

Composition of ultraphytoplankton in the central North Atlantic

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ABSTRACT: In the central North Atlantic Ocean, the ultraphytoplankton averaged over depth and season comprises 78% *Prochlorococcus* spp., 12% *Synechococcus* spp. and 10% eukaryotic algae by numbers. However, on the basis of light scatter (a substitute for carbon biomass), the average percentages are 11, 2 and 87% respectively. On the basis of red fluorescence (a substitute for chlorophyll biomass), the average percentages are 28, 11 and 61% respectively. On the basis of primary productivity, the average percentages are 19, 13 and 68% respectively. Thus, eukaryotic algae dominate the biomass and productivity of ultraphytoplankton.

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

INTRODUCTION

In oligotrophic oceans, most, though not all, of the phytoplankton are small and might be termed ultraplankton (*sensu* Shapiro & Guillard 1986). Of these, prochlorophytes (*Prochlorococcus* spp.) are generally the most abundant, cyanobacteria (*Synechococcus* spp.) rank second, and the taxonomically diverse group of eukaryotic algae (Prasinophyceae, Prymnesiophyceae, Chrysophyceae, Pelagophyceae, Bacillariophyceae and others) number the least. On the other hand, on average, prochlorophytes (ca 0.7 μm) are smaller than cyanobacteria (ca 1 μm), which in turn are smaller than many, though not all, of the eukaryotic ultraplankton (ca 0.7 to 5 μm).

To rank these 3 ultraphytoplankton groups in order of dominance by carbon biomass ($\mu\text{g C ml}^{-1}$), chlorophyll biomass ($\mu\text{g chl ml}^{-1}$) or primary productivity ($\mu\text{g C ml}^{-1} \text{ h}^{-1}$), it is necessary to weight the mean cellular carbon ($\mu\text{g C cell}^{-1}$), mean cellular chlorophyll ($\mu\text{g chl cell}^{-1}$) or mean cellular carbon uptake ($\mu\text{g C cell}^{-1} \text{ h}^{-1}$) of each group by the numerical abundance (cells ml^{-1}). Since abundance and cell size are ranked in opposing order, it is not obvious how biomass and productivity would be ranked. Simply stated, one large cell can be viewed as the equivalent of many small cells (Goldman 1988, Margalef 1994).

For ultraphytoplankton, flow cytometric analysis and sorting are able to yield all the information necessary for an initial, though inexact, solution to this problem (Li et al. 1993b, Li 1994a, b). Measurements can be routinely made of cell abundance, light scatter per cell (a substitute for volume per cell, and thus also a substitute for carbon per cell) and red fluorescence per cell (a substitute for chlorophyll *a* per cell). Less routinely, measurements can also be made of ^{14}C uptake per cell by flow sorting phytoplankton that have been radiolabelled over the course of standard incubation experiments (Li 1994b). The greatest uncertainty in this approach lies in the use of substitute variables because they are not necessarily related in a linear fashion to the variables of true interest. In this paper, the methods and assumptions described recently (Li et al. 1993b, Li 1994a, b) are used to rank the importance of *Prochlorococcus*, *Synechococcus*, and eukaryotic ultraphytoplankton in the central North Atlantic Ocean.

METHODS

Phytoplankton were taken from Niskin bottles deployed on 27 hydrocasts made in 1992 (September to October) and in 1993 (May to June) on transects from

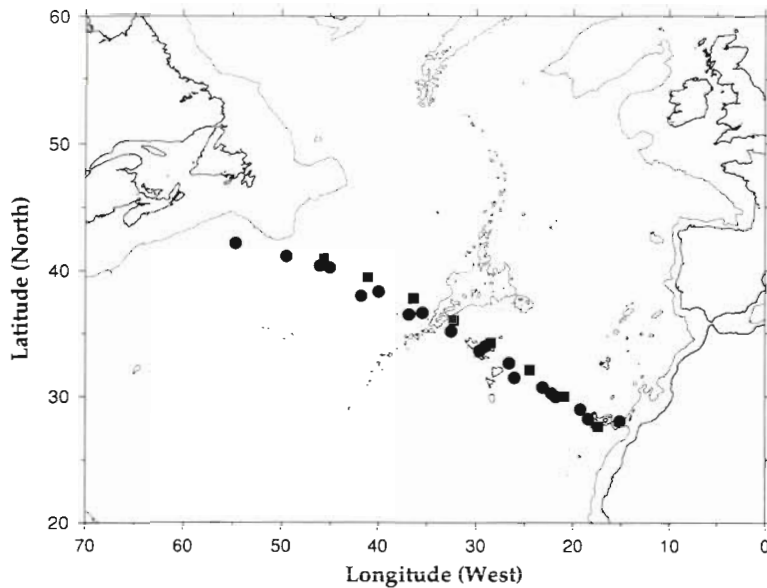


Fig. 1. Locations where samples were collected in September-October 1992 (●) and in May-June 1993 (■). Dotted lines indicate 2000 m bathymetric contour

the continental slope in the west to the Canary Islands in the east (Fig. 1).

Measurements of cell abundance, fluorescence per cell, and forward (narrow angle) light scatter per cell were made by flow cytometric analysis based on 488 nm excitation light (FACSort[tm], Becton Dickinson). Samples of 0.25 to 0.50 ml were freshly analysed shortly after removal from the Niskin bottles. Fluorescence at wavelengths longer than 650 nm (FL3 instrument channel) was ascribed to chlorophyll *a*; fluorescence centred at 585 ± 21 nm was ascribed to phycoerythrin. Light scattered over a range of half-cone angles from <1 to 10° was collected by a solid state silicon detector with spectral response from 300 to 1100 nm (specifications from manufacturer). Measurements of fluorescence and light scatter were collected using logarithmic amplification and recorded in relative units in a 4-decade range spanned by 256 channels. Data were extracted from list-mode format using LYSYS II[tm] software (Becton Dickinson). To ensure that only phytoplankton were enumerated, particles with red fluorescence weaker than that emitted by *Prochlorococcus* spp. were rejected. The identification of *Prochlorococcus* spp., *Synechococcus* spp., and eukaryotic ultraphytoplankton from cytometric signatures was based on interactive analysis of multiple bivariate scat-

terplots as described explicitly by Olson et al. (1993).

In flow cytometric analyses of phytoplankton, it is common practice to regard forward light scatter as an index of cell size (Olson et al. 1993). Light scatter is a measurement of particle cross sectional area, but this measurement may also be influenced by particle shape and refractive index. Nevertheless, Olson et al. (1989) have demonstrated a strong empirical correlation between forward light scatter and Coulter volume using a wide variety of algae including calcifying strains of coccolithophores. These authors suggested that, at the low angles used by the flow cytometer to measure forward scatter, the influences of cell shape and refractive index are minor. Relative measurements of forward light scatter were here calibrated using beads of various size

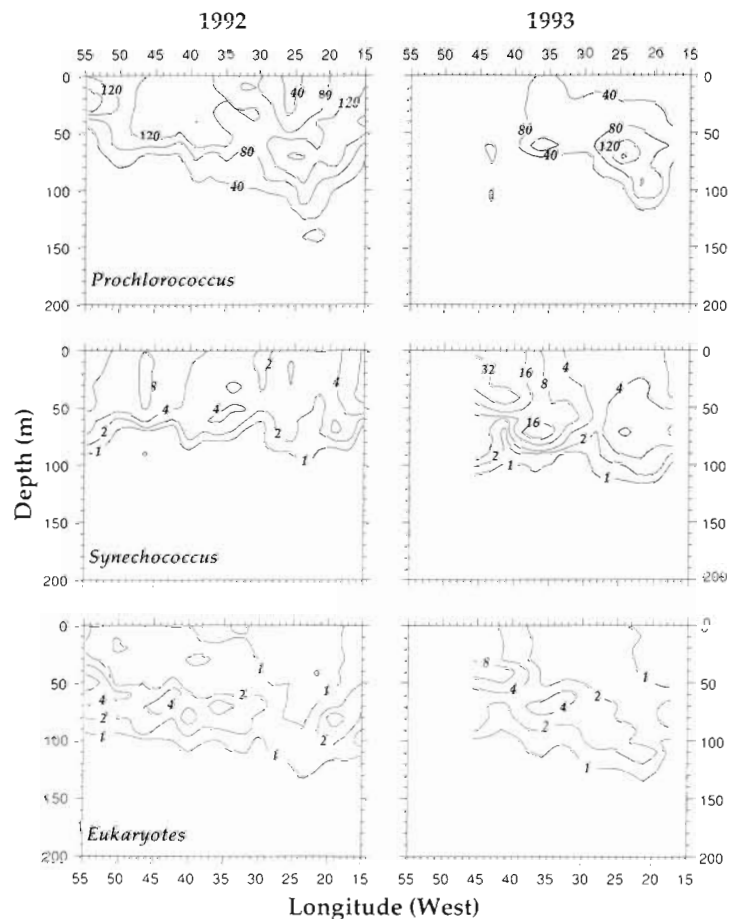


Fig. 2. *Prochlorococcus* spp., *Synechococcus* spp., and eukaryotic algae. Depth and longitudinal distributions of ultraphytoplankton abundance (10^3 cells ml^{-1}) in September-October 1992 (left column) and in May-June 1993 (right column)

and material composition (Polysciences #18859, 18860, 18604, 18861, 18862; Duke Scientific #R600, R0100, R0290, 268, 361; Coulter fluorospheres 5.43 μm , 8.55 μm , 9.64 μm). Expressed as equivalent spherical diameter, the useful range encompassed by the light scatter measurements in the present study was from 0.44 to 6.8 μm . Autofluorescing cells having an equivalent spherical diameter larger than 6.8 μm , if present in a sample of ≤ 0.5 ml, were detected, but their numbers were collectively accumulated in the highest data acquisition channel regardless of size. Here, equivalent spherical diameter may be thought of in the following way: a particle (cell) of undetermined shape and undetermined refractive index has scattered light in such a way that the intensity is the same as that of a certain size bead which has a spherical shape and a synthetic composition. That 'certain size' is not necessarily the same as cell diameter estimated by microscopy.

Cell-specific rates of ^{14}C uptake were determined on cells sorted by flow cytometry after simulated *in situ* 8 h incubations with added $\text{NaH}^{14}\text{CO}_3$. Details of the protocol have been described (Li 1994).

RESULTS AND DISCUSSION

A seasonal difference was evident in the abundance of each ultraphytoplankton group, but the major distributional features across 40° of longitude were broadly similar between seasons (Fig. 2). Subsurface maxima were conspicuous for *Prochlorococcus* and eukaryotes across much of the central ocean, but the depth of the maxima deepened from west to east (which was also a north to south excursion of ca 10° latitude), except in the vicinity of the Canary Islands where the maxima became shallower. *Synechococcus* tended to be uniformly distributed in the upper mixed layer with abundances higher in the west than in the east. These ocean-wide observations at 2 times of the year (Fig. 2) complement and augment other North Atlantic data (Olson et al. 1990a, b). Based on these observations, it appears that, in spite of spatial variability, there are persistent features that characterize the depth and seasonal distributions of ultraphytoplankton in the central North Atlantic.

These features are summarized as transect-wide averages and indicated in depth profiles (Fig. 3) representing late summer-early fall (1992) and late spring-early summer (1993). A most notable feature was the increase of *Pro-*

chlorococcus in late summer coincident with decreases of both *Synechococcus* and eukaryotes. The late summer situation approached what Campbell & Vaulot (1993) suggest is typical for subtropical open-ocean regions: namely, that *Prochlorococcus* is abundant in the surface mixed layer at a concentration more than half that at the sub-surface maximum and that the depth-integrated abundance of *Prochlorococcus* greatly exceeds that of *Synechococcus*. Across the central North Atlantic in late spring (1993), average depth-integrated abundances (10^6 cells cm^{-2}) were 462 for *Prochlorococcus*, 85 for *Synechococcus*, and 29 for eukaryotes. However, in late summer (1992), there were more *Prochlorococcus* (1031), fewer *Synechococcus* (32), and fewer eukaryotes (21).

The central question is now posed: which of the 3 groups dominates the ultraphytoplankton? There are 4

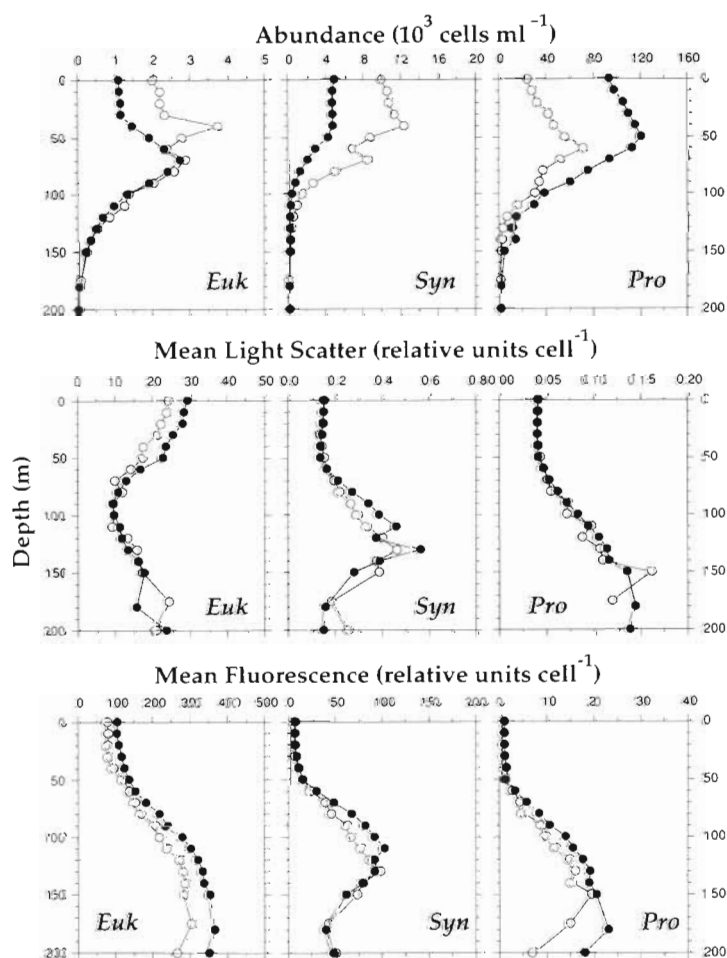


Fig. 3. Eukaryotic algae (*Euk*), *Synechococcus* spp. (*Syn*) and *Prochlorococcus* spp. (*Pro*). Transect-wide mean values for ultraphytoplankton in September-October 1992 (\bullet) and in May-June 1993 (\circ). Top panels show cell abundance (10^3 cells ml^{-1}); middle panels show mean forward light scatter cell^{-1} (relative units); bottom panels show mean red fluorescence cell^{-1} (relative units)

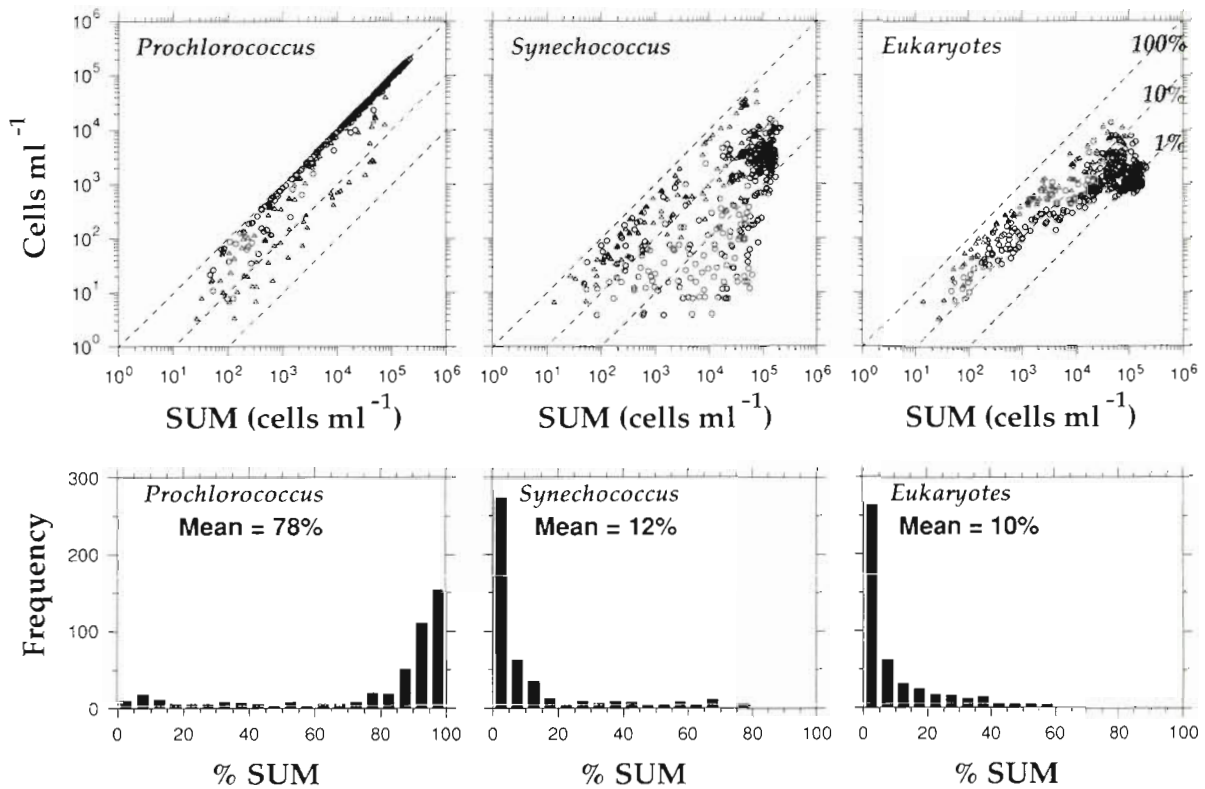


Fig. 4. *Prochlorococcus* spp., *Synechococcus* spp., and eukaryotic algae. Contribution to total ultraphytoplankton expressed in cell abundance ml^{-1} seawater. Top panels show the scatter relationship between each group and sum of the 3 groups; circles: 1992 samples, triangles: 1993 samples. Dashed diagonal lines indicate 100, 10 and 1% of the sum. Bottom panels show frequency distributions of the percentage contribution by each group to the sum (SUM). Mean percentage for each group is indicated and was calculated from the entire data encompassing all depths and both years

available measures on which to establish a ranking: cell numbers, carbon biomass, chlorophyll biomass, and primary productivity. For cell numbers, a simple indicator is:

$$\frac{n_i}{\sum_i n_i} \quad (1)$$

where n is abundance (cells ml^{-1}) and i refers to *Prochlorococcus*, *Synechococcus*, or eukaryotic ultraphytoplankters. Over the entire data set pooled from all depths and both seasons, the mean composition by numbers was 78% *Prochlorococcus*, 12% *Synechococcus*, and 10% eukaryotes (Fig. 4).

For carbon biomass, the indicator is based on the weighted measurement of mean light scatter per cell (\bar{s}_i) which is assumed to be a substitute for mean cell carbon (Li 1994a):

$$\frac{n_i \times \bar{s}_i}{\sum_i n_i \times \bar{s}_i} \quad (2)$$

The product of n_i and \bar{s}_i signifies total light scatter in unit volume seawater due to ultraphytoplankton group

i . On this basis, the pooled data indicate a distribution of 11% *Prochlorococcus*, 2% *Synechococcus*, and 87% eukaryotes (Fig. 5).

Similarly, for chlorophyll biomass, the indicator is based on the weighted measurement of mean red fluorescence per cell (\bar{f}_i) which is assumed to be a substitute for mean cell chlorophyll (Li 1994a):

$$\frac{n_i \times \bar{f}_i}{\sum_i n_i \times \bar{f}_i} \quad (3)$$

The product of n_i and \bar{f}_i signifies total fluorescence in unit volume seawater due to ultraphytoplankton group i . On this basis, the pooled data indicate a distribution of 28% *Prochlorococcus*, 11% *Synechococcus*, and 61% eukaryotes (Fig. 6).

For primary productivity, the indicator is based on the actual measurement of ^{14}C uptake rate per cell (p_i) obtained by flow sorting (Li 1994b):

$$\frac{n_i \times p_i}{\sum_i n_i \times p_i} \quad (4)$$

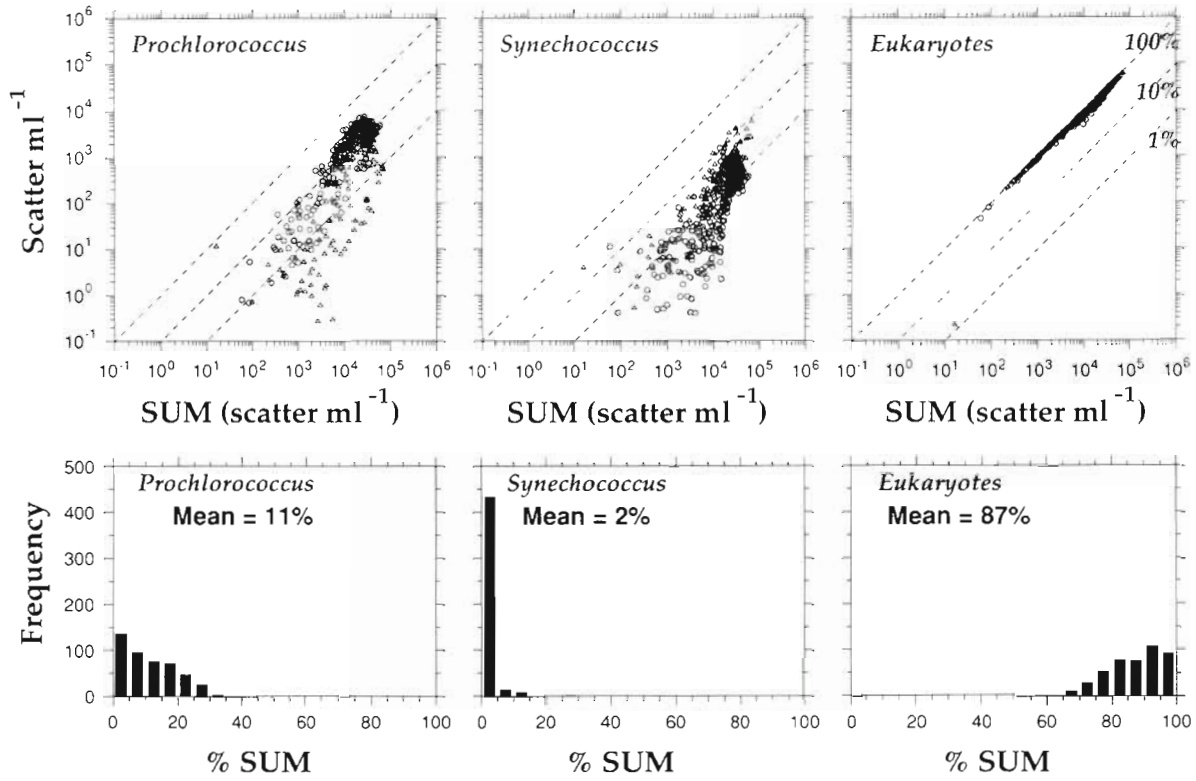


Fig. 5. *Prochlorococcus* spp., *Synechococcus* spp., and eukaryotic algae. Contribution to total ultraphytoplankton expressed in total light scatter ml^{-1} seawater. See Fig. 4 for further explanations

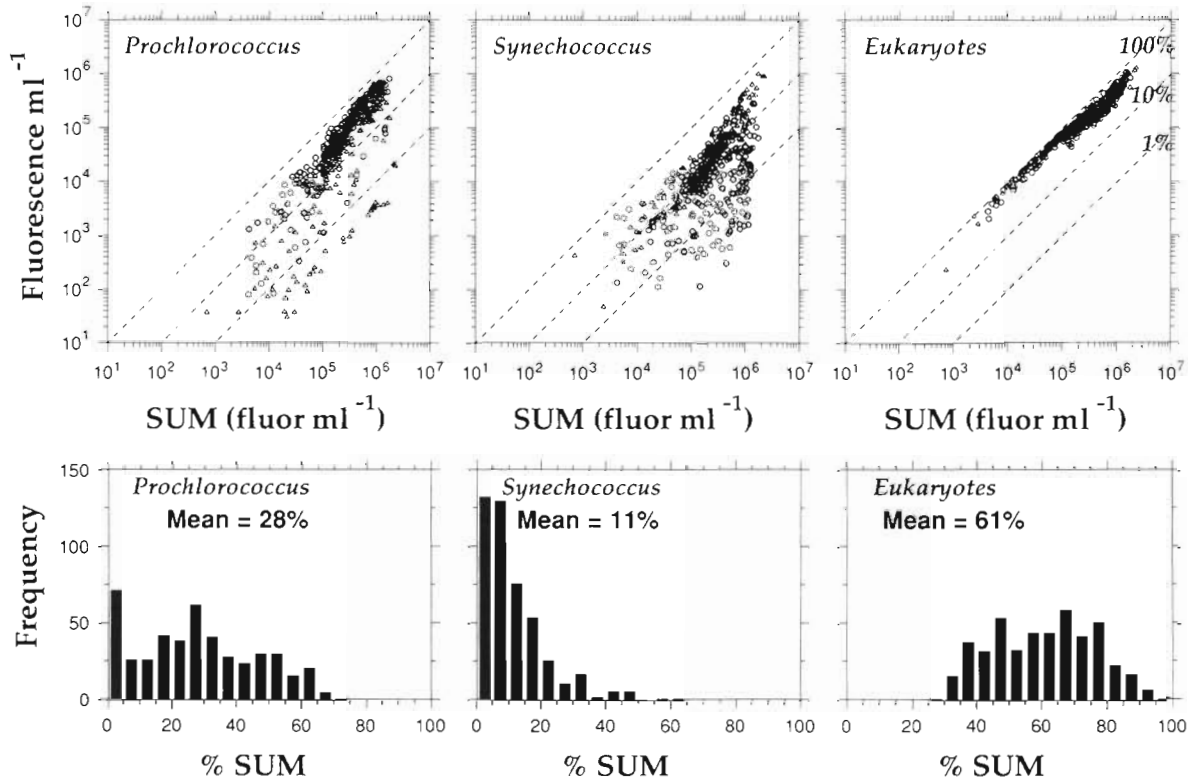


Fig. 6. *Prochlorococcus* spp., *Synechococcus* spp., and eukaryotic algae. Contribution to total ultraphytoplankton expressed in total red fluorescence (fluor) ml^{-1} seawater. See Fig. 4 for further explanations

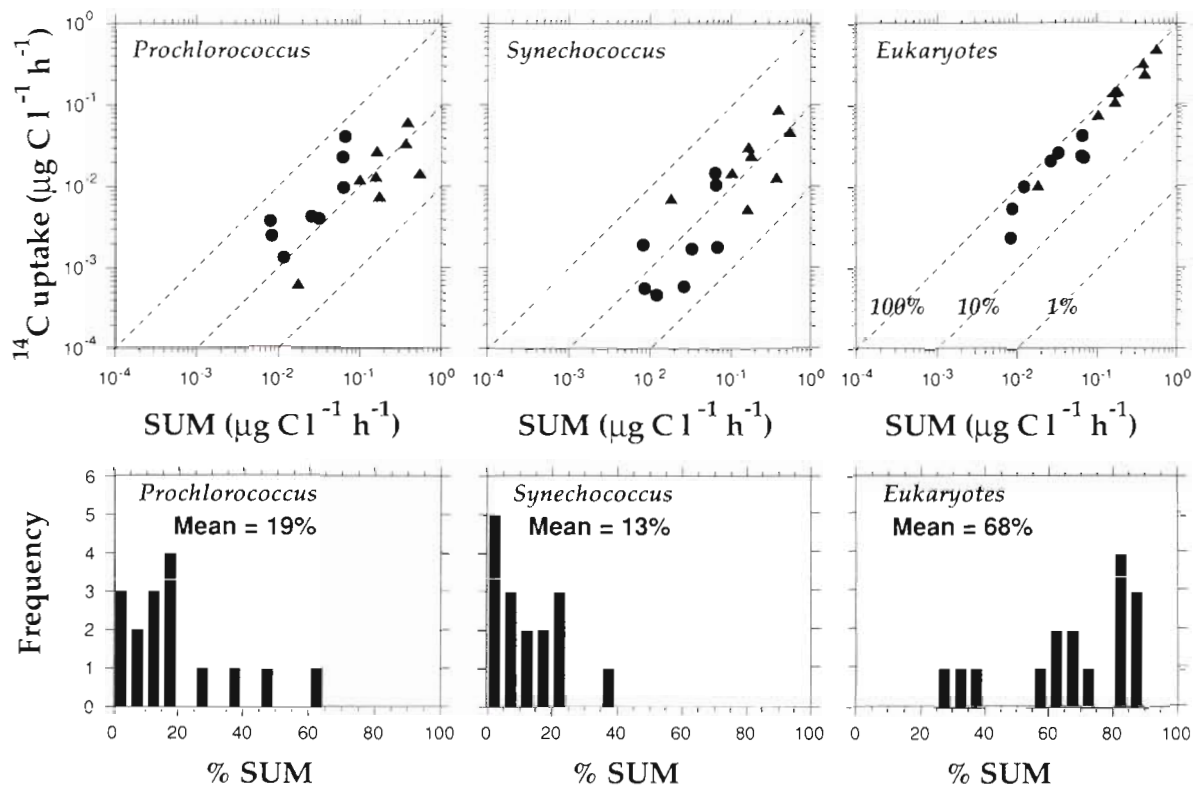


Fig. 7 *Prochlorococcus* spp., *Synechococcus* spp., and eukaryotic algae. Contribution to total ultraphytoplankton expressed in rate of ^{14}C uptake ml^{-1} seawater. See Fig. 4 for further explanations

The product of n_i and p_i is the ^{14}C uptake rate in unit volume seawater due to ultraphytoplankton group i . On this basis, the pooled data indicate a distribution of 19% *Prochlorococcus*, 13% *Synechococcus*, and 68% eukaryotes (Fig. 7).

These results can be summarized as follows. In the central North Atlantic, at most depths in either of the seasons in question, *Prochlorococcus* were almost always the most abundant ultraphytoplankton; however, the major portion of ultraplankton biomass and productivity was usually attributable to the eukaryotic cells.

Three lines of independent evidence are available to corroborate the results presented here. First, when carbon biomass of each group is estimated on the basis of cellular carbon content implied by cell size, rather than on the basis of flow cytometric light scatter, the same conclusion is reached, i.e. that eukaryotes dominate the ultraphytoplankton in the North Atlantic (Li & Wood 1988, Li et al. 1992, 1993a), in the equatorial Pacific (Eppley et al. 1992), and in the subarctic Pacific (Booth et al. 1993). Further, DuRand et al. (1994) determined that nanophytoplankton (mostly 2 to 5 μm cells) made the greatest contribution, via light scattering, to changes in beam attenuation which is a bulk optical property of seawater.

Second, when chlorophyll biomass of *Prochlorococcus* is estimated from the concentration of divinyl chlorophyll a which is a chemotaxonomic marker for this genus, the average percentage contribution to total chlorophyll a is 29% in the Sargasso Sea (Goericke & Repeta 1993, Goericke & Welschmeyer 1993), comparable to the present mean value of 28% for *Prochlorococcus* fluorescence (Fig. 6). In spite of the close similarity in these percentages, it should be noted that if only the data of 1992 (when *Prochlorococcus* were more abundant) are considered, *Prochlorococcus* fluorescence represented 37% of the total fluorescence. This mean value is less than 49%, which is the mean high performance liquid chromatography (HPLC)-measured contribution of divinyl chlorophyll a to total chlorophyll a over the central North Atlantic sampled in late summer 1992 (E. J. H. Head unpubl.). This discrepancy could be explained if the *in vivo* fluorescence yield per unit chlorophyll is less for *Prochlorococcus* than for other phytoplankton. There is some indirect evidence that this may be so (Li et al. 1993b). Even if a high percentage is accepted for the contribution of *Prochlorococcus* to pigment biomass (particularly in late summer), the eukaryotic ultraplankton remain a very significant component throughout the year.

A third aspect is the consideration of primary productivity. Two independent methods for estimating *Prochlorococcus* productivity yield percentage contributions similar to the present mean value of 19% (Fig. 7). In one method, *Prochlorococcus* productivity was measured by the incorporation of ^{14}C into divinyl chlorophyll *a* (Goericke & Welschmeyer 1993), and approximately 25% of total productivity was ascribed to this genus in the Sargasso Sea. In a second method, *Prochlorococcus* productivity was estimated from cell cycle analysis of DNA frequency histograms (Vaulot et al. 1994), and from 7 to 19% of total productivity was ascribed to this genus in the equatorial Pacific.

In the central North Pacific at station ALOHA near Hawaii, which typifies the subtropical open ocean, the abundance structure of the ultraphytoplankton assemblage is most extremely dominated by *Prochlorococcus*. There, the depth-integrated abundances (10^6 cells cm^{-2}) are about 2000 for *Prochlorococcus*, 15 for *Synechococcus*, and 15 for eukaryotes (Campbell & Vaulot 1993). This situation corresponds to a distribution of phytoplankton carbon biomass of 64% *Prochlorococcus*, 4% *Synechococcus*, and 32% eukaryotic algae (Campbell et al. 1994a). The percentage of chlorophyll *a* represented by the prochlorophyte marker divinyl chlorophyll *a* is also dominant at ALOHA, being 70% at the deep chlorophyll maximum layer (Letelier et al. 1993). Furthermore, *Prochlorococcus* at ALOHA accounts for a higher mean percentage (44%) of total productivity than elsewhere studied (Vaulot 1995). It thus appears that when *Prochlorococcus* abundance reaches (Campbell & Vaulot 1993) or exceeds (Veldhuis & Kraay 1993) about 200000 cells ml^{-1} , the biomass and productivity of this genus become very significant or even dominant. This was not the case observed in the 2 transatlantic transects.

Eukaryotic ultraphytoplankters are extremely diverse in a taxonomic sense (Hooks et al. 1988, Campbell et al. 1994b), but many of them are flexible enough to pass through membrane filter pores smaller than the cell diameter (Shapiro & Guillard 1986). The number and diversity of cells found in the filtrate of 2 μm Nuclepore membrane are very similar to the number and diversity in unfiltered seawater along these North Atlantic transects (Li 1994). Although measurements of size-fractionated chlorophyll or ^{14}C uptake rate often indicate large contributions from the picoplankton (<2 μm) to total phytoplankton, such measurements are unable to partition the biomass and productivity amongst *Prochlorococcus*, *Synechococcus*, and eukaryotic algae. In this regard, the apparent dominance of eukaryotes in the ultraplankton of the North Atlantic means that this taxonomically diverse assemblage is a crucial determinant of the carbon flux in this oceanic region.

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