

PlanktoMetrix – a computerized system to support microscope counts and measurements of plankton

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Abstract

We developed a computerized image-analysis system, PlanktoMetrix, the first system to conduct all steps of conventional microscope-based phytoplankton and zooplankton analyses (counting, measuring sizes, entering data, computations, storage in database) simultaneously using real-time digital imaging. The microscope field that displays the sample is continuously scanned by a digital camera and screened on a computer monitor, on which cell counts and measurements of linear dimensions are made by mouse clicks. When the microscope tasks are completed, computations of species abundances, estimates of biovolume per individual, species biomass per unit volume, and total assemblage biomass concentration are made automatically and stored into a database. All raw and computed data are exportable to common spreadsheet platforms. PlanktoMetrix offers the production of high-quality data in less time, with lower user fatigue and fewer typing errors; therefore, more time can be devoted to data analysis rather than generation. Furthermore, PlanktoMetrix allows collecting organism size data regularly, thus offering plankton ecologists a tool for following seasonal, ontogenetic, and other well-documented but generally ignored changes in plankton size and morphology. An example of PlanktoMetrix-generated cell size time series shows that the dinoflagellate *Peridiniopsis elpatiewskyi* undergoes a distinct annual cycle with larger cells in winter and smaller cells in summer. PlanktoMetrix is distributed free to interested users and will likely be available in the future as an open-source platform.

Key words: biovolume estimation, database, length-weight regressions, linear dimensions, phytoplankton, plankton enumeration, zooplankton

Introduction

Species composition and biomass of planktonic communities are key parameters for understanding aquatic food webs and assessing biodiversity and water quality, emphasizing the need for accurate estimates of the abundances and biomass concentrations of the microscopic organisms living in water. Traditionally these estimates involve microscopy by experts who can identify these organisms. For phytoplankton and microzooplankton (e.g., ciliates, rotifers), the sedimentation chamber and inverted microscope method (Lund et al. 1958, Utermöhl

1958) is most widely used and remains the preferred method for phytoplankton biodiversity studies (Wetzel and Likens 2004). Larger zooplankton are often enumerated under a dissecting microscope using a Sedgewick Rafter cell, plankton wheel, or Bogorov tray (Edmondson 1957), or under an inverted microscope, similar to phytoplankton enumeration.

Over the last decade, advanced optical technologies such as flow cytometry, FlowCAM (Fluid Imaging Technologies), Zooscan (BioTOM SA), and other optical counting systems (e.g., Sprules et al. 1998, Herman et al. 2004) have been evolving to replace traditional manual

microscope analyses with semi-automated and automated approaches. These technologies are rapidly developing but remain limited by issues relating to image capture and identification (discussed later) and, like microscopy, are still based primarily on morphological taxonomy. Molecular methods (e.g., real-time qPCR, microarrays) are also being developed for tracking planktonic organisms in their environment (Zamor et al. 2012, Penna and Galluzzi 2014). Although these methods offer major advances in taxonomic accuracy and sample through-put rates over standard microscope analyses, further development is needed before molecular methods can replace conventional microscopy across the broad taxonomic diversity typical in natural plankton assemblages.

Following sample collection, preservation, and preparation, conventional microscopic analysis of plankton samples consists of 3 steps: (1) organisms are identified and counted under the microscope to the lowest taxonomic resolution desired; (2) linear dimensions of representative individuals of each taxon are measured and recorded; and (3) count and measurement data are entered into computer files and are subjected to various computations. Typically, counts are converted to abundance data (individuals of each taxon per unit volume of water), and biovolume (or mass) per individual (henceforth “biovolume”) is estimated for each taxon based on taxon-specific size–biovolume equations for phytoplankton (e.g., Rott et al. 1981, Hillebrand et al. 1999) or length–weight regressions for zooplankton (e.g., Culver et al. 1985). Abundance and biovolume data are then combined to compute biomass per unit volume of water for each taxon and total biomass concentration for the entire plankton assemblage. These analyses are expensive because only trained experts can conduct the counts and measurements and because the determinations are time consuming, usually requiring several hours of microscopy per sample, depending on diversity and complexity of the assemblage. Transferring data manually to computer spreadsheets takes additional time and introduces possible typing errors. A system that simplifies these procedures, shortens the time required for analyses, and reduces potential human error is therefore an important contribution.

Various software packages are currently available to assist users in microscopic analyses of plankton (e.g., Sprules et al. 1981, Mills and Confer 1986, Hamilton 1990, Gosselain et al. 2000), but most are limited to 1 or 2 of the 3 steps (identification, measurement, data entry, and computation) and require the technician to continually shift viewing between the microscope and a computer keyboard, a process further complicated for eyeglass wearers. Our objective was to address the need to conduct all 3 steps of microscopic plankton analysis simultaneously on a single monitor using a single software program. PlanktoMetrix

(PMX), based in principle on CAPAS (Hambricht and Fridman 1994), was developed with the experience of decades of monitoring freshwater phytoplankton and zooplankton in Lake Kinneret, Israel, and designed with attention to the aspects of plankton enumeration that contribute to time consumption, error, and user fatigue in both sample and data analyses. Recently, a new version of the program, PMX-II, was released.

Overview of PlanktoMetrix

The hardware components required for using PMX are a microscope equipped with a digital camera and a Macintosh computer. Specifically, these are (1) a high-quality microscope (light or inverted) equipped with a photo-tube and C-mount (or F-mount, depending on specific camera requirements); (2) a digital microscopy camera supporting the IIDC/DCam standard and IEEE-1394 (FireWire) computer interface, with at least 1 MegaPixel per image and capable of delivering 10 or more uncompressed images per second; and (3) an Apple Macintosh (Mac) computer capable of running system software OS X v10.9.5 (Mavericks) or later. A high-quality display is recommended if a Mac without internal display is used (e.g., Mac Mini or Mac Pro). Because new Mac computers no longer come with a FireWire socket, a Thunderbolt to FireWire converter is also required.

PlanktoMetrix is a regular Mac application, without external components or drivers and no installer. It is installed by dragging the icon into the Applications folder on the Mac. Its working files, stored in the Documents folder, are database files composed of tables that store the count and size data, organized by samples. They also contain a Species Catalog, an Equation Catalog, and a Sample Details Window, containing information required for calculating the abundance of cells (or colonies or animals), median and mean biovolume per individual, and biomass concentration for each taxon in each water sample analyzed. A more comprehensive description of the database and its components is given in the Supplemental material.

Counting a sample begins by opening a new Sample Window, where sample and sampling details are entered through a series of dialog boxes, including the size of each subsample being analyzed, in either volume or areal units (Supplemental Fig. S3). In phytoplankton analysis via sedimentation chambers and an inverted microscope, individual fields-of-view can be considered subsamples. For zooplankton analyses, the entire volume of a plankton wheel may be the subsample size.

From the Sample Details Window, the user proceeds to the Microscope Window (Fig. 1) where all counts and measurements are performed. When the microscope field is viewed on the monitor, a species list (or a predefined subset list) appears on the right-hand side of the screen alongside

the microscope view (Fig. 1). Individuals of a given taxon are counted by highlighting the appropriate taxon in the list followed by individual mouse clicks for each individual of that taxon in the field of view. Colonial and filamentous phytoplankton species (or eggs per female in zooplankton) are counted as individual “colonies,” with the number of cells in the colony entered in a special box, so that both numbers of cells and colonies are recorded. Measurements for biovolume estimates are conducted in a similar manner, except that multiple mouse clicks are used to delineate linear dimensions required for a given taxon-specific equation. Throughout the counting and measuring process, the number of individuals counted per taxon is automatically updated and presented in the species list, next to the taxon name. An important aspect of PMX is that through a menu choice by the user, each new field of view (or strip, or other form of subsample) is recorded by the user during the analysis to ensure appropriate final sample-weighted calculations. Additional features can be evoked (Table 1), many during mid-count, to minimize time and effort by the user while maximizing data quantity and quality.

When analysis of a sample is completed, the user informs PMX that the analysis is complete and, on exiting

the microscope window, all calculations are tabulated automatically in the background and displayed for proofing and export. PlanktoMetrix outputs are a user-determined series of data tables containing raw counts and measurements, summary tables with mean linear dimensions, biovolumes, densities, biomass concentrations, and percent of total biomass for each taxon in a sample. These tables can be sorted and organized as required prior to export as .csv files.

Insights gained

Since 2005, PMX has been used routinely at the Kinneret Limnological Laboratory, Israel Oceanographic and Limnological Research, to monitor phytoplankton, zooplankton, and ciliates in Lake Kinneret. To date, PMX has produced a database of ~8000 samples analyzed for taxonomic composition, abundance, and biomass concentration. This database, with hundreds to thousands of individual linear measurements for each taxon spanning a decade of annual cycles, has provided insight into plankton dynamics never before available using standard plankton enumeration. For example, plotting a record for the dinoflagellate *Peridiniop-*

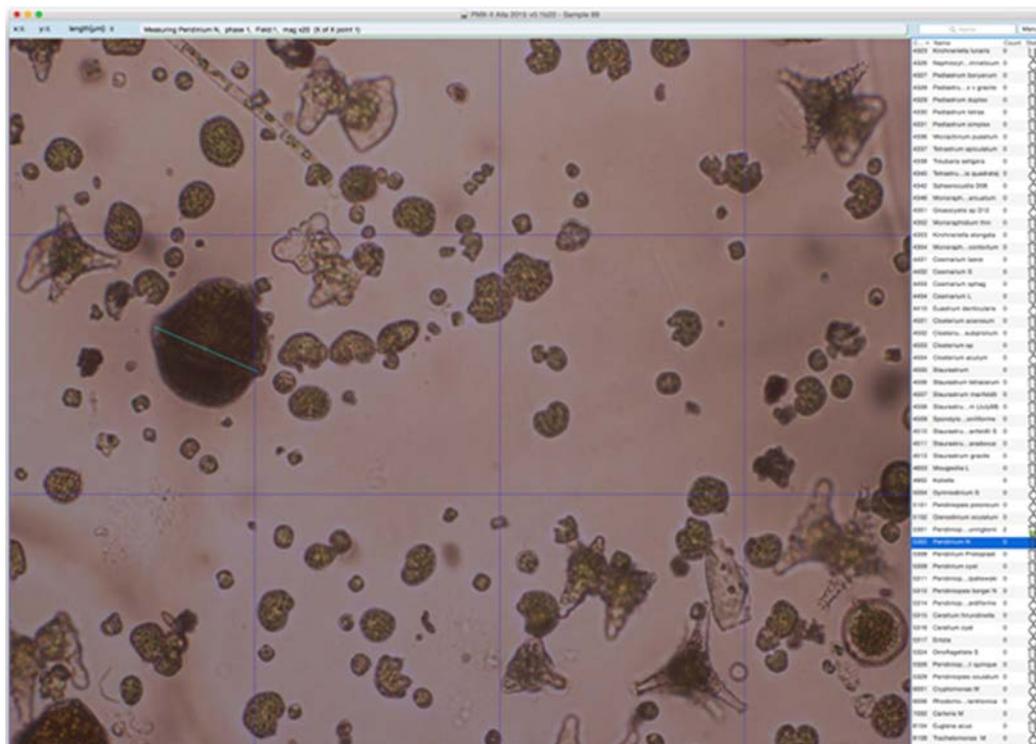


Fig. 1. The Microscope Window of PlanktoMetrix as seen on a computer screen. The main part is the microscope image, in this case of a water sample with phytoplankton, measuring a cell of *Peridinium gatunense* (largest dark cell on screen; line across cell). A 100 × 100 μm grid (optional) is superimposed. On the right a searchable list of codes and species names is provided from which to select the species to be counted and measured (highlighted). For each taxon, the number of individuals already counted is shown along with its counting scheme (count only: counter icon; measure and count: beaker icon). As measurements are made, the linear dimensions are reported in the top row above the microscope image, which also tells the user which phase and field of view are currently being counted.

Table 1. Optional features of PlanktoMetrix.

Feature	Description
Grid	Option to create a grid of a desired size (in μm) on the Microscope Window
Magnification	Option to change magnification during counting and measuring for taking more accurate measurements
Curved objects	Ability to measure curved objects using multiple linear mouse-clicks
Colonies	Option to enter the number of cells in the colony
Colonies	Option to measure only one cell in a colony
Abundant species	Option to stop counting the more abundant species after a pre-determined number of individuals was reached but continue counting the less abundant species in additional subsamples
Default biovolume or mass	Option to use a default biovolume or mass per individual for computations if no measurements are being conducted
Limited measurements	Option to stop measuring a species after a pre-determined number of individuals were measured, but continue to count it
Phases	Option to count the sample in several phases, each phase with a different subsample size (usually for applying different magnifications for different size organisms)
Sorting	All input and output tables are sortable
Searching	Tables are searchable by a variety of relevant categories

sis elpatiewskyi from Lake Kinneret demonstrates that cell size undergoes a typical annual cycle of larger cells in winter and smaller cells in summer (Fig. 2). Similar results were obtained for other dinoflagellate species occurring in Lake Kinneret. These seasonal fluctuations in cell size should be considered during routine monitoring of phytoplankton biomass in natural environments. Analysis of long-term zooplankton dynamics in Lake Kinneret, made possible by new PMX-based analysis of preserved zooplankton sample archives, has also changed a long-

accepted view of morphological constancy in crustacean zooplankton taxa (Hambright 2008). This zooplankton analysis revealed a decline in body size of the 3 dominant cladoceran genera during the 1980s and 1990s, interpreted as intensification of predation by planktivorous fish, an analysis that was previously impossible when zooplankton biomass was computed from abundance multiplied by a fixed mass per species or developmental stage. Other insights that can be obtained by applying PMX include the variability in the size and number of cells per colony or

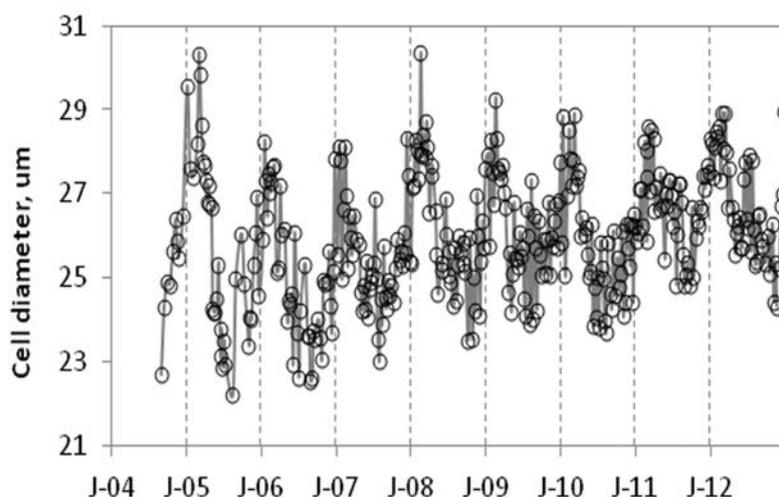


Fig. 2. Time series of cell diameter of the dinoflagellate *Peridiniopsis elpatiewskyi* in lake Kinneret, 2004–2012. Symbols are mean values of ~10 measurements on each sampling date. Dashed lines mark 1 January of each year. A total of 2292 measurements were made using PlanktoMetrix. The typical seasonal fluctuations in cell diameter imply that applying a default biovolume value to compute biomass concentrations over time is inappropriate for this species. Furthermore, the emerging seasonal pattern of cell diameter evokes new research questions.

filament in phytoplankton; the number and size of eggs per female in zooplankton life history studies (Remmel et al. 2011); more accurate assessments of mass-specific grazing and nutrient mineralization rates that vary with zooplankton body size (Hambright et al. 2007); and the temporal changes in the ratio between cell diameter and cell length in centric diatoms. We suspect many other hidden changes in plankton size and morphology have gone undetected. PlanktoMetrix enables real-time measurement and detection of subtle, yet important morphological changes in phytoplankton, zooplankton, and possibly other microscopic particles in water.

Concluding remarks

Microscopy remains a method of choice for many planktologists, especially those interested in biodiversity or those dealing with large-scale monitoring and research programs requiring knowledge of plankton abundances and diversity. Conventional microscopy is tedious, time consuming, and labor intensive. PlanktoMetrix includes all the steps of conventional microscope-based phytoplankton and zooplankton analyses (counting, measuring sizes, entering data, computations, storage in database) simultaneously and offers the production of higher-quality data in less time, with fewer typing errors and lower user fatigue. Ultimately, more time can be devoted to data analysis rather than generation. PMX-generated data can lead to new discoveries of interspecific and intraspecific size-related phenomena in plankton.

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Supplementary Material

Supplementary material is available for download via the Inland Waters website, <https://www.fba.org.uk/journals/index.php/IW/article/viewFile/965>

Supplementary information