeleya	8	single cell	Planktoniella	4	single cell
Biddulphia	8	single cell	Pleurosigma	9	single cell
Campylodiscus	8	single cell	Porosira	3	single cell
Cerataulina	3	single cell	Proboscia	3	single cell
Chaetoceros	8	single cell	Psammodictyon	9	single cell
Climacosphenia	20	single cell	Pseudonitzschia	9	single cell
Cocconeis	3	single cell	Pseudosolenia	9	single cell
Corethron	21	single cell	Rhabdonema	7	single cell
Coscinodiscus	3	single cell	Rhaphoneis	9	single cell
Coscinosira	3	single cell	Rhizosolenia	3	single cell
Cyclotella	3	single cell	Skeletonema	3	single cell
Cylindrotheca	16	single cell	Stauroneis	8	single cell
Cymatopleura	7	single cell	Stephanodiscus	3	single cell
Cymbella	4	single cell	Stictocyclus	3	single cell
Dactylosolen	3	single cell	Striatella	8	colony
Detonula	3	single cell	Surirella	4	single cell
Diatoma	8	single cell	Synedra	7	single cell
Dimerogramma	8	single cell	Tabellaria	7	single cell
Diploneis	8	single cell	Thalassionema	7	single cell
Ditylum	11	single cell	Thalassiosira	3	single cell
Ellerbeckia	3	single cell	Thalassiothrix	7	single cell
Epithemia	12	single cell	Toxarium	3	single cell
Eucampia	8	single cell	Trachyneis	8	single cell
Eunotia	12	single cell	Triceratium	11	single cell
Fragilaria	8	single cell	Tropidoneis	8	single cell
Fragilariopsis	8	single cell			
Gomphonema	13	single cell			
Grammatophora	8	single cell			
Hantzschia	7	single cell			
Guinardia	3	single cell			
Gyrosigma	9	single cell			
Haslea	8	single cell			
Hemiaulus	3	single cell			
Hyalodiscus	1	single cell			
Lauderia	3	single cell			

Appendix 2 - Continued.

genus	shape	C.U.	genus	shape	C.U.
Chlorophyceae					
Actinastrum	14	single cell	Cochlodinium	2	single cell
Ankistrodesmus	14	single cell	Dinophysis	4	single cell
Carteria	1	single cell	Diplopelta	4	single cell
Chlamydomonas	1	single cell	Diplopsalis	18	single cell
Chlorogonium	14	single cell	Glenodinium	3	single cell
Coelastrum	1	single cell or colony	Gonyaulax	14	single cell
Crucigenia	10	single cell	Gymnodinium	4	single cell
Crucigeniella	2	single cell	Gyrodinium	4	single cell
Desmodesmus	2		Goniodoma	1	single cell
Gonium	2	single cell	Heterocapsa	14	single cell
Kirchneriella	14	single cell	Heterodinium	14	single cell
Micractinium	1	single cell	Katodinium	18	single cell
Monoraphidium	14	single cell	Lingulodinium	4	single cell
Pediastrum	8	colony	Mesoporos	8	single cell
Planktonema	3	single cell	Minuscula	18	single cell
Scenedesmus	2	single cell	Nematodinium	4	single cell
Schroederia	14	single cell	Oblea	1	single cell
Tetraedon	7	single cell	Oxytoxum	2	single cell
Tetrapedia	1	single cell	Oxyphysis	14	single cell
Tetrastrum	1	single cell	Oxyrrhis	2	single cell
Treubaria	5	single cell	Peridinium	4	single cell
Chrysophyceae			Phalacroma	4	single cell
Calycomonas	5	single cell	Pheopolykrikos	4	single cell
Chrysococcus	2	single cell	Podolampas	4	single cell
Dinobryon	2	single cell	Polykrikos	4	single cell
Mallonomas	2	single cell	Pronoctiluca	18	single cell
Monochrysis	2	single cell	Prorocentrum	4	single cell
Ochromonas	18	single cell	Protoceratium	1	single cell
Paulinella	5	single cell	Protoperidinium	14	single cell
Spumella	18	single cell	Ptychodiscus	4	single cell
Cryptophyceae			Pyrocystis	2	single cell
Leucocryptos	18	single cell	Pyrophacus	4	single cell
Chroomonas	18	single cell	Scrippsiella	4	single cell
Cryptomonas	2	single cell	Torodinium	2	single cell
Hillea	2	single cell	Warnowia	2	single cell
Plagioselmis	18	single cell	**Dictyochophyceae**		
Rhinomonas	18	single cell	Apedinella	2	single cell
Rhodomonas	18	single cell	Dictyocha	1	single cell
Dinophyceae			Parapedinella	1	single cell
Akashiwo	4	single cell	Pseudopedinella	6	single cell
Alexandrium	4	single cell			
Amphidinium	4	single cell			
Amphisolenia	3	single cell			
Blastodinium	4	single cell			
Ceratium	22	single cell			

Appendix 2 - Continued.

genus	shape	C.U.	genus	shape	C.U.
Cyanophyceae			**Prymnesiophyceae**		
Anabaenopsis	3	N° of 100 µm filaments	Acanthoica	2	single cell
Anabaena	3	N° of 100 µm filaments	Anoplosolenia	14	single cell
Aphanizomenon	3	N° of 100 µm filaments	Calciopappus	5	single cell
Coelosphaerium	2	colony	Calciosolenia	4	single cell
Chroococcus	1	single cell	Chrysochromulina	2	single cell
Coccoid cyanobacteria	1	single cell	Coccolithus	1	single cell
Gomphosphaeria	1	single cell	Coronosphaera	1	single cell
Gloeocapsa	1	single or colony	Emiliania	1	single cell
Filamentous cyanobacteria	3	N° of 100 µm filaments	Gephyrocapsa	1	single cell
Lyngbya	3	N° of 100 µm filaments	Halopappus	2	single cell
Merismopedia	10	single cell	Lohmannosphaera	1	single cell
Microcystis	1	part of colony	Ophiaster	2	single cell
Nodularia	3	N° of 100 µm filaments	Phaeocystis	19	single cell
Nostoc	3	single	Pontosphaera	1	single cell
Oscillatoria	3	N° of 100 µm filaments	Prymnesium	18	single cell
Phormidium	3	N° of 100 µm filaments	Rhabdosphaera	1	single cell
Snowella	2	colony	Syracosphaera	1	single cell
Spirulina	3	N° of 100 µm filaments	**Trebouxiophyceae**		
Synechococcus	1	single cell	Lagerheimia	3	single cell
Trochiscia	1	single cell	Oocystis	2	single cell
Woronichinia	1	colony	Stichococcus	3	single cell
Euglenophyceae			**Xanthophyceae**		
Astasia	4	single cell	Meringosphaera	1	single cell
Euglena	19	single cell	Rhizochloris	1	single cell
Eutreptia	17	single cell	**Zygnematophyceae**		
Eutreptiella	17	single cell	Closterium	8	single cell
Lepocinclis	14	single cell	Cosmarium	3	single cell
Phacus	8	single cell	Mougeotia	3	single cell
Strombomonas	4	single cell	Staurastrum	15	single cell
Trachelomonas	4	single cell	**others classes**		
Prasinophyceae			Bicosta	3	single cell
Aulacomonas	2	single cell	Ebria	1	single cell
Halosphaera	1	single cell	Hermesinum	1	single cell
Mamiella	2	single cell	Pseudobodo	1	single cell
Micromonas	2	single cell			
Pachysphaera	2	single cell			
Pterosperma	1	single cell			
Pyramimonas	5	single cell			
Tetraselmis	2	single cell			

