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Estimating time series phytoplankton carbon biomass: Inter-lab comparison of species identification and comparison of volume-to-carbon scaling ratios

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ABSTRACT

An inter-calibration exercise was conducted to assess the performance of six phytoplankton taxonomists working within the Danish National Aquatic Monitoring and Assessment Program (DNAMAP). For species abundance and cell volume, a 2-fold difference was found among different estimates for subsamples from the same sample, which in turn cascaded into large differences in the species-specific carbon biomass contribution. The mean total carbon biomass estimated showed high variability (CV 43%) among the six taxonomists, but large variations were present within results produced by individual taxonomists (CV 8–50%), and one of the taxonomists produced significantly lower estimates than the others. Using data from phytoplankton time series samples, we also assessed the effect using a table of species-specific cell volumes versus cell volume measurements from a sample on carbon biomass values. For an example, the older cell-volume-to-carbon conversion method with fixed carbon-conversion constants was compared to the more recent approach of scaling biovolume to carbon biomass based on established regressions. We found that the regression between community biomass estimated by the old method versus the more recent equation yielded a slope close to 1, thus indicating general similar community biomass estimated between the methods. Type II regression suggested a high degree of variability in the estimates (17%). The highest degree of uncertainty was found by type II linear regression, when we compared the community biomass of diatoms estimated by cell sizes measured by sample to diatom community biomass estimated from cell sizes from a table of fixed cell sizes. In this analysis variation among methods for carbon estimation of individual samples was as high as 114%. Therefore, we recommend that, particularly for diatoms, cell volumes should be determined from the sample, or that table values be based on monthly estimates for at least the dominant diatom species for each study area.

1. Introduction

Performing taxonomical identification, cell volume measurements and cell carbon estimates are key components of phytoplankton monitoring programs. In particular, in light of ongoing and forecasted climate change, phytoplankton time series have become a valuable tool in understanding how marine foodwebs respond to climate drivers, underpinning the importance of precise and accurate cell volume and cell abundance estimates and of a reliable conversion of cell volume into species and community biomass. Identifying species is challenging and time consuming and the number of qualified taxonomists are decreasing globally. In this regard, active monitoring programs around the world are very important as they are the grounds for maintaining and educating future phytoplankton taxonomists with high level expertise. Within these programs, inter-calibration workshops are conducted to train taxonomists and compare their identification and counting performance. However, the outcomes of such workshops are often published in the grey literature in local languages and never reach a broader audience (Dürselen et al., 2014). So far, we have only been

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able to identify a few studies that have quantitatively addressed the performance of plankton taxonomists. One of the oldest works was by Lund et al. (1958), who noted that the number of cells counted in a given sample is an important source of bias in the analysis of plankton. In another study, skilled taxonomists were given images of different *Dinophysis* spp. and asked to identify the species (Culverhouse et al., 2003). Not surprisingly, it was observed that even skilled taxonomists made mistakes. In another inter-comparison of zooplankton data from Longhurst Hardy Plankton Recorder hauls, large disagreements were identified among six expert taxonomists (Culverhouse et al., 2014). Although the performance of the taxonomists is a very important issue, large differences can also emerge from different ways of analyzing the samples, including the number of cells counted and the size of the sample examined (Zingone et al., this volume). Particularly the volume of the sample examined constitutes a major source of variability in the detection and quantification of rare species and related diversity assessments, which would require the examination of about 1 L of sample (Rodríguez-Ramos et al., 2014). This is in contrast to the settling chamber volume of <50 mL and still smaller volumes are examined, which currently seem to be standard for phytoplankton analyses due to time constraints (Anon, 2014; Olenina et al., 2006).

Besides proper species identification and cell size measurement, applying carbon-to-cell volume conversion factors to obtain carbon biomass for phytoplankton species and the community is also a challenge. Assigning carbon as a common currency is particularly important because it allows the comparison of various phytoplankton data sets in time and space, and allows the quantitative assessment of the relationships between different trophic levels of the marine foodwebs. Over the past few decades, a series of papers have published relationships between cell volume and carbon content for phytoplankton (Mullin et al., 1966; Strathmann, 1967; Verity et al., 1992; Montagnes et al., 1994; Menden-Deuer and Lessard, 2000). These efforts have resulted in a series of cell-volume to cell-carbon relationships. The historical component where each decade has its own cell volume to cell carbon factor poses a problem for researchers who work with large data sets, such as those analyzed elsewhere in this volume (Harrison et al., this volume). In addition, time series often span across multiple decades and are often restricted to the method that was the state-of-the-art at the time that the program was launched. Hence, at present, it is unclear how these different relationships compare and to what extent potential differences in these conversion factors cascade into observed shifts in phytoplankton community carbon biomass in the analysis of decadal time series. Moreover, in some monitoring programs, cell sizes are binned into different size classes (Olenina et al., 2006). In other cases, a fixed cell size of each taxonomical entity is used, while in other programs, cell sizes are determined in the sample that is being analyzed (Edler, 1979).

This study had two objectives: i) comparing phytoplankton species abundance and biomass estimates obtained on subsamples from the same sample by six different taxonomists, and ii) using time series data in some of the carbon-to-biovolume scaling methods available in the literature. First, we assessed the comparability and reproducibility of species identification, counting and cell volume estimates among phytoplankton specialists (taxonomists). Second, we investigated how the biomass estimate from time series data is affected by different cell volume-to-carbon relationships. Thus, the ultimate aim was to identify possible limitations that need to be taken into account when comparing phytoplankton time series where phytoplankton experts and biomass calculation methods that were used in the time series have changed over time.

Table 1
Scaling parameters used in converting cell volume to carbon biomass. The parameters *a* and *b* refer to the scaling constants in Eq. (1).

Reference	*a*	*b*
Menden-Deuer and Lessard (2000) *	0.216	0.939
Menden-Deuer and Lessard (2000) †	0.288	0.881
Montagnes et al. (1994)	0.109	0.991

* For all species other than diatoms.
† For diatoms.

2. Methods

2.1. Phytoplankton sample analysis

Plankton samples were fixed in acid Lugol's solution (2% final concentration), and cells were measured and counted using an inverted microscope (Utermöhl, 1958). The chlorophyll-containing mixotrophic ciliate *Mesodinium rubrum* at times was very abundant and it was included in the estimate of phytoplankton biomass. The analysis followed the general guidelines given in the Danish National Aquatic Monitoring and Assessment Program (DNAMAP) (Anon., 2014). Briefly, at least 50 cells and preferably >100 cells of the dominant species were counted, with a total of at least 500 specimens counted. The biovolume of at least 10 cells of the dominant species was determined in each sample using appropriate geometrical models (Olenina et al., 2006). Cell volumes of species that contributed less biomass were obtained from a standard table derived from DNAMAP. The samples were analyzed within three months after collection.

2.2. Carbon biomass estimates

Cell carbon was estimated by applying either of two methods. The first method applied fixed volume-to-carbon conversion factor of 0.13 pg C μm^{-3} for thecate dinoflagellates and other phytoplankton (Edler, 1979), whereas the cell volume was corrected for the water vacuole by multiplying the plasma volume of diatoms by 0.11 pg C μm^{-3} (Strathmann, 1967). It must be noted that this method does take into account that in diatoms the plasma volume decreases relative to the water vacuole, thus yielding a non-linear increase in carbon per cell, with increasing cell size. The method by Menden-Deuer and Lessard (2000) accounted for the water vacuole of diatoms by applying different scaling parameters for diatoms and non-diatoms (marked by a superscript in Table 1).

The second method, which instead reproduces the non-linear increase in diatom carbon with size, utilized the power functions proposed by Menden-Deuer and Lessard (2000) and Montagnes et al. (1994), where cell carbon (C_c, pg C $cell^{-1}$) is estimated from the cell volume (V_c) according to:

Table 2
Mean community carbon biomass ± standard deviation of the triplicate samples analyzed by the six taxonomists.* indicates statistical significant difference (see text for details on test). Carbon biomass was determined using Menden-Deuer and Lessard (2000).

Taxonomist	Mean biomass (μg C L^{-1})	CV (%)
1	291 ± 24	8
2	230 ± 59	26
3	85 ± 25*	29
4	328 ± 104	32
5	387 ± 131	34
6	436 ± 220	50
Grand mean	293 ± 125	43