



PERGAMON

Deep-Sea Research II 48 (2001) 1983–2003

DEEP-SEA RESEARCH
PART II

www.elsevier.com/locate/dsr2

Phytoplankton population dynamics at the Bermuda Atlantic Time-series station in the Sargasso Sea

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Abstract

Phytoplankton populations were analyzed using flow cytometry in monthly samples at the Bermuda Atlantic Time-series Study (BATS) station in the Sargasso Sea from 1989–1994 for picoplankton (*Synechococcus* and *Prochlorococcus*) and from 1992–1994 for eukaryotic phytoplankton in order to better understand the mechanisms that dictate seasonal and inter-annual patterns in the phytoplankton community. The eukaryotic phytoplankton were dominated by populations of small nanoplankton (mostly 2–4 µm diameter), though populations of coccolithophores and sometimes pennate diatoms also could be distinguished. Flow cytometric measurements of population abundances, individual cell light scattering (which can be related to cell size), and chlorophyll fluorescence were made. *Synechococcus* and the eukaryotic phytoplankton reached their greatest concentrations during the spring bloom each year when the water column was deeply mixed and nutrients were detectable in surface waters. The maximum cell concentration for *Prochlorococcus* was in the summer and fall of each year, with a deeper sub-surface maximum than *Synechococcus*. Picoplankton chlorophyll fluorescence and estimated cell size were greater at depth than near the surface, and were lowest in midsummer for both *Synechococcus* and *Prochlorococcus*. In the summer and fall, *Prochlorococcus* cells were often smallest at mid-depth, even when fluorescence per cell and cell concentration were lower at the surface. For the eukaryotes (including coccolithophores), cell concentrations were high during the spring in both 1992 and 1993, and in fall 1992. At these times, mean cell size and fluorescence were low. Improved size and carbon estimates were made and it was found that the estimated contribution of phytoplankton carbon to total particulate organic carbon, integrated over the upper 200 m, averaged 33% (range 21–43%) with no pronounced seasonal pattern. © 2001 Elsevier Science Ltd. All rights reserved.

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1. Introduction

The seasonal physical, chemical, and biological properties in the Sargasso Sea have been well studied, with biweekly samplings at Hydrostation 'S' since 1954 (Schroeder and Stommel, 1969). Deep mixing in the winter vents cold, nutrient-rich, 18°C water to the surface. In the summer, the water column stratifies and the shallow surface mixed-layer is nutrient poor throughout the rest of the year. The resulting annual cycle of phytoplankton abundance and production, driven by the physical and chemical properties of the water column, was first described by Menzel and Ryther (1960, 1961). The spring phytoplankton bloom occurs shortly after the venting of the 18°C water and is followed by a chlorophyll maximum near the bottom of the euphotic zone when the surface waters are stratified. Production is highest in the winter and early spring and is dependent on vertical mixing.

Monthly sampling began at the Bermuda Atlantic time-series study (BATS) site in 1988, and the seasonal patterns of biogeochemistry have been described (Michaels et al., 1994) and an overview of the US Joint Global Ocean Flux Study (JGOFS) given (Michaels and Knap, 1996). Additional studies have focused on various aspects of the planktonic organisms and their controls at the BATS site, including the heterotrophic bacteria (Carlson et al., 1996), photosynthetic picoplankton (Olson et al., 1990a), microbial size spectra (Gin et al., 1999), diatoms (Nelson and Brzezinski, 1997), and optical properties and light availability (Siegel et al., 1995). Taxonomic identifications of phytoplankton and HPLC pigment analysis have been made at nearby Hydrostation 'S', where Prymnesiophyceae and Pelagophyceae were the most abundant groups of eukaryotic phytoplankton (Andersen et al., 1996). The biomass of bacteria and picophytoplankton (prokaryotic and eukaryotic) was investigated in September 1988 in the Sargasso Sea (Li et al., 1992), and it was found that the phytoplankton and bacteria had approximately equal contributions (near 10%) to total particulate organic carbon (POC). Caron et al. (1995) found that microbial carbon (including phototrophs and heterotrophs) was 55% of POC in March–April 1990 and 24% of POC in August 1989 near the BATS site in the Sargasso Sea.

Phytoplankton are an important component of biogeochemical cycling in the oceans, and the contribution of phytoplankton carbon is an important input for biogeochemical models. From flow cytometric measurements of samples from the BATS site, we describe seasonal and, where possible, inter-annual variations in the phytoplankton assemblage from prokaryotic picoplankton to eukaryotic phytoplankton. Through measurements of cell concentrations and estimates of cell size, coupled with carbon conversion factors, we can estimate phytoplankton contribution to particulate organic carbon. In addition, since specific phytoplankton groups can be identified (*Synechococcus*, *Prochlorococcus*, coccolithophores, and other eukaryotic phytoplankton), the phytoplankton community structure and the contributions of different groups to phytoplankton carbon can be determined.

2. Methods

2.1. Sample collection

Water samples for phytoplankton analysis were collected approximately monthly at the Bermuda Atlantic time-series site (nominal location 31°50'N, 64°10'W), from November 1989 through

November 1994. Samples were collected using Niskin or Go-Flo bottles at up to 12 depths from the surface to 250 m. Duplicate aliquots of 2-ml water samples were preserved with glutaraldehyde (1 or 0.1%) and stored in liquid nitrogen until analysis (May 1991 until November 1994) (Vaulot et al., 1989) or sent unpreserved in a cooler on icepacks to WHOI for immediate analysis (November 1989–April 1991) (see Olson et al., 1990a for comparison of fresh vs. stored samples). From February 1992 to January 1994, aliquots of 50 ml were preserved (1 or 0.1% glutaraldehyde) and liquid nitrogen frozen for analysis of the eukaryotic phytoplankton.

2.2. Flow cytometry analysis

A Coulter EPICS or a Becton Dickinson FACScan flow cytometer was used for sample analysis. Forward light scattering (FLS), red fluorescence from chlorophyll (CHL, 660–700 nm for EPICS, > 650 nm for FACS), and orange fluorescence from phycoerythrin (PE, 540–630 for EPICS, 564–606 nm for FACS) were measured after excitation by a 488 nm laser beam. Fluorescent beads (3.79 or 0.57 μm “Fluoresbrite”, Polysciences, Inc., Warrington, PA) were used as internal standards and for calibration of the above parameters between the two instruments. In addition, selected samples were analyzed on both instruments, also for cross-calibration. Flow cytometry (FCM) data were analyzed using “Cyto-PC” or “Cyto-Win” software (D. Vaulot, Station Biologique, Roscoff, France). The small-volume samples were analyzed using a FACScan modified for high sensitivity (Dusenberry and Frankel, 1994) or a high-sensitivity set-up of the EPICS flow cytometer and populations of *Synechococcus* and *Prochlorococcus* were identified from their characteristic flow cytometric signatures (Olson et al., 1990a, b). The larger volume samples were analyzed using an EPICS flow cytometer modified for larger volume through-put (Olson et al., 1993). Populations of eukaryotic phytoplankton (mostly 2–4 μm diameter cells, including cells up to 30 μm), pennate diatoms, and coccolithophores were distinguished from their characteristic light scattering and fluorescence (Olson et al., 1989).

2.3. BATS core measurements

Temperature, nutrients (nitrate + nitrite), particulate organic carbon (POC), and primary productivity were from the core measurements of the BATS database (<http://www.bbsr.edu/bats/bats.html>). These data as well as details of the methods used are available in published form (Knap et al., 1993, 1992–1997).

2.4. Volume and carbon conversion factors

For these data we have directly related forward light scattering to cell size using empirically determined calibrations. In addition to cell size, forward light scattering is also influenced by cell refractive index and cell shape. For laboratory cultures of reasonably spherically shaped cells, we have always seen a strong correlation between FLS and cell size (Olson et al., 1989; DuRand, 1995), despite small changes in refractive index (DuRand and Olson, 1998).

For the picoplankton, carbon biomass was determined by first converting mean FLS to cell size. This was done by fitting a power relationship to laboratory calibrations between *Synechococcus* FLS and diameter ($\text{FLS} = a \cdot \text{Diameter}^b$, $n = 8$, $r^2 = 0.90$; for 4 strains of *Synechococcus* at

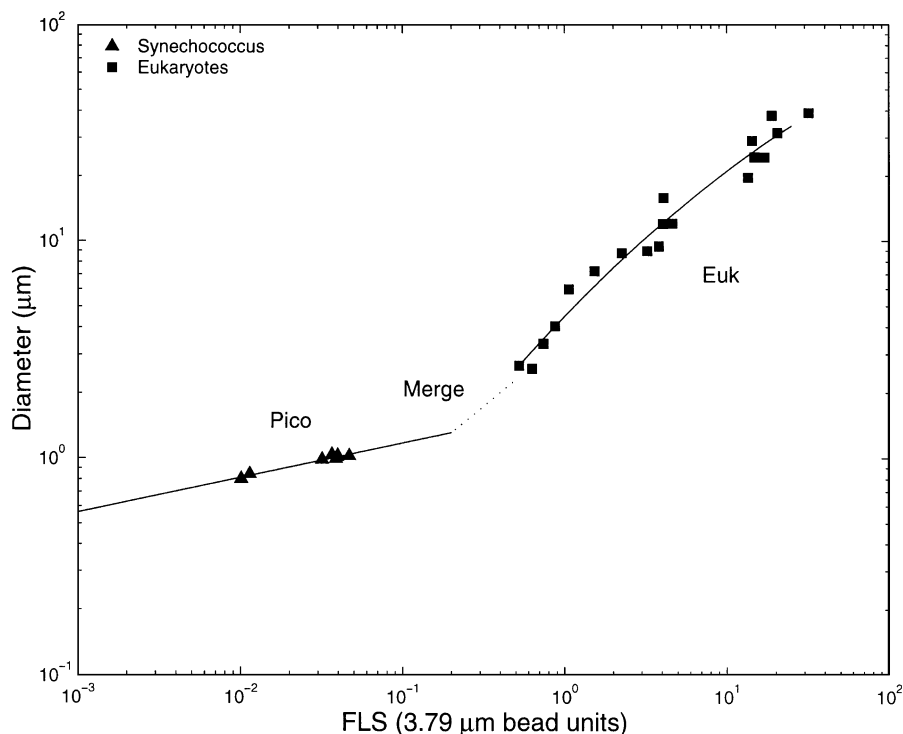


Fig. 1. Laboratory-culture-based calibration between flow cytometric forward light scattering (in 3.79 μm bead units) and cell diameter. There are separate calibrations for 'pico' (based on *Synechococcus* cultures) and 'euk' based on cultures of eukaryotic phytoplankton. The 'merge' calibration smooths the transition over the range of FLS where no culture calibration data were available. Note that this calibration was determined specifically for the configuration of the flow cytometer used in this study, though it was quite similar to that determined by a different method on a different EPICS flow cytometer (Cavender-Bares, 1999).

2 different times of day; Fig. 1) (DuRand, 1995). The exponent was found to be 5.4, which is reasonably close to that determined by Mie light scattering theory, which predicts that FLS should vary with diameter to the sixth power for picoplankton-sized cells (Morel and Bricaud, 1986). The resulting cell size was then converted to carbon using $325 \text{ fg C } \mu\text{m}^{-3}$, determined from CHN analysis of the same *Synechococcus* cultures. Previous studies have determined carbon conversion factors from $220 \text{ fg C } \mu\text{m}^{-3}$ (Fry, 1988) to $470 \text{ fg C } \mu\text{m}^{-3}$ (Verity et al., 1992) for picoplankton. Carbon per cell was then multiplied by cell concentration to obtain carbon biomass for each picoplankton group.

For the eukaryotic phytoplankton, 256-channel distributions of flow cytometric FLS and CHL were projected for each cell type (pennate diatoms, coccolithophores, and eukaryotic phytoplankton). The FLS distributions were first converted to biovolume using a laboratory-derived calibration performed on a number of different exponentially growing phytoplankton cultures (DuRand, 1995; Olson and Shalapyonok, unpublished data; Fig. 1). There were 19 cultures ranging in size from *Pycnococcus provasoli* (2.6 μm diameter) to *Gymnodinium sanquinum* (40 μm) and the resulting second-order polynomial fit for the log data had an r^2 of 0.97. For the calibration used,

the phytoplankton were analyzed live on a Coulter Multisizer to determine cell volume and preserved (0.1% glutaraldehyde) and frozen in liquid nitrogen before flow cytometric analysis, with the exception of the 8 largest cultures, which were analyzed live on both instruments. For the lower end of the FLS distribution (FLS for the eukaryotes in the same range as some of the larger *Synechococcus*), the picoplankton calibration between FLS and cell size was used (see above). For the range of the FLS distribution where no culture calibration data were available (diameters from about 1.3–2.3 μm), a power relationship was created to smooth the transition from the picoplankton calibration to the larger eukaryote calibration (Fig. 1).

The resulting distribution of biovolume per cell was converted to a distribution of carbon per cell using the empirical relationship: $\log \text{carbon (pg/cell)} = 0.94 \log \text{Vol } (\mu\text{m}^3) - 0.6$ (Eppley et al., 1970). The mean of the carbon per cell distribution was then multiplied by cell concentration to obtain carbon biomass for a particular group of eukaryotic phytoplankton. Means of the cell diameter and cell volume distributions also were calculated for each cell type. Data for the pennate diatoms were combined with those of the eukaryotic phytoplankton, since we only saw large populations of pennates in May 1993. Throughout this paper, “eukaryotic phytoplankton” does not include coccolithophores unless specified; coccolithophore data are presented separately. The cell concentration data are available on the accompanying compact disc.

2.5. Data analysis

For the contour plots presented, data were interpolated to a regular grid using the triangle-based linear interpolation method available in MATLAB software (The MathWorks, Inc.).

For each regression analysis presented, the standardized residuals were examined and, when necessary, x and y variables were log-transformed before regression. For all relationships both variables were uncontrolled and subject to error, thus Model II regressions (geometric mean [reduced major axis] regressions) were performed and the 95% confidence limits of the slope and correlation coefficient (r) determined as in Sokal and Rohlf (1981).

3. Results and discussion

3.1. Physical and chemical conditions

The depth of the thermocline ranged from less than 20 m during the summer to more than 250 m during the winter. Nitrate concentrations were usually below $0.1 \mu\text{mol kg}^{-1}$ in surface waters. Surface nitrate concentrations reached $0.1 \mu\text{mol kg}^{-1}$ only in February or March of each year (except 1990) and were closely related to the depth of mixing (Michaels and Knap, 1996).

3.2. Cell concentrations

In most years, *Prochlorococcus* reached maximum concentrations of $1.5\text{--}2.6 \times 10^5 \text{ ml}^{-1}$ in the summer and fall (typically August to November) but were less abundant in winter and spring (typically March and April) (Fig. 2). The maximum cell concentration was often near 60–80 m deep, with high concentrations extending to nearly 200 m. The maximum concentration of *Prochlorococ-*

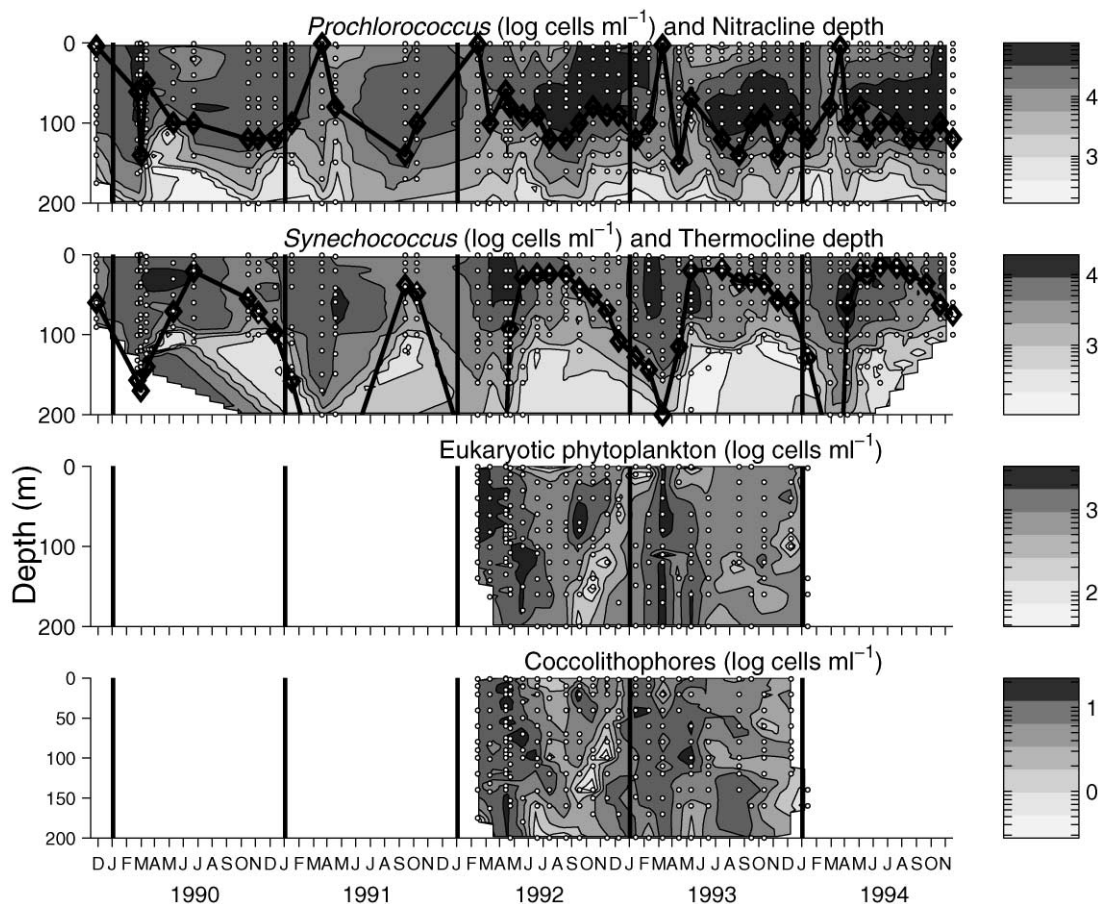


Fig. 2. *Prochlorococcus* and *Synechococcus* cell concentrations from November 1989 to November 1994 and eukaryotic phytoplankton and coccolithophore cell concentrations from February 1992 to January 1994 in the upper 200 m at BATS. The depth of the nitracline is plotted in the *Prochlorococcus* panel and the depth of the thermocline is plotted on the *Synechococcus* panel. In all panels, the small circles mark where samples were collected. Note that the scale is different for each panel.

cus tended to be just above the nitracline depth. In the spring of 1990, when the depth of the nitracline was never shallower than 60 m, concentrations of *Prochlorococcus* were greater in the surface waters than in spring of the other years when nitrate was present near the surface at concentrations $> 0.1 \mu\text{mol kg}^{-1}$. The *Prochlorococcus* cell concentration integrated over the upper 200 m was significantly correlated with the nitracline depth ($r = 0.54$, $n = 45$, $p < 0.001$, see Fig. 2). The depth of the *Prochlorococcus* median cell concentration was also significantly correlated with the nitracline depth ($r = 0.48$, $n = 45$, $p < 0.001$, see Fig. 2), which was seen by Olson et al. (1990a) previously in the Sargasso Sea.

Synechococcus cell concentrations reached maxima of $3.3\text{--}5.6 \times 10^4 \text{ ml}^{-1}$ in the spring (March–May) depending on the year. The highest cell concentrations were restricted to the mixed layer; the lower extent of *Synechococcus* cells tended to track the thermocline depth, extending

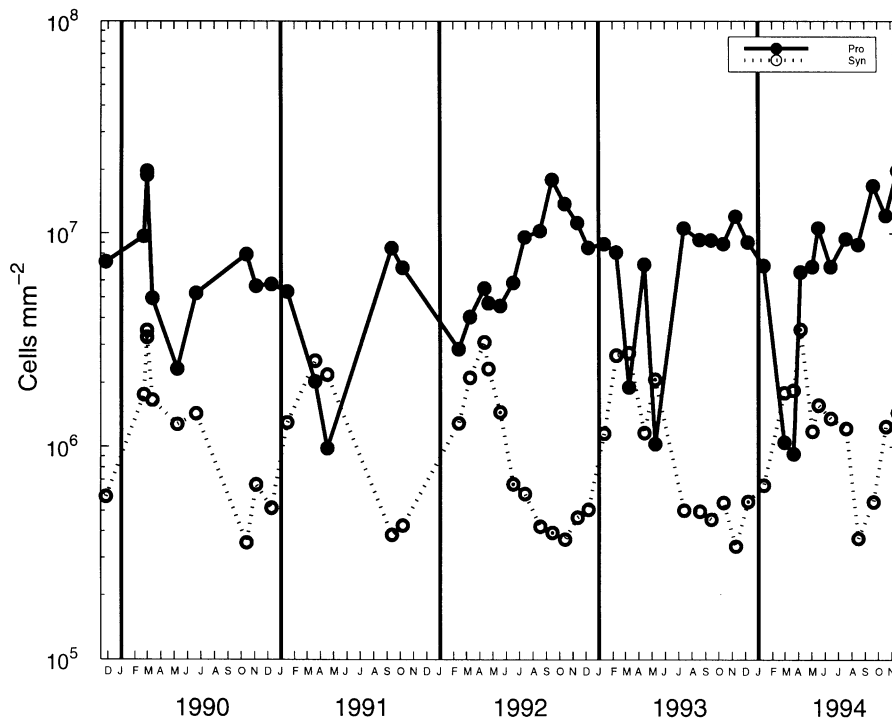


Fig. 3. Time series of integrated cell concentrations (cells mm^{-2}) for *Prochlorococcus* and *Synechococcus*. Data are integrated over the upper 200 m.

deepest in the spring due to deep mixing. The depth of the *Synechococcus* median cell concentration was significantly correlated with the thermocline depth ($r = 0.52$, $n = 45$, $p < 0.001$).

Depth-integrated cell concentrations of the two picoplankton showed opposing seasonal patterns, with *Prochlorococcus* peaking in the fall each year (near September) while *Synechococcus* were at a minimum (Fig. 3), and *Synechococcus* peaking in March–April during the spring bloom while *Prochlorococcus* were at a minimum. Concentrations of *Prochlorococcus* were always greater than those of *Synechococcus*, except during the spring bloom in 3 of the 5 years (1991, 1993 and 1994). The spring of 1990 was unusual in that mixing did not extend as deeply as in other years, and nitrate concentrations never reached $0.1 \mu\text{mol kg}^{-1}$ in the surface waters during the spring bloom. In this year alone, both *Prochlorococcus* and *Synechococcus* bloomed during the spring.

These seasonal patterns are similar to those reported by Olson et al. (1990a) at BATS from May 1988 to July 1989. They speculated that *Prochlorococcus* has a stricter requirement for nitrate (or other covarying nutrients) than does *Synechococcus*. The restriction of *Synechococcus* to shallower depths than deep-adapted *Prochlorococcus* may also be the result of light limitation imposed by its less efficient accessory pigments (Moore et al., 1995).

Campbell et al. (1997) used flow cytometry to examine the phytoplankton in the subtropical North Pacific at Station ALOHA (JGOFS–HOT Station) from December 1990–March 1994. They found similar seasonal patterns in *Prochlorococcus* and *Synechococcus* concentrations to those at BATS: the timing of peaks in *Synechococcus* followed that of the deepest mixed layer depths and the

shoaling of the nitracline; the maximum *Prochlorococcus* generally occurred when the nitracline was deepest. However, Campbell et al. found that *Prochlorococcus* was always dominant in the picoplankton in terms of abundance, with integrated cell concentrations from 10^7 to nearly $3.5 \times 10^7 \text{ mm}^{-2}$ (compared to 10^6 to $2 \times 10^7 \text{ mm}^{-2}$ for BATS, Fig. 3). *Synechococcus* integrated cell concentrations at HOT ranged from 10^5 to $5 \times 10^5 \text{ mm}^{-2}$, much lower than those at BATS ($\sim 4 \times 10^5$ to $\sim 4 \times 10^6 \text{ mm}^{-2}$).

An alternative controlling mechanism for picoplankton species succession, copper toxicity, has been recently proposed by Mann et al. (1997), who showed that *Prochlorococcus* is more susceptible to copper than *Synechococcus*. Copper activities appear to be lower in the Pacific than in the Atlantic (e.g., Coale and Bruland, 1990; Moffett, 1995), which might explain why *Prochlorococcus* dominates the picoplankton in the Pacific (Mann, pers. comm.). Since free cupric ion concentration in the surface waters of the Sargasso Sea is higher in summer than in winter, copper toxicity also might explain the absence of *Prochlorococcus* from BATS surface waters in summer. Based on available observations of free cupric ion concentration profiles (Moffett, 1995), however, it does not explain the disappearance of *Prochlorococcus* during the deep mixing period in the early spring at BATS. It is possible that deep mixing could create conditions favoring *Synechococcus* over the two ecotypes of *Prochlorococcus* (Moore et al., 1998; Moore and Chisholm, 1999), which may be more narrowly restricted in light-level range. Deep mixing might entrain low-light-adapted *Prochlorococcus* from deep in the euphotic zone into surface waters where they may not be able to survive even transient exposure to high light intensities; at the same time, the reduced average light intensity could be too low for the high-light-adapted ecotype.

Another possibility is that temperature is an important controlling factor, since *Prochlorococcus* is most abundant in the fall when the temperature of the euphotic zone is at its maximum (BATS core temperature data) and less abundant in the spring when deep mixing occurs and the euphotic zone temperature is coldest. However, we do observe *Prochlorococcus* growing deep in the water column in October when the temperature there is 19°C (near the temperature during deep mixing).

Cell concentrations for the eukaryotic phytoplankton were high in February 1992 and March 1993, with concentrations reaching 4000–6000 cells ml^{-1} and extending from 100 or 200 m (respectively) to the surface waters (Fig. 2). There were also high concentrations of eukaryotic phytoplankton evident at 60–120 m depth (which did not extend to the surface) at other times of the years. Goericke (1998) used HPLC pigment analysis to study the phytoplankton community at the BATS site in 12 vertical profiles over 2 years (1985–1986). He found that the community was dominated by prymnesiophytes, pelagophytes, and cyanobacteria, while other groups such as diatoms, dinoflagellates, prasinophytes, chlorophytes, and cryptophytes were never abundant during his sampling. However, in other years it has been observed that the spring bloom was dominated by diatoms (Hulburt et al., 1960). Michaels et al. (1994) describe the phytoplankton community at the BATS site from December 1989–June 1990 based on HPLC pigment data. They report that diatoms were a minor part of the biomass during the early spring bloom (early March 1990) and that chrysophytes (pelagophytes, sensu Andersen et al., 1993) and prymnesiophytes were present throughout the bloom. Andersen et al. (1996) used taxonomic identification of phytoplankton and HPLC pigment analysis at nearby Hydrostation ‘S’ and determined that prymnesiophytes and pelagophytes were the most abundant groups of eukaryotic phytoplankton in July 1992.

Coccolithophore cell concentrations reached 50 ml^{-1} , though concentrations were usually less than 20 ml^{-1} , with the greatest abundances in March–April (Fig. 2). These data tend to be lower than those of Haidar and Thierstein (2000), who determined the standing stocks and taxonomic composition of coccolithophores at Hydrostation “S” (half-way between BATS and Bermuda) in 32 vertical profiles from January 1991 to January 1994. They found a maximum concentration of near $100 \text{ cells ml}^{-1}$ during late winter to spring and a minimum concentration during the summer. The average cell concentration they determined was 20 cells ml^{-1} . They report that *Emiliania huxleyi* was the dominant coccolithophore in the spring bloom, which occurred a few weeks after the seasonal advection of nitrate.

3.3. Cell size and fluorescence

Prochlorococcus cell diameter (estimated from forward light scattering) averaged $0.68 \mu\text{m}$ (range $0.53\text{--}0.94$), and *Synechococcus* cell diameter averaged $0.87 \mu\text{m}$ (range $0.74\text{--}1.22$). Except during the deeply mixed period in spring, *Prochlorococcus* and *Synechococcus* were larger and had higher chlorophyll fluorescence at depth than in the upper water column (Figs. 4 and 5). Diameter minima for both picoplankton groups occurred in the upper waters during the summer months, whereas larger cells were present near the spring bloom of each year. The seasonal variations in diameter at a given depth (40 m, for example) were equivalent to changes in cell volume of 2.4-fold for *Prochlorococcus* and 2-fold for *Synechococcus*. The variations in flow cytometric chlorophyll fluorescence were even greater with depth, indicating that depth variations in chlorophyll due to photoacclimation are greater than those in cell size.

These seasonal patterns in picoplankton cell size appear to be related to the depth of the nitracline. When the nitracline is shallow (and the thermocline is deep) there are more nutrients in the upper waters and the picoplankton cells are larger and have greater fluorescence. In the summer and fall when the thermocline is shallow, levels of nitrate + nitrite are never detectable in the upper 80 m and thus the cells are smaller with lower fluorescence.

Some of these differences could be due to succession between dominance by the two ecotypes of *Prochlorococcus*, which are adapted for growth at different light intensities and have different forward light scattering (Moore et al., 1998; Moore and Chisholm, 1999). In addition, cells are on average larger when they grow faster (unpublished data), so perhaps the cells are growing faster when the nutrient levels are higher.

Similar trends in flow cytometric scattering and fluorescence have been seen previously in the Sargasso Sea (Olson et al., 1990a), and similar trends in single-cell fluorescence have been seen in the Pacific (Campbell et al., 1997). The changes in chlorophyll fluorescence with depth have been attributed to photoacclimation, as phytoplankton that grow deeper in the water column have higher pigment per cell to compensate for the decreased light levels (Olson et al., 1990a; Campbell et al., 1997). These increases reflect increases in cell size, as well as increases in fluorescence per cell, deeper in the water. Goericke and Welschmeyer (1998) noted that the increase in chlorophyll *a* with depth could be almost entirely attributed to photoacclimation, since phytoplankton carbon did not vary with depth. From analysis of phytoplankton on an individual-cell basis, we find that carbon per cell (based on FLS) does increase with depth, but that chlorophyll fluorescence per cell increases even more.

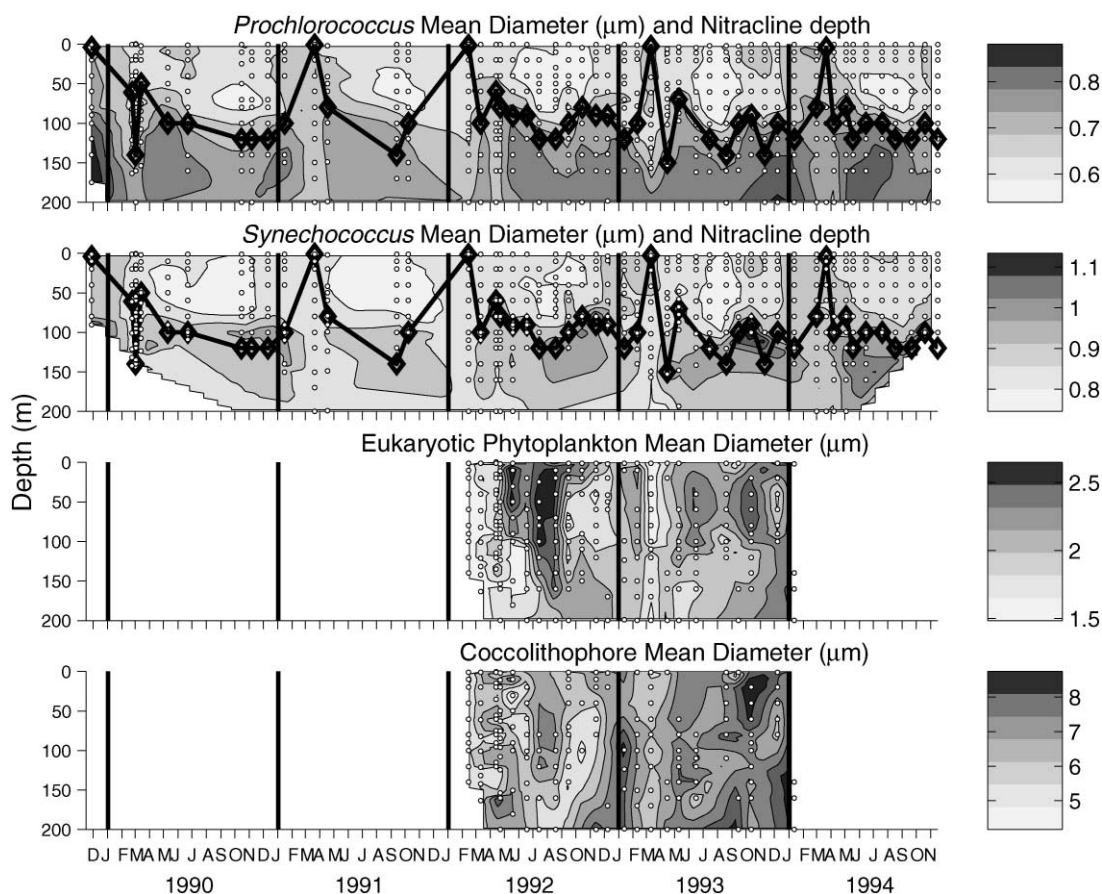


Fig. 4. Phytoplankton diameter (estimated from flow cytometric forward light scattering) for *Prochlorococcus*, *Synechococcus*, eukaryotic phytoplankton and coccolithophores. The depth of the nitracline is also plotted on the upper two panels. In all panels, the small circles mark where samples were collected. Note that the scale is different for each panel.

Variations in mean cell size for the populations of eukaryotic phytoplankton and the coccolithophores likely reflect shifts in species with season or depth. Maxima in cell size for the eukaryotic phytoplankton occur in April, June and July in 1992 and in June and October 1993 (Fig. 4). Strong features in eukaryote cell size and fluorescence (Fig. 5) are in general inversely related to cell concentration (Fig. 2), suggesting blooms of small cells. In both years in which measurements of eukaryotes are available, cell concentration maxima in the spring occur at the same time as minima in cell diameter and fluorescence.

Patterns in cell size for the coccolithophores are less clear, but each spring, when cell concentration is highest, the mean cell diameter is low (5–6 µm). This would be consistent with blooming of *E. huxleyi*, a relatively small coccolithophore, as observed by Haidar and Thierstein (2001) at Hydrostation “S”.

It should be noted that the time of sampling is not taken into account in any of these analyses. No attempt was made to control the time of day at which the samples were collected; however,

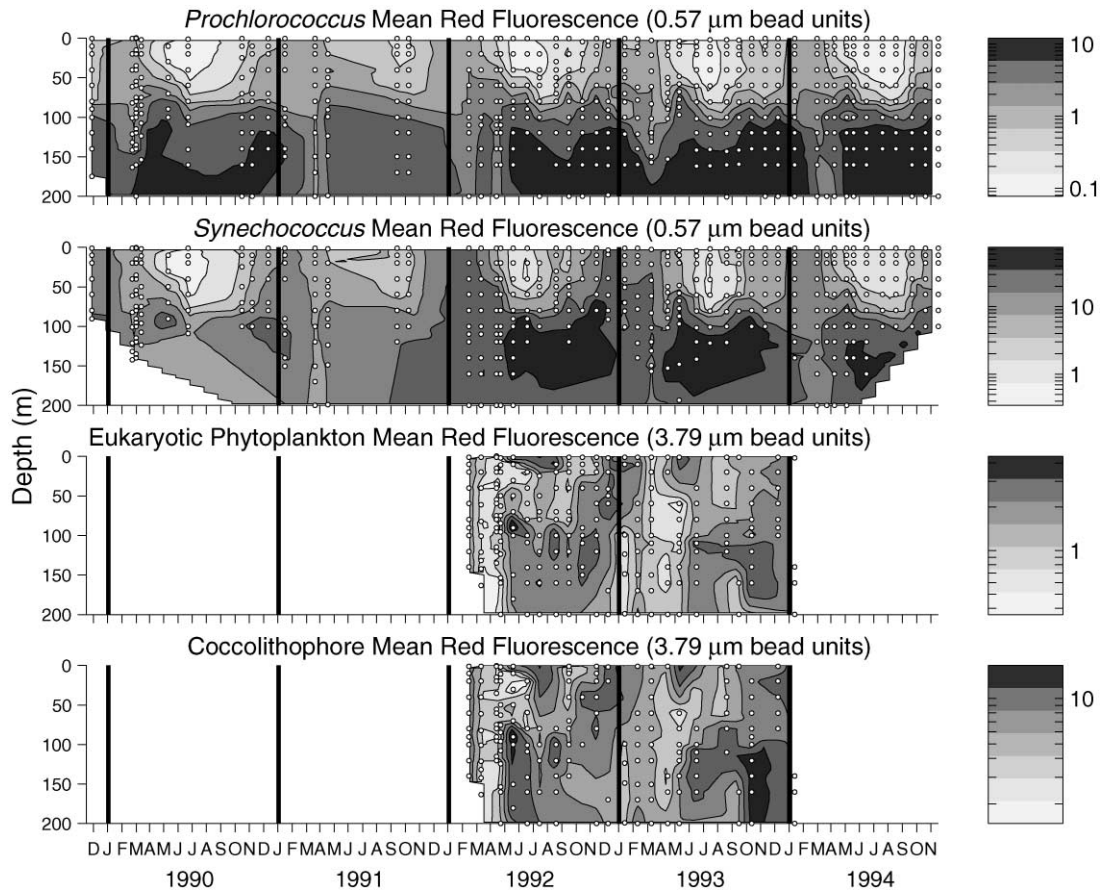


Fig. 5. Mean single-cell red fluorescence for *Prochlorococcus*, *Synechococcus*, eukaryotic phytoplankton and coccolithophores. Note that units are relative to fluorescence microspheres and are different in the upper two panels than in the lower two panels due to the use of different flow cytometers. In all panels, the small circles mark where samples were collected.

nearly 70% of the CTD casts were performed between 1100 and 1600 h, with 50% of them between 1100 and 1400 h. If the cells are synchronized in their growth and division patterns, then there may be diel variations (between dawn and dusk) up to two-fold in volume and even greater in flow cytometric CHL, as previously seen for these phytoplankton groups in the Sargasso Sea (DuRand, 1995; Dusenberry, 1995). The variations expected to occur within the few hours when the majority of the sampling was done would be smaller in magnitude.

3.4. Phytoplankton carbon

The estimated amount of carbon contained in the different phytoplankton groups shows seasonal and depth patterns similar to those of cell concentrations (Fig. 6). This indicates that patterns in total carbon were determined more by abundance than changes in cell size. The mean

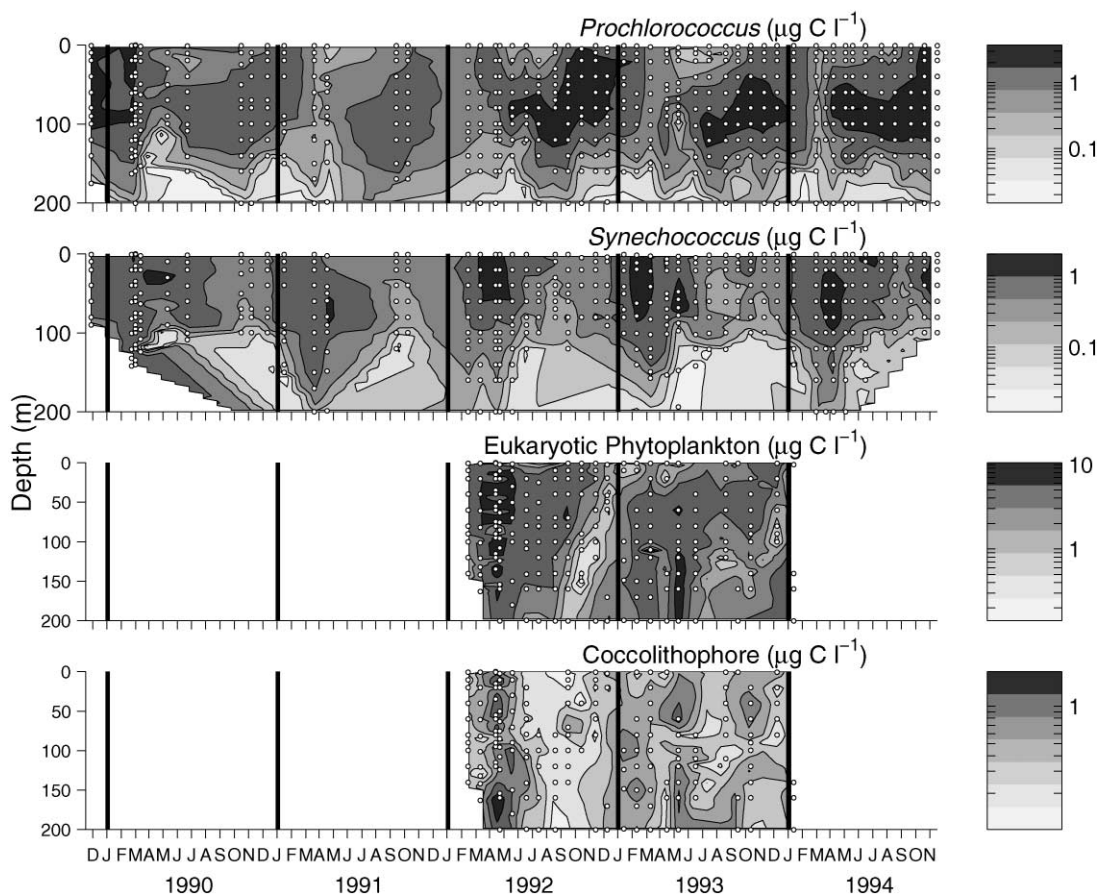


Fig. 6. Phytoplankton carbon ($\mu\text{g l}^{-1}$) for *Prochlorococcus*, *Synechococcus*, eukaryotic phytoplankton and coccolithophores. In all panels, the small circles mark where samples were collected. Note that the scale is different for each panel.

carbon concentrations reached $10 \mu\text{g C l}^{-1}$ for *Prochlorococcus*, $5.9 \mu\text{g C l}^{-1}$ for *Synechococcus*, $24 \mu\text{g C l}^{-1}$ for the eukaryotic phytoplankton, and $3.3 \mu\text{g C l}^{-1}$ for the coccolithophores.

The estimated carbon biomass of the *Prochlorococcus* and *Synechococcus*, integrated over the upper 200 m, tended to have opposite seasonal patterns, with *Synechococcus* slightly dominant during the spring bloom in each year when *Prochlorococcus* biomass was at its minimum (Fig. 7). *Prochlorococcus* biomass greatly exceeded that of *Synechococcus* in the summer and fall. These seasonal trends were evident each year of the dataset, though the summer/fall blooms of *Prochlorococcus* were greatest in 1992 and 1994, the two years in which the thermocline in the summer was shallower than in other summers. Other contributing factors are that in 1991 the *Prochlorococcus* concentration was not as high and in 1993, though the cell concentration was high, *Prochlorococcus* had only a subsurface maxima. As with the cell concentration data (Fig. 3), an exception occurred during the spring of 1990 (when the nitrate concentration never reached $0.1 \mu\text{mol kg}^{-1}$ in the surface waters) and *Prochlorococcus* as well as *Synechococcus* bloomed. Campbell et al. (1997)

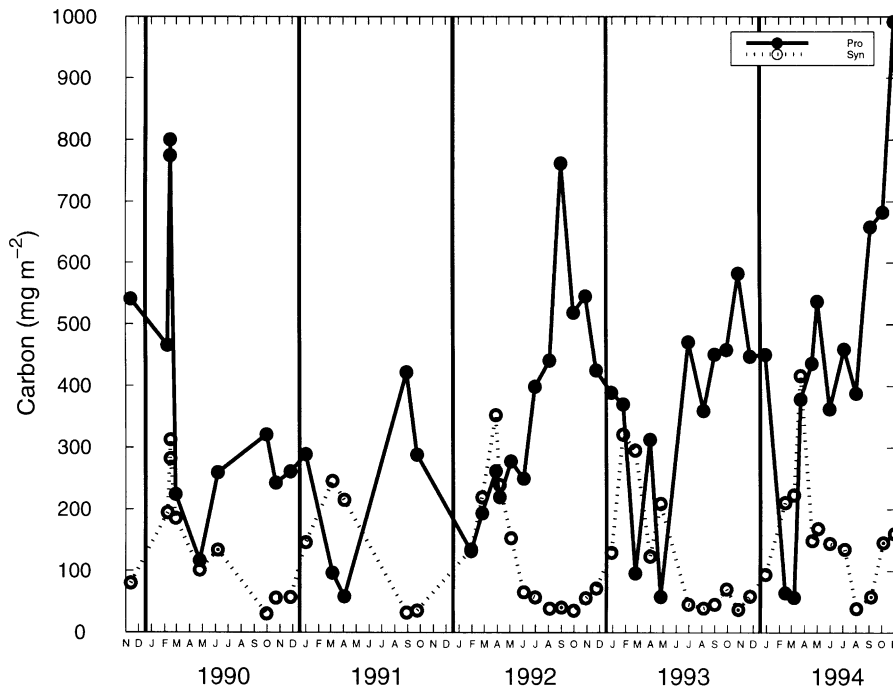


Fig. 7. Time series (November 1989–November 1994) of estimated carbon biomass of picoplankton (Pro = *Prochlorococcus*, Syn = *Synechococcus*). Data are integrated over the upper 200 m.

found that *Synechococcus* was never a large percentage of the biomass at the HOT site. This is in contrast to the results reported here for BATS, where integrated *Synechococcus* biomass often equaled or exceeded that of *Prochlorococcus* during the spring bloom.

For February 1992 to January 1994, the estimated carbon biomass of prokaryotic and eukaryotic phytoplankton groups was determined and compared to the BATS core data for particulate organic carbon (POC) (Fig. 8A). The total phytoplankton carbon as a percent of POC, integrated over the upper 200 m, was an average of 33% (from 23 months of data over 2 years), with a range from 21 to 43% and no clear seasonal patterns. Our values tend to be higher than data from Goericke (1998), who determined using the chlorophyll-labeling method that phytoplankton carbon was about 10% of POC in August 1985 at BATS. We found that there was year-to-year variability, with 35% in August 1992 and 24% in August 1993.

The integrated phytoplankton carbon biomass was subdivided into the contributions of *Prochlorococcus*, *Synechococcus*, coccolithophores, and other eukaryotic phytoplankton (Fig. 8B). *Prochlorococcus* had its greatest contribution to phytoplankton carbon from June 1992 to February 1993 and in July 1993 to January 1994, with values between 24 and 50%. During the other months (including the spring bloom in 1992), the values ranged from 4 to 17%. An apparent anomaly occurred in April 1993, when *Prochlorococcus* carbon was 35% of phytoplankton carbon. This peak is due to high cell concentration, as seen in the integrated cell concentrations (Fig. 3). One possible explanation is that the nitracline was unusually deep for this time of year, which would favor growth of *Prochlorococcus*. The *Synechococcus* contribution to phytoplankton carbon

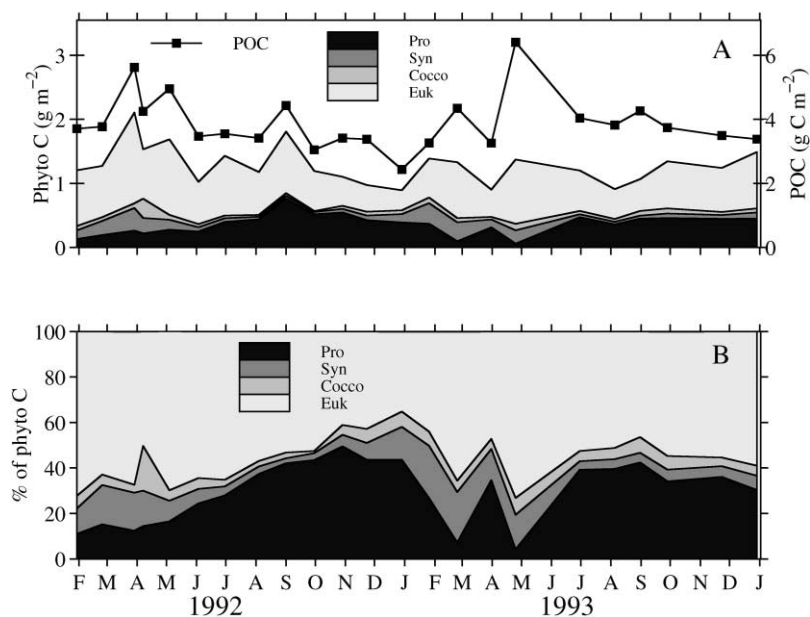


Fig. 8. Time series (February 1992–January 1994) of (A) particulate organic carbon (POC, BATS core data) and estimated contributions of different phytoplankton groups to flow cytometrically derived phytoplankton carbon and (B) the percent contribution of different phytoplankton groups to total integrated phytoplankton carbon. Pro = *Prochlorococcus*, Syn = *Synechococcus*, Cocco = coccolithophores, Euk = other eukaryotic phytoplankton. Data are integrated over the upper 200 m.

was greatest in February–April 1992 (11–17%) and in January–May 1993 (14–23%); at other times it was always less than 9% of phytoplankton carbon. Together, the two groups of prokaryotic picoplankton contributed 20–58% to phytoplankton carbon.

The contribution of the non-coccolithophore eukaryotic phytoplankton ranges from 35 to 73% of phytoplankton carbon, reaching maxima near the spring bloom each year. The data for coccolithophore carbon are quite variable with time and range from 1 to 7% (with an extreme maximum of 20% in April 1992). Thus the phytoplankton carbon tends to be dominated by eukaryotic phytoplankton during the spring blooms, but with *Prochlorococcus* carbon nearly reaching or slightly exceeding the eukaryotic contribution during the summer/fall months. Campbell et al. (1997) determined the contributions of the heterotrophic bacteria, *Prochlorococcus*, *Synechococcus*, picoeukaryotes, and 3–20 μ m algae to total carbon at the HOT Station and found that *Prochlorococcus* dominated the autotrophic components during the 40-month sampling period (except at one sampling time in late 1993 when the 3–20 μ m algae were dominant).

The relationship between our estimates of phytoplankton carbon and POC measurements are shown with a Model II regression of the log transformed data (Fig. 9). Even though a significant fraction of POC would be expected to be non-phytoplankton carbon, POC appears to be a good predictor of phytoplankton carbon, based on the small standard error of the slope and the narrow 95% confidence limits of the slope. When phytoplankton carbon is separated into picoplankton carbon and eukaryotic phytoplankton (including coccolithophore) carbon, it can be seen that POC is a better predictor of eukaryotic phytoplankton carbon than it is of picoplankton carbon. This is

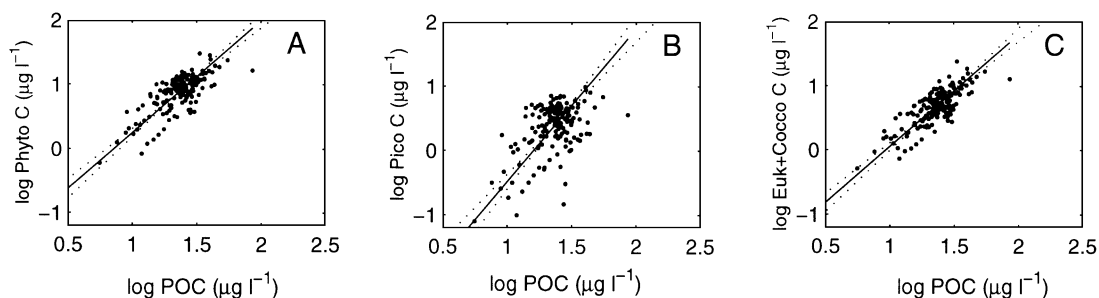


Fig. 9. Relationship between flow cytometrically derived (A) total phytoplankton carbon, (B) picoplankton carbon, and (C) eukaryotic phytoplankton carbon (including coccolithophores) and measured particulate organic carbon (POC) from the BATS database. For each, Model II linear regressions on the log-transformed data are shown along with 95% confidence limits of the slope. The equations, standard errors of the slope, and correlation coefficients (r) are: (A) $\log Y = 1.743 \log X - 1.496$ (s.e. = 0.09, $r = 0.75$), (B) $\log Y = 2.383 \log X - 2.867$ (s.e. = 0.158, $r = 0.52$), (C) $\log Y = 1.737 \log X - 1.682$ (s.e. = 0.082, $r = 0.80$).

because the largest variations in POC are during the spring blooms when the eukaryotic phytoplankton are relatively more important. The picoplankton tend to be more important in the summer/fall, due to *Prochlorococcus*, but there are no large peaks in POC seen then.

The relationship between primary productivity (PP, BATS core data) and our estimates of phytoplankton carbon, with a Model II regression of the log-transformed data, has a small standard error of the slope and tight 95% confidence limits of the slope (Fig. 10). This indicates that phytoplankton carbon is a reasonable predictor of PP, despite any uncoupling of production and biomass by high grazing rates.

The Model II regression between the fraction of biomass contained in the picoplankton, *Prochlorococcus* and *Synechococcus*, and integrated primary productivity has a negative slope (Fig. 11). Thus when picoplankton dominate, primary production is low, a relationship that is well established (see Chisholm, 1992).

3.5. Variables and approaches in estimating phytoplankton carbon

The determinations of phytoplankton carbon biomass presented here are dependent on the choice of biovolume and carbon conversion factors, both of which are subject to significant uncertainty. Caron et al. (1995) showed, for example, that microbial biomass could vary nearly three-fold depending on whether “low” or “high” published factors were used to convert biovolume to carbon. In an earlier study with a portion of this same data set, Gin et al. (1999) struggled with this issue. We have tried to improve on this and other previous approaches by: (1) accounting for cell size variations (including the picoplankton) by applying our calibrations to individually measured cells; (2) using a picoplankton carbon conversion factor derived from measurements of several strains of *Synechococcus* (see Methods); and (3) expanding our empirical FLS to size calibration by including cells up to 40 μm in diameter.

In most previous studies, carbon content of *Prochlorococcus* and *Synechococcus* has been assumed to be a constant. Previous determinations of *Prochlorococcus* carbon have assumed a diameter of 0.6 μm and then converted to carbon using a factor of 470 $\text{fg C } \mu\text{m}^{-3}$ from Verity et al.

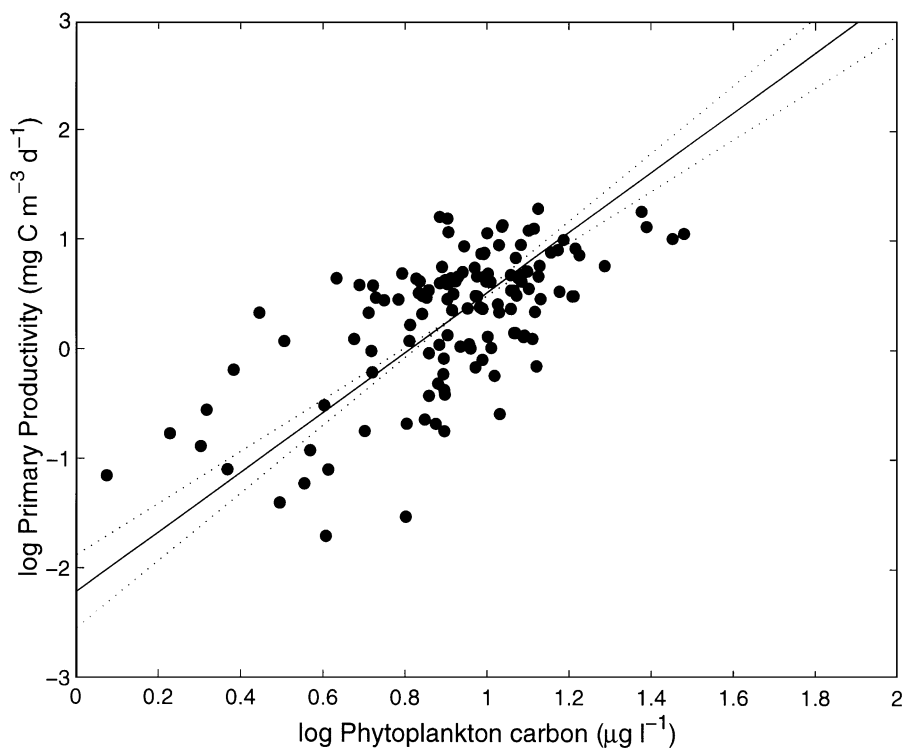


Fig. 10. Relationship between measured primary productivity values from the BATS database and flow cytometrically derived phytoplankton carbon. A model II linear regression on the log-transformed data is shown along with 95% confidence limits of the slope ($\log Y = 2.74 \log X - 2.22$; s.e. of slope = 0.184, $r = 0.64$).

(1992) to get 53 fg C per cell (Campbell et al., 1994, 1997) or assumed a diameter of 0.8 μm and converted to carbon using a factor of 220 $\text{fg C } \mu\text{m}^{-3}$ from Booth (1988) to get 59 fg C per cell (Li et al., 1992). In previous determinations of phytoplankton carbon biomass (Li et al., 1992; Campbell et al., 1994, 1997), *Synechococcus* has been assigned 250 fg C per cell, based on laboratory studies on strain WH7803 by Kana and Glibert (1987). This strain, though isolated from the Sargasso Sea, is similar to coastal strains in its pigment types and is larger than typical open-ocean strains of *Synechococcus* (Waterbury et al., 1986; Olson et al., 1990b).

Our flow cytometry data indicate that *Prochlorococcus* and *Synechococcus* increase in size with depth (see also Olson et al., 1990a; Gin et al., 1999), and this pattern has been documented using direct measurement techniques (Burkhill et al., 1993; Sieracki et al., 1995). We took this variability into account in our phytoplankton carbon estimates, and the resulting average values were 56 fg C cell^{-1} for *Prochlorococcus* (S.D. 20) and 112 fg C cell^{-1} for *Synechococcus* (S.D. 26). Thus our values for *Prochlorococcus* fall in the range of previous estimates. Our *Synechococcus* carbon contents, which we believe to be based on improved calibrations for open-ocean strains, are lower than those from most previous studies.

For converting volume to carbon in eukaryotic phytoplankton, we used the relationship determined by Eppley et al. (1970), which includes data from both Mullin et al. (1966) and from

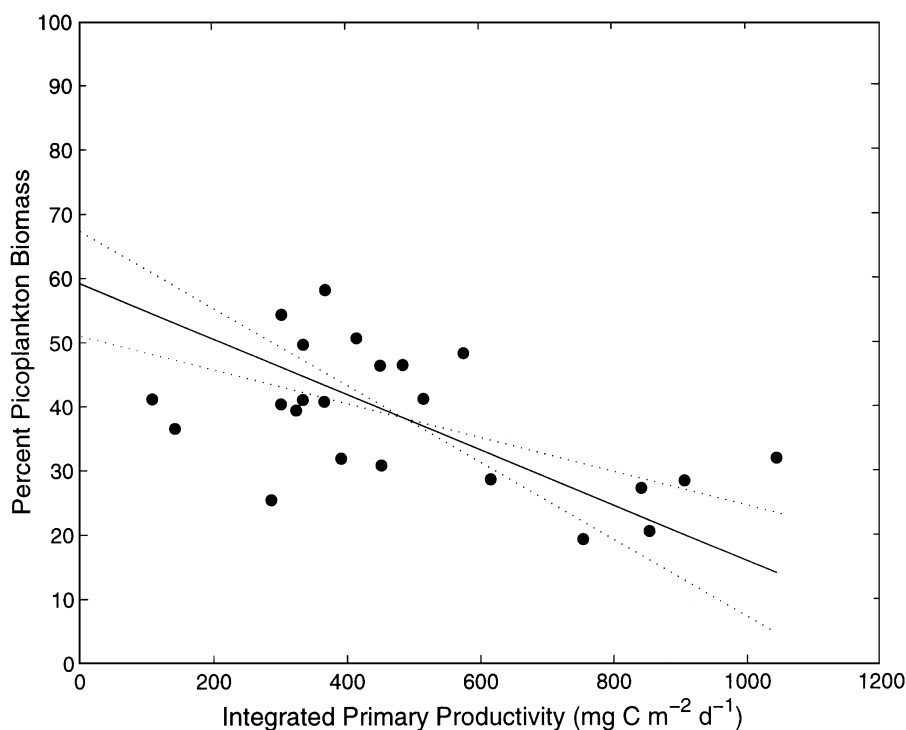


Fig. 11. Relationship between the percent of the total phytoplankton carbon in the picoplankton fraction (*Prochlorococcus* and *Synechococcus*) and primary productivity. Both are integrated to the depth of available primary productivity data (usually to 150 m). A model II linear regression is shown along with 95% confidence limits of the slope ($Y = -0.043 X + 59.3$; s.e. of slope = 0.008, $r = -0.52$).

Strathmann (1967). We chose this equation to be consistent with other workers in the JGOFS program (specifically Arabian Sea). A more recent empirical calibration between carbon and volume, used by Gin et al. (1999), is that of Verity et al. (1992): $\text{pg C} = 0.433 (\text{Biovolume})^{0.863}$. Our values for eukaryotic phytoplankton carbon (including coccolithophores) are an average of 85% (range of 73–113%) of those determined using the equation of Verity et al. (1992).

There are a number of uncertainties in the POC data that could contribute to the relationship between POC and phytoplankton carbon. Experiments conducted at BATS indicate that the reported values underestimate total POC by 10–50% due to the settling of particles below the Niskin bottle spout (Gundersen, in prep cited in Michaels and Knapp, 1996), though it is likely that we are also not sampling these large particles with flow cytometry. Li et al. (1992) determined that a correction for 47% of bacteria passing through GF/F filters resulted in estimated POC values that were 6% higher. Campbell et al. (1994) corrected the Hawaii Ocean Time-series (HOT) station ALOHA POC data for a 60% loss of heterotrophic bacteria and a 15% loss of *Prochlorococcus* and found that the correction made a difference of about 20% for values of POC integrated over 200 m. However, we have never observed significant numbers of *Prochlorococcus* passing through GF/F filters (unpublished data; Chavez et al., 1995).

Gin et al. (1999) investigated the bacteria and phytoplankton concentration and biomass size spectra from a small subset of this data set. They found, as we did, that eukaryotic phytoplankton were the major constituent of the phytoplankton, but the contribution of the larger cells was estimated to be much greater than in this study. We believe this is mostly due to the choice of conversion factors. The conversion between FLS and cell size used by Gin et al. for eukaryotic phytoplankton (using culture data from DuRand, 1995) tended to overestimate the size of the largest cells, as compared to the improved calibration we used here, which included larger phytoplankton cells. For the picophytoplankton, Gin et al.'s conversion from FLS to cell size led to similar estimates of size for *Prochlorococcus*, but larger estimates for *Synechococcus* than those reported here. In addition, different cell size-to-carbon conversions were used for all phytoplankton types in the two studies. Gin et al. used equations from Verity et al. (1992), which gives 45% higher carbon per volume for the picoplankton ($470 \text{ fg } \mu\text{m}^{-3}$ compared to our $325 \text{ fg } \mu\text{m}^{-3}$) and on average higher carbon estimates for the eukaryotic phytoplankton (see above). Gin et al.'s conversion factors in many cases resulted in phytoplankton carbon estimates as high as or higher than total measured POC. That is not the case here, due to the improvements we have made in the conversions.

4. Conclusions

Despite uncertainties in a number of parameters calculated in this study, a reasonably coherent picture of the seasonal changes in phytoplankton abundance and carbon biomass can be constructed. The integrated cell concentrations of the prokaryotic picoplankton generally showed opposing patterns, with *Prochlorococcus* maxima occurring during the late summer/fall and minima occurring during the spring bloom when *Synechococcus* peaked. Despite their opposing patterns in abundance, the sizes of both picoplankton cell types were smaller in the summer and larger during the spring bloom. The eukaryotic phytoplankton cell concentration was largest during the spring bloom, as was their contribution to phytoplankton carbon, even though the mean cell size was smallest at that time. The eukaryotic carbon contribution was almost always larger than that of the picoplankton, although during the late summer/fall the picoplankton were close to half, largely due to *Prochlorococcus*. Even though there were strong seasonal patterns in phytoplankton community structure, the phytoplankton contribution to particulate organic carbon was relatively constant, averaging 33%.

Acknowledgements

We thank the technical staff of the BATS program for sample collection. We are grateful to Erik Zettler, Nianzhi Jiao, Nicole Poulton, Laurel Schaidler, Karina Gin, Alexi Shalapyonok and Heidi Sosik for their contributions to the sample and data analysis. We also thank two anonymous reviewers for their helpful comments. This work was supported by NSF grants OCE-9014724 (to RJO) and OCE-9012117 (to SWC).

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