LIMNOLOGY
AND
OCEANOGRAPHY

September 2003
Volume 48
Number 5

Limnol. Oceanogr., 48(5), 2003, 1721–1731
© 2003, by the American Society of Limnology and Oceanography, Inc.

Elemental composition of marine *Prochlorococcus* and *Synechococcus*: Implications for the ecological stoichiometry of the sea

S. Bertilsson[1] *and O. Berglund*[2]
Department of Civil and Environmental Engineering, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

D. M. Karl
School of Ocean and Earth Science and Technology, University of Hawaii, Honolulu, Hawaii 96822

S. W. Chisholm
Department of Civil and Environmental Engineering, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

Abstract

The elemental composition of marine cyanobacteria is an important determinant of the ecological stoichiometry in low-latitude marine biomes. We analyzed the cellular carbon (C), nitrogen (N), and phosphorus (P) contents of *Prochlorococcus* (MED4) and *Synechococcus* (WH8103 and WH8012) under nutrient-replete and P-starved conditions. Under nutrient-replete conditions, C, N, and P quotas (femtogram cell^{-1}) of the three strains were 46 ± 4, 9.4 ± 0.9, and 1.0 ± 0.2 for MED4; 92 ± 13, 20 ± 3, and 1.8 ± 0.1 for WH8012; and 213 ± 7, 50 ± 2, 3.3 ± 0.5 for WH8103. In P-limited cultures, they were 61 ± 2, 9.6 ± 0.1, and 0.3 ± 0.1 for MED4; 132 ± 6, 21 ± 2, and 0.5 ± 0.2 for WH8012; and 244 ± 21, 40 ± 4, and 0.8 ± 0.01 for WH8103. P limitation had no effect on the N cell quota of MED4 and WH8012 but reduced the N content of WH8103. The cellular C quota was consistently higher in P-limited than in nutrient-replete cultures. All three strains had higher C : P and N : P ratios than the Redfield ratio under both nutrient-replete and P-limited conditions. The C : N molar ratios ranged 5–5.7 in replete cultures and 7.1–7.5 in P-limited cultures; C : P ranged 121–165 in the replete cultures and 464–779 under P limitation; N : P ranged 21–33 in the replete cultures and 59–109 under P limitation. Our results suggest that *Prochlorococcus* and *Synechococcus* may have relatively low P requirements in the field, and thus the particulate organic matter they produce would differ from the Redfield ratio (106C : 16N : 1P) often assumed for the production of new particulate organic matter in the sea.

The marine cyanobacterium *Prochlorococcus* is a small photosynthetic prokaryote (diameter, 0.5–0.8 μm) that is ubiquitous in the euphotic zone of tropical and subtropical oceans (40°S–40°N; Chisholm et al. 1988; Partensky et al. 1999). Abundances in warm surface waters are typically $>10^5$ cells ml^{-1}, and cells are found as deep as 200 m below the surface (Campbell et al. 1994; Partensky et al. 1999). These features make *Prochlorococcus* the numerically dominant oxygenic phototroph in the oceans. Its close relative, *Synechococcus*, is also important in subtropical ecosystems. Although concentrations are typically an order of magnitude lower than those of *Prochlorococcus* (Campbell et al. 1994; DuRand et al. 2001), their larger average cell size (diameters of 0.6–2.1 μm; Herdman et al. 2001) makes them approximately equal in terms of global photosynthetic biomass. *Synechococcus* cells are usually limited to the upper 100 m of the water column (Partensky et al. 1999) but have a broader latitudinal distribution than *Prochlorococcus*; they are not limited to warm oceanic waters but can be encountered at temperatures as low as 2°C (Shapiro and Haugen 1988).

[1] Corresponding author (Stebe@ebc.uu.se). Present address: Department of Evolutionary Biology/Limnology, Uppsala University, SE-75236 Uppsala, Sweden.

[2] Present address: Department of Ecology/Limnology, Lund University, SE-223 62 Lund, Sweden.

Acknowledgments

We thank John Waterbury for kindly making the axenic *Synechococcus* strains available to us, Tom Gregory for skillful analysis of total P using the MRP method, Crystal Shih for laboratory work in a preliminary study of C : N : P in *Prochlorococcus*, and Lisa Moore and Stephanie Shaw for initial discussions on experimental design.

This research was supported in part by the K & A Wallenberg foundation (postdoctoral fellowships to S.B. and O.B.) and in part by the U.S. National Science Foundation (grants to S.W.C. and D.M.K.) and the Department of Energy (grant to S.W.C.).

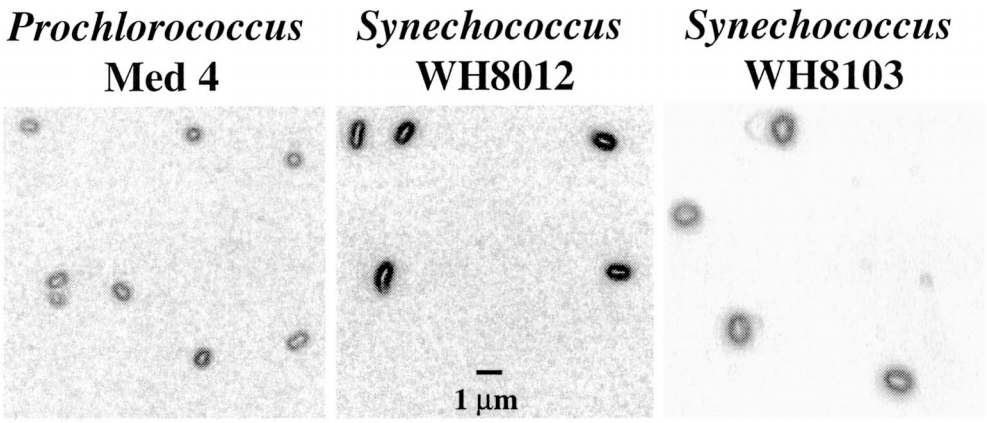


Fig. 1. Morphology and size of nutrient-replete *Synechococcus* WH8012, WH8103 and *Prochlorococcus* MED4. Cells are stained with 4′,6′-diamidino-2-phenylindole and have been delineated using the edge detection operator in the Scion Image 4.0.2 software.

The marine carbon cycle is a key component of the global cycling of carbon (Field et al. 1998). The nutritional requirements of marine phytoplankton, which supply the photosynthetically fixed carbon (C) for higher trophic levels (i.e., net primary production), have played a central role in dictating the elemental composition of seawater over evolutionary time (Redfield 1934) and influence the dynamics and availability of nitrogen (N), phosphorus (P), and other potentially limiting nutrients in the oceans (Falkowski et al. 1998; Tyrrell 1999).

Marine eukaryotic plankton typically conform to the Redfield ratio (molar C : N : P of 106 : 16 : 1; Redfield 1958), a stoichiometric relationship that is characteristic for bulk particulate matter in oceanic environments (Copin-Montegut and Copin-Montegut 1983; Falkowski 2000). This empirically derived ratio, which reflects the average biochemical composition of the major food-web components in oceanic environments, is frequently used in biogeochemical models to estimate ocean productivity and export production and also to differentiate N limitation from P limitation in the ocean (Codispoti 1989; Falkowski 1997; Falkowski et al. 1998; Tyrrell 1999). The C : N : P ratio, especially the C : P ratio, also sets a constraint on carbon sequestration (Michaels et al. 2001).

Even though the average C : N : P of oceanic particulate matter is close to the Redfield ratio, selected regions, depths, and seasons vary considerably in this regard (Copin-Montegut and Copin-Montegut 1983; Hebel and Karl 2001). This local and temporal variation in the elemental composition of particles could be linked to the composition and structure of the food webs (Geider and La Roche 2002). Oceanic food webs are typically dominated by marine microalgae and heterotrophic bacteria (Campbell et al. 1994; Gasol et al. 1997), and previous studies have shown that not only do the cellular C : N : P ratios of both bacteria and phytoplankton vary between different organisms but also that the elemental composition of individual species may vary extensively as a function of environmental constraints (Herbert 1961; Rhee 1973; Geider and La Roche 2002; Vrede et al. 2002). The physiological responses causing changes in cellular C : N : P stoichiometry of microorganisms involve systematic changes in the cellular content of quantitatively important biopolymers such as proteins, nucleic acids, carbohydrates, lipids, and polyphosphate (Herbert 1961; Geider and La Roche 2002). However, the available information is almost exclusively based on studies that involved a few cultured heterotrophic bacteria or eukaryotic microalgae, whereas very little information is available on the regulation of C : N : P in marine cyanobacteria (Cuhel and Waterbury 1984; Geider and La Roche 2002).

Because the collective biomass of *Prochlorococcus* and *Synechococcus* is often a significant fraction of the total biomass in oligotrophic ecosystems (e.g., ~50% at Sta. ALOHA [A Long-term Oligotrophic Habitat Assessment]; Karl and Dobbs 1998), it would help to understand the bounds of their C : N : P ratios when trying to interpret the dynamics of these ratios in ecosystems where these organisms dominate. Because axenic cultures of *Prochlorococcus* MED4 recently became available (Saito 2000; Moore et al. 2002), this is now possible. The goal of the work reported herein was to obtain robust estimates of the cellular content of C, N, and P in *Prochlorococcus* and two strains of *Synechococcus*, to help assess and model their role in the flux of carbon and other nutrients through oceanic food webs.

Materials and methods

Axenic *Prochlorococcus* MED4 was derived from the original MED4 strain (isolated by D. Vaulot) as described in Saito (2000). The axenic strains of *Synechococcus* WH8103 and WH8012 were obtained from the Cyanobacterial Culture Collection at the Woods Hole Oceanographic Institution (courtesy of John Waterbury). *Prochlorococcus* MED4 belongs to the high-light adapted (low B/A) clade 1 and were isolated from the Mediterranean Sea, whereas the two *Synechococcus* strains belong to the closely related marine *Synechococcus* cluster A and were isolated from the Sargasso Sea (Waterbury et al. 1986; Rocap et al. 2002). All three strains used in this study have a rod-shaped to spherical morphology but vary in size (*Prochlorococcus* MED4 < *Synechococcus* WH8012 < *Synechococcus* WH8103; Fig. 1).

Despite the apparent morphological similarities and close phylogenetic relationships, pigmentation is very different in the three strains; for example, *Prochlorococcus* lack phycobiliproteins but instead synthesize divinyl-chlorophyll *a* and *b* (Partensky et al. 1999), and the *Synechococcus* strains differ in their relative content of phycouribilin and phycoerythrin (Rocap 2002). Furthermore, *Synechococcus* WH8103 is motile, whereas the other two strains lack any form of swimming motility (Waterbury 1986; Rocap 2002).

All cultures were maintained at 22°C in constant light at 30–40 μmol quanta m^{-2} s^{-1}. Cool-white fluorescent lamps were used for all incubations. Batch cultures were grown in 25 ml Sargasso Sea–based medium added to 50-ml borosilicate glass tubes with 25 mm inner diameter (Moore et al. 2002). Filter-sterilized and autoclaved seawater was amended with filter-sterilized stock solution of NH_4Cl to a final concentration of 800 μmol L^{-1}, and a stock solution of NaH_2PO_4 was added to either 50 or 1 μmol L^{-1} to obtain media with molar N : P ratios of 16 and 800, respectively. A filter-sterilized trace-metal stock solution was added to the following final concentrations: 1.17 μmol L^{-1} ethylene diaminetetraacetic acid, 8 nmol L^{-1} zinc, 5 nmol L^{-1} cobalt, 90 nmol L^{-1} manganese, 3 nmol L^{-1} molybdenum, 10 nmol L^{-1} selenium, 10 nmol L^{-1} nickel, and 1.17 μmol L^{-1} iron.

All cultures were acclimated to the irradiance conditions and the respective growth medium (P-replete and P-limited) for a minimum of three transfers before the start of the experiment. Eighteen replicate cultures were set up for each medium and organism combination, and the growth of each culture was monitored on a daily basis by noninvasive in vivo chlorophyll fluorescence using a Turner 10AU fluorometer (Turner Designs). The P-replete cultures were harvested during the midexponential growth phase (after 7–9 d), and the P-limited cultures were harvested during the early stationary growth phase (12–14 d) (Fig. 2). Nine replicate cultures (three pooled samples of three tubes) were collected on combusted Whatman GF/F filters (2 h at 450°C) for the analysis of particulate C and N, and six single cultures were harvested in a similar way for the analysis of particulate P. All filtrations were done under low vacuum (<10 kPa) in an acid-washed and rinsed filtration funnel made of glass. Additional filtrations with 25–75 ml of 0.2-μm-filtered media from the harvested cultures were done as controls for adsorption of dissolved nutrients to the Whatman GF/F filters. All filters were dried at 60°C (1–2 d) and stored in the dark in a desiccator until analysis. The three remaining cultures for each treatment were used to monitor growth of the various cultures after the end-point sampling for particulate C, N, and P.

To determine the number of cells captured on each filter, 1-ml samples were collected before harvesting the cultures and in all filtrates for subsequent analysis by flow cytometry and epifluorescence microscopy. Samples were preserved in cryovials by first adding 0.2-μm-filtered glutaraldehyde to a final concentration of 0.125%, followed by rapid freezing and subsequent storage in liquid nitrogen. The filtration efficiency (percentage of cells captured on filters) was >99% for *Synechococcus* WH8103, >97% for *Synechococcus* WH8012, and >95% for *Prochlorococcus* MED4, indicating a highly efficient harvesting of cells by low vacuum filtration

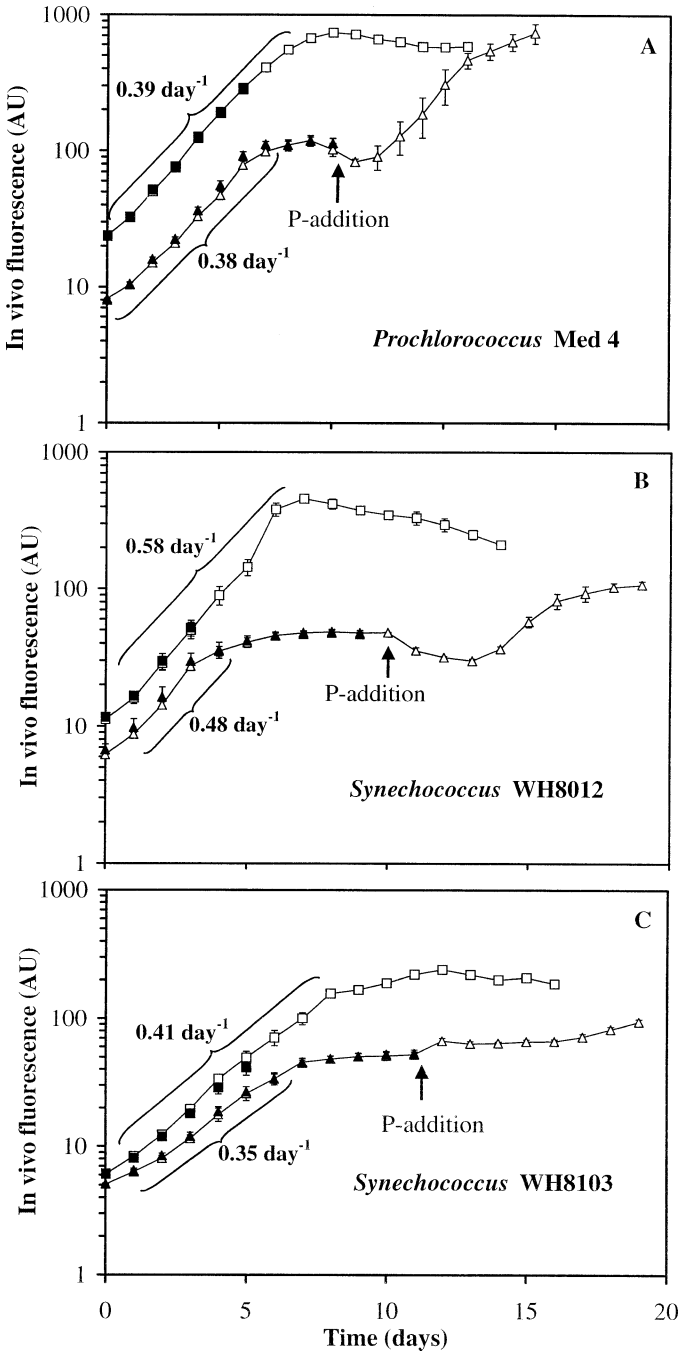


Fig. 2. Growth curves for (A) *Prochlorococcus* MED4, (B) *Synechococcus* WH8012, and (C) *Synechococcus* WH8103 based on in vivo chlorophyll fluorescence. Parallel growth curves are shown for cyanobacteria growing in P-replete (squares) and P-limited (triangles) medium. Black symbols indicate fluorescence of cultures used for CNP analysis (n = 15), and white symbols indicate fluorescence of triplicate cultures used for monitoring growth after harvesting cells for CNP analysis. Error bars represent the standard deviation (often hidden in symbol). Cultures used for CNP analyses were harvested after 4–12 d, as indicated by the disappearance of the solid symbols in the graphs. The triplicate cultures used to monitor growth in the P-limited cultures were amended with P during the late stationary growth phase, to assess P limitation. The growth rate in the exponential growth phase (indicated by brackets) is shown for each treatment ($r^2 > 0.97$, $n > 4$).

through combusted Whatman GF/F filters. To verify that the cyanobacterial cultures were axenic, subsamples were aseptically added at 1:5 (v:v) to Marine Purity Broth (17 g AC broth [Difco], 8 g $MgSO_4 \times 7H_2O$, 20 g NaCl, and 1.5 g $CaCl_2 \times 2H_2O$ in 1 liter of distilled water). No growth was detected during the 1-week incubation at 22°C, whereas positive controls had visible growth of heterotrophic bacteria in <2 d.

The chlorophyll fluorescence in the P-limited cultures without any P addition typically decreased during the late stationary phase (data not shown). We used the triplicate cultures remaining after the end-point filtrations to verify the P limitation of cell growth in the supposedly P-limited cultures by doing simple rescue experiments. Cultures were spiked with an additional 25 μmol L^{-1} NaH_2PO_4 during the plateau phase, and the growth response was monitored daily by in vivo fluorescence. In addition, samples for flow cytometry were collected 8 d after the amendment. The P additions caused an increase in chlorophyll fluorescence (Fig. 2). The increase in chlorophyll fluorescence was accompanied by an increase in cell concentrations of 770% for *Prochlorococcus* MED4 and 284% for *Synechococcus* WH8012, indicating strong P limitation for these cells. The concentration of *Synechococcus* WH8103 cells only increased by 10% during the 8 d after the P addition, whereas the corresponding chlorophyll fluorescence increased twofold. This may indicate a delayed growth response of the P-starved *Synechococcus* WH8103 to externally added nutrients but nevertheless supports a general P limitation in these cultures.

Cell concentrations were analyzed on a modified FACScan (Becton Dickinson) flow cytometer. Cells were separated from externally added fluorescent microspheres (0.47 μm diameter, Fluoresbrite; Polysciences) in a log/log plot of side scatter and chlorophyll fluorescence using a long-pass emission filter (>650 nm). Microspheres were added to final concentrations of 8×10^4 or 3×10^5 beads ml^{-1}, depending on the expected density of the cultures, and cell counts were normalized to externally added beads to obtain cell concentrations. In addition, a regular FACScan flow cytometer (Becton Dickinson) set to a low flow rate (12 μl min^{-1}) was used to measure the forward-angle light scatter (FALS) of cells stained with the nucleic acid stain Syto 13 (del Giorgio et al. 1996). Externally added fluorescent microspheres (1.58 μm diameter, Fluoresbrite; Polysciences) were added as an internal reference.

Selected samples from the P-replete cultures were stained with 4′,6′-diamidino-2-phenylindole (DAPI; Porter and Feig 1980). Cells were delineated using the edge detection operator in Scion Image 4.0.2 software (Scion Corp., Frederick, Maryland). The same software was used to roughly estimate the length and width of 20–100 cells. The average diameter for *Prochlorococcus* MED4 was 0.73 μm, and *Synechococcus* WH8012 and WH8103 had average diameters of 0.87 and 1.17 μm, respectively.

Particulate C and N were analyzed on a CN analyzer (model NA1500 NC; Fisons Instruments) using acetanilide (71.09% C and 10.36% N) as the reference material. All samples were corrected for the background of C and N caused by the adsorption of dissolved compounds to the filters. This background was approximately equal for all treatments and organisms (for 75 ml filtered medium; 31 ± 6 μg C and 8 ± 1 μg N $filter^{-1}$, $n = 10$).

Particulate P was analyzed colorimetrically after an initial hydrolysis of the cellular material. To ensure that hydrolysis and subsequent detection of cellular P was complete, two independent methods were used: (1) flow injection analysis of phosphate using the stannous chloride method on a FIAstar autoanalyzer after an initial hydrolysis of the sample with potassium peroxysulfate and heating at 125°C for 30 min (standard method SIS 028127; Valderrama 1981) and (2) analysis of soluble reactive P using molybdenum blue spectrophotometry after sequential combustion of the samples at 475–500°C for 3 h and acid hydrolysis in 0.5 M HCl at 80–90°C for 1.5 h (Hebel and Karl 2001). For both methods, samples were background-corrected for adsorption of dissolved P to the combusted GF/F filters in the different treatments. The average background for P-limited cultures was 0.09 μg $filter^{-1}$ (<32% of sample signal), whereas the background for P-replete cultures was higher; 0.25 μg $filter^{-1}$ (<22% of sample signal; both values for filtration of 25 ml medium). The two methods were in excellent agreement (regression slope 1.03, $r^2 = 0.94$, $n = 24$). The molybdenum blue spectrophotometry method has been used extensively for analysis of total P in marine plankton (Goldman et al. 1979; Björkman 2000; Hebel and Karl 2001), and we therefore use these total P values consistently throughout the present article.

Results

As expected, cultures reached the stationary phase at much lower fluorescence in P-limited (N:P, 800) than in P-replete (N:P, 16) cultures (Fig. 2). The growth rates achieved during exponential growth were slightly lower in the low-P media for all three strains, but the differences were not statistically significant (*F*-tests, $P > 0.05$).

The C content of *Prochlorococcus* MED4 grown under P-replete (45.8 fg C $cell^{-1}$) and P-limited (60.9 fg C $cell^{-1}$) conditions was similar to previously published estimates obtained using both volumetric methods, dry weight estimates, and direct analysis of particulate carbon with corrections for contaminating heterotrophic bacteria (Table 1). For all three organisms, the C content was consistently lower in P-replete cultures than in the corresponding P-limited cultures harvested in the early stationary phase (Table 2).

Flow cytometrically derived FALS is related to cell volume (Robertson et al. 1998) and thus should be related to cellular C content. When we compared the average FALS for cells from our six treatments (three different cyanobacteria, two different growth conditions), we observed a significant linear correlation between FALS and the average carbon content of the cells (Fig. 3), which was independent of growth condition or strain.

The impact of growth conditions on cellular N content was more variable, with insignificant effects on the cellular N in *Prochlorococcus* MED 4 and *Synechococcus* WH8012, whereas the P-replete cells of the larger *Synechococcus* WH8103 had a higher N content than the P-limited stationary-phase cells (Table 2).

Table 1. Published estimates of cellular carbon content of *Prochlorococcus* sp.

Reference	fg C cell^{-1}	Analytical method
Cailliau et al. 1996	49 ± 9	POC, non-axenic cultures, corrected for presence of contaminating bacteria*
Campbell et al. 1994	53	Average cell size assumed (0.6 μm diameter), volume to carbon conversion (470 fg C μm^{-3})
Claustre et al. 2002	17–38	POC, axenic culture (high irradiance)
Durand et al. 2001	54	Cell size determination and volume to carbon conversion (325 fg C μm^{-3})†
Li et al. 1992	59	Average cell size assumed (0.8 μm diameter), volume to carbon conversion (220 fg C μm^{-3})
Moore 1997	61–94	Primary production estimates*
Shaw 2001	78	Dry weight determination and assumed carbon content of 50% in biomass*
Veldhuis and Kraay 1990	124	Average cell size assumed (0.6 μm diameter), volume to carbon conversion
This work	46–61	Particulate carbon, exponentially growing or P-limited axenic cultures*

* Analysis based on cultures of *Prochlorococcus* MED4.
† Flowcytometric analysis of volume.

In contrast, the cellular P content was consistently three- to fourfold lower in the P-limited cultures than in P-replete cultures (Table 2). These results indicate that the content of P-rich cell constituents such as RNA and DNA may be depleted to very low levels in response to decreased nutrient availability.

The molar ratios of cellular C : N were similar for all three organisms. Exponentially growing cells were slightly enriched in N relative to the Redfield ratio of 6.6 (Redfield 1958), whereas cells from P-limited cultures had a molar C : N slightly above this ratio (Fig. 4A). These C : N values are both close to reports on the average composition of marine particulate matter and the average composition of a wide range of nutrient-replete marine phytoplankton cultures (Table 3).

All three strains had a low relative content of cellular P. The molar ratios of both C : P and N : P in the nutrient-replete cultures were at the higher end of the spectrum or exceeded previously reported values for nutrient-replete marine phytoplankton (Table 3; Fig. 4B,C). P-limited cells were even more depleted in P, with a molar C : P ratio of 464–779 and a molar N : P ratio of 59–109, which suggests that P-starved cells can alter their relative content of P-rich constituents substantially (Fig. 4B,C).

Table 2. Cellular carbon, nitrogen, and phosphorus in axenic cultures of *Prochlorococcus marinus* MED4 and *Synechococcus* WH8012 and WH8103 in P-replete or P-limited conditions. Cell concentrations are given for each culture at the time of cell capture for elemental analysis. Arithmetic means of triplicate samples are presented with standard deviation between brackets. Significance levels (unpaired *t*-test) are given for differences between P-replete and P-limited growth conditions for each strain and element (degrees of freedom, 4).

Organism condition	Cell concentration (10^6 cells ml^{-1})	Carbon*	Nitrogen*	Phosphorus*
Prochlorococcus MED4				
P-replete	110 (5)	45.8 (4.0)	9.4 (0.9)	0.98 (0.19)
P-limited	56 (4)	60.9 (1.8)	9.6 (0.07)	0.34 (0.08)
Student's *t*	—	$p < 0.01$	NS†	$p < 0.01$
Synechococcus WH8012				
P-replete	54 (11)	92.4 (13.3)	20.0 (2.7)	1.84 (0.13)
P-limited	49 (10)	132 (6.2)	20.6 (2.0)	0.47 (0.17)
Student's *t*	—	$p < 0.01$	NS	$p < 0.01$
Synechococcus WH8103				
P-replete	11 (0.6)	213 (7.3)	50.2 (1.8)	3.34 (0.51)
P-limited	31 (4)	244 (20.7)	39.8 (3.8)	0.81 (0.01)
Student's *t*	—	$p < 0.1$	$p < 0.05$	$p < 0.01$

* In fg cell^{-1}.
† Not significant ($p > 0.1$).

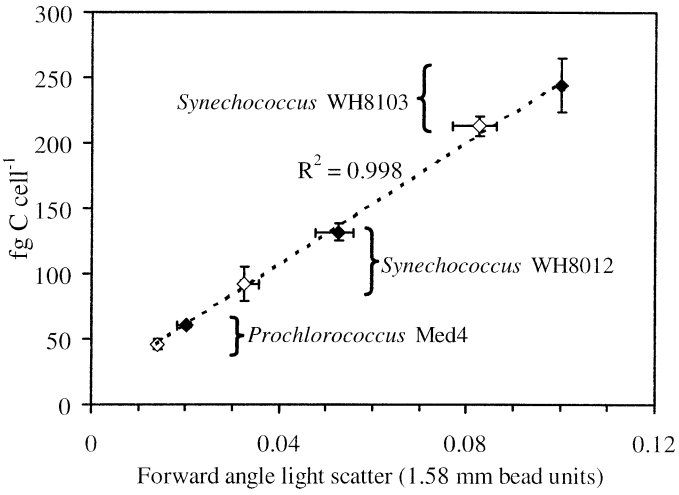


Fig. 3. The correlation between forward-angle light scatter (FALS) and analyzed cellular carbon content for cultures of *Prochlorococcus* MED4, *Synechococcus* WH8012, and *Synechococcus* WH8103. Black symbols represent P-limited cultures, and white symbols represent exponentially growing cells in P-replete medium. Each data point represents the average cellular C content for triplicates of a single treatment, with error bars denoting standard deviations. FALS is reported as the median of the average cyanobacterial FALS intensity of Syto 13–stained cells normalized to 1.58 μm fluorescent microspheres in triplicate cultures. Error bars represent the maximum–minimum range (n = 3), and the line represents a linear regression of all data points.

Discussion

Nutrient stoichiometry—The most striking feature of the C : N : P ratios of these cyanobacterial strains is the low cellular P content relative to C and N. In a recent survey of the C : N : P variability in marine microalgae, Geider and La Roche (2002) reported molar C : P ratios of 27–135 and molar N : P ratios of 7–19 for various nutrient-replete phytoplankton cultures. Their survey was based on data from eukaryotic phytoplankton, but no data were available for marine cyanobacteria, and some of the lower C : P and N : P ratios are likely due to the accumulation of polyphosphate or other P-rich compounds. The corresponding elemental ratios for nutrient-replete *Prochlorococcus* and *Synechococcus* reported here range 121–165 for C : P and 21–33 for N : P (Fig. 4, Table 3). Hence, our results indicate that marine *Prochlorococcus* and *Synechococcus*, which often dominate phytoplankton communities in oligotrophic waters, have very low P requirements. The low cellular P is in agreement with a previous study of C : N : P in *Synechococcus* WH7803, in which the molar ratio of C : P was 115 in exponentially growing cells (Table 3; Cuhel and Waterbury 1984). A recently completed study, conducted using X-ray microanalysis, also supported the result of low cellular P requirements in *Prochlorococcus* and *Synechococcus* (Table 3; Heldal et al. 2003).

The average C : P and N : P ratios of marine particulate matter is typically lower than our estimates for marine cyanobacteria (Table 3). An extensive survey of >500 pairwise analyses of these elements in particulate matter from diverse

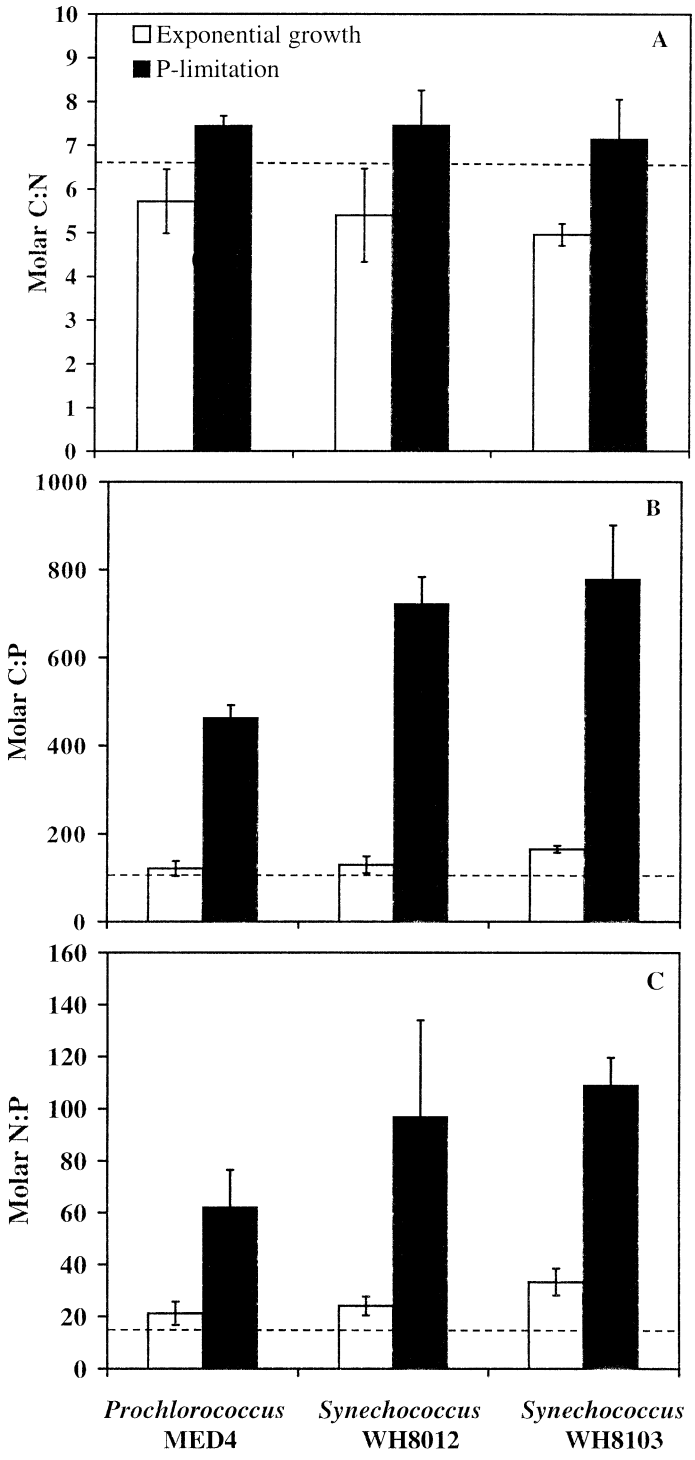


Fig. 4. Molar ratios of (A) C : N, (B) C : P, and (C) N : P in three cultured marine cyanobacteria. Bars represent average elemental ratios of triplicate cultures with cells growing either exponentially in P-replete medium (white bars) or cells experiencing severe P limitation (black bars). Error bars represent the combined standard deviation, calculated to account for the propagation of errors. The dashed line represents the Redfield ratio (Redfield 1958).

Table 3. Elemental ratios (molar C : N, C : P, and N : P) for marine phytoplankton cultures, bacteria, and particulate organic matter. Arithmetic means are presented, with standard deviation between brackets.

Sample	C : N	C : P	N : P	Reference
Prochlorococcus Med4				
P-replete	5.7 (0.7)	121 (17)	21.2 (4.5)	This study
P-limited	7.4 (0.2)	464 (28)	62.3 (14.1)	
Synechococcus WH8012				
P-replete	5.4 (1.1)	130 (19)	24.0 (3.6)	This study
P-limited	7.5 (0.8)	723 (61)	96.9 (37)	
Synechococcus WH8103				
P-replete	5.0 (0.2)	165 (8)	33.2 (5.2)	This study
P-limited	7.1 (0.9)	779 (122)	109 (10.5)	
Exponential* *Prochlorococcus* (6 strains)	8.5–9.9	156–215	15.9–24.4	Heldal et al. 2003
Exponential* *Synechococcus*				
WH8103	10	150	15	Heldal et al. 2003
WH7803	8.9	113	13.3	
Exponential *Synechococcus* WH7803	7	115	16.4	Cuhel and Waterbury 1984
Marine phytoplankton cultures†				
Average	7.7 (2.6)	75 (31)	10.1 (3.9)	Geider and La Roche 2002
Range	4–17	27–135	5–19	
Marine particulate matter‡				
Average	7.3 (1.7)	114 (45)	16.4 (6.2)	Geider and La Roche 2002
Range	3.8–12.5	35–221	5–34	
Marine bacterial cultures				
Exponential	3.8–6.3	26–50	5.5–7.9	Vrede et al. 2002
P-limited	7.7–12	176–180	16–19	

* Cells cultured in PCR-S11 medium (Partensky et al. 1999), sampled in exponential phase.
† Meta-analysis of nutrient-replete eukaryotic marine phytoplankton cultures.
‡ Meta-analysis of marine particulate matter.

oceanic environments (Indian Ocean, Atlantic Ocean, Southern Ocean, and Mediterranean Sea) demonstrated an average C : P ratio of 103 and an average N : P ratio of 16.4, even though there was a considerable variability between the different sites (Copin-Montegut and Copin-Montegut 1983). Hence, it appears that either marine picocyanobacteria are not well represented in the analyzed particulate fractions or that other organisms or particles rich in P counterbalance the signal from the low-P cyanobacteria. Both of these explanations are possible, but neither seems likely for the vast low-nutrient open-ocean gyres that dominate our planet.

First, particulate matter is an operational definition, and most analyses of particulate C : N : P ratios have used filters with nominal pore sizes of 0.7–1.2 μm, which may be more or less efficient in capturing the smaller unicellular microorganisms such as *Prochlorococcus* and *Synechococcus.* In agreement with earlier studies (Chavez et al. 1995), our results show that gentle filtration through a glass-fiber filter with a nominal pore size of 0.7 μm efficiently captures even small *Prochlorococcus* cells (diameter, 0.7 μm).

Second, next to marine picocyanobacteria, heterotrophic prokaryotes represent a dominant biomass component of marine food webs (Campbell et al. 1994; Gasol et al. 1997). Studies of indigenous freshwater bacterioplankton and cultured aquatic bacteria have suggested that these organisms may be enriched in phosphorus (Table 3; Fagerbakke et al. 1996; Vrede et al. 2002), which suggests that they would be able to counterbalance the high C : P and N : P ratios in cyanobacteria. This hypothesis is supported by the often tight spatial and temporal coupling between these two groups of organisms in oceanic systems (Li et al. 1992); for example, cyanobacteria and other primary producers supply the energy and nutrients required for heterotrophic growth of bacteria, ''the microbial loop'' (Azam et al. 1983). However, a recent study of indigenous aquatic bacteria collected in the Sargasso Sea (Gundersen et al. 2002) suggested that the cellular ratios of C : N : P in marine bacteria are close to the Redfield ratio of 106 : 16 : 1, and, if these ratios are representative for bacterioplankton in a wider range of open-ocean systems, the balancing effect would be minimal.

The cellular C : N in the nutrient-replete cultures of *Prochlorococcus* and *Synechococcus* varied between 5 and 5.7 (Table 3). These ratios are within the observed range of C : N for nutrient-replete cultures of marine eukaryotic phytoplankton (Table 3) and are also consistent with reported values of C : N in two different marine *Synechococcus* strains (range, 4.4–8.7; Biddanda and Benner 1997; Lourenço et al. 1998). The largest proportion of cellular N in phytoplankton

is bound in protein and free amino acids (>70%), and a detailed study of internal N pools in a strain of marine *Synechococcus* showed that proteinaceous material contributes between 74% and 84% of total cellular N, whereas the cellular N contained in other organic biomolecules such as pigments and nucleic acids is minor (<3%) (Lourenço et al. 1998).

The C : N of *Prochlorococcus* and *Synechococcus* harvested in the early stationary phase in P-limited cultures was also close to the Redfield ratio (Table 3, Fig. 4A). The slightly higher C : N ratio in the stationary growth phase agrees with earlier observations of *Synechococcus* batch cultures (Biddanda and Benner 1997; Lourenço et al. 1998) and may suggest an intracellular depletion of amino acids, proteins, and/or phycobiliproteins as cells enter the stationary phase. An alternative explanation could be an intracellular accumulation of C-rich biomolecules that do not contain N (e.g., lipids or carbohydrates). The latter explanation is supported by the nearly constant fraction of cellular N associated with proteinaceous material in the exponential and stationary growth phase of seven cultured phytoplankton, including a marine *Synechococcus* strain (Lourenço et al. 1998) and the higher C content of the P-limited cells in our study (Table 2). Still, the cellular N content in both *Prochlorococcus* and *Synechococcus* appears to be relatively stable, even under contrasting nutrient conditions, and this agrees with observations of the C : N ratios of marine particulate matter, which are much more constrained than elemental ratios involving phosphorus (Geider and La Roche 2002). Still, earlier studies of eukaryotic phytoplankton have demonstrated that N starvation may result in a threefold higher cellular C : N ratio than nutrient-replete conditions (Goldman et al. 1979; Geider and La Roche 2002), and we may expect a more dramatic change in cellular C : N ratios in response to N-limited conditions.

Nutrient limitation and cellular phosphorus—As one would expect from many early studies of P limitation in eukaryotic phytoplankton (e.g., Goldman et al. 1979; Tett et al. 1985), cells harvested from P-limited cultures had high cellular C : P and N : P ratios (Table 3) and a three- to fourfold decrease in the absolute amount of P per cell compared with P-replete cultures (Table 2). This reflects the ability of phytoplankton to dramatically lower their cellular content of P when this resource is scarce in the surrounding medium. This could be accomplished by lowering the cellular content of P-rich cell components such as nucleic acids (e.g., RNA and DNA).

A closer look at the biochemical composition of the P-starved cultures of *Prochlorococcus* MED4 and *Synechococcus* WH8012 indicates that the cellular P content is close to the minimum level possible in these cultures. A single copy of the 1.66-Mb genome of *Prochlorococcus* MED4 (http://www.jgi.doe.gov) contains 0.18 fg P (assuming 1 P per nucleotide in the double-stranded genomic DNA). Hence, for cells containing a single copy of the genome, >50% of the total measured cellular phosphorus would be bound in DNA (Table 2). The exact genome size of *Synechococcus* WH8012 is not known, but that of its close relative *Synechococcus* WH8102 is 2.4 Mb (http://www.jgi.doe.gov) and would contain 0.26 fg phosphorus if we make the same assumptions. Because the average P-starved *Synechococcus* WH8012 cell contains 0.47 fg P cell^{-1}, genomic DNA is probably the dominant pool of cellular P in these cells as well, and this is in agreement with a previous study that demonstrated RNA/DNA ratios close to 1 for a marine *Synechococcus* strain (Lourenço et al. 1998).

Cellular carbon and cell volume—Previous surveys have indicated that *Prochlorococcus* is not only abundant in the tropical and subtropical oceans but also constitute a major biomass component in these habitats (Li et al. 1992; Campbell et al. 1994; Karl and Dobbs 1998; Du Rand et al. 2001). The biomass estimates have mostly been based on *Prochlorococcus* cell volumes and subsequent volume to C transformations (Veldhuis and Kraay 1990; Li et al. 1992; Campbell et al. 1994). This approach has great uncertainty, because it is known that the level of cellular C per cell volume for marine bacteria varies between 33 and 241 fg C μm^{-3} among different microbial isolates and within single populations growing under different environmental conditions (Vrede et al. 2002). Studies of naturally occurring bacterial cells collected from oceanic surface waters have suggested an even larger variability in cellular C content in situ (24–537 fg C μm^{-3}, Gundersen et al. 2002). The uncertainties are also apparent for *Prochlorococcus,* in which the estimated C content of cells with the same average diameter (0.6 μm) ranges from 53 fg C cell^{-1} (Campbell et al. 1994) to 124 fg C cell^{-1} (Veldhuis and Kraay 1990).

In a later study, Caillau et al. (1996) directly analyzed the particulate organic C of cells in a set of nonaxenic cultures of a single *Prochlorococcus* strain using a regression-based approach to correct for the C contributed from contaminating heterotrophic bacteria. The obtained estimate of 49 fg C cell^{-1} is likely more robust, but the analysis relies on certain critical assumptions—for example, that the cellular C content is independent of light conditions and growth rate. Despite these large uncertainties in the various extrapolation factors used, our chemically measured C content in *Prochlorococcus* is similar to many of the earlier estimates (Table 1). The volume of the exponentially growing *Prochlorococcus* MED4, roughly determined from epifluorescence microscopy of DAPI-stained cells, was ~0.20 μm^3, indicating a volume to C transformation factor ~226 fg C μm^{-3}. The average cell volume estimated for the P-replete *Synechococcus* WH8012 and WH8103 was 0.38 and 0.91 μm^3, with corresponding volume to C conversion factors of 260 and 270 fg C μm^{-3}. Hence, in the present study, cell volume could be used to predict the C content of the nutrient replete unicellular picocyanobacteria, even though there were some differences between the three strains. We also observed a linear correlation between FALS and cellular C content (Fig. 3). This strong linear correlation is not surprising per se, because FALS is positively related to both cell diameter and the refractive index of the analyzed particle—two factors that are also likely to covary with cellular C content. A similar linear relationship between FALS and cellular C content has been observed in a previous study of eight different *Prochlorococcus* isolates in which C content was estimated from photosynthetic rate measurements (Moore 1997). Here

we extend this observation with direct measurements of carbon per cell for both nutrient-replete and P-limited cultures. The robust relationship between FALS and C per cell suggests that FALS could be used, with the proper calibrations, to directly compare the relative biomass of *Prochlorococcus* and *Synechococcus* in oceanic surface waters and also to estimate their absolute biomass.

Heldal et al. (2003) used transmission electron microscopy and X-ray microanalysis to determine the size and cellular carbon content of six *Prochlorococcus* isolates harvested during the late exponential phase. They report an average of 30 fg C $cell^{-1}$, which is somewhat lower than our results but close to the values used by Björkman et al. (2000) in their investigation of the central North Pacific gyre. This lower cellular C content could at least partly be explained by the smaller size of some cells analyzed in the Heldal et al. (2003) study. Nevertheless, the estimated volume to C transformation factors in their study ranged 140–280 fg C μm^{-3}, compared with our result of 226 fg C μm^{-3}, which indicates considerable variability in volumetric C content between strains.

Cells in the P-limited cultures always had a higher C content than exponentially growing cells (Table 2), and this higher C content appears to be coupled to an increase in size (Fig. 3). One putative explanation for this observation could be an inability of the P-limited cells to divide due to a deficiency in the P needed for chromosome replication with a resulting cell cycle arrest or an intracellular accumulation of energy-rich organic compounds low in N and P (e.g., lipids) when nutrients are scarce.

Potential ecological implications—There are several important ecological considerations that derive from these new data on the bioelemental compositions of *Synechococcus* and *Prochlorococcus* growing under different nutrient conditions. First and foremost are the extremely low P cell quota requirements for both species, especially *Prochlorococcus.* The P requirements are low both in absolute terms and with respect to the C and N cell quotas, yielding new biomass with C : P and N : P ratios that are well in excess of those anticipated for other microorganisms, especially under P-limited conditions. Although we have not made an effort to determine the subcellular distribution of P, calculations indicate that nearly one half of the total cell P is contained in genomic DNA; this is a minimum estimate based on the assumption that there is only a single complement of DNA. If there were no other P demands for phospholipids or polysaccharides, this would limit the RNA content to—at most—a pool equivalent to DNA or a RNA : DNA ratio of 1. In most prokaryotic microorganisms, the RNA : DNA ratio is $\gg 1$, even for slow-growing cells, and the ratio generally increases with increasing specific growth rate (Maaløe and Kjeldgaard 1966). Slow growth coupled to low P-requirements would be an advantage under conditions of chronic nutrient limitation, and this would preadapt *Prochlorococcus* for survival in the hyperoligotrophic midocean gyres where they appear to dominate. Such an adaptation could explain the inverse relationship between soluble reactive P and the abundance of *Prochlorococcus* that has been observed in the North Atlantic Ocean, paralleled by a positive correlation between this indicator of bioavailable P and bacterial abundance (Cavender-Bares et al. 2001).

A low P cell quota may have other, more cryptic, ecological advantages that could also promote survival in selected habitats. For example, P limitation and low relative P cellular stoichiometry may influence the top-down control of microbial populations by altering the feeding rates and efficiencies of microbial grazers such as protozoans. Elemental imbalances between producers and grazers can affect both the consumer population and the ecosystem as a whole (Elser and Hassett 1994). It is well known that many grazers of small particles selectively remove or retain particles of greatest nutritive benefit. By virtue of growing with a low P content relative to C and N, *Prochlorococcus* cells with a low P content may be a less desirable particulate matter food resource, and this may result in selection against removal by particle consumers, provided that there is a better source of food available to them. In nature it is likely that other environmental variables, such as light quality and quantity, might also affect the ecological stoichiometry of marine seston (Urabe et al. 2002); in our laboratory studies, we applied a constant light flux of 30–40 μmol quanta m^{-2} s^{-1}, so this remains to be tested.

In addition, both the frequency and pattern of viral infection may be influenced by a low intracellular P content. In previous P-limited laboratory studies of *Synechococcus,* cyanophages established a nonlethal lysogenic association with host cells (Wilson et al. 1996) compared with P-replete cultures. This results from the high demand for viral P during the lytic cycle. By maintaining a low cell quota of P, even when external P supplies are nonlimiting, *Prochlorococcus* would retain an intracellular P limitation, which could extend lysogeny and protect the species from lytic infections. The tradeoff of slow growth potential for long-term survival may be a key ecophysiological strategy that is common in open-ocean species such as *Prochlorococcus.*

Finally, the very high C : P and N : P ratios that we observed for the P-limited cultures of *Synechococcus* and *Prochlorococcus* in the present study (C : P $>$ 400 and N : P $>$ 60) are unlike any particulate matter ratios reported for the open sea. This suggests that there are other living and nonliving components in marine ecosystems that are dominated by these species that might effectively shift the stoichiometric balance toward a Redfield ratio reconciliation or that the marine ecosystems studied to date have not yet been forced to the physiological limits (e.g., P limitation) that we observed in these laboratory experiments; without any direct proof, we suspect the latter. In most oceanic habitats, severe P limitation never sets in because there is consecutive nutrient colimitation by N or a required trace element such as iron. What our data imply is that *Prochlorococcus* and *Synechococcus* can, under certain growth conditions, produce new biomass with a bioelemental stoichiometry well in excess of the canonical Redfield ratio. We could envision a warm, dust (iron)–rich future ocean that was trace element sufficient and N_2 fixation conducive. Under these hypothetical conditions, species like *Prochlorococcus* and *Synechococcus* would thrive, dominate, and ultimately establish a new P-depleted, relative to C and N, stoichiometric balance that would have numerous and far-reaching ecological con-

sequences from changes in the structure of the food web to enhanced carbon sequestration into the mesopelagic and abyssal zones as high C : P ratio particulate organic matter is removed by the ocean's biological carbon pump.

References

AZAM, F., T. FENCHEL, J. G. FIELD, J. S. GRAY, L. A. MEYER-REIL, AND F. THINGSTAD. 1983. The ecological role of water-column microbes in the sea. Mar. Ecol. Prog. Ser. **10:** 257–263.

BIDDANDA, B., AND R. BENNER. 1997. Carbon, nitrogen, and carbohydrate fluxes during the production of particulate and dissolved organic matter by marine phytoplankton. Limnol. Oceanogr. **42:** 506–518.

BJÖRKMAN, K., A. L. THOMSON-BULLDIS, AND D. M. KARL. 2000. Phosphorus dynamics in the North Pacific subtropical gyre. Aquat. Microb. Ecol. **22:** 185–198.

CAILLIAU, C., H. CLAUSTRE, F. VIDUSSI, D. MARIE, AND D. VAULOT. 1996. Carbon biomass and gross growth rates as estimated from ^{14}C pigment labelling, during photoacclimation in *Prochlorococcus* CCMP 1378. Mar. Ecol. Prog. Ser. **145:** 209–221.

CAMPBELL, L., H. A. NOLLA, AND D. VAULOT. 1994. The importance of *Prochlorococcus* to community structure in the central North Pacific Ocean. Limnol. Oceanogr. **39:** 954–961.

CAVENDER-BARES, K. K., D. M. KARL, AND S. W. CHISHOLM. 2001. Nutrient gradients in the western North Atlantic Ocean: Relationship to microbial community structure and comparison to patterns in the Pacific Ocean. Deep-Sea Res. I **48:** 2373–2395.

CHAVEZ, F. P., AND OTHERS. 1995. On the chlorophyll a retention properties of glass-fiber GF/F filters. Limnol. Oceanogr. **40:** 428–433.

CHISHOLM, S. W., R. J. OLSON, E. R. ZETTLER, R. GOERICKE, J. WATERBURY, AND N. WELSCHMEYER. 1988. A novel free-living prochlorophyte abundant in the oceanic euphotic zone. Nature **334:** 340–343.

CLAUSTRE, H., AND OTHERS. 2002. Diel variations in *Prochlorococcus* optical properties. Limnol. Oceanogr. **47:** 1637–1647.

CODISPOTI, L. A. 1989. Phosphorus vs. nitrogen limitation of new and export production, p. 377–394. *In* W. Berger, V. Smetacek, and G. Wefer [eds.], Productivity of the ocean: Present and past, Dahlem workshop report 44. Wiley.

COPIN-MONTEGUT, C., AND G. COPIN-MONTEGUT. 1983. Stoichiometry of carbon, nitrogen and phosphorus in marine particulate matter. Deep-Sea Res. **30:** 31–46.

CUHEL, R. L., AND J. B. WATERBURY. 1984. Biochemical composition and short term nutrient incorporation patterns in a unicellular marine cyanobacterium *Synechococcus* (WH7803). Limnol. Oceanogr. **29:** 370–374.

DEL GIORGIO, P. A., D. B. BIRD, Y. T. PRAIRIE, AND D. PLANAS. 1996. Flow cytometric determination of bacterial abundance in lake plankton with the green nucleic acid stain Syto 13. Limnol. Oceanogr. **41:** 783–789.

DURAND, M. D., R. J. OLSON, AND S. W. CHISHOLM. 2001. Phytoplankton population dynamics at the Bermuda Atlantic Time-series station in the Sargasso Sea. Deep-Sea Res. II **48:** 1983–2003.

ELSER, J. J., AND R. P. HASSETT. 1994. A stoichiometric analysis of the zooplankton-phytoplankton interactions in marine and freshwater ecosystems. Nature **370:** 211–213.

FAGERBAKKE, K. M., M. HELDAL, AND S. NORLAND. 1996. Content of carbon, nitrogen, oxygen, sulphur and phosphorus in native aquatic and cultured bacteria. Aquat. Microb. Ecol. **10:** 15–27.

FALKOWSKI, P. G. 1997. Evolution of the nitrogen cycle and its influences on the biological sequestration of CO_2 in the ocean. Nature **387:** 272–275.

———. 2000. Rationalizing elemental ratios in unicellular algae. J. Phycol. **36:** 3–6.

———, R. BARBER, AND V. SMETACEK. 1998. Biogeochemical controls and feedbacks on ocean primary production. Science **281:** 200–206.

FIELD, C. B., M. J. BEHRENFELD, J. T. RANDERSON, AND P. G. FALKOWSKI. 1998. Primary production of the biosphere: Integrating terrestrial and oceanic components. Science **281:** 237–240.

GASOL, J. M., P. A. DEL GIORGIO, AND C. M. DUARTE. 1997. Biomass distribution in marine planktonic communities. Limnol. Oceanogr. **42:** 1353–1363.

GEIDER, R. J., AND J. LA ROCHE. 2002. Redfield revisited: Variability of C : N : P in marine microalgae and its biochemical basis. Eur. J. Phycol. **37:** 1–17.

GOLDMAN, J. C., J. J. MCCARTHY, AND D. G. PEAVEY. 1979. Growth rate influence on the chemical composition of phytoplankton in oceanic waters. Nature **279:** 210–215.

GUNDERSEN, K., M. HELDAL, S. NORLAND, D. A. PURDIE, AND A. H. KNAP. 2002. Elemental C, N, and P cell content of individual bacteria collected at the Bermuda Altantic Time-series Study (BATS) site. Limnol. Oceanogr. **47:** 1525–1530.

HEBEL, D. V., AND D. M. KARL. 2001, Seasonal, interannual and decadal variations in particulate matter concentrations and composition in the subtropical North Pacific Ocean. Deep-Sea Res. II **48:** 1669–1695.

HELDAL, M., D. J. SCANLAN, S. NORLAND, F. THINGSTAD, AND N. H. MANN. 2003. Elemental composition of single cells of various strains of marine *Prochlorococcus* and *Synechococcus* using X-ray microanalysis. Limnol. Oceanogr. **48:** 1732–1743.

HERBERT, D. 1961. The chemical composition of micro-organisms as a function of their environment. Symp. Soc. Gen. Microbiol. **11:** 391–416.

HERDMAN, M., R. W. CASTENHOLZ, J. B. WATERBURY, AND R. RIPPKA. 2001. Form-genus XIII. *Synechococcus*, p. 508–512. *In* D. R. Boone, R. W. Castenholz, and G. M. Garrity [eds.], Bergey's manual of systematic bacteriology. V. 1, 2nd ed. Springer.

KARL, D. M., AND F. C. DOBBS. 1998. Molecular approaches to microbial biomass estimation in the sea, p. 29–84. *In* K. Cooksey [ed.], Molecular approaches to the study of the ocean. Chapman & Hall.

LI, W. K. W., P. M. DICKIE, B. D. IRWIN, AND A. M. WOOD. 1992. Biomass of bacteria, cyanobacteria, prochlorophytes and photosynthetic eukaryotes in the Sargasso Sea. Deep-Sea Res. **39:** 501–519.

LOURENÇO, S. O., E. BARBARINO, U. M. LANFER MERQUEZ, AND E. AIDAR. 1998. Distribution of intracellular nitrogen in marine microalgae: Basis for the calculation of specific nitrogen-to-protein conversion factors. J. Phycol. **34:** 798–811.

MAALØE, O., AND N. O. KJELDGAARD. 1966. Control of macromolecular synthesis. W. W. Benjamin.

MICHAELS, A. F., D. M. KARL, AND D. G. CAPONE. 2001. Elemental stoichiometry, new production and nitrogen fixation. Oceanography **14:** 68–77.

MOORE, L. R. 1997. Physiological ecology of *Prochlorococcus:* A comparison of isolates from diverse oceanic regimes. Ph.D. thesis, Dept. of Civil and Environmental Engineering, Massachusetts Institute of Technology.

———, A. F. POST, G. ROCAP, AND S. W. CHISHOLM. 2002. Utilization of different nitrogen sources by the marine cyanobacteria, *Prochlorococcus* and *Synechococcus.* Limnol. Oceanogr. **47:** 989–996.

PARTENSKY, F., W. R. HESS, AND D. VAULOT. 1999. *Prochlorococcus*, a marine photosynthetic prokaryote of global significance. Microbiol. Mol. Biol. Rev. **63:** 106–127.

PORTER, K. G., AND Y. S. FEIG. 1980. The use of DAPI for identifying and counting aquatic microflora. Limnol. Oceanogr. **25:** 943–948.

REDFIELD, A. C. 1934. On the proportions of organic derivatives in sea water and their relation to the composition of plankton, p. 176–192. *In* R. J. Daniel [ed.], James Johnstone memorial volume. Univ. Press of Liverpool.

———. 1958. The biological control of chemical factors in the environment. Am. Sci. **46:** 205–221.

RHEE, G.-Y. 1973. A continuous culture study of phosphate uptake, growth rate and polyphosphate in *Scenedesmus* sp. J. Phycol. **9:** 495–506.

ROBERTSON, B. R., D. K. BUTTON, AND A. L. KOCH. 1998. Determination of the biomass of small bacteria at low concentrations in a mixture of species with forward light scatter measurement by flow cytometry. Appl. Environ. Microbiol. **64:** 3900–3909.

ROCAP, G., D. L. DISTEL, J. B. WATERBURY, AND S. W. CHISHOLM. 2002. Resolution of *Prochlorococcus* and *Synechococcus* ecotypes by using 16S–23S ribosomal DNA internal transcribed spacer sequences. Appl. Environ. Microbiol. **68:** 1180–1191.

SAITO, M. 2000. The biogeochemistry of cobalt in the Sargasso Sea. Ph.D. thesis, Massachusetts Institute of Technology/Woods Hole Oceanographic Institute.

SHAPIRO, L. P., AND E. M. HAUGEN. 1988. Seasonal distribution and temperature tolerance of *Synechococcus* in Boothbay Harbor, Maine. Estuar. Coast. Shelf Sci. **26:** 517–525.

SHAW, S. L. 2001. The production of non-methane hydrocarbons by marine plankton. Ph.D. thesis, Massachusetts Institute of Technology.

TETT, P., S. I. HEANEY, AND M. R. DROOP. 1985. The Redfield ratio and phytoplankton growth rate. J. Mar. Biol. U.K. **65:** 487–504.

TYRRELL, T. 1999. The relative influences of nitrogen and phosphorus on oceanic primary production. Nature **400:** 525–531.

URABE, J., M. KYLE, W. MAKINO, T. YOSHIDA, T. ANDERSEN, AND J. J. ELSER. 2002. Reduced light increase herbivore production due to stoichiometry effects of light/nutrient balance. Ecology **83:** 619–627.

VALDERRAMA, J. C. 1981. The simultaneous analysis of total nitrogen and total phosphorus in natural waters. Mar. Chem. **10:** 109–122.

VELDHUIS, M. J. W., AND G. W. KRAAY. 1990. Vertical distribution and pigment composition of a picoplanktonic prochlorophyte in the subtropical North Atlantic: A combination study of HPLC-analysis of pigments and flow cytometry. Mar. Ecol. Prog. Ser. **68:** 121–127.

VREDE, K., M. HELDAL, S. NORLAND, AND G. BRATBAK. 2002. Elemental composition (C, N, P) and cell volume of exponentially growing and nutrient-limited bacterioplankton. Appl. Environ. Microbiol. **68:** 2965–2971.

WATERBURY, J. B., S. W. WATSON, F. W. VALOIS, AND D. G. FRANKS. 1986. Biological and ecological characterization of the marine unicellular cyanobacterium *Synechococcus.* Can. Bull. Fish. Aquat. Sci. **214:** 71–120.

WILSON, W. H., N. G. CARR, AND N. H. MANN. 1996. The effect of phosphate status on the kinetics of cyanophage infection in the oceanic bacterium *Synechococcus* sp. WH7803. J. Phycol. **32:** 506–516.

Received: 30 September 2002
Accepted: 24 March 2003
Amended: 3 April 2003