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RESEARCH ARTICLE

BioCalc: a software tool for the calculation of biovolume of phytoplankton samples

BioCalc: uma ferramenta de software para o cálculo do biovolume em amostras fitoplanctônicas

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Resumo A estimativa do biovolume celular, baseada em medidas de dimensões lineares da forma, adaptadas a modelos geométricos padronizados, é importante para o estudo da ecologia do fitoplâncton. Contudo, cálculos demorados são demandados para a estimativa do biovolume a partir de medidas lineares. Neste trabalho, uma ferramenta de software simples é apresentada para auxiliar no cálculo do biovolume das amostras de fitoplâncton, usando modelos geométricos e observações microscópicas convencionais. O software BioCalc implementa as fórmulas mais recentes disponíveis na literatura para calcular o biovolume celular com base em um conjunto de 31 formas geométricas.

Palavras-Chave: biovolume, fitoplâncton, morfometria, software.

Abstract The estimate of cell biovolume, based on measurements of linear dimensions of shapes adapted to standard geometric models, is important for the study of phytoplankton ecology. However, time-consuming calculations are required for the estimation of biovolume from linear measurements. In this paper, a simple, user-friendly multiplatform software tool is presented to aid in the calculation of the biovolume of phytoplankton samples, using geometric models and conventional microscopic observations. The BioCalc software implements the most recent formulae available in the literature for calculating cell biovolume on the basis of a set of 31 geometric shapes.

Keywords: biovolume, phytoplankton, morphometrics, software.

Introduction

Water covers most of the planet Earth. In aquatic environments, phytoplanctonic algae are the main primary producers. Currently, with the degree of degradation of these environments, the use of algae and several of their attributes for monitoring or evaluating environmental quality is increasing and justified by the advantages that these organisms present. However, one of the major difficulties in the use of phytoplanctonic algae is the fact that they present an immense diversity of shapes and sizes, which makes interspecific comparison difficult and often inappropriate.

Knowledge of the structure of phytoplankton communities is of great importance in limnological studies, in order to understand the dynamics of these communities and their interactions with the abiotic environment, as well as to allow the monitoring of environmental conditions that can be inferred from attributes like species composition and biomass (Vadrucci *et al.,* 2007; Fonseca *et al.,* 2014). Whereas species composition require floristic inventories and taxonomic identification, biomass can be evaluated more directly by an estimate of the biovolume or cell volume, based on measurements of linear dimensions and adaptation of shapes to standard geometric models (Hillebrand *et al.,* 1999; Sun & Liu 2003; Saccà 2017). So it becomes possible to make independent comparisons of shape or size. Perhaps the simplest and most useful way to do this is by an estimate of the biovolume, using linear measurements according to the geometric shape of organisms, which can be done with a common microscope available in all laboratories that deal with this question. For these calculations, there are formulae, some complex enough that may require precious time to calculate. But this time would be saved by taking the measurements and automating the biovolume calculations. These biovolume measurements can be used according to the objectives of each study, whether from the population, specific or general point of view of the whole group studied.

In general lines, calculation of the biovolume involves taking measurements of linear dimensions (e.g. length, width, diameter, height, etc.) from cells of each species (in µm) and the calculation of the volume of each cell, using a geometric formula which better represents the cell shape and calculation of mean cell volume for the respective species (in μm^3). The biovolume (in $mm^3\ L^{-1}$) per taxon and sample is calculated by multiplying the mean cell volume (in μm^3) of the taxon by the number of counted cells (cells mL^{-1} or cells L^{-1}). Assuming that the phytoplankton cells have a density equivalent to that of water ($1\ mm^3\ L^{-1} = 1\ mg\ L^{-1}$), then the biovolume (in $mm^3\ L^{-1}$) of phytoplankton cells can be converted in wet weight (in mg L^{-1}) or carbon content (in mgC L^{-1}).

Several computer programs have been developed to aid in the calculation of the biovolume of phytoplanktonic or bacterial samples, using automatic or semi-automatic methods of image analysis (Fry & Davies, 1985; Bjørnsen, 1986; Bloem *et al.,* 1995; Blackburn *et* *al.,* 1998; Carpentier *et al.,* 1999; Zeder *et al.,* 2011). However, the most general and direct method for calculating cell volume is based on the morphometric approach described above, using conventional observations from optical microscopy (Hillebrand *et al.,* 1999, Sun & Liu, 2003), and no software is available for performing such calculations.

In this paper, a user-friendly computer program is presented to aid in the calculation of the biovolume of phytoplankton samples, using geometric models and conventional microscopic observations.

Materials and Methods

For implementing the calculation of biovolume, the formulas developed by Hillebrand *et al.* (1999), updated by Sun & Liu (2003), were applied.

The software, named BioCalc, was designed to operate as a 32-bits and 64-bits native application, in PC-compatible microcomputers, running under Microsoft Windows and GNU/Linux operating systems.

The development environment adopted was Lazarus (www.freepascal.lazarus.org), based on the Free Pascal language and available for MS-Windows, GNU/Linux and Mac OS operating systems. The choice was guided by taking into account the resources provided by this software tool, as the easy design of graphic user interfaces, language flexibility, efficiency and reliability of the compiler and multiplatform rapid application development.

Results

In its initial version, the BioCalc software uses an intuitive interface, consisting of a simple form with a selection list of morphotypes and specific fields for the entry of measurements for each morphotype (Fig. 1).

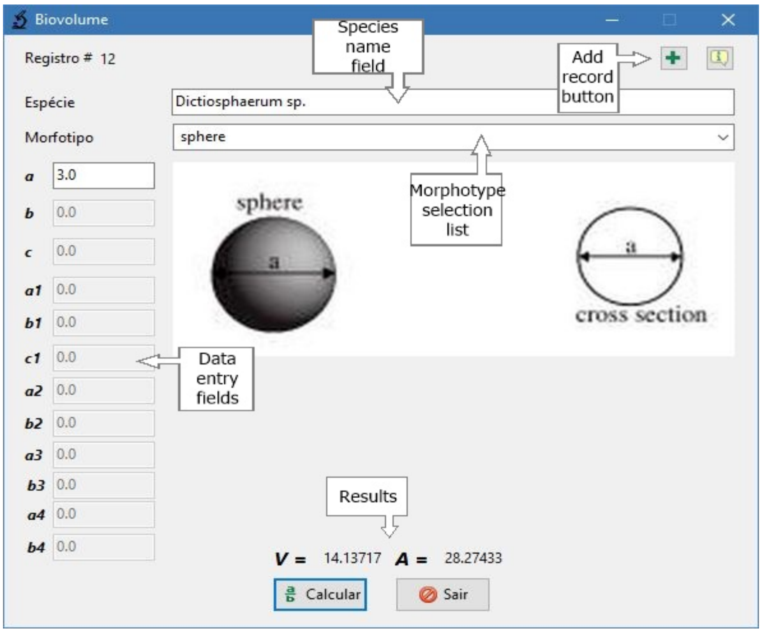
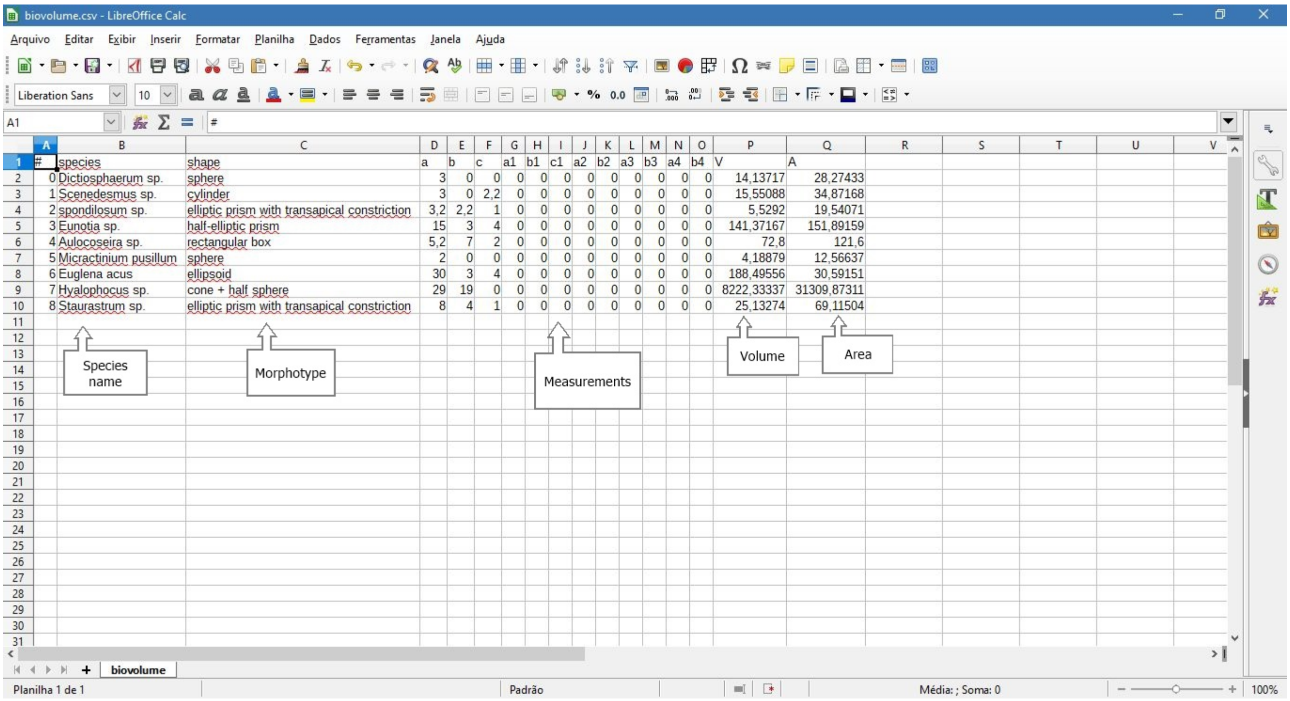


Figure 1 – Screenshot of the program for the calculation of biovolume.

For each morphotype chosen in the selection list, the program automatically enables the respective fields for the entry of measurements (in micrometers) from the cell corresponding to the selected morphotype. The "Calculate" button allows the user to confirm the introduced data and the volume and area are then calculated.

All the measurements entered for each cell, as well as the calculated values of area and volume, are stored in a text-delimited file, automatically created and maintained by the program during its execution. This file is a table, where each line represents a cell and each column corresponds to the measurements taken on each cell, according to its morphotype, with two additional columns corresponding to the values calculated for area and volume of the respective cell. The file can be opened by any electronic spreadsheet software (Microsoft Excel, LibreOffice Calc, etc.), for performing statistical analyses and creating plots (Fig. 2).



#	species	shape	a	b	c	a1	b1	c1	a2	b2	a3	b3	a4	b4	V	A
0	Dictiosphaerum sp.	sphere	3	0	0	0	0	0	0	0	0	0	0	0	14,13717	28,27433
1	Scenedesmus sp.	cylinder	3	0	2,2	0	0	0	0	0	0	0	0	0	15,55088	34,87168
2	spondilosum sp.	elliptic prism with transapical constriction	3,2	2,2	1	0	0	0	0	0	0	0	0	0	5,5292	19,54071
3	Eunotia sp.	half-elliptic prism	15	3	4	0	0	0	0	0	0	0	0	0	141,37167	151,89159
4	Aulocoseira sp.	rectangular box	5,2	7	2	0	0	0	0	0	0	0	0	0	72,8	121,6
5	Micractinium pusillum	sphere	2	0	0	0	0	0	0	0	0	0	0	0	4,18879	12,56637
6	Euglena acus	ellipsoid	30	3	4	0	0	0	0	0	0	0	0	0	188,49556	30,59151
7	Hyalophocus sp.	cone + half sphere	29	19	0	0	0	0	0	0	0	0	0	0	8222,33337	31309,87311
8	Staurastrum sp.	elliptic prism with transapical constriction	8	4	1	0	0	0	0	0	0	0	0	0	25,13274	69,11504

Figure 2 – Data file created by the program with the results from calculation of biovolume.

Discussion

Estimates of biomass are fundamental in studies of plankton ecology. Different methods have been proposed with this aim, based on geometric models or computational algorithms. Geometric models combined with observations by optical microscopy provide a direct and effective approach for the calculation of cell volume of phytoplankton samples. In this context, an interactive and multiplatform software tool for personal computers offers a simple alternative to this calculation.

The development of a version for mobile devices (tablets and cellphones running the Android operating system) will widen the possibilities of application of this tool, facilitating its use in field conditions and contributing to expedite ecological studies of plankton in the most remote regions, as the Amazon Basin and other tropical river basins.

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