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Comparative physiology of *Synechococcus* and *Prochlorococcus*: influence of light and temperature on growth, pigments, fluorescence and absorptive properties

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ABSTRACT: *Prochlorococcus marinus* is abundant and widespread throughout the world's oceans and always co-occurs geographically with the marine cyanobacterium *Synechococcus*. In the Atlantic Ocean, these 2 picoplankters exhibit different spatial and seasonal distributions. In order to better understand the ecology of these species, we measured growth and photoacclimation responses including fluorescence excitation [$F^*_{ph}(\lambda)$] and *in vivo* absorption [$a^*_{ph}(\lambda)$] spectra over a range of growth irradiances for *P. marinus* (clone SS120) and *Synechococcus* WH8103, both isolated from the Sargasso Sea. To explore the physiological diversity of *P. marinus*, we measured the physiological responses of another *P. marinus* clone, MED4, isolated from the Mediterranean Sea. Growth rate as a function of temperature was also examined for all 3 clones. *P. marinus* SS120 and *Synechococcus* WH8103 have different temperature optima for growth, but these do not explain the different latitudinal distributions in the North Atlantic. *P. marinus* SS120 is adapted for growth at low light intensities relative to *Synechococcus* WH8103, which is consistent with the relative depth distribution of *P. marinus* and *Synechococcus* in the field. The light-dependent growth response of *P. marinus* MED4 is more similar to *Synechococcus* WH8103 than to *P. marinus* SS120. The unique pigment content of *P. marinus* (which contain divinyl chlorophylls *a* and *b*) results in maximal absorbance in the blue wavelengths. The high total chl *b*/chl *a* ratio of *P. marinus* SS120 enables it to absorb more light, grow faster than *Synechococcus* WH8103 (and *P. marinus* MED4) at low light intensities, and presumably to outcompete *Synechococcus* in the deep euphotic zone. At high growth irradiances, *P. marinus* SS120 contains measureable amounts of normal (monovinyl) chl *b*, whereas this pigment was not found in *P. marinus* MED4 at any growth irradiance. Photoacclimative changes in pigment ratios, and not package effect, account for most of the changes in $a^*_{ph}(\lambda)$ and $F^*_{ph}(\lambda)$ with light intensity for all 3 picoplankters. At high light intensities, zeaxanthin contributes substantially to $a^*_{ph}(\lambda)$ in the blue, but appears to transfer little or no excitation energy to the reaction centers, based on $F^*_{ph}(\lambda)$ measurements. For *P. marinus*, high absorption in the blue due to divinyl chl *a* and *b* relative to normal chl *a* and *b*, absorption due to zeaxanthin, and small cell size result in unusually high a^*_{ph} (blue) relative to a^*_{ph} (red).

KEY WORDS: *Prochlorococcus marinus* · *Synechococcus* · Light · Absorption · Pigments · Divinyl chlorophyll *a*

INTRODUCTION

Prochlorococcus marinus (Chisholm et al. 1992) is ubiquitous throughout the euphotic zone in tropical and subtropical oceans and contributes substantially to photosynthetic biomass and primary production (Chisholm et al. 1988, Campbell & Vaulot 1993, Goericke & Welschmeyer 1993). It has a unique suite of pigments, which includes divinyl chlorophyll *a* (chl a_2) as the principal light-harvesting pigment, and divinyl chl *b* (chl b_2), zeaxanthin, α-carotene and a chl *c*-like pigment as the main accessory pigments (Goericke &

Repeta 1992). Based on flow cytometric signatures and/or the presence of chl a_2, *P. marinus* has been found in the Atlantic Ocean (Neveux et al. 1989, Olson et al. 1990, Veldhuis & Kraay 1990), the tropical and subtropical Pacific (Chavez et al. 1991, DiTullio et al. 1992, Campbell & Vaulot 1993), the Mediterranean Sea (Vaulot et al. 1990, Vaulot & Partensky 1992), and the Red Sea (Veldhuis & Kraay 1993). *Synechococcus* has always been found in regions where *P. marinus* is present, although *P. marinus* often extends to lower depths (Olson et al. 1990, Campbell & Vaulot 1993, Veldhuis & Kraay 1993). *P. marinus* is usually found in abundances reaching 10^4 to 10^5 cells ml^{-1} (Olson et al. 1990, Vaulot et al. 1990, Chavez et al. 1991, Campbell & Vaulot 1993, Veldhuis & Kraay 1993) and can contribute up to 65% of total chl *a*, i.e. the sum of chl a_1 and chl a_2 (Veldhuis & Kraay 1990, Goericke & Welschmeyer 1993). Goericke & Welschmeyer (1993) measured the growth rate of *P. marinus* by measuring the incorporation of ^{14}C into chl a_2. Surface layer growth rates of *P. marinus* ranged from 0.1 to 0.5 d^{-1} and did not vary systematically over the seasons; growth rates at the subsurface chlorophyll maximum (SCM) ranged from 0.04 to 0.16 d^{-1}. These authors also calculated that the seasonally averaged contribution of *P. marinus* to the total primary productivity in the Sargasso Sea was 25%.

Prochlorococcus marinus has been observed in the North Atlantic only when surface water temperatures were above 15°C, suggesting that low temperature can influence the *P. marinus* distribution (Olson et al. 1990, Veldhuis et al. 1993). In the North Atlantic, *P. marinus* blooms after *Synechococcus* does (after the onset of spring stratification) and establishes a subsurface abundance maximum usually associated with the deep chlorophyll maximum (Olson et al. 1990). *P. marinus* is generally present throughout the euphotic zone during the winter, fall and spring, but during the early summer the majority of cells are largely restricted to the SCM (Olson et al. 1990). In the North Pacific (station ALOHA), *P. marinus* is uniformly abundant in the surface of the euphotic zone and declines in numbers with depth during all seasons (Campbell & Vaulot 1993). *Synechococcus* populations in both the North Atlantic and North Pacific are uniformly abundant in the surface and have no subsurface maximum yearround (Olson et al. 1990, Campbell & Vaulot 1993). *P. marinus* populations are more abundant and extend deeper in the water column than *Synechococcus* populations throughout most of the year in the oligotrophic North Atlantic (Olson et al. 1990, Veldhuis & Kraay 1993) and Pacific (Campbell & Vaulot 1993).

Prochlorococcus marinus seems to be particularly well adapted to growth in low light, as determined by laboratory studies (Partensky et al. 1993) and distributions observed in the field. At the 1.6% light level in the Sargasso Sea, *P. marinus* was found to grow twice as fast as other phytoplankton, based on ^{14}C incorporation into chl a_2 and chl a_1 (Goericke & Welschmeyer 1993). Morel et al. (1993) suggested that the ability of *P. marinus* to dominate the algal population in the deeper euphotic zone may be due, in part, to the optical properties of *P. marinus*. Because of its small size, it has a higher absorption efficiency than *Synechococcus*, even though both are well suited for absorbing the blue light available in oligotrophic waters (Morel et al. 1993).

In stratified waters, photoacclimation (used here to describe reversible light-induced alterations in the physiological or morphological characteristics of a population) of *Prochlorococcus marinus* populations is reflected by a change in cellular concentrations of chl a_2 and in mean cellular fluorescence (Olson et al. 1990, Veldhuis & Kraay 1990, 1993, Campbell & Vaulot 1993). Goericke & Repeta (1993) reported dramatic changes of the ratio of chl b_2/chl a_2 with depth at a station in the southern Sargasso Sea, ranging from 0.1 in the surface layer to 3.0 below the SCM.

These field observations led us to examine how light and temperature affect the growth rates of *Prochlorococcus marinus* and *Synechococcus* and to analyze the effects of light on the pigment composition of the cells. While differences in nutrient utilization are undoubtedly a critical environmental determinant, this factor could not be studied as axenic cultures have not yet been established for these species. In addition, we examined the extent of physiological differences between 2 *P. marinus* clones isolated from different parts of the world: the Sargasso Sea and the Mediterranean Sea.

METHODS

Culture conditions and growth measurements. Clonal cultures of WH8103, a high phycourobilin (PUB) *Synechococcus* strain isolated from the Sargasso Sea (obtained from John Waterbury, Woods Hole Oceanographic Institution, MA, USA), and *Prochlorococcus marinus* isolated at 120 m from the Sargasso Sea (*P. marinus* SS120; designated CCMP-1375 at the Center for the Culture of Marine Phytoplankton, Bigelow Laboratory for Ocean Sciences, West Boothbay Harbor, ME, USA) and from the surface of the Mediterranean Sea (*P. marinus* MED4; CCMP-1378) were maintained in a modified K/10 medium (Chisholm et al. 1992) supplemented with 50 µM urea and 10 nM $NiCl_2$. For measuring growth rate as a function of temperature, cultures were grown in glass test tubes in an aluminum temperature-gradient bar on a 14 h

light:10 h dark cycle at 90 µE m^{-2} s^{-1}, incident from below. For light experiments, cultures were grown in 250 ml polycarbonate bottles on a 14 h light:10 h dark cycle at 24 ± 1°C. Different light levels and quality were generated using cool white fluorescent lamps in combination with neutral density (Rosco #3402, 3403, 3404) and blue (Rosco #69) filters. The blue filter, which transmits maximally at 440 nm with half-maximum transmission width of 130 nm, provides a light environment similar to that found in the deep euphotic zone of the Sargasso Sea (Jerlov 1976). The growth irradiance (µE m^{-2} s^{-1}) for the white and blue light regimes was matched using a recently calibrated quantum scalar irradiance meter (Biospherical QSL-100, San Diego, CA, USA). Of the white light irradiance, 47% is comprised of wavelengths between 380 and 540 nm, corresponding approximately to the wavelengths of light provided by the blue filters. Thus, the cells grown in white light had approximately half the energy in the blue wavelengths as the cells grown only in blue light of comparable total irradiance.

Cultures were acclimated to each irradiance level for several generations (until the mean fluorescence per cell was constant) before data was collected. Replicates for all growth experiments represent serial transfers from the same 'parent' culture. Cells were harvested in exponential growth phase for the pigment analyses, *in vivo* absorption spectra, and fluorescence excitation and emission spectra.

Growth rate was determined by sampling each culture at the same time of day over several generations using a Becton-Dickinson FACScan (San Jose, CA) flow cytometer to enumerate the cells. This instrument employs an argon-ion laser with 488 nm line as the excitation source. Fluorescence emission was obtained using a long-pass filter (>650 nm) for chlorophyll (red fluorescence) and a narrow band-pass filter (585 nm, half-maximum transmission width = 42 nm) for phycoerythrin (orange fluorescence). Mean chlorophyll fluorescence per cell is presented relative to standard fluorescent beads (0.57 µm diameter; Polysciences, Inc., Warrington, PA, USA). Data were analyzed using CYTOPC software (Vaulot 1989).

Pigment measurements. *Prochlorococcus marinus* and *Synechococcus* WH8103 cultures (25 to 75 ml) were collected on 25 mm Whatman GF/F filters using vacuum pressure of <75 mm Hg. Filters were stored in liquid nitrogen for 1 to 6 mo. For pigment analysis by reverse-phase high-pressure liquid chromotography (RP-HPLC), filters were extracted as described in Goericke & Welschmeyer (1993). All samples were analyzed on a C-18 column-based RP-HPLC system using a Rainin Dynamax 10 cm 3 µm C-18 column; the following solvents: A (MeOH:aqueous ammonium acetate, 85:15), B (MeOH), and C (acetone); and a linear ternary gradient (time; % solvent A, % solvent B, % solvent C): (0 min; 100, 0, 0), (5 min; 6, 92, 2), (12 min; 0, 100, 0), (15 min; 0, 90, 10), (18 min; 0, 40, 60), (20 min; 0, 20, 80), (24 min; 0, 20, 80), with a flow rate of 1.5 ml min^{-1}. The coefficient of variation for replicate analyses on this system was concentration dependent; for the major pigments discussed here it was in the range 1 to 5%.

Selected samples were also analyzed on a C-8 column-based RP-HPLC system (Goericke & Repeta 1993). On this system, chl a_1 is well separated from chl a_2 and its stereoisomer chl a_2'. Chl b_1 and chl b_2 are partially separated. The chromatographic systems were calibrated with zeaxanthin, α-carotene, chl b_2, and chl a_2 isolated from low-light cultures of *Prochlorococcus marinus* (clone MED4), and with chl a_1 and chl b_1 isolated from spinach. Pigments were quantified (Waters 990 photodiode array detector) using integrated absorbance at 440 nm and the extinction coefficients given by Goericke & Repeta (1993). The chl b_1/chl b_2 concentration ratio was determined for all samples from the ratio of the integrated absorbances at 468 and 478 nm (Goericke & Repeta 1993).

All pigments were identified based on retention time and on-line Vis-spectra. Mass spectra of chl a_2, chl b_1, and total chl *b*, i.e. the sum of chl b_1 and chl b_2, were recorded using liquid secondary ion mass spectrometry (LSIMS) with a VG dynamic LSIMS probe, acetone as a solvent and nitrobenzylalcohol as a matrix with polyethyleneglycol 600 and 1000 as an internal standard. Samples were ionized with a cesium ion gun operated at 35 kV. Pigments for this analysis were isolated from a higher plant and from pooled extracts of high-light cultures of *Prochlorococcus marinus* SS120.

Absorption spectra. An *in vivo* absorption spectrum for cells concentrated on a filter was run for each sample using a Beckman DU-7 (Irvine, CA) single-beam spectrophotometer following methods outlined by Mitchell & Kiefer (1988). Absorption spectra of cells collected on filters, rather than spectra of whole cell suspensions, were used in order to compare lab results to field results. Scans were run with a 2 nm bandpass at 600 nm min^{-1}. All samples were filtered using Whatman GF/F filters at low vacuum pressure (<75 mm Hg), placed on a glass slide, and run within 2 to 3 min of filtration to avoid potential artifacts (Stramski 1990). A GF/F filter saturated with culture medium was used as the blank, and the optical density of the filter sample (OD_f) at 750 nm was subtracted from all spectra to obtain $OD_f(\lambda)$.

In order to correct the absorption spectra of filter samples for the effects of pathlength amplification, a β-correction algorithm was empirically derived for our spectrophotometer using *Synechococcus* WH8103 and

Prochlorococcus marinus, following published methods (Mitchell & Kiefer 1988, Mitchell 1990). Each species was concentrated by centrifugation (11 000 rpm, 19 000 × *g*, 30 min, 10 °C), and serial dilutions were made to cover a range of optical densities. Absorption spectra for these cell suspensions were obtained with an opal diffuser (Shibata 1958). For the same culture, there were no differences in shape between the absorption spectrum obtained using an opal diffuser and that obtained using an integrating sphere (H. Sosik & L. R. Moore unpubl. data). For each absorption spectrum of a cell suspension, a corresponding spectrum was determined using an equivalent pathlength (clearance area of filter multiplied by the length of the spectrophotometer cuvette) of cells collected on a filter.

The relationship between the $OD_f(\lambda)$ and the optical density of cells in suspension, $OD_s(\lambda)$, fits a second-order polynomial:

$$OD_s(\lambda) = a\, OD_f(\lambda) + b\, [OD_f(\lambda)]^2 \quad (1)$$

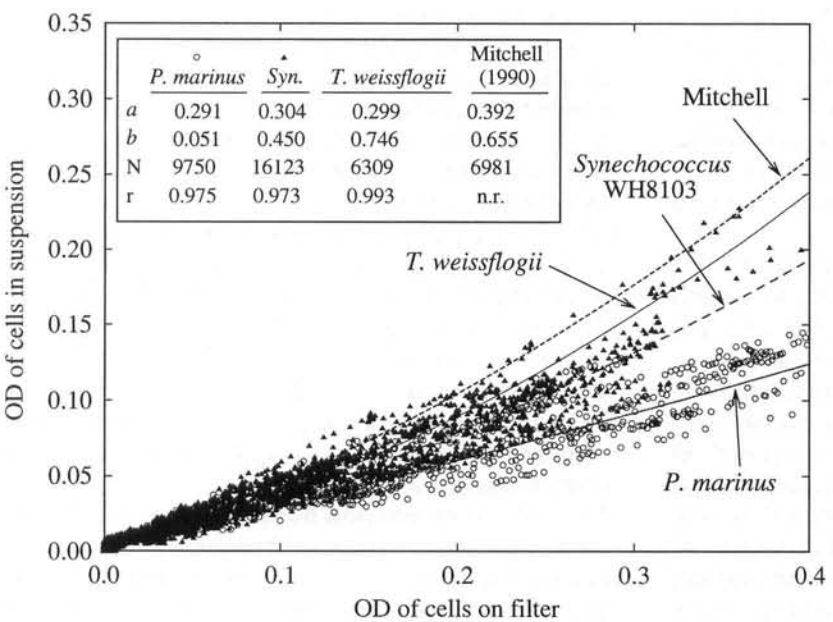
where a and b are the coefficients (inset, Fig. 1). The *in vivo* absorption spectrum for each phytoplankton culture was calculated as follows:

$$a^*_{ph}(\lambda) = 2.3\, A_f\, [OD_s(\lambda)] / V_f\, C_i \quad (2)$$

where A_f is the area of the cells on the filter (m^2), V_f is the volume of cells filtered (ml), $OD_s(\lambda)$ is the optical density of the suspension obtained by applying the correction algorithm (Eq. 1) to the measured values of $OD_f(\lambda)$, C_i is the amount of chl a_1 or chl a_2 in the sample (mg ml^{-1}), and the constant 2.3 converts units from log of base 10 to natural log (Mitchell & Kiefer 1988).

The relationship between OD_f and OD_s obtained for *Synechococcus* WH8103 differed (up to 30% at $OD_f = 0.4$) from that found previously for other phytoplankton species, including an unidentified strain of *Synechococcus* (Mitchell 1990) and *Synechococcus* WH7803 (Cleveland & Weidemann 1993). The relationship found for *Prochlorococcus marinus* differed even more, up to 50% lower OD_s is obtained when the optical density of cells filtered onto a GF/F filter reaches 0.4. To verify that the different β correction found for *Synechococcus* WH8103 and *P. marinus* in this study was not due to any systematic or procedural error, absorption of *Thalassiosira weissflogii* cells was also measured on our system and found to be consistent with that found by other researchers for other phytoplankton (Fig. 1) (Mitchell 1990, Cleveland & Weidemann 1993). A greater correction for *Synechococcus* WH8103 cells, and the even smaller *P. marinus* cells, may result from their small size. It is likely that the cells become embedded within the filter matrix rather than creating a layer on top of the filter, and the interaction of the filter scattering and the cell scattering (as small as it is; Morel et al. 1993) might increase the pathlength of amplification, similar to a thicker filter pad (cf. Mitchell 1990). Culture conditions such as nutrients and light which affect cell size might influence the extent of the β correction; however, this effect deserves more study.

The unique β correction of *Prochlorococcus marinus* and *Synechococcus* WH8103 becomes particularly important when estimating photosynthetic quantum yield. Depending on the optical density of the sample, if the β-correction algorithm for other phytoplankton is used



	○ *P. marinus*	▲ *Syn.*	*T. weissflogii*	Mitchell (1990)
a	0.291	0.304	0.299	0.392
b	0.051	0.450	0.746	0.655
N	9750	16123	6309	6981
r	0.975	0.973	0.993	n.r.

Fig. 1. Relationship between the optical density (*OD*) of cells measured in suspension and on a filter for *Synechococcus* WH8103 (▲) and *Prochlorococcus marinus* (○) from this study compared to the relationship obtained for *Thalassiosira weissflogii* in this study (individual data points not shown) and obtained by Mitchell (1990) for a collection of phytoplankton species, including *Synechococcus*. Inset: coefficients for Eq. (1) obtained by fitting N data points to a second-order polynomial. r: correlation coefficient; n.r.: not reported

for *P. marinus* (e.g. Partensky et al. 1993), the absorption spectrum could be overestimated by as much as a factor of 2 in the blue, resulting in a higher spectrally weighted chl *a*-specific absorption and, consequently, a lower photosynthetic quantum yield. Thus, the relatively high maximum quantum yields calculated by Partensky et al. (1993) would actually be *higher* if samples with $OD_f > 0.1$ were used and our *P. marinus*-specific β correction were used.

Spectral reconstruction. Whole-cell absorption spectra [$a_{ph}(\lambda)$; m^{-1}] were reconstructed and compared to *in vivo* absorption spectra as follows (Mann & Myers 1968, Bidigare et al. 1987, 1989a, b):

$$a_{ph}(\lambda) = \Sigma\,[a_i(\lambda)]\,c_i \quad (3)$$

where $a_i(\lambda)$ is the spectral specific absorption coefficient for each pigment, *i*, and c_i is the volume-based concentration (mg m^{-3}) of pigment *i*. The individual pigment-specific absorption coefficients [$a_i(\lambda)$] (Fig. 2) were determined by normalizing the HPLC-generated absorption spectra to the weight-specific absorption coefficient (Goericke & Repeta 1993), multiplying by 2.3 to convert from log of base 10 to natural log units, and shifting the wavelength of the pigment spectra as follows: (1) chl a_2 $a_i(\lambda)$ was shifted to the red by 6 nm for wavelengths greater than 480 nm; (2) zeaxanthin $a_i(\lambda)$ was shifted to the red by 10 nm; (3) the $a_i(\lambda)$ for α-carotene and the unknown carotenoid were shifted by 6 nm to the red; and (4) no shift was made for chl b_2 and the chl *c*-like $a_i(\lambda)$. The wavelength shifts were the best matches for the *in vivo* absorption maxima (see Fig. 11).

Fluorescence spectra. Because a spectrofluorometer was not readily available, samples for fluorescence excitation and emission spectra were fixed with 0.125% glutaraldehyde (Tousimis, Rockville, MD, USA) and frozen in liquid nitrogen for later analysis (Vaulot et al. 1989). A SLM-Aminco (Champagne-Urbana, IL, USA) SPF-500 spectrofluorometer with a Xenon arc lamp as the excitation source was used for the fluorescence excitation and emission spectra. Excitation spectra were determined by measuring fluorescence emission at 680 nm (the wavelength of maximum emission for both *Prochlorococcus marinus* and *Synechococcus* WH8103) as a function of excitation wavelength. Measurements were obtained in 1 nm increments (2 nm excitation band width) from 400 to 660 nm in 'ratio mode' which corrects for spectral variation due to the lamp. A media blank was subtracted from each spectrum. Quantum corrected excitation spectra were obtained by measuring the absorption and fluorescence excitation spectra of pure chl a_1, chl b_1 and phycoerythrin (Sigma, St. Louis, MO, USA), calculating the ratio of fluorescence excitation to absorption as a function of wavelength for these standards, and correcting the spectrum of each sample by dividing by this ratio. The rhodamine-B method of Melhuish (1962) was not appropriate for the spectrofluorometer used in this study.

To evaluate the influence of preservation on the analysis, spectra run on fresh aliquots and thawed aliquots which had been fixed and frozen were compared. For *Prochlorococcus marinus* SS120, the amplitude of the fluorescence excitation peak at 450 nm (due to chl a_2) did not change, whereas the peak at 480 nm (due predominantly to chl b_2) was reduced by 18% in the fixed and frozen sample relative to the fresh sample. In *P. marinus* MED4, the chl a_2 and chl b_2 fluorescence excitation peaks were reduced 22 and 12%, respectively, by the preservation process. Although these differences were significant, they did not obscure the systematic intraspecies changes in fluorescence spectra which accompanied changes in light intensity. In contrast, the changes in fluorescence excitation due to preservation for *Synechococcus* WH8103 (40% decrease in both phycoerythrin peaks with a concomitant increase of 27% in the chl a_1 fluorescence excitation peak) were too large to allow meaningful interpretation of the trends.

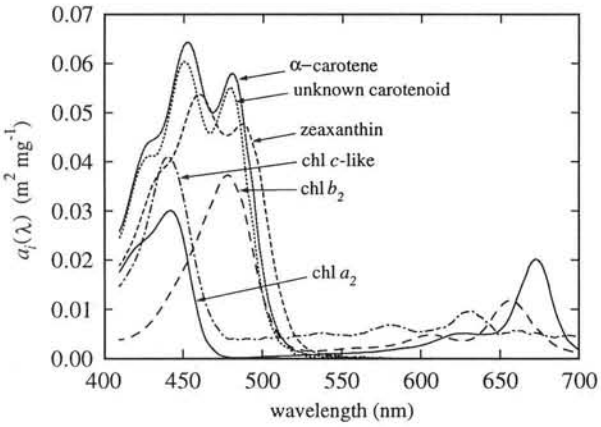


Fig. 2. Pigment-specific absorption coefficients [$a_i(\lambda)$] for the different pigments in *Prochlorococcus marinus*, wavelength shifted to match the *in vivo* absorption peaks. Each pigment is designated by a different line type

RESULTS AND DISCUSSION

Temperature optima

The 2 *Prochlorococcus marinus* clones had the same optimal growth temperature of 24 °C, but neither would grow at 28 °C, the optimum for *Synechococcus* WH8103 (Fig. 3). Between 15 and 22 °C, however, the growth rates were quite similar for all 3 strains. *Synechococcus* WH8103 would not grow below 15 °C under the conditions used in this study, differing from results previously

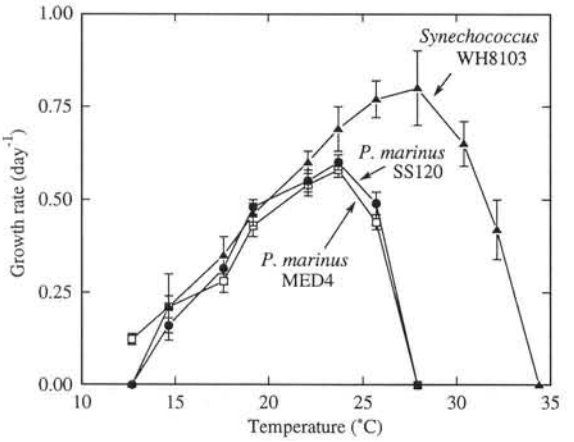


Fig. 3. Growth rate as a function of temperature for 3 oceanic picoplankters: *Prochlorococcus marinus* SS120 (●), *P. marinus* MED4 (□) and *Synechococcus* WH8103 (▲). Cultures were maintained at 93 μE m^{-2} s^{-1} under a 14 h light:10 h dark regime. Error bars are 1 SD from the mean based on 2 or 3 cultures

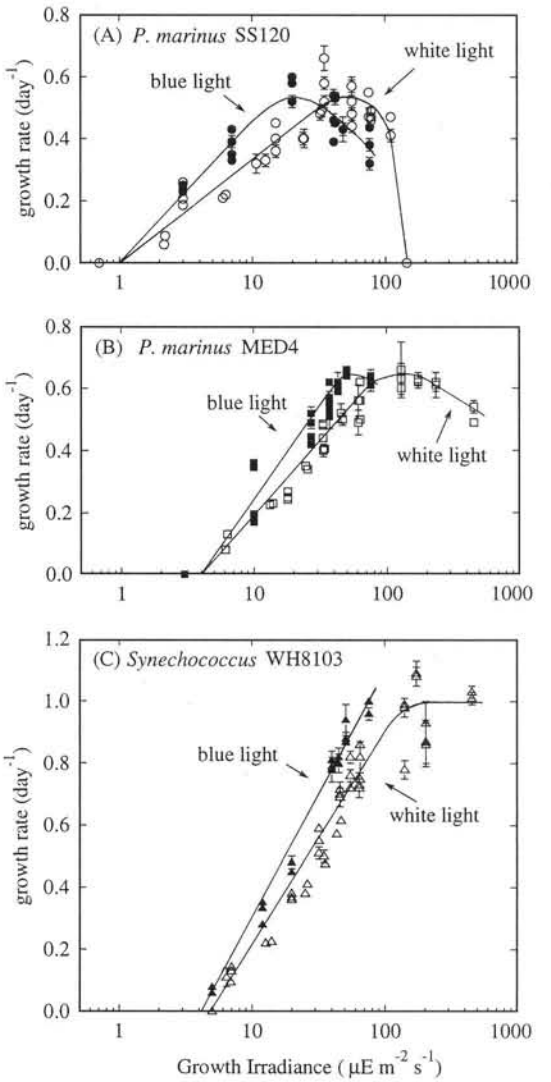


Fig. 4. Growth rate (± SD) as a function of both white and blue light irradiance. Cultures were maintained at 24.5 °C under a 14 h light:10 h dark regime. Cultures were adapted to the growth irradiance for at least 10 generations. Open symbols: white-light-grown cells; closed symbols: blue-light-grown cells. (A) *Prochlorococcus marinus* SS120, (B) *P. marinus* MED4, (C) *Synechococcus* WH8103

reported for several other marine *Synechococcus* clones (Waterbury et al. 1986, Castenholtz & Waterbury 1989). The relatively low upper-limit growth temperature for the 2 *P. marinus* clones most likely is strain-specific, since *P. marinus* has been detected at water temperatures as high as 28 °C in the equatorial and south Pacific (R. Olson, E. Zettler, J. Dusenberry & B. Binder unpubl. data), the north Pacific (Campbell & Vaulot 1993) and the Red Sea (Veldhuis & Kraay 1993).

Of the 3 clones examined in this study, only *Prochlorococcus marinus* MED4 grew at a temperature of 12.5 °C, comparable to that of the Mediterranean Sea in wintertime (Vaulot et al. 1990). The lower temperature limit for growth of *P. marinus* SS120 is consistent with the observation that *P. marinus* has only been detected in the North Atlantic when surface temperatures are greater than 15 °C (Olson et al. 1990, Veldhuis et al. 1993). However, the differences in optimal growth temperature cannot account for the difference in latitudinal and seasonal distributions of *P. marinus* and *Synechococcus* seen in the North Atlantic (Olson et al. 1990, Goericke & Welschmeyer 1993, Veldhuis et al. 1993). The depth-integrated abundance of *P. marinus* is the same as that of *Synechococcus* during the winter in the North Atlantic but is greater than that of *Synechococcus* at other times of the year when the surface temperature ranges from 22 to 27 °C (Olson et al. 1990).

Light-dependent growth rates

The light-saturated, maximum growth rates (μ_{max}) for the 2 *Prochlorococcus marinus* clones were similar: 0.53 ± 0.06 d^{-1} for *P. marinus* SS120 (Fig. 4A) and 0.63 ± 0.06 d^{-1} for *P. marinus* MED4 (Fig. 4B). The μ_{max} for *Synechococcus* WH8103 was higher (1.0 ± 0.1 d^{-1}) (Fig. 4C). *P. marinus* SS120 (which was isolated from 120 m) had a significantly lower compensation light intensity (I_{comp}) for growth (the 95% confidence interval about I_{comp} did not overlap with those of *P. marinus* MED4 and *Synechococcus* WH8103) and grew faster at lower light levels than both *P. marinus* MED4 (which was isolated from the surface; cf. Partensky et al. 1993) and *Synechococcus* WH8103 (Table 1). *P. marinus* SS120 had positive growth rates at light intensities about 5 times lower than *Synechocccus*

Table 1. Light-dependent growth parameters (μE m^{-2} s^{-1}) for *Prochlorococcus marinus* and *Synechococcus* cultures grown in white and blue light. I_{comp}: compensation light level at which no growth occurs (= *x*-intercept for a linear fit to the light-limited region of the μ vs log(I) growth curve). Values in parentheses are the 95% confidence interval about I_{comp}. I_{max}: light level at which growth rate reaches a maximum; I_{inhib}: irradiance at which growth rate is first inhibited due to excess light

		I_{comp}	I_{max}	I_{inhib}
P. marinus SS120	White	1 (0.4–2.4)	37 ± 8	110
	Blue	1 (0.4–1.4)	17 ± 3	50
P. marinus MED4	White	4 (2.6–6.7)	90 ± 13	450
	Blue	4 (2.0–5.9)	49 ± 8	80
Synechococcus WH8103	White	5 (3.5–8.4)	142 ± 21	>450
	Blue	4 (3.5–6.1)	>80	>80

WH8103 (Fig. 4A, C). At the I_{comp} for *Synechococcus* WH8103, for example, *P. marinus* SS120 grew at about 0.3 d^{-1}. At 10 μE m^{-2} s^{-1} of blue light (typical of the wavelengths which penetrate deep in oligotrophic waters), the growth rate of *P. marinus* SS120 was about twice that of the other 2 picoplankters. These results are consistent with field observations which have shown that *P. marinus* extends deeper into the euphotic zone than *Synechococcus* (Chisholm et al. 1988, Olson et al. 1990, Campbell & Vaulot 1993, Veldhuis et al. 1993) and that *P. marinus* grows faster than other phytoplankton by a factor of 2 at the 1.6% light level in the Sargasso Sea (Goericke & Welschmeyer 1993).

Growth of *Prochlorococcus marinus* SS120 was inhibited at light intensities greater than 37 μE m^{-2} s^{-1}, and it did not grow at 140 μE m^{-2} s^{-1}, even after a several day adjustment period employing small incremental increases in irradiance (as suggested by Kana & Glibert 1987). Photoinhibition of this strain at relatively low growth irradiances is not consistent with the distribution of *P. marinus* in the Sargasso Sea, where they are present (Olson et al. 1990) and growing (Goericke & Welschmeyer 1993) in the surface waters when light intensities are in excess of 200 μE m^{-2} s^{-1}. In the surface layer during the winter, growth of *P. marinus* is comparable to the growth rates of the other phytoplankton, and during the summer, *P. marinus* grow at a rate corresponding to 60% of the growth rate of the other phytoplankton (Goericke & Welschmeyer 1993). The difference between our laboratory data and the field data could be explained by physical mixing (which could modulate the light regime experienced by these cells in the field), or by the existence of 2 (or more) different strains of *P. marinus* in the Sargasso Sea — one adapted for growth at high light, and one (e.g. *P. marinus* SS120) adapted for growth deep in the euphotic zone.

The light-dependent growth response of *Prochlorococcus marinus* MED4 has characteristics distinct from *P. marinus* SS120 and is consistent with characteristics expected for a high-light-adapted strain. The I_{max} for *P. marinus* MED4, for example, was more than twice that for *P. marinus* SS120 and more similar to the I_{max} for *Synechococcus* WH8103 (Table 1). *P. marinus* MED4 was photoinhibited only at the highest growth irradiance tested (450 μE m^{-2} s^{-1}). Thus, the differences between *P. marinus* MED4 and *P. marinus* SS120 could reflect differences between populations collected from different *depths* rather than different geographical locations.

For all 3 picoplankters, light-limited growth rates in blue light were higher than at the same irradiance of white light (Fig. 4). These differences can be explained if we consider the portion of photon flux density in the white light corresponding to the band of transmittance of the blue filters (47% of the white light irradiance is between 380 and 540 nm). This band of wavelengths includes the wavelengths of absorption by the major photosynthetic pigments: chl a_1, chl a_2, chl b_2, and PUB. The reduced growth rates of cells grown in white light relative to those in blue light can be explained solely on the basis of reduced levels of photosynthetically usable radiation: the graphs 'collapse' onto one another when the white light irradiance is expressed in terms of blue photon flux density (data not shown).

Cellular pigment content

Concentrations of chlorophylls and carotenoids were determined in light-limited and light-saturated cultures of all 3 picoplankton clones. The major pigments present in *Synechococcus* WH8103 were chl a_1, zeaxanthin and β-carotene, and the major pigments of low-light cultures of *Prochlorococcus marinus* SS120 and *P. marinus* MED4 were chl a_2, chl b_2, a chl *c*-like pigment, zeaxanthin, α-carotene, and an unknown carotenoid, consistent with previous reports (Guillard et al. 1985, Goericke & Repeta 1992). Analysis of the pigments on the C-18 column-based RP-HPLC system also revealed traces of β-carotene in the 2 *P. marinus* clones.

The analysis of high-light cultures of *Prochlorococcus marinus* SS120 on the C-8 column-based HPLC system revealed 2 partially separated chl *b* peaks (Fig. 5a) with on-line absorbance spectra typical of chl b_1 and chl b_2 (cf. Goericke & Repeta 1993). Chl a_1 was undetectable in these cultures, which would have eluted at 28.9 min, well separated from chl a_2 and its stereoisomer chl a_2' (Fig. 5a). We isolated chl a_2 and total chl *b* from high-light cultures of the *P. marinus* clone SS120 and recorded their mass spectra to prove

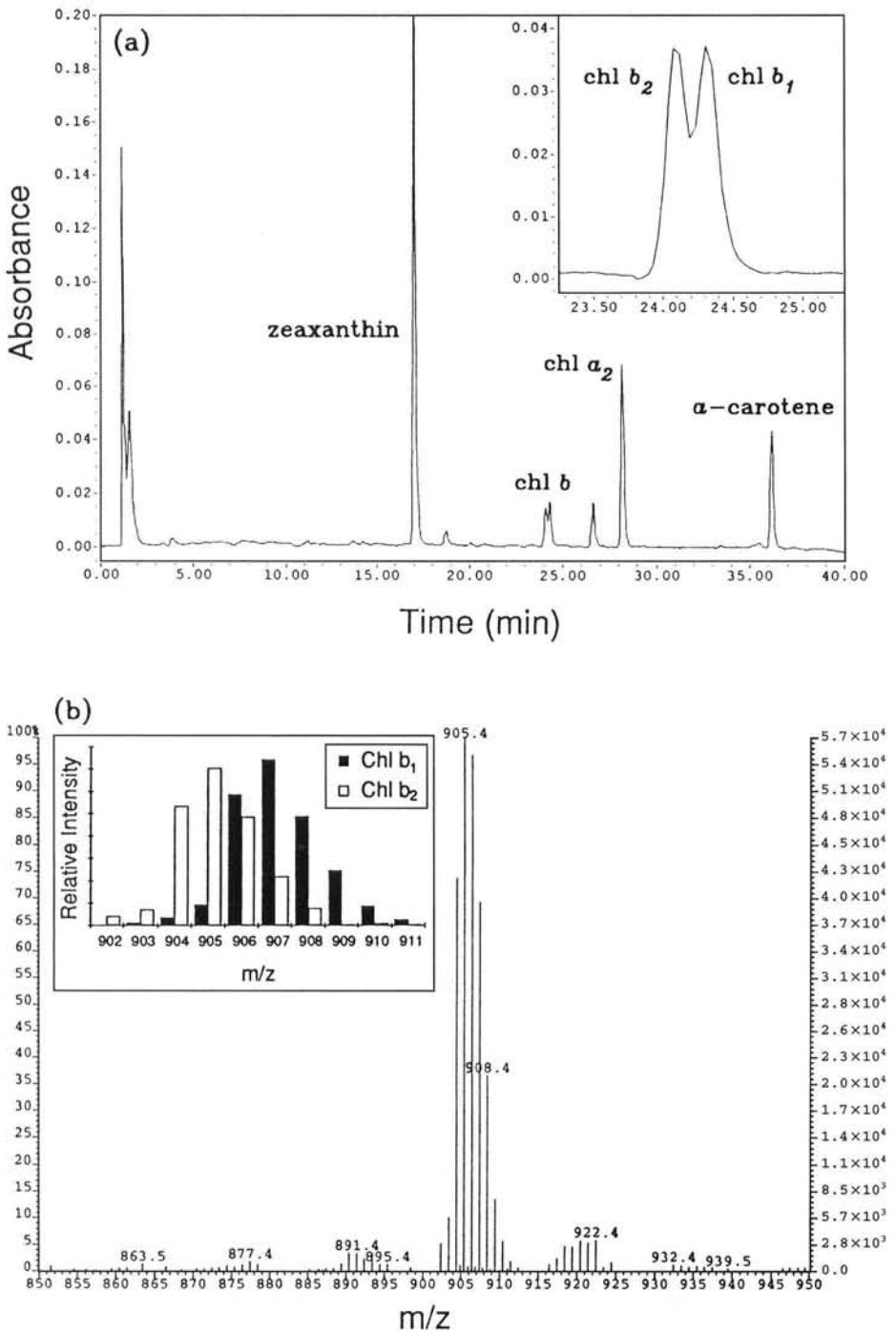


Fig. 5. (a) System II chromatogram (absorbance at 440 nm) of the pigments of the *Prochlorococcus marinus* SS120 grown at 75 µE m^{-2} s^{-1} under white light. Inset: partial separation of chl b_1 and chl b_2 when absorbance is measured at 473 nm. (b) Mass spectrum of total chl *b* isolated from high-light cultures of *P. marinus* clone SS120. The cluster at *m/z* 905 to 907 is due to chl b_1 and chl b_2 as demonstrated from the deconvoluted spectra (inset) which are identical to the spectra of chl b_1 (*m/z* 907) and chl b_2 (*m/z* 905)

the presence of these pigments and the absence of chl b_2 allomers. The major ion in the mass spectrum of chl a_2 was *m/z* 891, consistent with its molecular ion. The dominant peak in the mass spectrum of total chl *b* was a cluster around *m/z* 903 to 909 (Fig. 5b); notably absent were major signals at *m/z* 921, 934 and 937, which would have corresponded to the allomers of chl b_2 (cf. Otsuki et al. 1987). Using least-squares analysis, the cluster at *m/z* 903 to 909 was deconvoluted into 2 chl *b* spectra with molecular ions at *m/z* 905 and 907 (Fig. 5b, inset), which correspond to the molecular ions of chl b_2 and chl b_1, respectively.

The relative concentrations of chl b_1 and chl b_2 were determined in all cultures by recording the ratio of the integrated total chl *b* absorbances at 468 and 478 nm (A_{468}/A_{478}). The ratios of the integrated absorbances at 468 and 478 nm were 1.229 ± 0.004 (standard deviation) for pure chl b_1 isolated from higher plants and

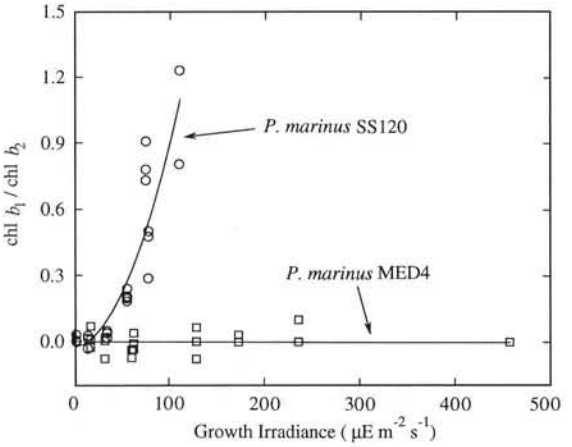


Fig. 6. Ratio of chl b_1 to chl b_2 in *Prochlorococcus marinus* SS120 (○) and *P. marinus* MED4 (□) grown under white light

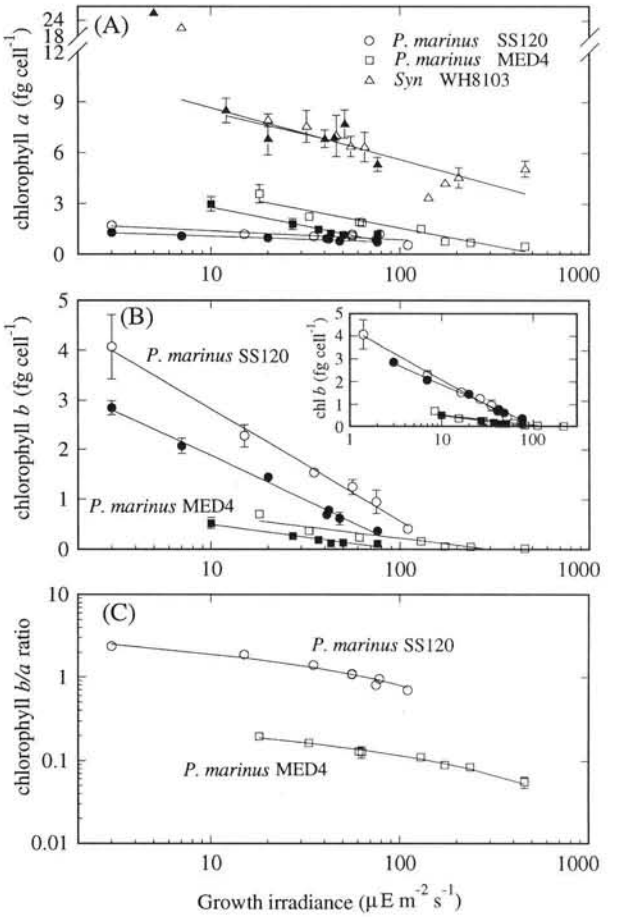


Fig. 7. (A) Cellular chl a_1 for *Synechococcus* and chl a_2 for *Prochlorococcus marinus* SS120 and *P. marinus* MED4. (B) Cellular chl *b* content of both *P. marinus* clones. The inset shows that the total chl *b* in the 2 *P. marinus* clones as a function of blue light and 'corrected' white light (see text) is the same. (C) Ratio of total chl *b* to chl a_2 as a function of white growth irradiance for the 2 strains. Symbols for all 3 panels are the same: *P. marinus* SS120 (○, ●), *P. marinus* MED4 (□, ■) and *Synechococcus* WH8103 (△, ▲); open symbols: white light; closed symbols: blue light. Error bars: 1 SD of the mean of 2 or 3 cultures

0.837 ± 0.004 for pure chl b_2 isolated from the corn mutant ON8147 (Bazzaz 1981). The chl *b* from low-light cultures of *Prochlorococcus marinus* SS120 (< 20 µE m^{-2} s^{-1}) had an average A_{468}/A_{478} ratio of 0.84 ± 0.008 (N = 13), a value not significantly different from the value of pure chl b_2 ($0.10 > p > 0.05$). The ratio increased with increasing growth irradiances, indicating that *P. marinus* SS120 has measurable amounts of normal chl b_1 at high light (Fig. 6). Chl b_1 was not detected in previous analyses of pigment content in the *P. marinus* isolate from the Sargasso Sea because the light level used in those studies (Goericke & Repeta 1992) was below that at which chl b_1 begins to be synthesized. In contrast, *P. marinus* MED4 does not have any detectable levels of chl b_1 at any growth irradiances (Fig. 6), as indicated by an average A_{468}/A_{478} ratio of 0.839 ± 0.016 (N = 42), a value not significantly different ($p > 0.25$) than the value of 0.837 for pure chl b_2. The presence of chl b_1 in *P. marinus* SS120 and its absence in *P. marinus* MED4 has been suggested by other researchers (Partensky et al. 1993). For the rest of this paper, references to chl *b* in the *P. marinus* data refer to the sum of chl b_1 and chl b_2 (i.e. total chl *b*) for *P. marinus* SS120 and to chl b_2 only for *P. marinus* MED4.

Cellular chl *a* increased with decreasing light intensity in all 3 picoplankton (Fig. 7A). The chl a_2 per cell of *Prochlorococcus marinus* was 2 to 6 times lower than the chl a_1 of *Synechococcus* WH8103 at all light intensities. *P. marinus* MED4 had about 2.5 times the chl a_2 per cell, but 3 to 10 times less chl *b* per cell (Fig. 7B), than *P. marinus* SS120, such that the sum of the chl a_2 and total chl *b* was similar for both clones (data not shown). The total chl *b*/chl a_2 ratio for *P. marinus* SS120 was 10 times higher than the chl b_2/chl a_2 ratio for *P. marinus* MED4 at all growth irradiances, but the percent change in the chl *b*/chl a_2 ratio over the growth irradiance range was the same (Fig. 7C).

Depth profiles of chl b_2/chl a_2 ratios in the field (Goericke & Repeta 1993) span a range which is greater than the ranges measured here for either of the 2 clones of *Prochlorococcus marinus*. The range of chl b_2/chl a_2 ratios measured in the deep euphotic zone of the Sargasso Sea is similar to the range of total chl *b*/chl a_2 ratio of *P. marinus* SS120 measured under blue and white light (0.4 to 2.4), whereas the range found in the surface waters of the Sargasso Sea is more similar to the range measured for *P. marinus* MED4 grown under blue and white light (0.05 to 0.2) (Goericke & Repeta 1993). As suggested by Goericke

& Repeta (1993), the sharp transition of chl b_2/chl a_2 ratios at depth may be due, in part, to changing populations of differently photoadapted (as opposed to photoacclimated) *P. marinus* populations: in the surface, the *P. marinus* population may consist of low chl b_2-type cells capable of growth at higher light, similar to *P. marinus* MED4; in the deeper euphotic zone, the population may shift to high chl b_2-type cells, similar to *P. marinus* SS120.

The chlorophyll content for all 3 picoplankters grown in blue light was lower than that of cultures grown in white light (Fig. 7A, B). When the white light irradiance was corrected for the amount of blue light wavelengths, however, the differences disappeared for the chlorophylls and other pigments (shown for total chl *b* only; Fig. 7B, inset). Thus, these *Prochlorococcus marinus* clones do not chromatically adapt to blue light. Rather, they respond to the amount of photosynthetically usable light energy available in both the blue and white light treatments.

Cellular concentrations of zeaxanthin varied by about a factor of 2 in the 2 *Prochlorococcus marinus* clones and *Synechococcus* WH8103 (Fig. 8A). The trends range from slightly increasing with irradiance in *P. marinus* SS120 (regression coefficient = 0.005 ± 0.001; $p < 0.001$) to no obvious variations with growth irradiance in *P. marinus* MED4 and *Synechococcus* WH8103, with the exception that low-light cultures of *Synechococcus* WH8103 have relatively high concentrations of zeaxanthin. These data confirm the results of Kana et al. (1988), who showed that cellular concentrations of zeaxanthin do not vary appreciably in nutrient-replete batch cultures of *Synechococcus* WH7803 grown under a wide range of white light intensities. In contrast to the results of Bidigare et al. (1989b) for *Synechococcus* WH7803, we found no difference in the average cellular zeaxanthin content for *P. marinus* and *Synechococcus* WH8103 cells grown in white light and those grown in blue light (blue light data not shown).

The content of zeaxanthin in *Synechococcus* WH8103 (3.3 ± 0.6 fg zeaxanthin cell^{-1}) was 1.7 times greater than that reported for *Synechococcus* WH7803 (Kana et al. 1988) and 3 times the amount in the *P. marinus* clones (Fig. 8A). However, when zeaxanthin content is normalized to estimated cell volume, there is no significant difference between *Synechococcus* WH8103 and *Prochlorococcus marinus*. [The volume of *Synechococcus* WH8103 (0.42 ± 0.03 µm^3 cell^{-1}) is estimated to be 3.2 times greater than that of *P. marinus* (0.13 ± 0.01 µm^3 cell^{-1}); Morel et al. 1993]. In addition, a significant, positive correlation ($p < 0.01$) was found between cellular zeaxanthin and the forward light scatter signal on the flow cytometer for all 3 organisms (data not shown). When zeaxanthin is normalized to forward light scatter and plotted against growth irradiance (Goericke pers. comm.), outliers disappear, in particular the low-light-grown *Synechococcus* WH8103 data points. These results point to the possibility of using zeaxanthin as a marker for prokaryotic biovolume in the field.

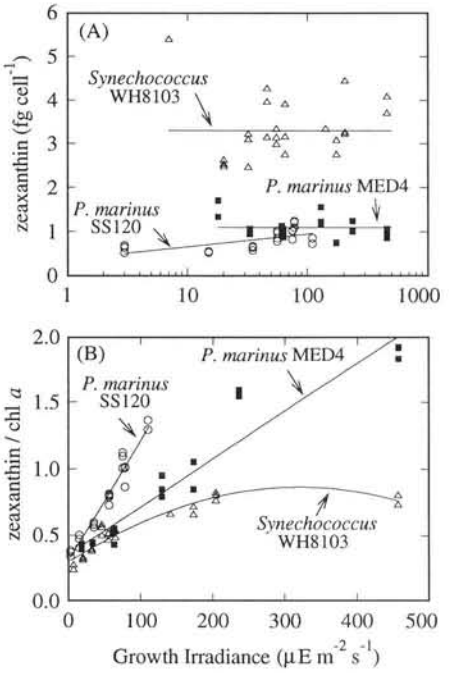


Fig. 8. Change in zeaxanthin and carotenes with change in white light intensity for *Synechococcus* WH8103 (△) and the 2 *Prochlorococcus marinus* clones, SS120 (○) and MED4 (■). (A) Zeaxanthin per cell, (B) ratio of zeaxanthin to chl *a*

For all 3 picoplankton clones, the zeaxanthin to chl *a* ratio increased (driven by photoacclimative changes in chl a) with increasing growth irradiance (Fig. 8B). At high light intensities, the zeaxanthin/chl *a* ratio was highest for *Prochlorococcus marinus* MED4 and began to level off for *Synechococcus* WH8103. *P. marinus* SS120 has its highest ratio of zeaxanthin/chl *a* (about 1.5) at light intensities at which its growth rate was photoinhibited (Fig. 4A).

The α-carotene in the *Prochlorococcus marinus* cultures and the β-carotene in the *Synechococcus* cultures covaried with chl *a* (data not shown) such that the ratio of carotene to chl *a* was constant over the entire irradiance range for all 3 organisms (0.26 ± 0.02 for *P. marinus* SS120, 0.13 ± 0.02 for *P. marinus* MED4, and 0.09 ± 0.01 for *Synechococcus* WH8103). It is likely that these carotenes are associated with the photosynthetic apparatus, as suggested for β-carotene in *Synechococcus* WH7803 (Kana et al. 1988). In addition, both the chl *c*-like pigment and an unknown carotenoid in the *P. marinus* cultures increased with decreasing light (data not shown), also suggesting that these 2 pigments are involved in photosynthesis.

Flow-cytometrically induced red fluorescence

As expected, the mean red fluorescence per cell increased with decreasing growth irradiance in all 3 organisms (Fig. 9A, B). *Prochlorococcus marinus* SS120 had a greater mean cellular red fluorescence at a given irradiance than *P. marinus* MED4 (Fig. 9A) and the change in fluorescence per unit chl *a* was larger in *P. marinus* SS120 than *P. marinus* MED4 (Fig. 10A). This difference is reduced when fluorescence was normalized to the sum of chl a_2 and total chl *b* (Fig. 10B), as expected since the sum of total chlorophylls is the same for the 2 strains. However, the difference is not totally eliminated by this normalization. Even if the red fluorescence is plotted against the sum of all the pigments except zeaxanthin, the difference in slopes still exists ($p < 0.001$). *P. marinus* SS120 has a greater change in red fluorescence per pigment content because it absorbs more light than *P. marinus* MED4 at 488 nm (see below), which is the wavelength of the laser line used by the flow cytometer and which

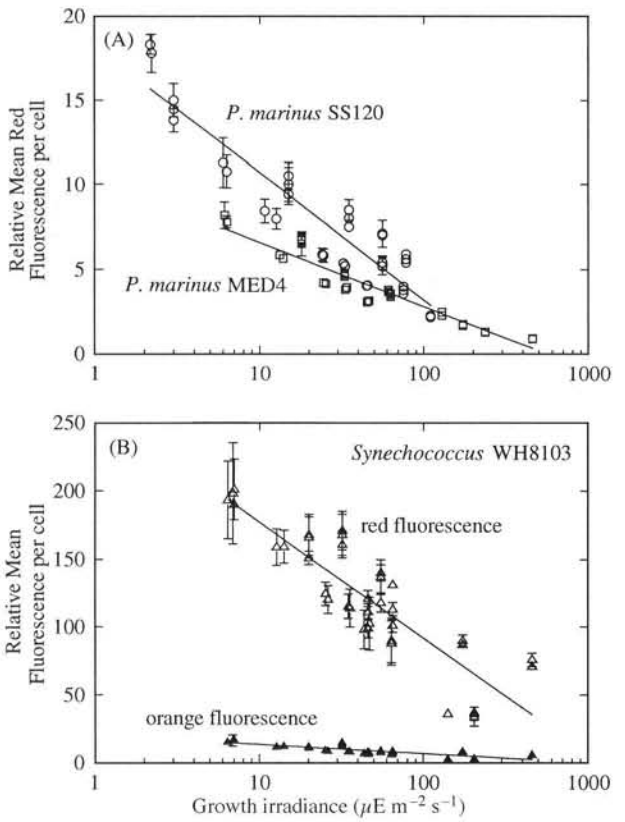


Fig. 9. Flow-cytometrically induced fluorescence per cell as a function of white growth irradiance. Symbols: the mean fluorescence of the population relative to standard beads; error bars: 1 SD of mean fluorescence. (A) *Prochlorococcus marinus* SS120 (○) and *P. marinus* MED4 (□), (B) *Synechococcus* WH8103. (△) mean red fluorescence per cell; (▲) mean orange (phycoerythrin) fluorescence per cell

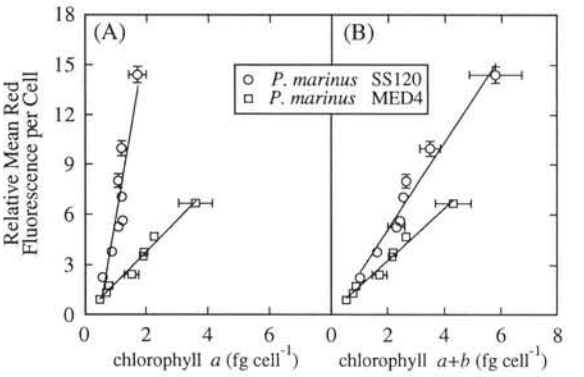


Fig. 10. Relative mean red fluorescence per cell as a function of chlorophyll for both *Prochlorococcus marinus* clones, SS120 (○) and MED4 (□) grown under white light intensity. (A) Cellular chl a_2, (B) sum of chl a_2 and total chl *b* per cell

is close to the *in vivo* absorption maxima of chl a_2. The linear relationship between the fluorescence and chl a_2, and the sum of chl a_2 and total chl *b* (Fig. 10), is indicative of a negligible pigment package effect in these small cells (Sosik et al. 1989, Veldhuis & Kraay 1990, Morel et al. 1993).

In the North Atlantic, the mean red fluorescence of *Prochlorococcus marinus* has been shown to increase sharply (about 5-fold) between the 2 and 3% light levels (Veldhuis & Kraay 1990). A similar sharp increase in fluorescence with depth was observed in the North Pacific between the 50 and the 120 m depths (Campbell & Vaulot 1993). These changes are greater than expected from photoacclimative increases in red fluorescence per cell for either *P. marinus* SS120 or *P. marinus* MED4 alone (Fig. 9A), but might be explained partially by a shift in population from a low chl b_2-type *P. marinus* population with relatively low mean red fluorescence in the surface waters to a high chl b_2-type *P. marinus* population with higher mean red fluorescence in the deep euphotic zone. This interpretation of the fluorescence data from field measurements is consistent with observations by other authors (Campbell & Vaulot 1993, Goericke & Repeta 1993, Veldhuis & Kraay 1993) and the possible existence of 2 (or more) strains of *P. marinus* which coexist in the same water column.

Chlorophyll-specific absorption

For all 3 picoplankters, the main blue absorption peak in the *in vivo* chl *a*-specific absorption (a^*_{ph}) spectrum decreased with decreasing growth irradiance, except for low-light cultures of *Prochlorococcus marinus* SS120 (Fig. 11A to C). As irradiance levels decreased, absorption due to total chl *b* (at 480 and 657 nm) increased relative to the chl a_2 absorption (at 449 and 673 nm) in

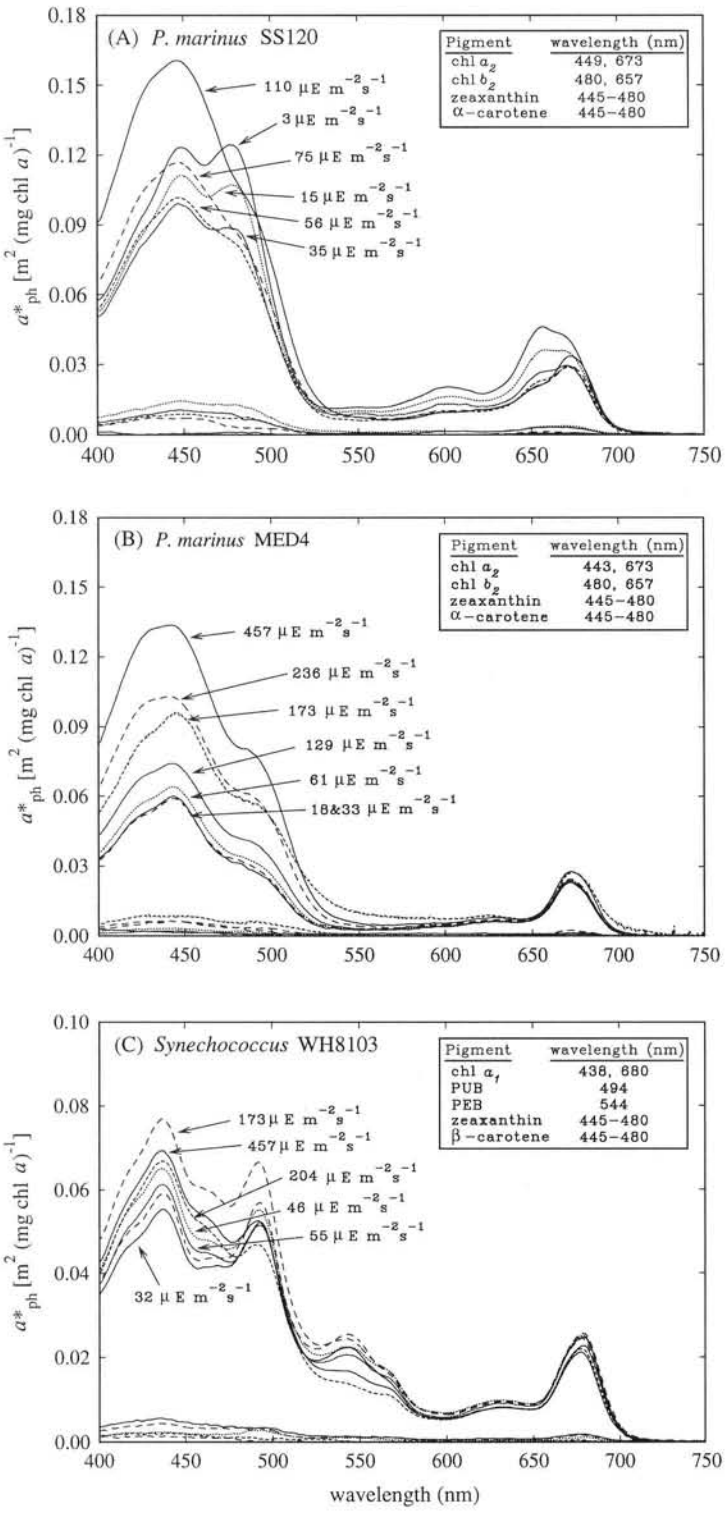


Fig. 11. *In vivo* chl *a*-specific absorption spectra $\{a^*_{ph}\ [m^2\ (mg\ chl\ a)^{-1}]\}$ for all 3 picophytoplankters. Wavelengths of maximum absorption for the various pigment peaks are listed in the insets (PEB: phycoerythrobilin; PUB: phycourobilin). Each line corresponds to an average spectrum of 2 or 3 cultures grown at different white-light irradiances. Lines at the bottom correspond to the standard deviation of each average spectrum. (A) *Prochlorococcus marinus* SS120, (B) *P. marinus* MED4, (C) *Synechococcus* WH8103

P. marinus SS120, resulting in an apparent increase in the height of the chl a_2 absorption peak in the blue as well as in the red (Fig. 11A). For *P. marinus* MED4, there was no change in the relative peak heights at 480 and 443 nm with changing growth irradiance (Fig. 11B) because the ratio of chl b_2 to chl a_2 is relatively small (Fig. 7C). The blue absorption of chl a_1 in *Synechococcus* WH8103 also decreased slightly as growth irradiance decreased (Fig. 11C), although not to the same extent and not as systematically as for *P. marinus* MED4. Overall, *P. marinus* SS120 had a higher a^*_{ph} in the blue wavelengths than *Synechococcus* WH8103.

To quantify the contribution of various pigments to the absorption spectra of *Prochlorococcus marinus*, absorption spectra were reconstructed from knowledge of the concentration of the individual pigments and their respective extinction coefficients. (Absorption spectra for *Synechococcus* WH8103 were not reconstructed because the phycobiliproteins were not measured.) Reconstructed absorption spectra for both *P. marinus* clones grown at high and low light levels closely matched the measured *in vivo* absorption spectra [$a_{ph}(\lambda)$, m^{-1}; not normalized to chl a_2] (Fig. 12A to D). The contribution by the individual pigments in each reconstructed spectrum reveals that the non-photosynthetic pigment, zeaxanthin, is a major contributor to absorption at both high and low irradiances in *P. marinus*. At the high growth irradiances, zeaxanthin absorption contributed 40 and 50 % to the total absorption at 449 and 443 nm in *P. marinus* SS120 and *P. marinus* MED4, respectively (Fig. 12A, C). High contributions of non-photosynthetic pigment absorption to total absorption could have important implications for estimates of primary productivity and photosynthetic quantum yield (Bidigare et al. 1987, 1989b).

At the lowest growth irradiances, the zeaxanthin contribution to the total absorption at 443 nm decreased to 30 % and chl a_2 increased to 51 % of the total in *Prochlorococcus marinus* MED4; chl b_2 contributed ≤5 % of the total absorption at 448 nm over all irradiance levels (Fig. 12C, D). The shoulder at 480 nm for *P. marinus* MED4 is due primarily to the second absorption peak of zeaxanthin, not chl b_2 (Fig. 12C, D). In low-light *P. marinus* SS120, the absorption peak at 449 nm is due to 42 % absorption by chl b_2,

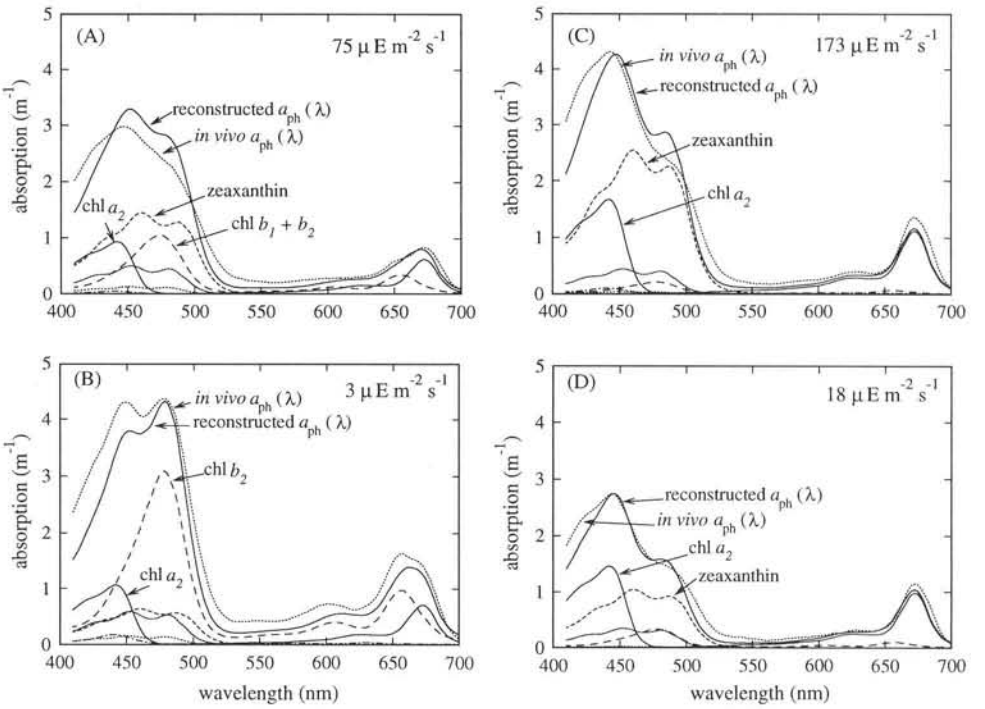


Fig. 12. Comparison of the *in vivo* absorption spectra, $a_{ph}(\lambda)$, m^{-1}, and respective reconstructed absorption spectra for high- and low-light-grown *Prochlorococcus marinus* cultures. Different pigments are designated by the same line types as used in Fig. 2. Only the pigments with the greatest contribution to the total absorption are indicated. (A) *P. marinus* SS120 grown at 75 µE m^{-2} s^{-1}, (B) *P. marinus* SS120 grown at 3 µE m^{-2} s^{-1}, (C) *P. marinus* MED4 grown at 173 µE m^{-2} s^{-1}, (D) *P. marinus* MED4 grown at 18 µE m^{-2} s^{-1}

20 % absorption by chl a_2, and only 15 % absorption by zeaxanthin (Fig. 12B). Thus, the large amount of absorption by chl b_2 at low growth irradiances keeps the absorption in the blue at relatively high values (Fig. 11A). For both *P. marinus* clones, absorption by α-carotene at the chl a_2 blue peak wavelength was 10 to 15 % and that due to an unknown carotenoid and the chl *c*-like pigment was less than 5 % over the range of growth irradiances (Fig. 12A to D).

The slight differences between the *in vivo* and the reconstructed absorption spectra could be due to several factors: (1) the wavelength shifts assumed for calculating $a_i(\lambda)$ may not be precisely correct; (2) we used the 662 and 653 nm extinction coefficients for chl a_2 and chl b_2, respectively (Goericke & Repeta 1993), which differ slightly from those measured by Shedbalkar & Rebeiz (1992); (3) imprecision in the correction of the filter absorption spectra may result in differences in the magnitude of absorption. Still, the close match between the reconstructed absorption spectra and the *in vivo* absorption spectra, in particular the lack of 'flattening' of absorption in the blue, is consistent with the findings of Morel et al. (1993) that the package effect in *Prochlorococcus marinus* is minimal. Bidigare et al. (1989b) concluded the same for *Synechococcus* WH7803 using similar methods.

The photoacclimative changes in chl *a* absorption peak height, quantified above using reconstructed absorption spectra, can also be illustrated by plotting the light-dependent ratio of chl *a* absorption in the blue relative to that in the red (B/R ratio; Mitchell & Kiefer 1988) (Fig. 13). For all 3 phytoplankters, the B/R ratio increased with increasing growth irradiance reflecting the trend in zeaxanthin to chl a ratio (Fig. 8B). *Prochlorococcus marinus* had a B/R ratio that increased to 5 as growth irradiance increased (see also Partensky et al. 1993), whereas 'typical' eukaryotic phytoplankton have B/R ratios of less than 2.5 (SooHoo et al. 1986, Maske & Haardt 1987, Mitchell & Kiefer 1988).

Several factors contribute to high B/R ratios in *Prochlorococcus marinus*. First, the ratios of accessory pigments (particularly chl b_2 and zeaxanthin) to chl a_2 are

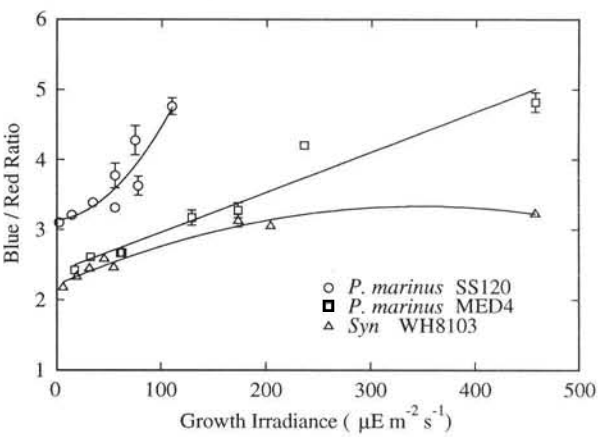


Fig. 13. B/R ratio (chl *a* absorption peak in the blue relative to that in the red) as a function of white growth irradiance. *Prochlorococcus marinus* SS120 (○), *P. marinus* MED4 (□), and *Synechococcus* WH8103 (△)

high. Secondly, the B/R ratio of pure chl a_2 and chl b_2 in solvent (diethyl ether) is about 15 and 50% higher than that for chl a_1 and chl b_1, respectively (see also Bazzaz 1981). Higher specific absorption in the blue wavelengths for chl a_2 and chl b_2 would provide an added advantage for *P. marinus* to absorb blue light relative to other phytoplankton. Thirdly, the small size of *P. marinus* results in a smaller package effect relative to most other phytoplankton and, hence, less flattening of the absorption spectrum in the blue region (Morel et al. 1993, Partensky et al. 1993).

High B/R ratios (> 3) from *in vivo* a^*_{ph} measurements (corrected for detrital absorption) have been observed in the Sargasso Sea (Bricaud & Stramski 1990) and off the coast of southern California (H. Sosik & B. G. Mitchell unpubl. data) and are likely due to the presense of *Prochlorococcus marinus*, as hypothesized by Bricaud & Stramski (1990). In fact, B/R ratio could possibly be used to detect the presence of *P. marinus* in the field when HPLC or flow cytometry is not available.

Chlorophyll *a*-specific fluorescence excitation

The fluorescence emission spectra for both *Prochlorococcus marinus* clones is a single peak at 680 nm, the same wavelength as for *Synechococcus* WH8103 (data not shown). The chl *a*-normalized fluorescence [$F^*_{ph}(\lambda)$] of *P. marinus* SS120 increased as growth irradiance decreased (Fig. 14A), opposite to the trend in $a^*_{ph}(\lambda)$. This increase was due in large part to the increase in chl b_2 relative to chl a_2 (Fig. 7C), such that below irradiances of 56 µE m^{-2} s^{-1} the contribution of chl b_2 to fluorescence excitation exceeded that of chl a_2 (Fig. 14A). In fact, the ratio of the total chl *b* F^*_{ph} peak at 480 nm to that of the chl a_2 F^*_{ph} peak at 443 nm (*F*-ratio; Mitchell & Kiefer 1988) is positively correlated ($r = 0.87$, $p < 0.01$) with the total chl *b*/chl a_2 ratio for *P. marinus* SS120 (data not shown). For *P. marinus* MED4, the differences between high- and low-light chl a_2 $F^*_{ph}(\lambda)$ peaks are small compared to differences of the chl a_2 a^*_{ph} peak. However, fluorescence emission due to chl b_2 absorption F^*_{ph} (480 nm) was apparent when *P. marinus* MED4 was grown at low irradiances (Fig. 14B).

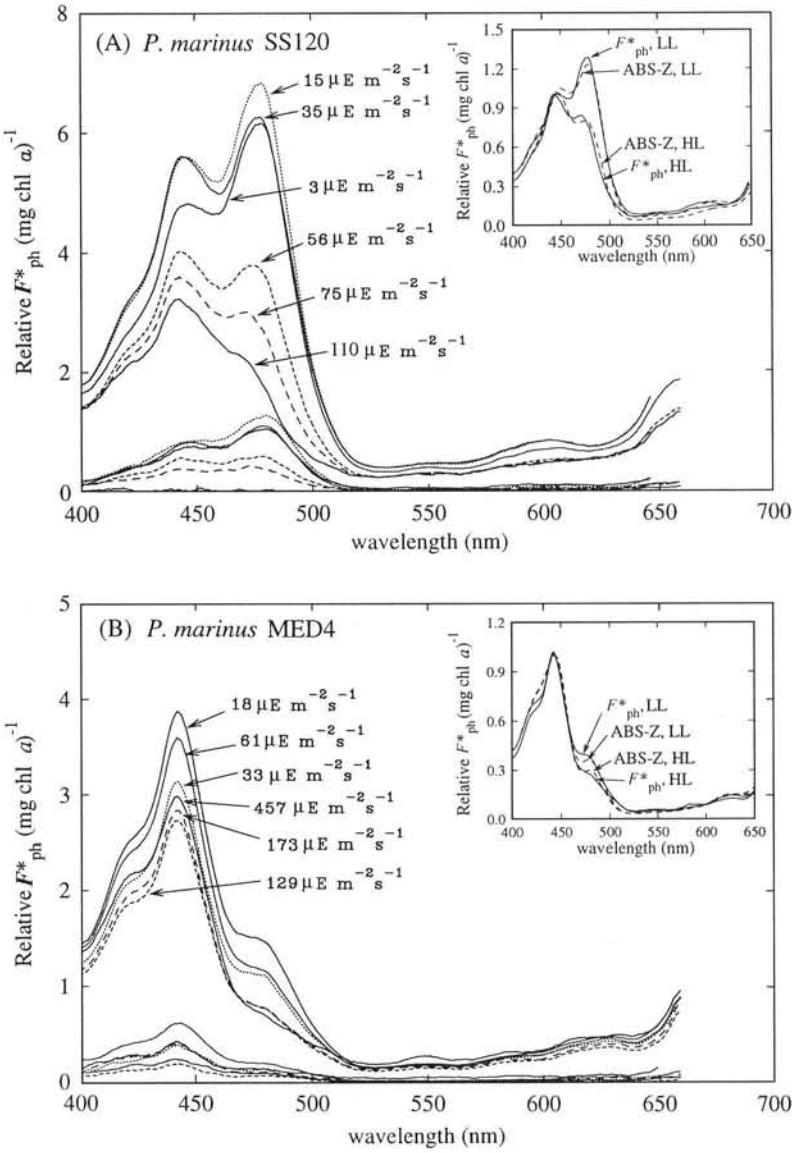


Fig. 14. Chl *a*-specific fluorescence excitation spectra at different growth irradiances. Fluorescence emission was measured at 680 nm. Each line corresponds to an average spectrum of 2 or 3 cultures grown at different white-light irradiances. Lines at the bottom (below a relative fluorescence of 1) correspond to the standard deviation of the average spectrum. (A) *Prochlorococcus marinus* SS120, (B) *P. marinus* MED4. Inset: comparison of $F^*_{ph}(\lambda)$ and chl *a*-specific reconstructed spectra, ABS-Z, generated by subtracting the zeaxanthin absorption from the reconstructed absorption and dividing by chl a_2 content at high (HL) and low (LL) light intensities. $F^*_{ph}(\lambda)$ was normalized to the chl a_2 peak of ABS-Z for easy comparison of spectral shapes. LL = 3 µE m^{-2} s^{-1} for *P. marinus* SS120 and 18 µE m^{-2} s^{-1} for *P. marinus* MED4; HL = 75 µE m^{-2} s^{-1} for *P. marinus* SS120 and 173 µE m^{-2} s^{-1} for *P. marinus* MED4

The opposite trend in the magnitude of the chl a_2 peak in fluorescence excitation spectra relative to the

absorption spectra can be explained more fully by considering the non-photosynthetic role of zeaxanthin (Kana et al. 1988, Bidigare et al. 1989b). The fluorescence excitation spectra should be spectrally similar to the absorption spectra only if the light absorbed by the accessory pigments (e.g. chl b_2) is transferred with high (and equal) efficiency to chl a_2. Pigments not connected with the photosynthetic apparatus will not be observed in the excitation spectrum. To verify that zeaxanthin does not contribute to chl a_2 fluorescence, we subtracted zeaxanthin absorption from the chl a-normalized reconstructed absorption spectra (ABS-Z) and compared this to the corresponding measured $F^*_{ph}(\lambda)$ at both high and low growth irradiances. The comparison revealed that ABS-Z spectra for both *Prochlorococcus marinus* clones was spectrally similar to the measured $F^*_{ph}(\lambda)$, even when considering spectral distortion in $F^*_{ph}(\lambda)$ due to the preservation process (see 'Methods'). Furthermore, the trend of decreasing absorption in the blue with decreasing irradiance (Fig. 11A, B) was eliminated for *P. marinus* MED4 and reversed for *P. marinus* SS120, as was seen for $F^*_{ph}(\lambda)$. This indicates that zeaxanthin does not contribute to fluorescence emission, consistent with the non-photosynthetic role of zeaxanthin suggested by other authors (Kana et al. 1988, Bidigare et al. 1989b).

CONCLUSIONS

Prochlorococcus marinus SS120 is adapted to growth at low light, relative to *Synechococcus* WH8103 (Fig. 4, Table 1). This result supports observations that *P. marinus* is a very abundant (Olson et al. 1990) and fast-growing (Goericke & Welschmeyer 1993) picoplankter in the deep euphotic zone of the Sargasso Sea. A high abundance of *P. marinus* has been observed in the deep euphotic zone in other oceanic regions as well: the eastern North Atlantic (Veldhuis et al. 1993), the Red Sea (Veldhuis & Kraay 1993), the North Pacific (Campbell & Vaulot 1993) and the equatorial Pacific (R. Olson, E. Zettler, J. Dusenberry & B. Binder unpubl. obs.). Thus, it is likely that strains similar to *P. marinus* SS120 are present in these locations as well.

Differences in seasonal and latitudinal distributions of *Prochlorococcus marinus* and *Synechococcus* in the Sargasso Sea cannot be explained by the temperature-dependent growth responses observed in this study (Fig. 3). Other factors, such as nutrient availability, are likely to be important environmental determinants. In the North Atlantic, *P. marinus* appear to be limited to regions with temperatures above 15 to 18 °C (Olson et al. 1990, Veldhuis et al. 1993). The presence of *P. marinus* strains other than those used in this study also may account for the different latitudinal and seasonal distributions observed in the North Atlantic.

The 2 clones of *Prochlorococcus marinus* used in this study differ significantly in their physiological responses to light. *P. marinus* MED4 is high-light adapted, exhibiting a light-dependent growth response similar to *Synechococcus* WH8103 (isolated from the surface of the Sargasso Sea), whereas *P. marinus* SS120 is low-light adapted. *P. marinus* MED4 has significantly reduced levels of chl b_2/chl a_2 relative to *P. marinus* SS120, which results in different absorption and fluorescence properties. Gene sequence analysis of the *rpoC1* RNA polymerase gene indicates that these 2 *P. marinus* clones are more divergent than 2 heterocyst-forming cyanobacteria from different morphological groups (Palenik & Haselkorn 1992), supporting the idea that these 2 *P. marinus* isolates are genetically distinct. On the other hand, these isolates are only 1.2% divergent in the slower-evolving 16S ribosomal RNA gene (E. Urbach unpubl. data), which suggests that these 2 clones are monophyletic. Analysis of 16S ribosomal RNA, *psbB* and combined *petB* and *petD* gene sequences has revealed that these 2 clones and other *P. marinus* isolates form a phylogenetic cluster (E. Urbach unpubl. data), suggesting that they should be considered a single genus, and possibly a single species. Further studies of the phylogenetic diversity of these organisms are needed to explore this issue.

It is possible that the differences in the physiological response to light between *Prochlorococcus marinus* SS120 and *P. marinus* MED4 are not due solely to the fact that they were isolated from 2 geographically distinct regions, but reflect the depth from which each was isolated. Several lines of evidence suggest that at least 2 strains of *P. marinus* may coexist at a given locality, and that these strains may be photoadapted for high and low light in ways that are similar to *P. marinus* MED4 and *P. marinus* SS120, respectively. (1) *P. marinus* are able to grow in the surface mixed layer of the Sargasso Sea (Goericke & Welschmeyer 1993), even though the Sargasso Sea *P. marinus* isolate is photoinhibited at high light. (2) Depth profiles of chl b_2/chl a_2 in the Sargasso Sea are suggestive of a high chl b_2-containing *P. marinus* at depth and a low chl b_2-containing *P. marinus* in the surface (Goericke & Repeta 1993). (3) At least 2 coexisting populations of *P. marinus* in the deep euphotic zone have been observed based on flow cytometric measurements in the North and South Pacific (Campbell & Vaulot 1993, B. Binder, R. Olson, J. Dusenberry & E. Zettler unpubl. obs.), the Red Sea (Veldhuis & Kraay 1993) and the Sargasso Sea (authors' unpubl. obs.).

The observed variability in chl *a*-specific absorption and fluorescence excitation spectra as a function of light is due to photoacclimative changes in pigment ratios

and not to pigment package effect, as evidenced by reconstructed absorption spectra. Our results differ from results for eukaryotic phytoplankton, where the package effect accounts for most of the changes in the blue region (chl *a* peak) of a^*_{ph} (Mitchell & Kiefer 1988, Berner et al. 1989). Reconstructed absorption spectra for *Prochlorococcus marinus* indicated that zeaxanthin is a major contributor to the magnitude and changes in the blue region of a^*_{ph} but does not contribute to chlorophyll emission, consistent with a non-photosynthetic role in these organisms (Kana et al. 1988, Bidigare et al. 1989b). Estimates of primary productivity and photosynthetic quantum yields could be incorrect by as much as 50% if the absorption of light due to zeaxanthin is included. The use of chl *a*-specific photosynthetically active absorption (a^*_{ps}) quantified from $F^*_{ph}(\lambda)$ to eliminate variability in a^*_{ph} due to non-photosynthetically active pigments has been suggested (Sosik & Mitchell unpubl. data). An additional error in the estimates of primary productivity and quantum yields may occur if an incorrect β-correction algorithm is used to correct absorption of cells on filters from waters dominated by picoplankton, in particular *P. marinus*.

In vivo a^*_{ph} in the blue for *Prochlorococcus marinus* is higher relative to that measured for eukaryotes (Maske & Haardt 1987, Mitchell & Kiefer 1988, Berner et al. 1989), resulting in a high B/R ratio, which could be used as an indicator of *P. marinus* when more reliable methods of detection, such as flow cytometry or HPLC, are unavailable. The high a^*_{ph} in the blue is due not only to high pigment-specific absorption by accessory pigments but also to the small cell size of *P. marinus* (Morel et al. 1993). The small cell size and chl b_2 content of *P. marinus*, particularly *P. marinus* SS120, are key features which give *P. marinus* a competitive advantage over *Synechococcus* and other phytoplankton in the deeper euphotic zone where nutrients are relatively available.

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LITERATURE CITED

Bazzaz, M. B. (1981). New chlorophyll chromophores isolated from a chlorophyll-deficient mutant of maize. Photobiochem. Photobiophys. 2: 199–207

Berner, T., Dubinsky, Z., Wyman, K., Falkowski, P. G. (1989). Photoadaptation and the 'package effect' in *Dunaliella tertiolecta* (Chlorophyceae). J. Phycol. 25: 70–78

Bidigare, R. R., Morrow, J. H., Kiefer, D. A. (1989a). Derivative analysis of spectral absorption by photosynthetic pigments in the western Sargasso Sea. J. mar. Res. 47: 323–341

Bidigare, R. R., Schofield, O., Prezelin, B. B. (1989b). Influence of zeaxanthin on quantum yield of photosynthesis of *Synechococcus* clone WH7803 (DC2). Mar. Ecol. Prog. Ser. 56: 177–188

Bidigare, R. R., Smith, R. C., Baker, K. S., Marra, J. (1987). Oceanic primary production estimates from measurements of spectral irradiance and pigment concentrations. Global biogeochem. Cycles 1: 171–186

Bricaud, A., Stramski, D. (1990). Spectral absorption coefficients of living phytoplankton and nonalgal biogenous matter: a comparison between the Peru upwelling area and the Sargasso Sea. Limnol. Oceanogr. 35(3): 562–582

Campbell, L., Holla, H. A., Vaulot, D. (1994). The importance of *Prochlorococcus* to community structure in the central North Pacific Ocean. Limnol. Oceanogr. (in press)

Castenholtz, R. W., Waterbury, J. B. (1989). Taxa of the Cyanobacteria in Bergey's 9th edn, Vol. III. Williams & Wilkins, Baltimore, p. 1727–1738

Chavez, F. P., Buck, K. R., Coale, K., Martin, J. H., DiTullio, G. R., Welschmeyer, N. A., Jacobson, A. C., Barber, R. T. (1991). Growth rates, grazing, sinking and iron limitation of equatorial Pacific phytoplankton. Limnol. Oceanogr. 36: 1816–1833

Chisholm, S. W., Frankel, S. L., Goericke, R., Olson, R. J., Palenik, B., Waterbury, J. B., West-Johnsrud, L., Zettler, E. R. (1992). *Prochlorococcus marinus* nov. gen. nov. sp.: a marine prokaryote containing divinyl chlorophyll *a* and *b*. Arch. Microbiol. 157: 297–300

Chisholm, S. W., Olson, R. J., Zettler, E. R., Goericke, R., Waterbury, J. B., Welschmeyer, N. A. (1988). A novel free-living prochlorophyte abundant in the oceanic euphotic zone. Nature 334: 340–343

Cleveland, J. S., Weidemann, A. D. (1993). Quantifying absorption by aquatic particles: a multiple scattering correction for glass-fiber filters. Limnol. Oceanogr. 38: 1321–1327

DiTullio, G. R., Hutchins, D. A., Bruland, K. W. (1992). Interaction of iron and major nutrients controls phytoplankton growth and species composition in the tropical North Pacific Ocean. Limnol. Oceanogr. 38: 495–508

Goericke, R., Repeta, D. J. (1992). The pigments of *Prochlorococcus marinus*: the presence of divinyl chlorophyll *a* and *b* in a marine procaryote. Limnol. Oceanogr. 37(2): 425–433

Goericke, R., Repeta, D. J. (1993). Chlorophylls *a* and *b* and divinyl chlorophylls *a* and *b* in the open subtropical North Atlantic Ocean. Mar. Ecol. Prog. Ser. 101: 307–313

Goericke, R., Welschmeyer, N. A. (1993). The marine prochlorophyte *Prochlorococcus* contributes significantly to phytoplankton biomass and primary production in the Sargasso Sea. Deep Sea Res. 40: 2283–2294

Guillard, R. R. L., Murphy, L. S., Foss, P., Liaaen-Jensen, S. (1985). *Synechococcus* spp. as likely zeaxanthin-dominant ultraphytoplankton in the North Atlantic. Limnol. Oceanogr. 30: 412

Jerlov, N. G. (1976). Marine optics. Elsevier Scientific Publ., New York, p. 231

Kana, T. M., Glibert, P. M. (1987). Effects of irradiances up to 2000 μE m^{-2} s^{-1} on marine *Synechococcus* WH7803 - I. Growth, pigmentation, and cell composition. Deep Sea Res. 34(4): 479–495

Kana, T. M., Glibert, P. M., Goericke, R., Welschmeyer, N. A. (1988). Zeaxanthin and β-carotene in *Synechococcus* WH7803 respond differently to irradiance. Limnol. Oceanogr. 33(6): 1623–1627

Mann, J. E., Myers, J. (1968). On pigments, growth, and photosynthesis of *Phaeodactylum tricornutum*. J. Phycol. 4: 349–355

Maske, H., Haardt, H. (1987). Quantitative *in vivo* absorption spectra of phytoplankton: detrital absorption and comparison with fluorescence excitation spectra. Limnol. Oceanogr. 32(3): 620–633

Melhuish, W. H. (1962). Calibration of spectrofluorometers for measuring corrected emission spectra. J. Optical Soc. Am. 52(11): 1256–1258

Mitchell, B. G. (1990). Algorithms for determining the absorption coefficient of aquatic particulates using the quantitative filter technique (QFT). SPIE Ocean Optics 1302: 137–148

Mitchell, B. G., Kiefer, D. A. (1988). Chlorophyll *a* specific absorption and fluorescence excitation spectra for light-limited phytoplankton. Deep Sea Res. 35(5): 639–663

Morel, A., Ahn, Y., Partensky, F., Vaulot, D., Claustre, H. (1993). *Prochlorococcus* and *Synechococcus*: a comparative study of their optical properties in relation to their size and pigmentation. J. mar. Res. 51: 617–649

Neveux, J., Vaulot, D., Courties, C., Fukai, E. (1989). Green photosynthetic bacteria associated with the deep chlorophyll maximum of the Sargasso Sea. C.r. Acad. Sci. Paris 308: 9–14

Olson, R. J., Chisholm, S. W., Zettler, E. Z., Altabet, M. A., Dusenberry, J. A. (1990). Spatial and temporal distributions of prochlorophyte picoplankton in the North Atlantic Ocean. Deep Sea Res. 37: 1033–1051

Otsuki, A., Watanabe, M. M., Sugahara, K. (1987). Chlorophyll pigments in methanol extracts from ten axenic cultured diatoms and three green algae as determined by reverse phase HPLC with fluorometric detection. J. Phycol. 23: 406–414

Palenik, B., Haselkorn, B. (1992). Multiple evolutionary origins of prochlorophytes, the chlorophyll *b*-containing prokaryotes. Nature 355: 265–267

Partensky, F., Hoepffner, N., Li, W. K. W., Ulloa, O., Vaulot, D. (1993). Photoacclimation of *Prochlorococcus* sp. (Prochlorophyta) strains isolated from North Atlantic and the Mediterranean Sea. Plant Physiol. 101: 285–296

Shedbalkar, V. P., Rebeiz, C. A. (1992). Chloroplast biogenesis: determination of the molar extinction coefficients of divinyl chlorophyll *a* and *b* and their pheophytins. Analyt. Biochem. 207: 261–266

Shibata, K. (1958). Spectrophotometry of intact biological materials. Absolute and relative measurements of their transmission, reflection and absorption spectra. J. Biochem. 45: 599–623

SooHoo, J. B., Kiefer, D. A., Collins, D. J., McDermid, I. S. (1986). *In vivo* fluorescence excitation and absorption spectra of marine phytoplankton: I. Taxonomic characteristics and responses to photoadaptation. J. Plankton Res. 8(1): 197–214

Sosik, H. M., Chisholm, S. W., Olson, R. J. (1989). Chlorophyll fluorescence from single cells: interpretation of flow cytometric signals. Limnol. Oceanogr. 34(8): 1749–1761

Stramski, D. (1990). Artifacts in measuring absorption spectra of phytoplankton collected on a filter. Limnol. Oceanogr. 35(8): 1804–1809

Vaulot, D. (1989). CYTOPC: processing software for flow cytometric data. Signal and Noise 2: 8

Vaulot, D., Courties, C., Partensky, F. (1989). A simple method to preserve oceanic phytoplankton for flow cytometric analyses. Cytometry 10: 629–635

Vaulot, D., Partensky, F. (1992). Cell cycle distributions of prochlorophytes in the northwestern Mediterranean Sea. Deep Sea Res. 39: 727–742

Vaulot, D., Partensky, F., Neveux, J., Mantoura, R. F. C., Llewellyn, C. A. (1990). Winter presence of prochlorophytes in surface waters of the northwestern Mediterranean Sea. Limnol. Oceanogr. 35: 1156–1164

Veldhuis, M. J. W., Kraay, G. W. (1990). Vertical distribution and pigment composition of a picoplanktonic prochlorophyte in the subtropical N. Atlantic: a combined study of HPLC-analysis of pigments and flow cytometry. Mar. Ecol. Prog. Ser. 68: 121–127

Veldhuis, M. J. W., Kraay, G. W. (1993). Cell abundance and fluorescence of picoplankton in relation to growth irradiance and nitrogen availability in the Red Sea. Neth. J. Sea Res. 31: 135–145

Veldhuis, M. J. W., Kraay, G. W., Gieskes, W. W. C. (1993). Growth and fluorescence characteristics of ultraplankton on a north-south transect in the eastern North Atlantic. Deep Sea Res. 40: 609–626

Waterbury, J. B., Watson, S. W., Valois, F. W., Franks, D. G. (1986). Biological and ecological characterization of the marine unicellular cyanobacterium *Synechococcus*. In: Platt, T., Li, W. K. W. (eds.) Photosynthetic picoplankton. Can. Bull. Fish Aquat. Sci. 214: 71–120

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