Ultraphytoplankton in the eastern Mediterranean Sea: towards deriving phytoplankton biomass from flow cytometric measurements of abundance, fluorescence and light scatter

W. K. W. Li¹, T. Zohary², Y. Z. Yacobi², A. M. Wood³

¹Biological Oceanography Division, Bedford Institute of Oceanography, Dartmouth, Nova Scotia, Canada B2Y 4A2
²The Yigal Allon Kinneret Limnological Laboratory, Israel Oceanographic and Limnological Research Ltd, PO Box 345, Tiberias 14102, Israel
³Department of Biology, University of Oregon, Eugene, Oregon 97403, USA

ABSTRACT: Prochlorophytes, cyanobacteria and eukaryotic ultraphytoplankton from the southern Levantine Basin of the eastern Mediterranean Sea were analyzed by flow cytometry to obtain measurements of cell abundance, relative cellular fluorescence and relative cellular light scatter. Assuming that fluorescence is a proxy for chlorophyll and that light scatter is a proxy for cellular carbon, phytoplankton biomass can be expressed as the sum (over all cell groups) of adaptive cellular characteristics (i.e. chlorophyll and carbon) weighted by cell abundance. On this basis, much of the carbon appeared attributable to eukaryotic ultraphytoplankton, but chlorophyll was more evenly partitioned such that the contributions from prochlorophytes and cyanobacteria were also significant. The subsurface chlorophyll maximum coincided with the maximum in total fluorescence but not with the maximum abundance of cells nor with the presumed maximum in the carbon biomass of ultraphytoplankton.

KEY WORDS: Chlorophyll · Cyanobacteria · Flow cytometry · Mediterranean · Prochlorophytes · Ultraphytoplankton

INTRODUCTION

The concentration of particulate chlorophyll a (chl a) is an important and easily measured variable. It has long served as an index of phytoplankton biomass and its vital role in biological oceanography is indisputable. Chl a is a bulk property of seawater. In common with other bulk measurements, chl a gives no indication of the relative contribution from constituents. For example, the same chl a value can be obtained from a sample containing a few large phytoplankton cells and from a sample containing many small cells. Thus, while the bulk measurement of chl a provides a good estimate of the pigment biomass of primary producers, it can mask many important differences that affect ecosystem structure and function. For this reason, as emphasised by Platt (1989) and Yentsch & Campbell (1991), the recovery of bulk properties from the details of the constituents is a most desirable goal in phytoplankton research.

To this end, we follow Shimada et al. (1993) in comparing bulk measures of chl a with the summed contributions of properties from single cells of the ultraphytoplankton measured by flow cytometry. The study was made on phytoplankton sampled from the southern Levantine Basin of the eastern Mediterranean Sea. The waters in this region are extremely oligotrophic (Berman et al. 1984), and most of the chl a is attributable to cells passing 10 µm membrane filters (Y. Z. Yacobi, T. Zohary, N. Kress, A. Hecht, R. D. Robarts, M. Waiser & W. K. W. Li unpubl.). As expected from observations elsewhere (Li et al. 1992a, b), the ultraphytoplankton here includes prochlorophytes, unicellular cyanobacteria, and various red-fluorescing pico- and nano-phytoplankton (A. M. Wood, J. Neveux, M. Sondergaard & Y. Z. Yacobi unpubl.; Yacobi et al.)
unpubl.). In the Mediterranean Sea, the microspectrofluorimetric results of Zaika & Yashin (1984) on abundant ‘red luminescing’ algae of 0.5 μm size preceded the more recent flow cytometric observations of prochlorophytes (Vaulot et al. 1990, Li et al. 1992b, Vaulot & Partensky 1992).

In this paper, we first formulate the concepts required to derive phytoplankton biomass (in terms of chl a and carbon) from flow cytometric measurements and then indicate the difficulties of achieving this goal. We follow by suggesting 2 interim approaches for examining the data at hand. Finally, we present our results for both approaches. In these waters, we conclude that the observed depth distribution of bulk chl a can be faithfully matched by joint considerations of cell abundance and pigment content of the ultraphytoplankton. Furthermore, we suggest that the subsurface chlorophyll maximum did not correspond with the maximum abundance of cells nor with the maximum in the carbon biomass of ultraphytoplankton.

CONCEPTUAL OUTLINE

Proceeding from the general ideas of Yentsch & Campbell (1991), we express the concentration of chl a (μg l⁻¹) at depth z as follows:

\[ \text{chl } a (z) = \sum_i n_i(z) \cdot f_i(z) \cdot \psi_i(z) \]  

(1)

where \( i \) refers to each recognizable group (i.e. prochlorophytes, cyanobacteria, photosynthetic eukaryotes); \( n = \text{cell concentration (cells l}^{-1}) \); \( f = \text{mean cellular fluorescence (relative fluorescence units cell}^{-1}) \); \( \psi = \text{chlorophyll content per unit fluorescence [μg (relative fluorescence unit}^{-1}] \). This is a formal statement of the approach presented by Shimada et al. (1993).

The concept of \( \psi \) denotes the use of \textit{in vivo} fluorescence as a measure of chlorophyll. Unfortunately, \( \psi \) is variable, both genotypically and phenotypically (Cullen 1992). Furthermore, the relationship between cellular chlorophyll content and flow cytometric fluorescence may be nonlinear (Sosik et al. 1989). The uncertainty involved in assigning values to \( \psi \) makes it difficult to directly implement Eq. (1).

Likewise, the concentration of phytoplankton carbon (C; μg l⁻¹) at depth z can be expressed as follows:

\[ C(z) = \sum_i n_i(z) \cdot s_i(z) \cdot \chi_i(z) \]  

(2)

where \( s = \text{mean cellular light-scatter (relative scatter units cell}^{-1}) \); \( \chi = \text{carbon content per unit of scatter [μg (relative scatter unit}^{-1}] \). As with fluorescence, a parameter (\( \chi \)) is needed to denote the use of cellular light scatter as a measure of carbon content. It is commonly accepted that cellular carbon content is directly related to cell volume (Strathmann 1967). Furthermore, strong empirical correlations between cellular light scatter and cell volume have been established using a variety of algae including calcifying strains of coccolithophores (Olson et al. 1989, Chisholm 1992). At the low angles used by the flow cytometer to measure forward scatter, the influences of cell shape and refractive index appear to be minor (Olson et al. 1989). We can therefore expect that flow cytometric light scatter is related (in a non-linear fashion) to cellular carbon; but the uncertainty involved in assigning values to \( \chi \), makes it difficult to directly implement Eq. (2).

To summarize: we have the quantities \( n, f, \) and \( s \) from flow cytometry; but not \( \psi \), and \( \chi \). We also have the quantity chl a measured by bulk fluorometry of solvent-extracted pigments. However, we do not have the quantity \( C \) which is difficult to estimate (for methods see Epplie et al. 1977) because there is no bulk chemical assay method. In this paper, we approach the problem in 2 ways. In turn, we assume that \( \psi_i(z) \) is the same for all \( i \) at each \( z \), and then that \( \psi_i(z) \) for each \( i \) is the same for all \( z \).

In the first approach, we make the simplifying assumptions that at a given depth, a single (unknown) value \( \Psi(z) \) can be taken to represent all the values of \( \psi_i(z) \); and that a single (unknown) value \( X(z) \) can be taken to represent all the values of \( \chi_i(z) \).

\[ \text{chl } a (z) = \Psi(z) \cdot \sum_i n_i(z) \cdot f_i(z) \]  

(3a)

\[ \text{chl } a (z) = \Psi(z) \cdot F(z) \]  

(3b)

\[ C(z) = X(z) \cdot \sum_i n_i(z) \cdot s_i(z) \]  

(4a)

\[ C(z) = X(z) \cdot S(z) \]  

(4b)

where \( F \) represents the summation in Eq. (3a) and \( S \) represents the summation in Eq. (4a). With this approach, we cannot ‘derive’ phytoplankton biomass from flow cytometric information; however it is a means of comparing the properties of particular phytoplankton groups with the bulk chlorophyll (see below).

In the second approach, we assume that for each cell group \( i \), a single (to be estimated) value of \( \psi_i \) which is independent of depth can be taken to represent all the values of \( \psi_i(z) \).

\[ \text{chl } a (z) = \sum_i n_i(z) \cdot f_i(z) \cdot \psi_i \]  

(5)

We use multiple regression analysis to estimate the parameters \( \psi_i \) from Eq. (5). This approach, like the first, does not ‘derive’ chl a from flow cytometric information but is useful in characterizing the group composition of bulk chlorophyll (see below). Gieskes & Kraay (1983) and Gieskes et al. (1988) presented this regression approach as a means to infer the contribu-
tion to total chlorophyll from various algal taxa determined by high-performance liquid chromatography (HPLC) analysis of pigments.

METHODS

The locations at which samples were collected for flow cytometric analysis are shown in Fig. 1. The stations labelled by alphabetical letters were sampled from the Turkish vessel 'Bilim'; those labelled by numerals were sampled from the Israeli vessel 'Shikmona'. Details of the cruise programme, methods of sample procurement and fluorometric assay of acetone-extracted chl a appear elsewhere (Yacobi et al. unpubl.). Samples for flow cytometric analysis were preserved by the method of Vaulot et al. (1989) and analyzed using the FACSort instrument (Becton Dickinson). Recently, new information indicates that paraformaldehyde may be the preservative of choice for picoplankton (Landry et al. 1993, Monger & Landry 1993. Sieracki & Cucci 1993) but we were unaware of this when we used glutaraldehyde according to Vaulot et al. (1989). Measurements of chlorophyll fluorescence and light scatter were recorded in relative units from 1 to 10^4. Using criteria described in previous studies by others (Olson et al. 1990a, Vaulot et al. 1990, Veldhuis et al. 1993) and ourselves (Li & Wood 1988, Li et al. 1992a, b), we classified the fluorescing particles into 3 groups: prochlorophytes, cyanobacteria and eukaryotic ultraphytoplankton. Cell abundance, mean fluorescence per cell, and mean light scatter per cell were extracted from listmode data using LYSYS II software (Becton Dickinson).

RESULTS

Regional variation of ultraphytoplankton

Generally, the depth distribution of ultraphytoplankton cells was similar throughout the study area (Fig. 2). Prochlorophytes ranged from 10^3 to 10^4 cells ml^-1 at the surface, and attained a subsurface peak between 70 and 100 m at concentrations from 1 to 4 x 10^4 cells ml^-1. The highest concentrations of both cyanobacteria and eukaryotes were found in the upper zone; about 10^3 to 10^4 cyanobacteria ml^-1 and about 500 eukaryotes ml^-1.

Two stations were exceptional: Stns 12 & 18. These were located within an anti-cyclonic eddy immediately southeast of Crete (Yacobi et al. unpubl.). Here, the water column was well-mixed to a great depth and this can be seen in the relatively uniform vertical distribution of all ultraphytoplankton cells down to about 130 m (Fig. 2).

Profiles of n, f, and s

The depth variations of n, f, s, n, f, and n, s are shown in 3 examples. The first (Fig. 3) refers to mean values along a horizontal transect at 33° 30' N (Stns 72, 76, 77, 80). The second was Stn 54 (Fig. 4) where the concentration of bulk chl a at the subsurface peak was double that along the aforementioned horizontal transect. The third was Stn 18 (Fig. 5) inside the eddy where prochlorophytes were much less abundant.

Three significant points emerge from inspection of these profiles. First, the range span for the product n, f, was generally comparable for each of the 3 groups. For example, along the horizontal transect (Fig. 3), n Prochl was large and f Prochl was small; conversely n Euk was small and f Euk was large. However, the products n Prochl . f Prochl and n Euk . f Euk were comparable. Second, n Euk . S Euk was always much larger than either n Cya . S Cya or n Prochl . S Prochl. That is to say, the much larger degree of light scattering by each eukaryotic cell carried more weight than the large numbers of prokaryotic cells. Third, the vertical variation of the products (n, f, and n, s) could be quite different from the variation of the component factors. For example, along the horizontal transect (Fig. 3), clear subsurface peaks in n, f, appeared for cyanobacteria and eukaryotes even though no such peaks appeared in the profiles of n, alone.
Profiles of \( F \) and \( S \)

The summed contribution from each of the 3 ultra-
phytoplankton groups is expressed as \( F \) (Eq. 3a, b) and
\( S \) (Eq. 4a, b). For each of the 3 examples (Figs. 3, 4 & 5), the depth variations in \( F \) and bulk chl \( a \) were closely
matched (Fig. 6A, C, E). However, the match between
\( S \) and chl \( a \) was poor (Fig. 6B, D, F). Profiles of \( S \) were
largely similar to profiles of \( n_{\text{Euk}} \cdot \text{shak} \), implicating
minor contributions from cyanobacteria and prochlor-
ophytes to overall phytoplankton carbon biomass.

Estimating \( \psi_j \) by regression

If we assume that \( \psi_j \) did not vary with depth (which
is likely incorrect), then \( \psi_j \) (which is independent of
depth) can be estimated for each station by regression
using measured values of chl \( a(z) \), \( n_j(z) \) and \( f_j(z) \)
(Eq. 5). The fit of data for individual stations was
extremely good \( (r^2 > 0.97, \text{Fig. 7}) \) and deteriorated only
slightly when all data were pooled \( (r^2 = 0.87, \text{Fig. 7}) \).
Using regression-estimated values of \( \psi_j \), the percent-
age contribution to chl \( a \) from each ultra-
phytoplankton group at a given depth was calculated as:

\[
\frac{[n_j(z) \cdot f_j(z) \cdot \psi_j]}{\left[ \sum_j n_j(z) \cdot f_j(z) \cdot \psi_j \right]} \times 100
\]

This analysis indicates dominance by cyanobacteria
above 100 m, dominance by prochlorophytes below
100 m, and significant intermediate contribution from
eukaryotes at all depths (Fig. 7).
DISCUSSION

The view of phytoplankton composition presented by flow cytometric analysis is based on cell abundances. In oligotrophic oceanic waters, cytometric signatures are generally dominated by large numbers of prochlorophytes and cyanobacteria. Eukaryotic ultradeckton are invariably present but in smaller numbers. There is a need to reconcile this view of the phytoplankton with the more common perspective derived from bulk measures of chlorophyll a. To date, various authors have expressed flow cytometric measurements of phytoplankton in terms of 'total fluorescence' (Demers et al. 1989, Li 1989, Perry & Porter 1989, Chisholm 1992) but only Shimada et al. (1993) have considered the relationship of this index to chlorophyll a. In this paper, we indicate explicitly how the estimates of abundance and pigment content for each cell group should be related to chlorophyll a (Eq. 1).

The recovery of bulk phytoplankton biomass on the basis of ultraphytoplankton is grounded on the following assumption: namely that there is no significant contribution to overall biomass from larger phytoplankton. Most of the chlorophyll-bearing cells in the oligotrophic waters of the eastern Mediterranean Sea are small (<10 μm). We have verified this directly (Yacobi...
et al. unpubl.) and the results are consistent with the general view that in waters of low total chl a (e.g. <1 μg l \(^{-1}\)), most (>85%) of the chl a is accounted for by the <10 μm fraction (Raimbault et al. 1988). This is not to say that larger cells do not occur. Indeed, many species of variously-sized phytoplankton have been observed in these waters (Kimor & Wood 1975, Kimor et al. 1987) and macroscopic phytoplankton colonies or mats are important agents of nutrient cycling in other oceanic regions (Carpenter & Romans 1991, Villareal et al. 1993). However, based on the inverse relationship between abundance and size of oceanic particles (e.g. Stramski & Kiefer 1991), it is expected that cells >10 μm would generally occur at concentrations less than about 1 to 10 per ml. Thus, when the sample volume is large (e.g. 2 to 4 l), sufficient numbers of the larger cells are collected and the estimates of biomass approach an upper value. However, oceanographic estimates of chl a are commonly derived from samples of smaller volumes (100 to 1000 ml). Our present concern is whether bulk measures of chl a made by filtering plankton contained in 250 ml can be represented by counts of cells contained in the 0.5 ml samples used for flow cytometric analyses.

As described earlier, it is not possible to derive chl a from Eq. (1) and C from Eq. (2): this is because \(\psi\) and \(\chi\) are not known. In lieu of this, we have calculated the 'total fluorescence', \(F\), and the 'total scattering', \(S\), which are the ratios chl a:Ψ (Eq. 3b) and C:X (Eq. 4b) respectively. The result from this analysis is that the depth variation of \(F\) matched that of chl a very well (Fig. 6A, C, E). Shimada et al. (1993) reported the same result for phytoplankton in the western Pacific Ocean. Possibly, the values of \(\psi\), for prochlorophytes, cyanobacteria and eukaryotic ultraplankton are not very different; or there are compensatory differences amongst the 3 values. Elsewhere, we

![Fig. 5. Ultraphytoplankton at Stn 18. See Fig. 3 legend for description](image)

![Fig. 6. Vertical distribution of (C) bulk chl a, (●) total fluorescence, F, and (▲) total scatter, S, along the horizontal transect at (A, B) 33°30' N, (C, D) Stn 54 and (E, F) Stn 18](image)
analysed Eq. (1) to examine the sensitivity of chl a (z) to relative changes in $\psi(z)$. For phytoplankton in the oligotrophic central North Atlantic, it appeared that a very large difference in $\psi$ (ca 500-fold) between cells with the least and most fluorescence could be tolerated without substantially altering the depth distribution of chl a (W. K. L. Li unpubl.). It is now important that the experiments of Sosik et al. (1989) on fluorescence yield of *Thalassiosira weissflogii*, *Hymenomonas carterae* and *Amphidinium carteri* be repeated on typical members of the prochlorophytes, cyanobacteria and eukaryotic ultraplankton.

For the sake of discussion, if we accept that a single value of $\Psi(z)$ [or $X(z)$] can be taken to represent all the values of $\psi_i(z)$ [or $\chi_i(z)$] at a given depth (Eqs. 3b & 4b), then the following important conclusions arise from our data. During October–November, the depth of the subsurface chl a maximum in the eastern Mediterranean was not the depth at which prochlorophytes, cyanobacteria or eukaryotic ultraplankton attained their maximum abundances. Instead it was the depth at which the combined contribution of fluorescence from all 3 groups together [i.e. $F(z)$] attained its maximum (Fig. 6A, C, E). We conclude that the subsurface chl a peak was not a maximum in the carbon biomass of ultraplankton because the profiles of $S(z)$ were distinctly different from those of chl a (z) (Fig. 6B, D, F). The subsurface chl a maximum was a co-manifestation of the vertical distribution of cells ($n_i$) and of depth-related (adaptive) variations in cellular pigment content (indexed by $f_i$).

Earlier, GilMartin & Revelante (1988) similarly pointed out that in the northern Adriatic Sea, the cell concentrations of picoplankton were maximal in the surface layers and not at the depth of the subsurface chl a maximum. Further, they similarly suggested that the difference between the vertical distributions of picoplankton chlorophyll and cell concentrations were due to depth-related differences in fluorescence intensities. However, this abbreviated report contained no description of prochlorophytes nor quantitative indices of cellular pigment or carbon content.

In stable oligotrophic environments, it is characteristic for the chl a maximum to be deeper than the maximum of phytoplankton carbon biomass (Steele 1964, Cullen 1982, Longhurst & Harrison 1989). Our results indicate that this apparently simple relationship is in fact a manifestation of the different degrees to which constituent ultraplankton groups adjust their cell contents of carbon and chlorophyll. The key to delineating various possible vertical patterns is to recognise that the cell groups contribute differently to total carbon and to chlorophyll. In the eastern Mediterranean, it appears as if much of the carbon is attributable to eukaryotic ultraplankton, but that chlorophyll is more evenly partitioned such that the contributions from prochlorophytes and cyanobacteria cannot be dismissed. In the tropical and subtropical western
Pacific studied by Furuya (1990), the subsurface chl a peak was ascribable, partly to increased cellular content of chl a, but primarily to an increase in phytoplankton carbon biomass. In this context, a significant feature in the Pacific data was the larger mean diameter of cells comprising the chl a maximum layer.

The profiles of $s_1(z)$, $n_1(z)$, $s_2(z)$, and $S(z)$ (Figs. 3 to 6) are the first in which we have explicitly examined the influence of cell size variation on the vertical distribution of ultraphytoplankton biomass. In previous studies (Li et al. 1992a, 1993), we addressed this issue only partially. Cell size was measured as Coulter cell volume (FACS Analyzer flow cytometer, Becton Dickinson) but was available only for the eukaryotic ultraplankton; prochlorophytes and cyanobacteria were too small so that their signals were indistinguishable from instrument noise. In these previous studies, the carbon content of eukaryotic ultraplankton was estimated using the Strathmann (1967) formula on measured Coulter volumes; but for prochlorophytes and cyanobacteria, depth-independent values of 59 and 250 fg C cell$^{-1}$ respectively were assumed. The present results (Figs. 3 to 5) and those of others (Olson et al. 1990a, b) clearly show depth-related variations in cellular light scatter of prochlorophytes and cyanobacteria which should not be ignored. The task remains to adopt the approach of Chisholm (1992) for converting measurements of light scatter to cellular carbon content.

The second way in which we examined our data was to assume $\psi_i(z)$ to be independent of depth. For each cell group $i$, a single parameter $\phi_i$ characterized the entire depth profile. Given the likelihood that this assumption may not be valid, this analysis must be viewed with care. Nevertheless, a cautious consideration is worthwhile because others have performed this analysis on HPLC pigment data giving interesting conclusions (Gieskes & Kraay 1983, Gieskes et al. 1988). This partitioning of chl a to constituent groups is different from a size fractionation of chl a using membrane filters because such filters do not achieve ‘clean’ separation of the cell groups. For example, the filtrate from a 1 $\mu$m Nuclepore membrane would contain prochlorophytes, cyanobacteria as well as small members of the eukaryotic picoplankton. Our regression analysis indicates a marked distinction between cyanobacteria and prochlorophytes, the former dominating the contribution to chl a near the surface, and the latter dominating the contribution to chl a at depth (Fig. 7). This concurs with the analysis of Gieskes (1991) for the oligotrophic Banda Sea.

In the future, when much more data are available, it may be possible to improve this regression approach by estimating $\psi_i(z)$ according to depth, thereby foregoing the assumption of depth-independent $\psi_i$. In other words, for data obtained from a given region amenable to characterization by a typical vertical structure and physiological parameters, it might be possible to extract, by regression, the values of $\psi_i(z)$ using an adequately large database of chl a(z), $n_i(z)$ and $f_i(z)$. With sufficient data on HPLC pigment fingerprints, Gieskes et al. (1988) were able to apply the regression approach to algal assemblages deemed similar by cluster analysis.

In conclusion, we have confirmed the occurrence and numerical dominance of prochlorophytes, cyanobacteria and eukaryotic ultraplankton in the oligotrophic waters of the Mediterranean Sea. In these waters, it appears that the vertical distribution of chl a can be explained on the basis of the combined contribution from depth-related variations in cell abundance and relative cellular pigment content. This is a promising step towards understanding the bulk properties of phytoplankton from a study of the properties of constituent cell groups.

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