Estimating time series phytoplankton carbon biomass:

Inter-lab comparison of species identification and comparison of volume-to-carbon scaling ratios

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Research Highlights

- Large variation among six taxonomists for carbon biomass estimation

- Different volume-to-carbon conversion methods do not on average produce large differences in total phytoplankton biomass estimates from time series
- Phytoplankton community biomass varies with cell volumes used in the conversion; differences between using fixed cell volume values and cell volumes determined for the sample varied up to 46-49%.
- Large variation in diatom community biomass was found when diatom community biomass was based on measured cell sizes compared to community biomass estimated from fixed cell sizes based on table values (114-109%.)
- Cell volume should be determined for diatoms by sample or alternatively by month to correct for the seasonal variation in their cell volume.

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 contributions. 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 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 newer equation yielded a slope close to 1, thus indication 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 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 (Menden-Deuer and Lessard, 2000; Montagnes et al., 1994; Mullin et al., 1966; Strathmann, 1967; Verity et al., 1992). 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 issue). 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.

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 & 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 & 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:

where a and b are characteristic scaling parameters (see the different scaling parameters in Table 1).

Phytoplankton carbon biomass (*Cb,* µg C L^{-1}) was calculated by summing the cell abundance determined with a microscope (*n*) by cell carbon (C_c,) estimated as outlined above for all taxonomical entities (*N*) accordingly to Eq. 2:

Carbon biomass values (*Cb*) obtained by the different methods were compared using a type II linear regression (uncertainty associated with both axes); however, we assumed that carbon biomass values were log-normal distributed, i.e. that the uncertainty of *Cb* scaled with the value of *Cb*. This regression was carried out by formulating the linear model for *Cb*, but fitting the model to log(*Cb*) using a non-linear regression.

2.3 Performance of phytoplankton taxonomists

Six expert taxonomists from three laboratories were invited to conduct an inter-calibration exercise. In this context, an expert taxonomist is defined as a person who has analyzed phytoplankton samples and submitted these data to the DNAMAP program. The expert taxonomists and the laboratories that deliver data to the DNAMAP program are regularly reviewed for their qualifications. As part of this process, a seawater sample was collected from the mixed surface layer in Ringkøbing Fjord, DK (56.6ºN; 9.1ºE) in September 2012. The sample was split and distributed among three laboratories to be analyzed by a total of six taxonomists working within the monitoring program. The analytical procedure was identical to the outline above, except that the expert taxonomists were asked to conduct their analysis in triplicate. After analysis, all data were stored in a database including information about phytoplankton species, abundances, cell sizes, etc. Differences in the estimated carbon biomass among taxonomists were investigated using a one-way ANOVA applied to log-transformed data followed by a pair-wise comparison using Tukey-Kramer test.

2.4 Time series analysis

We analyzed the time series, including hydrochemistry data, from a single station located at Northern Little Belt (55.67°N°, 10.09°E), which is part of the DNAMAP. This station has been sampled bimonthly with >500 phytoplankton samples analyzed since 1990. We restricted the analysis to the period 2005-2014 (208 samples/data points), because cell volumes for specific species were occasionally missing in the earlier data. In this analysis, *Cb* was calculated using the methods outlined by Menden-Deuer & Lessard (2000), Montagnes et al. (1994), Strathmann (1967), and Edler (1979). To this end, we applied either a set of measured cell sizes from a table derived from the DNAMAP program, or we used cell sizes of the most common species measured from the samples.

2.5 Monthly biovolumes

Samples from 22 stations in DNAMAP were analyzed to describe seasonal variations in the biovolume of 12 common diatom species. All these stations are located around 54-56°N, and have an average annual seawater temperature around 10˚C and a salinity ranging between 7 and 31 across stations (Conley et al., 2000). Monthly biovolume means as well as their standard errors were estimated for the 12 diatom species selected for this study.

3. Results

3.1 Performance of phytoplankton taxonomists

In the inter-calibration exercise, the six different phytoplankton taxonomists identified between 24 and 52 species in the subsamples examined, and estimated a mean total phytoplankton community biomass that varied from 85 to 436 µg C L^{-1}, i.e. by more than 5-fold (Table 2). Interestingly, the coefficient of variation (i.e. variation among triplicates) for individual taxonomist results ranged up to 50%. Differences among taxonomists' results were significant (one-way ANOVA; $F_{5,12}$=14.03, P<0.008). Pair-wise comparison of the taxonomists revealed that taxonomist #3 estimated significantly lower biomass in the samples than the other five taxonomists (Tukey-Kramer pairwise comparison of means; p<0.05).

All taxonomists found that the three main taxonomical groups were bacillariophyceans (proportion=69% ±12%), cryptophyceans (proportion=13% ±2%) and dinophyceans (proportion=11% ±7%), and these three groups contributed on average 93% of the total biomass. Other taxonomical groups were much less abundant (Fig. 1).

A large variation among taxonomists' estimates was also found for cell density (Fig. 2A) and cell volume (Fig. 2B) of the most dominant species. However, it is interesting to observe that cell volume results, for some species were less variable within triplicates than among taxonomists, whereas the opposite was true for other species (e.g. *Mesodinium rubrum*, Fig. 2).

The taxonomists disagreed on the ranking of the most important species in terms of biomass (Fig. 3). While all taxonomists identified *Ditylum brightwellii* as the dominant species, *Cerataulina pelagica* made a substantial contribution to the phytoplankton biomass for five out of six taxonomists (Fig. 3) who reported it as the second most abundant species. Among the most common 8 species, only one diatom (*Leptocylindrus danicus*), and two dinoflagellates (*Prorocentum triestinum* and *P. micans*) were found by all 6 taxonomists.

3.2 Comparison of carbon estimation methods

Carbon biomass (*Cb*) values estimated using cell volumes from reference tables were compared to *Cb* estimated from measured cell volumes (Fig. 4a), and there was overall good agreement between the two methods (Table 3; Fig. 4). *Cb* estimated following Menden-Deuer & Lessard (2000) on the two types of cell volume estimates scaled identically (the slope was not significantly different from 1), but carbon biomass from measured cell volumes was on average 3 µg C L^{-1} lower. However, differences between the two methods for individual samples were typically 46-49% (Table 3). Since diatoms were the dominant taxonomical group and because their cell volume varies seasonally, the same comparison was made for diatoms only (Fig. 4b). We found that the biomass of diatoms was on average approximately 5% higher

when measured cell volumes were used in the biomass estimate. However, the difference between the two methods for calculating diatom biomass of individual samples was much larger than for the total community (~109 - 114%). When *Cb* was estimated based on Menden-Deuer & Lessard (2000), it yielded on average similar community biomass than *Cb* estimates based on Montagnes et al. (1994). In this analysis was individual samples uncertainty around 46-49% (Table 3, Fig. 4C). Finally, *Cb* calculated following Menden-Deuer & Lessard (2000) was on average similar to *Cb* estimated with a fixed carbon conversion value proposed by Strathmann (1967) and Edler (1979) (Fig. 4D; Table 3). The uncertainty in this analysis was surprisingly low among the methods for individual samples (17%).

3.3 Time series analysis by date

Comparing the carbon biomass (*Cb*) calculated using fixed cell sizes versus cell sizes estimated from the sample occasionally revealed systematic differences between the two methods (Fig. 5A). Overall, there was good agreement between the two methods when the sample biomass was low, although *Cb* obtained from a table of cell sizes (sizes derived from the DNAMAP program) yielded periodically very high peaks that were not captured when *Cb* was estimated using measured cell sizes from the sample (e.g. biomass peaks in Fig. 5A). Comparing *Cb* values estimated following Menden-Deuer & Lessard (2000) to those obtained following Montagnes et al. (1994) over time showed a reasonably good agreement between the two methods (Fig. 5B). However, in few cases, biomass peak was found by the method by Montagnes et al. (1994) but not by the method following Menden-Deuer & Lessard (2000).

3.4 Monthly biovolumes

Calculations of biovolume based on cell measurements for 12 centric diatoms produced values varying by 2-3 fold over the year, while statistically significant differences were found

in log-transformed cell volumes by month for all species (one-way ANOVA; $P<0.0003$). There was considerable seasonal variation, but generally the largest biovolume values were observed in the winter/spring, while smaller values were observed in late summer for these 12 diatoms (Fig. 6).

4.0 Discussion

There were surprisingly large variations in the estimates of phytoplankton community carbon provided by the 6 taxonomists for the same sample. However, because of the large variation between replicates analyzed by the same taxonomist, carbon biomass estimates from only one of the phytoplankton experts deviated significantly from the others. These variations were found to be a product of varying cell size and cell abundance estimates (i.e. differences in the sub-samples). Moreover, bigger cell sizes and larger abundances gave the best agreement amongst the taxonomists. For example, the very abundant and easily distinguishable cells of *Prorocentrum micans* and *Cerataulina pelagica* yielded similar biomass (Fig. 3). In contrast, the less abundant species were not found by all taxonomists, supporting the observation by Rodríguez-Ramos et al. (2014). There can be multiple reasons for these patterns. Firstly, the number of cells counted affects the counting statistics (Dürselen et al., 2014; Lund et al., 1958). Secondly, the fatigue of the taxonomist may play an important role in the analysis (Culverhouse, 2007). Thus, the more cells that are counted, the higher the precision is for the abundance estimate. Related to counting statistics is also the sample volume which affects the precision in the estimate of the abundance of rare species (Rodríguez-Ramos et al., 2014; Woodcock et al., 2006). Generally, the way a sample is handled from its collection, including randomizing, subsampling, settling and observation methods, and obviously the experience of the taxonomist,

may all effect the results with the light microscope, thus affecting the comparability of the data obtained (Zingone et al., this volume).

One of the aims of phytoplankton community analyses is to relate the biomass of species, genera or plankton classes using carbon as the common currency, to assess environmental or climate change.

The monthly estimates of the cellular biovolume (Fig. 6) showed large seasonal variation among diatoms that varied ~2-fold over the year for all the investigated species (Fig. 6). These dynamics are driven primarily by the life cycle of diatoms due to cell size reduction during cell division and maximum size restoration by auto-enlargement or following sexual reproduction. In fact, their cell size can be used to track recurring sexual reproduction events in some cases (D'Alelio et al., 2010). Seasonal changes in environmental variables such a light, nutrients and temperature are also important drivers of size changes throughout the year. For example, cell sizes are reduced in response to lowered light (Thompson et al., 1991), limitation of nutrients such as N and P (Davidson et al., 2002; Edwards et al., 2011; Harrison et al., 1990), whereas Si limitation increases the cell size in diatoms (Harrison et al., 1976). Temperature also affects phytoplankton cell size, but the literature remains inconclusive on this matter (Atkinson et al., 2003; Montagnes and Franklin, 2001; Thompson et al., 1992). Based on a literature review, Atkinson et al. (2003) found that body size scales inversely with increasing temperature by 2.5% per degree. Assuming a mean temperature of 8 ºC and a maximum temperature of approx. 18 ºC (representative for the area where the data were collected), the effect of temperature would result in a biovolume decrease of approximately 28% from spring to summer. This only accounts for about 50% of the observed decrease in biovolume, which highlights the relevance of biological factors and their modulation by multiple factors mirrored by the cell volume variations of the 12 listed diatom species over the year. For all these reasons, diatom biovolume variations remain one of the main sources of uncertainty when estimating phytoplankton community carbon (Table

3, Fig. 4B). Therefore we suggest that variation may be reduced if cell volumes are determined during sample analysis since this would help to correct for the significant seasonal changes in diatom cell volume.

Conversion of biovolume into carbon biomass is important because a common currency allows the analysis and modeling of carbon pathways and comparisons across spatial and temporal scales. To our knowledge, this study is the first attempt to compare different cell volume-to-carbon conversion methods that are used to calculate phytoplankton community carbon biomass.

The use of standard cell size tables to assess phytoplankton biovolume throughout the year disregards the species-specific cell size seasonal variation and is likely to produce erroneous biomass estimates for different times of the year. We acknowledge that often it is not feasible to estimate cell size in every sample taken, but ignoring seasonal dynamic in cell sizes may ultimately lead to a misrepresentation of changes in the planktonic food web, with the possibility of overlooking important structural food web changes. This potential risk is supported by our seasonal cell volume comparison of using fixed cell size versus measured cell sizes over time (Fig. 4B). The variability may very well be driven by the species dynamics and the 2-fold variation in the diatom cell volume (Fig. 5). Our analysis also shows that the fixed cell size tables (often determined only once in many cases) estimates higher peak abundance in comparison with the cell sizes measured in the sample.

Our results show that carbon biomass estimates following Menden-Deuer & Lessard (2000) and Montagnes et al. (1994) show good correspondence between methods (Table 3, Fig. 4B, 5B). In fact, when we compared the method of Menden-Deuer & Lessard (2000) to Strathmann (1967) and Edler (1979), we found very little uncertainty (17%) among the methods (Table 3, Fig. 4C). This analysis therefore suggests that older data series based on fixed factor volume to

biomass conversion in fact are well comparable to the more modern approach by Menden-Deuer & Lessard (2000).

During the past decades, several approaches have emerged to circumvent the issues with microscopy analysis outlined above. Among these are pigment analysis using pigments by HPLC (Mackey et al., 1996), FlowCAM analysis (Jakobsen and Carstensen, 2011; Sieracki et al., 1998) and various flow cytometers combined imagining technologies (Dubelaar et al., 2004; Premazzi et al., 1992; Sosik and Olson, 2007). It is beyond the scope of the current paper to describe the technologies behind these methods, but the conclusion from those studies is that imagining technologies are powerful for sizing cells. Furthermore, the volume capacity for processing is also increasing with the advancement in technology, providing more representative samples and improving the overall statistical performance. Yet, images collected with these technologies still need to be evaluated by a skilled taxonomist. Some steps toward replacing taxonomists using automated expert systems are showing promising results (Alvarez et al., 2012), but taxonomical quality control is still needed. In addition to automated expert systems, specialized flow cytometers already have revealed their potential in terms of high resolution sampling (Thyssen et al., 2008), yet these systems also need to be supported and ground-truthed by microscopy analysis, or even combined with molecular techniques.

In conclusion, differences in species identification, cell volume measurements and carbon estimates among taxonomists present a major challenge and stress the need for continuous training and frequent inter-calibration workshops, even for experienced taxonomists. Even within the triplicates that were analyzed by the same taxonomist, there was a rather large deviation among sample counts for several species (Fig. 2). This variation was due to a combination of heterogeneity among the three subsamples and uncertainty induced by the taxonomist. Therefore, caution should be taken in using species-specific carbon biomass estimates. Moreover, many time series extend across several decades and the samples have been analyzed by many different

taxonomists, underlining the need for continuous training between out-going and in-coming taxonomists. Fixed cell sizes from the literature increases the chance of producing incorrect biomass peaks especially for diatoms in the time series (Fig. 5a). However, monitoring programs are often limited by resources. One solution to account for seasonal changes in cell sizes is to develop local tables of monthly cell size for the dominant species for each study area, instead of using one single cell measurement for the entire year or the whole time series. Using locally determined monthly carbon/cell volume conversion factors is somewhat analogous to frequently running standards in a QA/QC chemical analysis. Moreover, the tools used to convert cell volumes into biomass need further development and there is a particular need to address how the water vacuole of diatoms influences the cellular carbon for a wide range of diatom cell sizes.

5.0 Acknowledgments

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Tables

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 & Lessard (2000)[1]	0.216	0.939
Menden-Deuer & Lessard (2000)[2]	0.288	0.881
Montagnes et al. (1994)	0.109	0.991

[1]For all species other than diatoms.
[2]For diatoms.

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 & Lessard (2000)

Taxonomist	Mean biomass ($\mu g\ C\ L^{-1}$)	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

Table 3. Parameter estimates for the type II linear regressions (y=a+bx) for different methods for calculating carbon biomass (first vertical column (parameter a) refers to the panels in Fig. 4). For each regression, the hypotheses of zero intercept and slope equal one are tested. The two last columns give the root mean squared error for the log-transform of the two regression variables and in parentheses the corresponding percent uncertainty (relative uncertainty was estimated as).

Panel	a	P(a=0)	b	P(b=1)	RMSE(x)	RMSE(y)
a	-2.511	<0.0162	0.969	<0.001	0.379 (46%)	0.402 (49%)
b	0.208	<0.001	1.047	0.003	0.760 (114%)	0.739 (109%)
c	2.242	0.009	0.96	<0.001	0.380 (46%)	0.392(48%)
d	2.754	<0.0001	1.015	<0.001	0.155 (17%)	0.155 (17%)

Figures

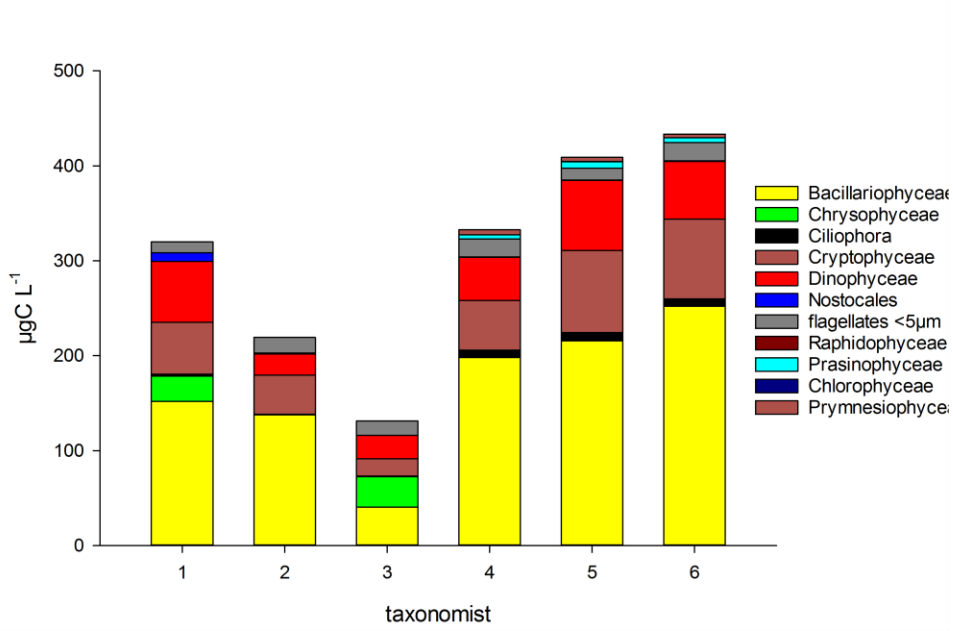

Fig 1. Phytoplankton community carbon biomass, divided into major taxonomical groups, determined on subsamples from a single sample by six taxonomists.

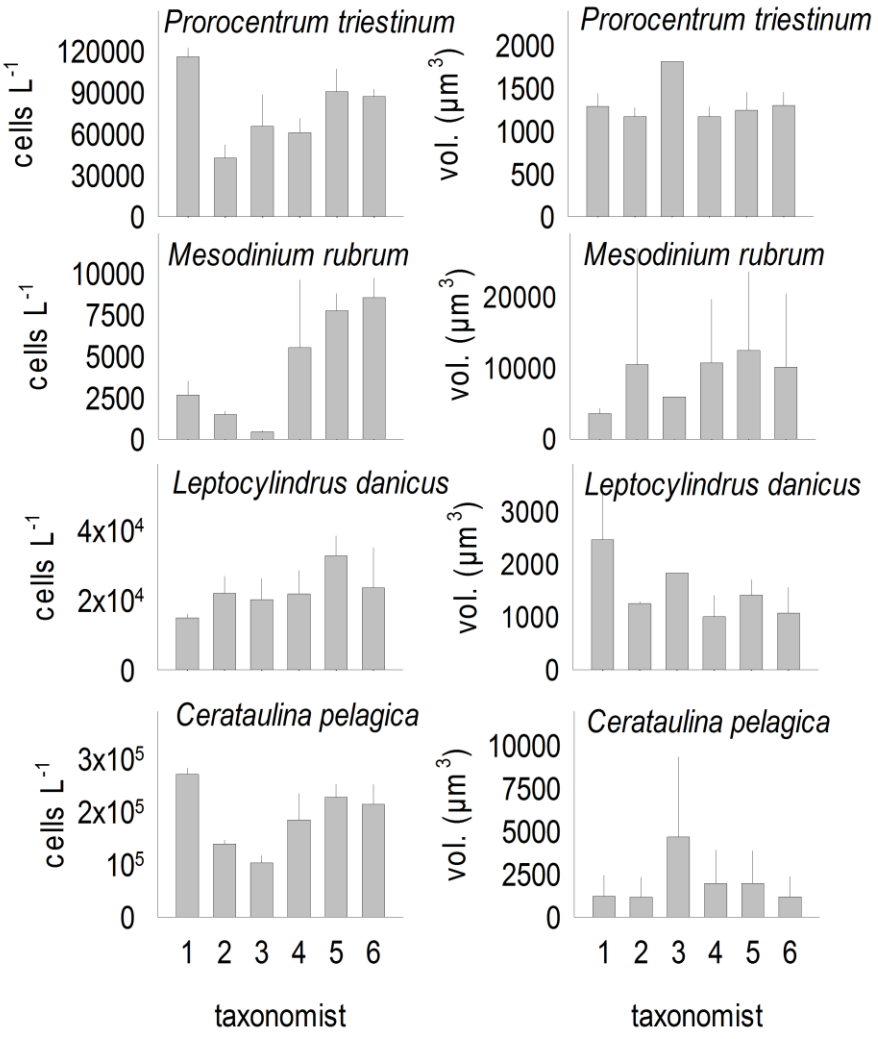

Fig. 2 Cell concentrations (cells L^{-1}) and cell volumes (μm^3) for some of the phytoplankton species determined by six taxonomists. Error bars are standard deviation of the means (n=3).

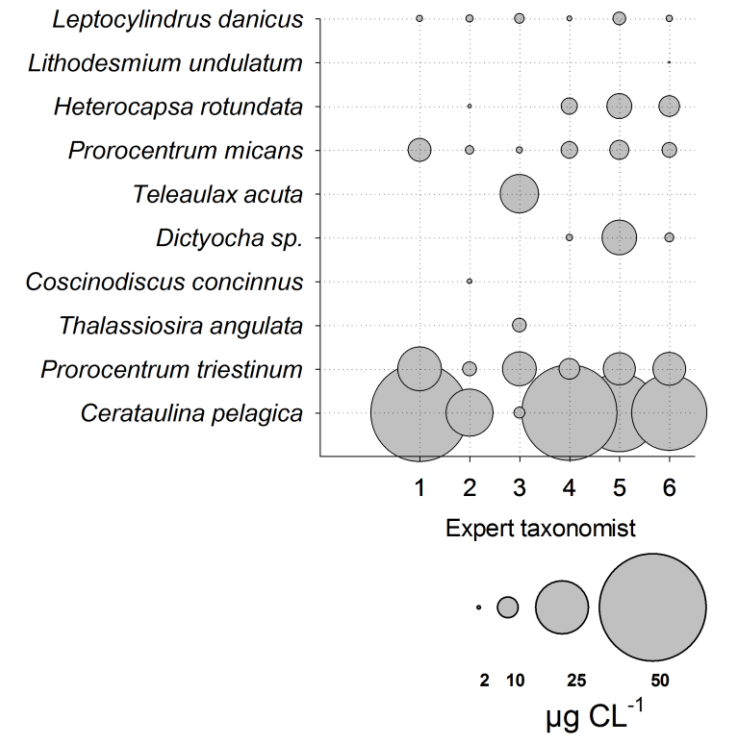

Fig. 3 Bubble plot comparing the cell carbon of 10 dominant species determined by six taxonomists. The most dominant species *Ditylum brightwellii*, which accounted for more than 50% of the sample biomass, is not shown.

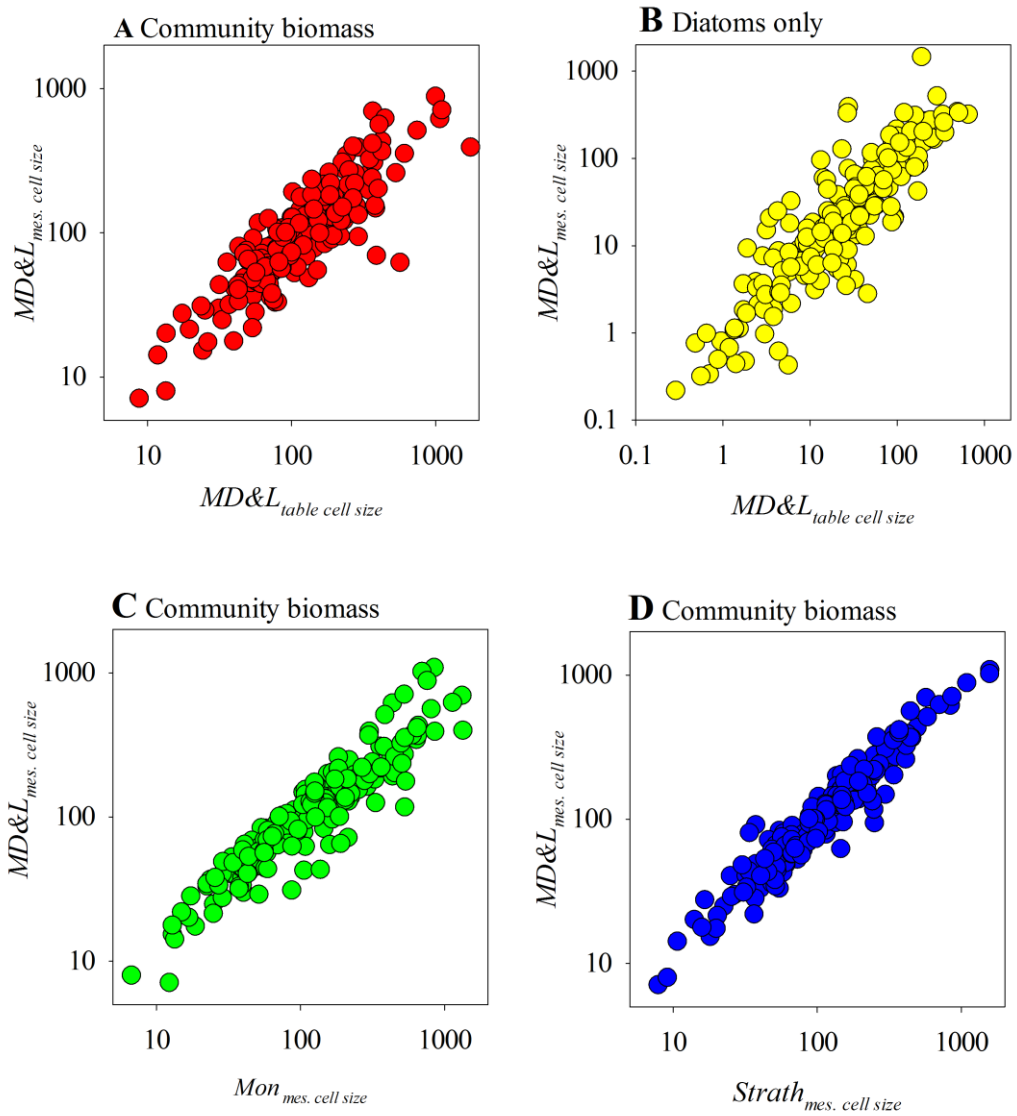

Fig. 4 Comparison of different methods for calculating carbon biomass (n=144). All axes are in units of µg C L^{-1}. a) Community carbon biomass using measured ($MD\&L_{mes.\ cell\ size}$) versus fixed cell ($MD\&L_{table\ cell\ size}$) volumes applying equations from Menden-Deuer & Lessard (2000). b) Same as a) for diatoms only. c) Community carbon biomass calculated using measured cell sizes ($MD\&L_{mes.\ cell\ size}$) following Menden-Deuer & Lessard (2000) ($MD\&L_{mes.\ cell\ size}$) versus Montagnes et al. (1994) ($Mon_{mes.\ cell\ size}$). d) Community carbon biomass using measured cell sizes applying Menden-Deuer & Lessard (2000) ($MD\&L_{var.\ cell\ size}$) versus Strathmann (1967) ($Strath_{mes.\ cell\ size}$). Linear type II regression parameters and statistics for the relationships are found in Table 3.

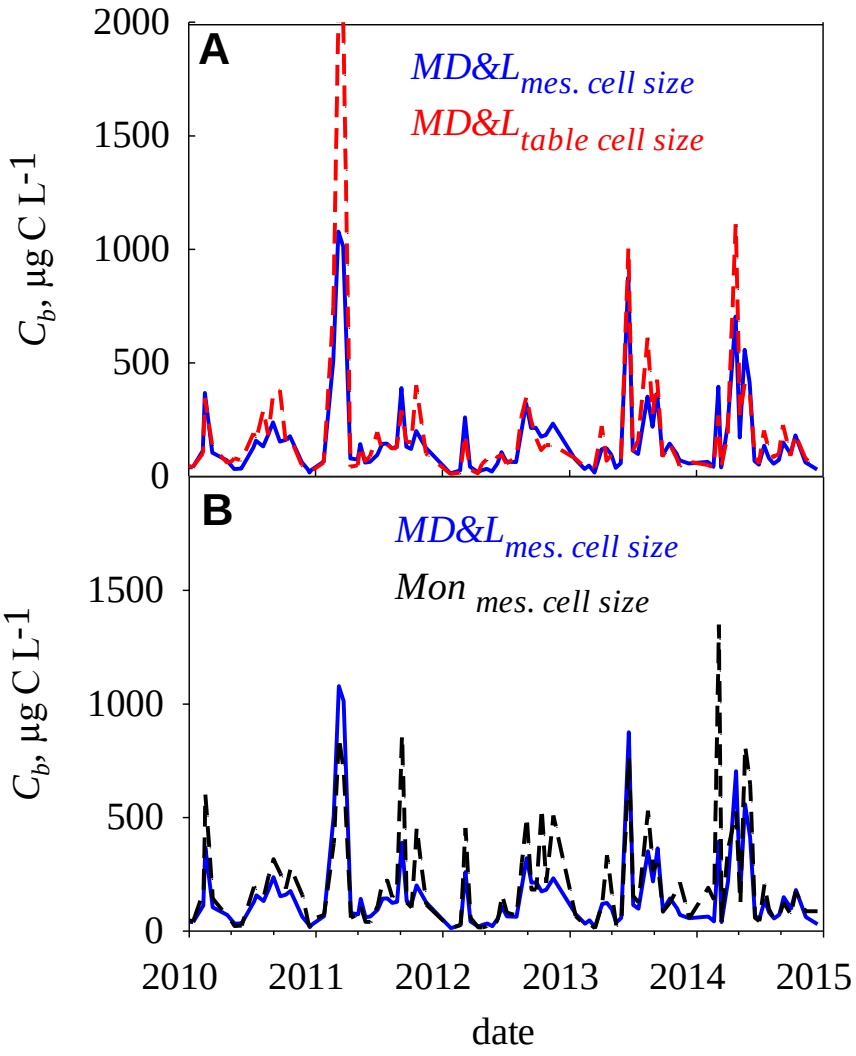

Fig. 5 Time series of phytoplankton community carbon biomass in the Northern Little Belt over a 5-year period. A) Using measured cell volumes (blue) and cell sizes from the DNAMAP table (red) applied to Menden-Deuer and Lessard (2000) B) Using measured cell volumes (blue) and cell sizes from the DNAMAP table (red) applied to Menden-Deuer and Lessard (2000) (blue) and Montagnes et al. (1994) (black).

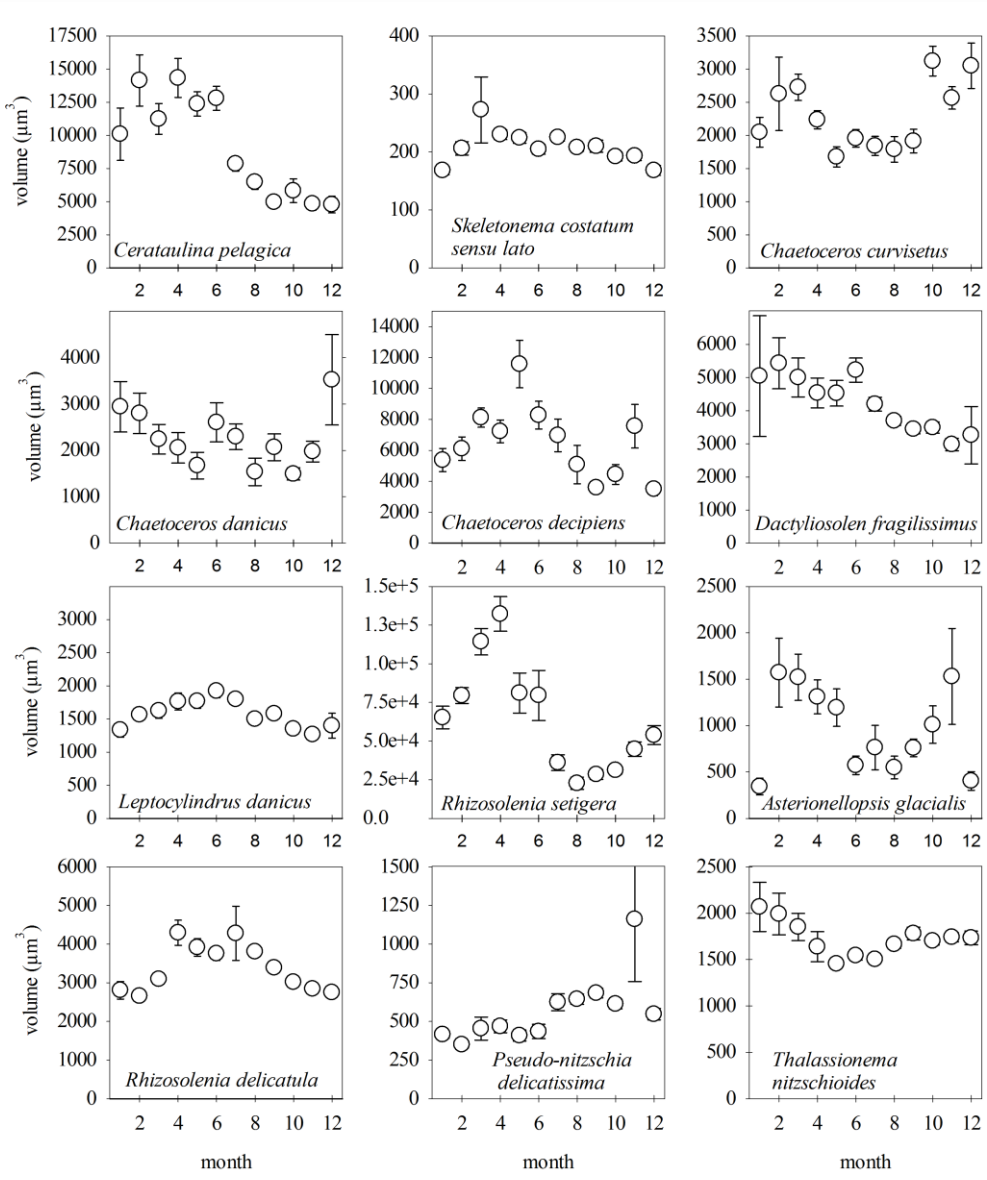

Fig. 6 Seasonal variation in biovolume (µm^3) for 12 diatom species from the Danish monitoring program (DNAMAP). Error bars are the standard deviation and n >20. The diatom *Skeletonema costatum sensu lato* is most likely *Skeletonema marinoi*, but we have no confirmation.

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