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Photophysiology of the marine cyanobacterium *Prochlorococcus:* Ecotypic differences among cultured isolates

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Abstract

Cultured isolates of *Prochlorococcus* from the Mediterranean Sea (MED4) and Sargasso Sea (SS120) have been shown to have dramatically different pigment composition and growth rate responses when grown over a range of irradiances. Moreover, analyses of field populations in the North Atlantic have shown that distinct ecotypes can coexist in the same water column. These and other observations have led to the hypothesis that *Prochlorococcus* is comprised of genetically distinct ecotypes that collectively expand the range of light intensities over which the genus can thrive. In this paper, we explore this hypothesis by comparing the photophysiology of 10 different *Prochlorococcus* isolates from diverse oceanographic regimes. We found that the 10 isolates could be grouped into two loose clusters based on their growth response to varying light intensity and their chlorophyll b/a_2 (Chl b/a_2) ratios. Although both groups photoacclimate when grown over a range of light intensities, isolates with distinctly higher Chl b/a_2 ratios (high B/A ecotype) reach maximal growth rates at lower irradiances ($I_{k,g}$), have high growth efficiencies (α_g), and are inhibited in growth at irradiances where isolates with low Chl b/a_2 ratios (low B/A ecotype) are growing maximally. High Chl b/a_2 ratios resulted in higher spectrally weighted average Chl a_2-specific absorption coefficient ($\bar{a}^*_{\text{chl}}$), Chl a_2-specific light-harvesting efficiency ($\alpha_{\text{chl}a}$), and quantum yield (ϕ_m) under low growth irradiances for the isolates of the high B/A ecotype relative to the others. The distinction between the high and low B/A ecotypes is supported by molecular phylogenies constructed using the 16S ribosomal ribonucleic acid (rRNA) gene. The physiological differences between the ecotypes most likely result in different relative distributions in a given water column and in fluctuations in their relative abundances as a function of seasonal dynamics and water-column stability.

Prochlorococcus, a Chl *b*-containing cyanobacterium (Palenik and Haselkorn 1992; Urbach et al. 1992; Pinevich et al. 1997), is the dominant phototroph found throughout the euphotic zone from temperate to tropical waters of the open oceans. Its vertical distribution spans from the surface to depths of 200 m, where the light level can be as low as 0.1% of surface irradiance (Chisholm et al. 1988; Olson et al. 1990*a*; Campbell and Vaulot 1993; Veldhuis and Kraay 1993; Lindell and Post 1995; Partensky et al. 1996; Campbell et al. 1997). *Prochlorococcus* populations exhibit unusually large changes in their ratio of Chl *b* to divinyl Chl *a* (Chl a_2) and flow cytometrically derived chlorophyll fluorescence per cell with depth (Olson et al. 1990*a*; Goericke and Repeta 1993; Partensky et al. 1996; Shimada et al. 1996; Campbell et al. 1997). In addition, bimodal frequency distributions of chlorophyll fluorescence per cell have been observed in the equatorial and North Pacific (Campbell and Vaulot 1993; Binder et al. 1996; Blanchot and Rodier 1996), the North Atlantic (Olson et al. 1991; McManus and Dawson 1994; Partensky et al. 1996; Moore et al. 1998), and the Red Sea (Veldhuis and Kraay 1993).

These features collectively suggested that at least two types of *Prochlorococcus* coexist in the oceans: one acclimated to the low light and high nutrient conditions of the deep euphotic zone and the other acclimated to high light and low nutrient conditions at the surface (Campbell and Vaulot 1993; Goericke and Repeta 1993; Partensky et al. 1996). Indeed, laboratory studies of two *Prochlorococcus* strains isolated from different oceans—MED4 from the Mediterranean Sea and SS120 from the Sargasso Sea—showed that the two strains were physiologically distinct. SS120, which was isolated from the deep euphotic zone, had a significantly higher Chl b/a_2 ratio and was better adapted for growth at lower light intensities than the surface isolate, MED4 (Partensky et al. 1993; Moore et al. 1995). Recently, we demonstrated that bimodal *Prochlorococcus* populations at two locations in the North Atlantic actually represented the coexistence of at least two physiologically and genetically distinct populations with high and low light-adapted characteristics similar to MED4 and SS120 (Moore et al.

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Table 1. Isolation information for *Prochlorococcus* isolates used in this study.

Isolate name	Location	Coordinates	Depth of isolation	Date isolated	Isolator
MIT9215*	Equatorial Pacific	0°, 140°W	Surface	Oct 92	B. Binder
MIT9201*	South Pacific	12°S, 145.4°W	Surface	Sep 92	B. Binder
MIT9202*	South Pacific	12°S, 145.4°W	79 m	Sep 92	B. Binder
MIT9302†	Sargasso Sea	34.8°N, 66.2°W	100 m	Jul 93	L. R. Moore
MIT9312†	Gulf Stream	37.5°N, 68.2°W	135 m	Jul 93	L. R. Moore
MED4‡	Mediterranean Sea	43.2°N, 6.9°E	5 m	Jan 89	D. Vaulot
MIT9211*	Equatorial Pacific	0°, 140°W	~83 m	Apr 92	R. Olson
MIT9303†	Sargasso Sea	34.8°N, 66.2°W	100 m	Jul 93	L. R. Moore
MIT9313†	Gulf Stream	37.5°N, 68.2°W	135 m	Jul 93	L. R. Moore
SS120*§	Sargasso Sea	29°N, 64.4°W	120 m	May 88	B. Palenik

* Isolates obtained by filter fractionation.
† Isolates obtained by sorting on flow cytometer.
‡ Same isolate as CCMP1378.
§ Same isolate as CCMP1375.

1998). In addition, phylogenetic analyses of 16S rDNA sequences for a number of *Prochlorococcus* isolates from diverse locations indicate that isolates with low Chl b/a_2 ratios formed a distinct cluster, which has been called the high light-adapted clade, whereas the other isolates, characterized by high Chl b/a_2 ratios, branched separately from this cluster (Moore et al. 1998; Rocap et al. 1998; Urbach et al. 1998).

Collectively, these observations led to the question of whether or not *all* isolates of *Prochlorococcus* could be characterized as either low or high light-adapted ecotypes based on their Chl b/a_2 ratio, growth response, and photosynthetic performance as a function of light intensity. We address this question here through a detailed comparative analysis of the photophysiology of 10 *Prochlorococcus* isolates from diverse oceans and depths.

Materials and methods

Isolation and culture conditions—Ten isolates of *Prochlorococcus* (Table 1) were examined for their physiological response to light availability. Isolation and culture conditions for the clonal isolates SS120 and MED4 have been described previously for their growth, pigment, and fluorescence data (Moore et al. 1995). Isolates MIT9302, MIT9303, MIT9312, and MIT9313 were obtained by sorting on a flow cytometer as described in Moore et al. (1998), and the four isolates from the Pacific Ocean, MIT9201, MIT9202, MIT9211, and MIT9215, were obtained by filter fractionation (as described in Chisholm et al. 1992). None of these eight isolates was rendered clonal by growing up from a single cell. However, they have been shown to have single 16S rDNA sequences (Moore et al. 1998; Rocap et al. 1998) and have always maintained a coherent flow cytometric signature. None of the isolates used in this study was axenic.

Batch cultures were grown in 25-mm glass test tubes on 14 : 10 light : dark (LD) cycle in Sargasso Sea water enriched with 10 μM NaH_2PO_4, 50 μM NH_4Cl, 100 μM urea, 1.17 μM EDTA, and the following trace metals: 8 nM Zn, 5 nM Co, 90 nM Mn, 3 nM Mo, 10 nM Se, 10 nM Ni, and 1.17 μM Fe (''PRO2'' media, a modification of K/10-Cu media outlined in Chisholm et al. 1992). The only exception to these conditions was for the growth, pigment, and fluorescence data for SS120 and MED4 taken from Moore et al. (1995), where the cultures were grown in 250-ml polycarbonate flasks in a medium that contained 10-fold higher concentration of EDTA (11.7 μM). Different light levels were obtained using cool-white fluorescent lamps in combination with layers of plastic neutral-density filters (Roscolux) and measured with a Biospherical QSL-100 light meter. Through a series of small incremental changes in irradiance levels (as suggested by Kana and Glibert 1987*a*), the cultures were acclimated to each irradiance level for at least 10 generations until balanced growth was established, as indicated by a constant chlorophyll fluorescence per cell. Various measurements were made during exponential phase once acclimation and balanced growth were established. Experiments made at irradiance levels where growth rate was zero were repeated to ensure that a zero growth was indeed real. All growth experiments were carried out at 24°C (±1), which is the optimal growth temperature for SS120 and MED4 (Moore et al. 1995) and is only slightly below the optimal growth temperature (25.5°C) for two Pacific isolates, MIT9211 and MIT9215 (Moore 1997).

Growth rate and flow cytometric measurements—Growth rate was determined by sampling each culture at the same time of day over several generations using a FACScan flow cytometer (Becton-Dickinson) to enumerate the cells. Mean fluorescence per cell was also determined flow cytometrically and is reported relative to 0.57-μm-diameter yellow-green, polystyrene microspheres (Polysciences). Data were analyzed using CYTOPC software (Vaulot 1989). As a means of comparing the light-dependent growth response between isolates, four parameters were used: μ_{max}, the light-saturated, maximum growth rate for the temperature and media conditions used; α_g, the growth efficiency under subsaturating light; and $I_{k,g}$, the light level at which μ_{max} is reached. The parameters were calculated as follows: μ_{max} from the average of the light-saturated growth rates, α_g from the initial slope of the μ vs. I_g curve, and $I_{k,g}$ from the ratio of μ_{max} to α_g.

Pigment measurements—Pigments were measured using high-pressure liquid chromatography (HPLC), as described in Goericke and Repeta (1993). Throughout this paper, we

use ''Chl *b*'' to refer to both monovinyl and divinyl Chl *b* (Chl b_1 and Chl b_2, respectively), as several isolates contain both (*see Results and discussion*). For pigment analyses, cultures were harvested from exponential growth phase by filtering a known volume (10–25 ml) onto 25-mm Whatman GF/F filters under low vacuum. Filters were stored in liquid nitrogen until extraction (as described in Goericke and Welschmeyer 1993) and analyzed on a C-8 column-based reverse-phase Beckman Gold HPLC system. All pigments were identified based on their relative retention times (Goericke and Repeta 1993) and quantified using integrated absorbance at 440 nm for Chl a_2, zeaxanthin, and α-carotene and 478 nm for Chl b_1 and Chl b_2. The chromatographic systems were calibrated with Chl b_1 extracted from spinach and Chl a_2 and Chl b_2 from *Prochlorococcus* cultures by collecting each pigment as it eluted off the HPLC, drying under N_2(g), resuspending in 90% acetone, quantifying spectrophotometrically using known extinction coefficients (Goericke and Repeta 1993), and then injecting a dilution series for each pigment on the HPLC system. For pigment analysis of cultures used in photosynthesis–irradiance experiments (P-I) (*see below*), Chl a_2 and Chl *b* were extracted as described above and quantified spectrophotometrically using the trichromatic equations of Jeffrey and Humphrey (1975).

P-I measurements—For P-I experiments, all 10 isolates were grown at 24°C on a 14 : 10-LD cycle at a low (~9 μmol Q m^{-2} s^{-1}) and relatively high growth irradiance (~70 μmol Q m^{-2} s^{-1}, except for MIT9303, where 52 μmol Q m^{-2} s^{-1} was used because its range of saturated growth is more narrow than the other isolates) using cool-white fluorescent lamps. Photosynthesis, cell counts by flow cytometry, and absorption measurements (*see below*) were made, and samples for pigment analysis were harvested during exponential growth phase. Measurements were performed at the same time of day (4–6 h into light cycle) for each culture to avoid diel effects. Photosynthesis was measured by incubating exponentially growing cells with $NaH^{14}CO_3$ (0.1 μCi ml^{-1}; specific activity between 10^5 and 2×10^5 DPM) at 23 ± 1°C for 45 min and was terminated by acidification, as described in Moore et al. (1998). Irradiances were obtained using very high output/daylight spectrum fluorescent bulbs attenuated with neutral-density filters. Chl a_2- and cell-normalized data were fitted to the equation of Platt et al. (1980) using the curve-fitting program in SigmaPlot (Jandel Scientific) to obtain photosynthesis parameters: maximum rate of photosynthesis, P_{max} (fg C (fg Chl a_2)$^{-1}$ h^{-1} or fg C $cell^{-1}$ h^{-1}), the initial slope of the P-I curve, α (fg C (fg Chl a_2)$^{-1}$ h^{-1} (μmol Q m^{-2} s^{-1})$^{-1}$ or fg C $cell^{-1}$ h^{-1} (μmol Q m^{-2} s^{-1})$^{-1}$), the irradiance at which photosynthesis is saturated, I_k (μmol Q m^{-2} s^{-1}), and the index of photoinhibition, I_b (μmol Q m^{-2} s^{-1}). The maximum quantum yield of photosynthesis, ϕ_m (mol C mol Q^{-1}) was calculated from the ratio of α_{chla} to $\bar{a}^*_{chl}$. The rate values presented here are gross photosynthesis, as respiratory losses were assumed to be small due to the short incubation times used (45 min). Measurements were carried out on duplicate cultures for each strain and treatment, and all values reported are the mean (±1 SE) of duplicate cultures.

Absorption measurements—An in vivo absorption spectrum was obtained for each culture used in the P-I experiments. A Beckman DU-640 spectrophotometer was used, with an opal diffuser placed between the suspended cells and the detector opening to minimize particle-scattering effects (when necessary, cultures were concentrated by centrifugation, as described in Moore et al. 1995). The spectrally weighted average Chl a_2-specific absorption coefficient, $\bar{a}^*_{chl}$ (m^2 (mg Chl a_2)$^{-1}$), was calculated over the photosynthetically available radiation (PAR) range (λ = 400–700 nm) as follows:

$$\bar{a}^*_{chl} = \frac{\int a^*_{chl}(\lambda) \cdot E(\lambda)\, d\lambda}{\int E(\lambda)\, d\lambda} \quad (1)$$

where $a^*_{chl}(\lambda)$ is the Chl a_2-specific absorption and $E(\lambda)$ is the energy spectrum (W nm^{-1}) of the daylight fluorescent lamps used for P-I experiments.

Results and discussion

Growth rate vs. irradiance—All *Prochlorococcus* isolates exhibited steep initial slopes of growth rate vs. irradiance curves and began to saturate at relatively low growth irradiances, such that differences between isolates are best observed by plotting irradiance on a log scale (Fig. 1A,B). The initial slope of the growth vs. irradiance on a linear scale, α_g, ranged from 0.013 to 0.027 d^{-1} (μmol Q m^{-2} s^{-1})$^{-1}$ (Table 2), which is on the high end of values reported for other phytoplankton species from diverse taxa (Langdon 1988; Iriarte and Purdie 1993; Milligan and Cosper 1997). Species with approximately twofold higher α_g values include the chlorophytes *Chlorella pyrenoidosa* (Bannister 1979, as reported in Langdon 1988) and *Pycnococcus provasolii* (Iriarte and Purdie 1993). The growth of all the *Prochlorococcus* isolates saturated at low irradiances, $I_{k,g}$ = 22–59 μmol Q m^{-2} s^{-1} (Table 2), and the range of irradiances over which they grew before growth was inhibited due to high light was relatively narrow, approximately two orders of magnitude. Most other species that have been studied are capable of growth over larger irradiance ranges and typically do not show inhibition at the relatively low irradiances seen for *Prochlorococcus.* For example, growth occurs over three orders of magnitude for the diatom *Phaeodactylum tricornutum* (Nelson et al. 1979) and the cyanobacterium *Synechococcus* WH7803 (Kana and Glibert 1987*a*).

There are distinct differences between *Prochlorococcus* isolates in their growth response to light intensity. At first glance, the growth rate curves of the 10 isolates may appear to form a continuum, particularly at subsaturating irradiances, but upon closer examination, they can be clumped into two distinct groups based on their $I_{k,g}$ (Table 2). One group, which did not show growth inhibition until light intensities exceeded ~250 μmol Q m^{-2} s^{-1} (Fig. 1A), consists of previously designated high light-adapted isolates MED4, MIT9312, and MIT9302 (Moore et al. 1995, 1998) and also includes three isolates from the Pacific, MIT9201, MIT9202,

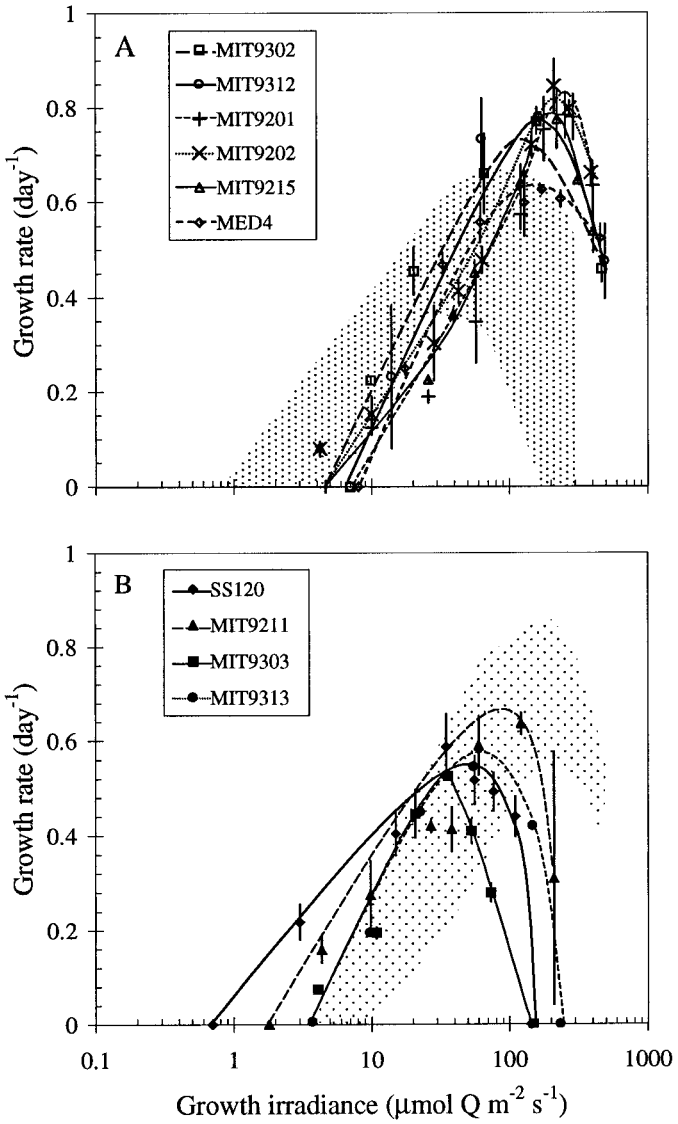

Fig. 1. Growth rate vs. irradiance curves for each isolate. (A) Data points are for high light-adapted isolates, and the shadow represents the range of data corresponding to the low light-adapted isolates. (B) Data points are for low light-adapted isolates, and the shadow represents the range of data corresponding to the high light-adapted isolates. Curves fitted by eye. Data for SS120 and MED4 are from Moore et al. (1995), and data for MIT9302, MIT9303, MIT9312, and MIT9313 are from Moore et al. (1998).

and MIT9215. The other group (isolates SS120, MIT9211, MIT9303, and MIT9313; Fig. 1B) exhibited $I_{k,g}$ values that were about half those of the high light-adapted isolates and (with the exception of MED4) had significantly lower μ_{max} values (Table 2). Under the growth conditions of this study, these four low light-adapted isolates were incapable of growth above ~200 μmol Q m^{-2} s^{-1}, where the other isolates were at or near maximum growth rates (compare Fig. 1A,B). At subsaturating light intensities, the responses of the four low light-adapted isolates were less uniform. Both the type strain SS120 and the Pacific isolate MIT9211 were able to grow at the lowest irradiances of any of the isolates; the Atlantic isolate MIT9303 had a very narrow range of light intensities over which it could grow (3.6–73 μmol Q m^{-2} s^{-1}); and the Atlantic isolate MIT9313 had an intermediate response between MIT9303 and MIT9211.

Table 2. Growth parameters from growth vs. light curves for each isolate. Maximum light-saturated growth rate, μ_{max}, in units of d^{-1}; growth efficiency under subsaturating light intensities, α_g, in units of d^{-1} (μmol Q m^{-2} s^{-1})$^{-1}$; and irradiance at which growth is saturated, $I_{k,g}$, in units of μmol Q m^{-2} s^{-1}.

Isolate	Mean μ_{max}	±SE	Mean α_g	±SE	Mean $I_{k,g}$	±SE
MIT9215	0.77	0.05	0.013	0.002	59	13
MIT9201	0.79	0.04	0.020	0.005	40	12
MIT9202	0.83	0.05	0.014	0.002	59	12
MIT9302	0.75	0.03	0.014	0.003	56	13
MIT9312	0.78	0.01	0.021	0.002	36	5
MED4	0.63	0.06	0.014	0.001	45	7
MIT9211	0.63	0.02	0.027	0.006	23	6
MIT9303	0.51	0.03	0.023	0.002	22	3
MIT9313	0.54	0.04	0.022	0.004	25	6
SS120	0.53	0.06	0.020	0.005	27	10

The grouping of the isolates based on light-dependent growth rate response is consistent with their molecular phylogenies, based on 16S rDNA sequences. The high light-adapted isolates form a distinct cluster among the marine cyanobacteria, which has been designated the high light-adapted clade (Moore et al. 1998; Rocap et al. 1998; Urbach et al. 1998). The four low light-adapted isolates branch separately from the high light-adapted clade in these trees but do not form a distinct cluster (Moore et al. 1998; Rocap et al. 1998; Urbach et al. 1998), which is consistent with their nonuniform growth response to light intensity. Similar phylogenetic patterns also have been obtained from sequence analyses of two ecologically relevant functional genes, *psbB* (which codes for the Chl *a*-binding antenna protein CP47; Urbach et al. 1998) and *ntcA* (which codes for the global nitrogen regulator NtcA; Lindell and Post unpubl. data).

The growth rates of *Prochlorococcus* reported here are consistent with estimates of growth rates in the field at comparable light intensities. Within and below the subsurface chlorophyll maximum (70–200 m), estimates of growth rates range between 0.04 and 0.39 d^{-1} for different oceans (Goericke and Welschmeyer 1993; Binder et al. 1996; Partensky et al. 1996; Liu et al. 1997). In our cultures, an I_g of 10 μmol Q m^{-2} s^{-1} (which corresponds to a depth of 118 m, assuming an attenuation coefficient of k = 0.045 m^{-1} and surface irradiance of I_o = 2,000 μmol Q m^{-2} s^{-1}) yielded growth rates ranging from 0.04 to 0.25 d^{-1} for the high light-adapted isolates and from 0.18 to 0.41 d^{-1} for the low light-adapted isolates (Fig. 1A,B). This suggests that growth rates reported for some deep populations in situ may represent a composite growth rate for multiple ecotypes.

The complete photoinhibition of the low light-adapted isolates grown at light intensities above ~200 μmol Q m^{-2} s^{-1} raises questions as to whether they could survive in a surface mixed layer. For example, in an upper mixed layer extending to 50 m, PAR can range from 210 to 2,000 μmol Q m^{-2} s^{-1} (assuming the same k and I_o as above); these light levels far

exceed the upper irradiance of growth for the low light-adapted isolates (Fig. 1B). Complete inhibition of growth due to high light exposure is not a common observation for most phytoplankton, although it has been found for oceanic species of dinoflagellates at ~800 μmol Q m^{-2} s^{-1} (Brand and Guillard 1981). *Prochlorococcus* spp. are found in surface waters, however, and estimates of in situ growth rates in surface waters reach 0.75 d^{-1} (Goericke and Welschmeyer 1993; Landry et al. 1995; Vaulot et al. 1995; Liu et al. 1997; Veldhuis et al. 1997; Shalapyonok et al. 1998), similar to the μ_{max} of the high light-adapted isolates (Table 2). This, in combination with the complete photoinhibition of the low light-adapted isolates, suggests that populations of *Prochlorococcus* in surface-stratified water columns are likely composed only of high light-adapted ecotypes.

Chl b/a$_2$ *ratio*—All the *Prochlorococcus* isolates photoacclimate, i.e., they increase their chlorophyll content as irradiance decreases. Increases in Chl *b* surpassed those of Chl a_2 such that the ratio of Chl b/a_2 increased as irradiance decreased, though to differing degrees for different isolates (Fig. 2A). The six high light-adapted isolates clustered together with low ratios of Chl b/a_2 (0.05–0.65), whereas the other four had the highest ratios (0.47–2.6). Among the low light-adapted isolates, SS120 and MIT9211 had the highest Chl b/a_2 ratios and were slightly separated from MIT9303 and MIT9313, suggesting that these four isolates do not form a single cluster, which is again consistent with the growth patterns and molecular phylogeny.

At any particular growth irradiance, the high light-adapted isolates have significantly lower Chl b/a_2 ratios than the low light-adapted isolates ($P < 0.01$), which, thus far at least, allows this parameter to serve as a distinguishing characteristic between these two groups, as long as the light history is known. Two clonal isolates from the northwestern Pacific, GP2 and SB, have low Chl b_2/a_2 ratios (0.17 and 0.13, respectively) when grown at low blue-green light (6 μmol Q m^{-2} s^{-1}; Shimada et al. 1996). From this, we would predict that these two isolates would have a high light-adapted growth response. Consistent with this characterization, phylogenetic relationships inferred from 16S rDNA sequences place these two isolates in the high light-adapted clade (Urbach et al. 1998). Chl b/a_2 ratios also have been instrumental in generating hypotheses about distributions of natural populations of *Prochlorococcus.* In situ estimates of Chl b/a_2 ratios in surface waters are typically <0.7 fg/fg (Goericke and Repeta 1993; Claustre and Marty 1995), consistent with the ratios characteristic of *Prochlorococcus* isolates capable of growth in higher light intensities. Ratios in the deep euphotic zone shift to values as high as 3 fg/fg (Goericke and Repeta 1993; Claustre and Marty 1995), spanning the range found for the low light-adapted isolates, strongly supporting the hypothesis that *Prochlorococcus* ecotypes partition the water column with respect to depth (Campbell and Vaulot 1993; Goericke and Repeta 1993; Moore et al. 1998).

Because the distinction between the low and high light-adapted groups is most clear based on their Chl b/a_2 ratios, and in keeping with the use of pigment differences to characterize different types of *Synechococcus* (e.g., high and low PUB types; *see* Waterbury et al. 1986), we will refer to high light-adapted *Prochlorococcus* as the low B/A ecotype and low light-adapted isolates as the high B/A ecotype throughout the rest of the paper.

Only four of the isolates—two low B/A (MIT9302 and MIT9312) and two high B/A (MIT9211 and SS120)—contain the more typical, monovinyl Chl b_1 in addition to divinyl Chl b_2, as has been previously reported for SS120 (Partensky et al. 1993; Moore et al. 1995) (data not shown). Chl b_1 was only present as maximal growth rates were achieved, and it increased relative to Chl b_2 as irradiances became photoinhibitory for both the low and high B/A isolates. In the former, Chl b_1 constituted 9–37% of the total Chl *b*, whereas it reached 71% of the total Chl *b* in MIT9211. Another interesting pigment anomaly is the presence of an unidentified pigment in MIT9303 and MIT9313, which elutes from the HPLC system 0.71 min after the zeaxanthin peak. This pigment (possibly the carotenoid parasiloxanthin; Goericke pers. comm.) was present only under low growth irradiances. Although pigment composition may not, by itself, be the best taxonomic criteria (Pinevich et al. 1997), the presence of additional pigments in some, but not all, members of each ecotype points more strongly to the possibility of subgroups within the high and low B/A ecotypes.

Chlorophyll fluorescence per cell—Because the difference in flow cytometrically derived chlorophyll fluorescence per cell was one of the first indications of the coexistence of different types of *Prochlorococcus* in natural populations (Campbell and Vaulot 1993; Veldhuis and Kraay 1993), we examined this parameter in the cultured isolates to see if it could also be used to distinguish between the two ecotypes. We found that although the two ecotypes could be loosely clustered according to this parameter, there was significant overlap, particularly at high light intensities (Fig. 2B). This is because the fluorescence signal is tightly correlated with total pigment content (Chl $a_2 + b_1 + b_2$) ($r^2 = 0.88$) and not as tightly with the Chl b/a_2 ratio. Thus, this parameter was not a distinguishing characteristic between the two groups.

The high B/A isolates, SS120, MIT9211, MIT9303, and MIT9313, all exhibited small but significant amounts of flow cytometrically induced orange (585 nm) fluorescence that increased with decreasing growth irradiance (data not shown). This is consistent with observations of the phycoerythrin (PE) genes in SS120 (Hess et al. 1996; Hess 1998) and MIT9303 (Ting et al. 1998). Furthermore, low levels of orange fluorescence have been reported for a natural population of *Prochlorococcus* in the bottom euphotic layer of the tropical Pacific, which have been attributed to the presence of small amounts of PE (Hess et al. 1996).

Absorption properties—As presented previously for isolates SS120 and MED4 (Morel et al. 1993; Moore et al. 1995), changes in the ratio of Chl b/a_2 are reflected in the in vivo Chl a_2-specific absorption spectra of all *Prochlorococcus* isolates (Fig. 3). When grown under low irradiance, a distinct peak associated with Chl $b_1 + b_2$ ($\lambda \cong 480$ nm) is apparent for the four high B/A isolates. Photoacclimative changes in the concentrations of the pigments are also apparent in the ratio of the total Chl *b* peak in the blue relative

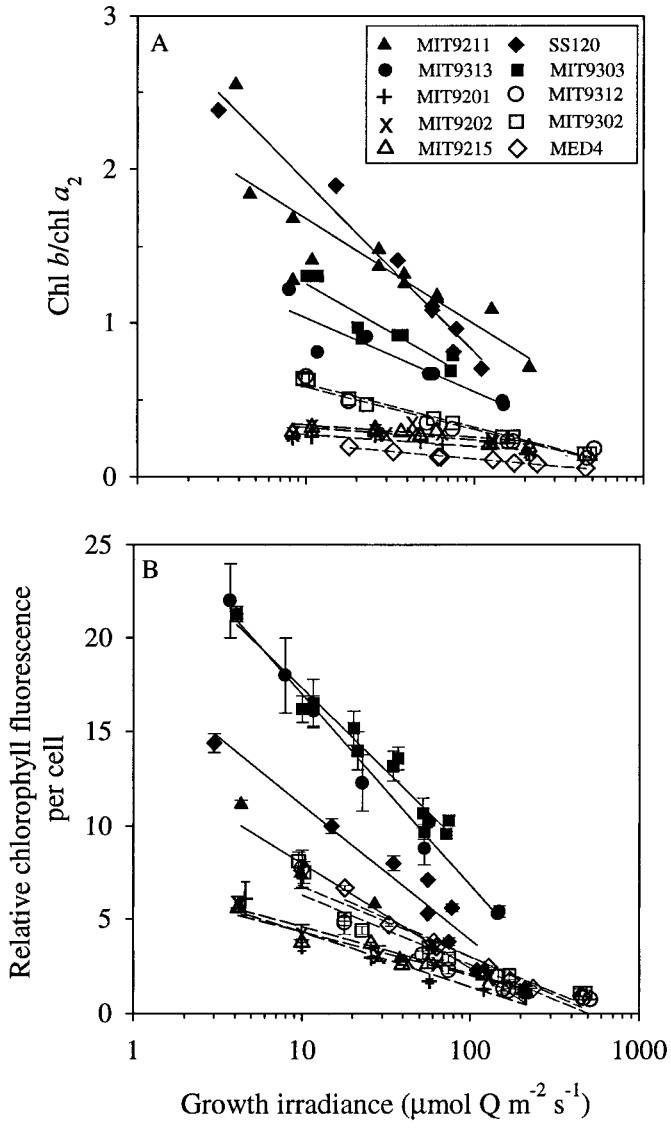

Fig. 2. Chl b/a_2 ratio (fg/fg) and relative mean chlorophyll fluorescence per cell as a function of growth light intensity. (A) Ratio of Chl b/a_2 (fg/fg) vs. irradiance. (B) Relative mean chlorophyll fluorescence per cell vs. irradiance. Values are means (±1 SE) of duplicate cultures. Data for SS120 and MED4 in both panels are from Moore et al. (1995). Symbols for each isolate are the same as in Fig. 1.

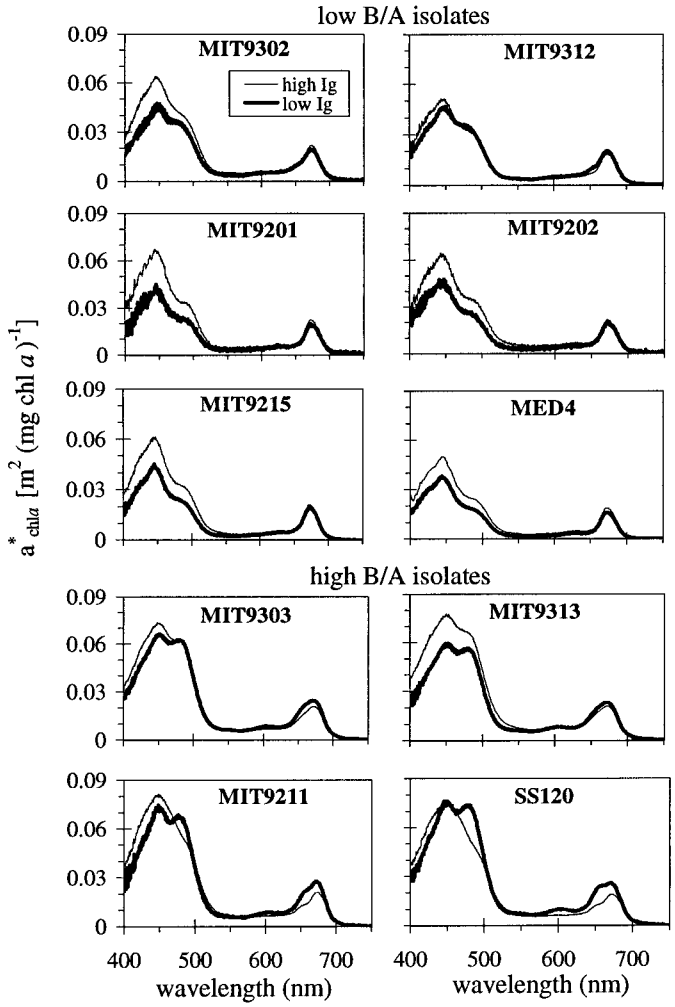

Fig. 3. Chl a_2-specific absorption spectra (m^2 (mg Chl a_2)$^{-1}$) for each isolate grown at two growth irradiances (I_g): low I_g = 9 μmol Q m^{-2} s^{-1} (thick lines) and high I_g = 70 μmol Q m^{-2} s^{-1} (52 μmol Q m^{-2} s^{-1} for MIT9303) (thin lines).

to the Chl a_2 blue peak (a_{480}/a_{449}) and the ratio of peak absorption of Chl a_2 in the blue relative to that in the red (a_{449}/a_{680}; Fig. 3). All *Prochlorococcus* isolates examined to date and a deep *Prochlorococcus* population in the Arabian Sea (Johnson et al. 1998) have values of a_{449}/a_{680} (B/R ratio) exceeding those observed for other phytoplankton (SooHoo et al. 1986; Maske and Haardt 1987; Mitchell and Kiefer 1988; Moore et al. 1995), allowing this parameter to be used as a rough indicator of the presence of these cells. As expected, the average Chl a_2-specific absorption coefficients spectrally weighted to the daylight lamps ($\bar{a}^*_{chl}$) are 1.6-fold higher for the four high B/A isolates relative to the low B/A isolates (average $\bar{a}^*_{chl}$ = 0.029 ± 0.003 vs. 0.018 ± 0.003 m^2 (mg Chl a_2) $^{-1}$, respectively; $P < 0.01$).

Photosynthesis characteristics—The P-I curves of the isolates examined in this study (Fig. 4) were similar to those reported previously for MED, SARG, and NATL1 (Partensky et al. 1993). Many, but not all, of the photosynthetic parameters reveal differences between the isolates consistent with the high and low B/A categorization. At low growth irradiance, the high B/A isolates are about twice as effective at utilizing available light energy as the low B/A isolates (average α_{chla} = 0.11 vs. 0.05 fg C fg Chl a^{-1} h^{-1} (μmol Q m^{-2} s^{-1})$^{-1}$; $P < 0.01$) (Fig. 5A). As has been shown for a natural *Prochlorococcus* population in the Arabian Sea (Johnson et al. 1998) and other cyanobacteria (Raps et al. 1983; Kana and Glibert 1987*b*), this can be attributed to an increase in energy transfer from the accessory pigments to Chl a_2, as indicated by a positive correlation between α_{chla} and the Chl b/a_2 ratio in this group (r^2 = 0.799; Fig. 6A). Partensky et al. (1997) have shown that this increased effi-

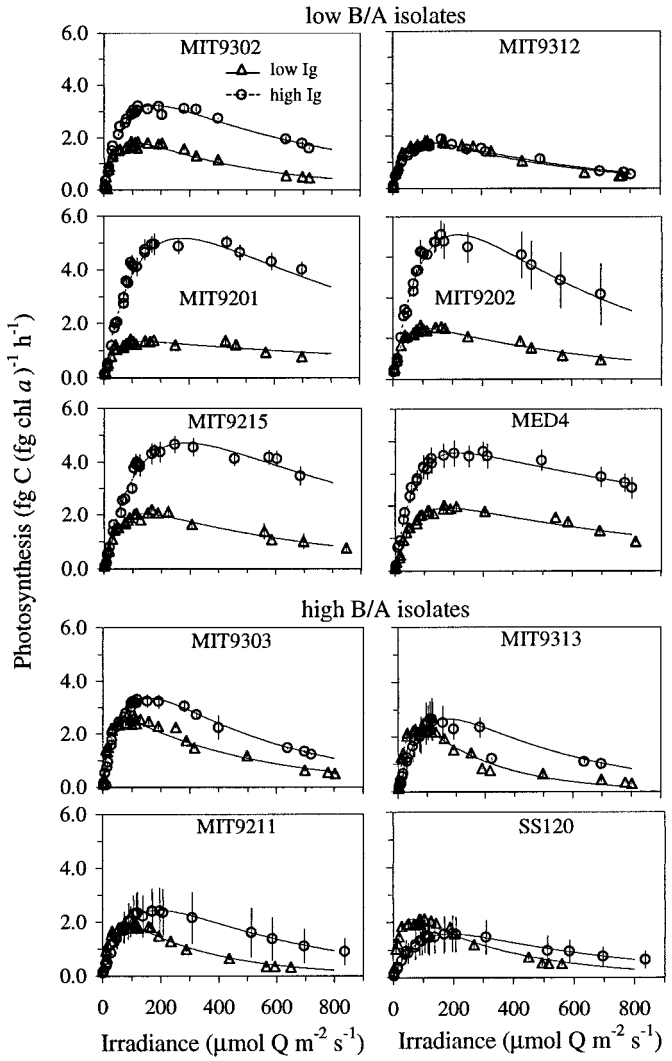

Fig. 4. Average Chl a_2-normalized P-I curves for each isolate grown at low and high growth irradiances: low I_g (triangles) = 9 μmol Q m^{-2} s^{-1} and high I_g (circles) = 70 μmol Q m^{-2} s^{-1} (52 μmol Q m^{-2} s^{-1} for MIT9303). Values are means ($\pm$1 SE) of duplicate cultures. Error is smaller than symbol size for data without error bars.

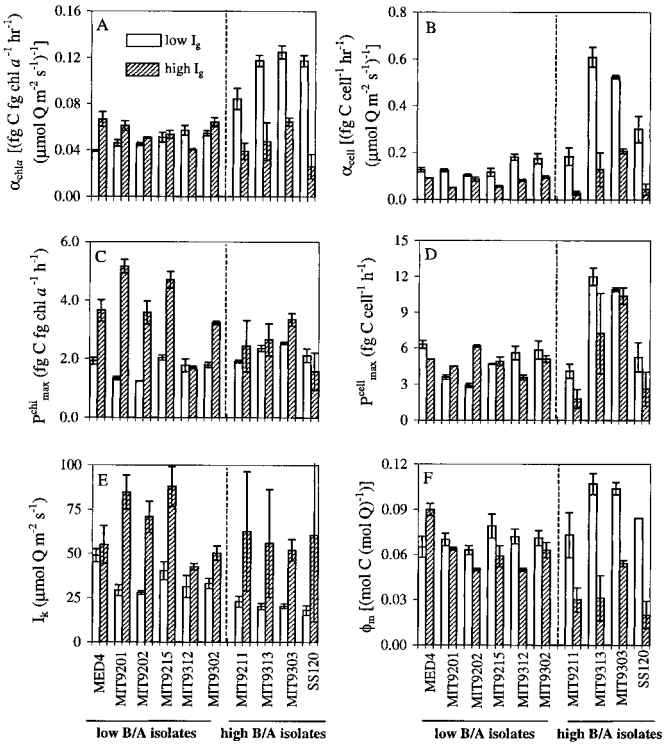

Fig. 5. P-I curve parameters for each isolate. Error bars are $\pm$1 SE of duplicate cultures. (A) α_{chla}. (B) α_{cell}. (C) P^{chl}_{max}. (D) P^{cell}_{max}. (E) $I_{k,g}$. (F) ϕ_m.

ciency is accomplished by an increase in the concentration of antenna complexes but not the concentration of photosystem I and II cores in SS120. When acclimated to high growth irradiances, α_{chla} of the high B/A isolates decreased, while that of the low B/A isolates stayed roughly the same (Fig. 5A). The regulation of α_{chla} for the low B/A isolates is difficult to assess based on P-I measurements because the changes are small; however, the same mechanisms of photoacclimation are apparently at play, though to a smaller degree (Partensky et al. 1997).

The overall range of photosynthetic efficiency values measured in this study (α_{chla} = 0.03–0.125 fg C fg Chl a^{-1} h^{-1} (μmol Q m^{-2} s^{-1})$^{-1}$) also is consistent with value ranges previously reported for *Prochlorococcus* (Partensky et al. 1993; Shimada et al. 1996) and *Synechococcus* (Alberte et al. 1984; Kana and Glibert 1987*b*; Bidigare et al. 1989; Shimada et al. 1996). Even though these values need to be reduced by ~20% for comparison with other studies in which cool-white fluorescent lamps were used, they still fall in the high end of values relative to most other phytoplankton (*see* Langdon 1988).

In contrast to the findings of Partensky et al. (1993), all the isolates had higher cell-specific light-harvesting efficiency (α_{cell}) when grown in low light than in high light (Fig. 5B). α_{cell} varied significantly among the high B/A isolates, with low light-grown MIT9313 and MIT9303 having the highest α_{cell} values ever reported for *Prochlorococcus* (Partensky et al. 1993; Shimada et al. 1996) or *Synechococcus* (Glibert et al. 1986; Kana and Glibert 1987*b*), again pointing to the extremely efficient light-harvesting capabilities of this organism. The variability in α_{cell} in all the isolates is well correlated with the total cellular chlorophyll content (Chl a_2 + b_1 + b_2), though the relationship differs for the low and high B/A isolates (Fig. 6B), reflecting different photoacclimative strategies between these two ecotypes.

Maximum photosynthetic rates normalized to Chl a_2 ($P^{chl\,a}_{max}$) varied significantly among the different isolates (Fig. 5C), ranging from 1.3 to 5.2 fg C fg Chl a^{-1} h^{-1}, and are comparable to values reported by others for *Prochlorococcus* (Partensky et al. 1993) and other picophytoplankton (Alberte et al. 1984; Johnsen et al. 1992). They were generally higher for cells grown at high light, and the differences between high and low light-grown cells were only pronounced among the low B/A isolates, except for MIT9312 (Fig. 5C). The lack of photoacclimative response of photosynthesis in

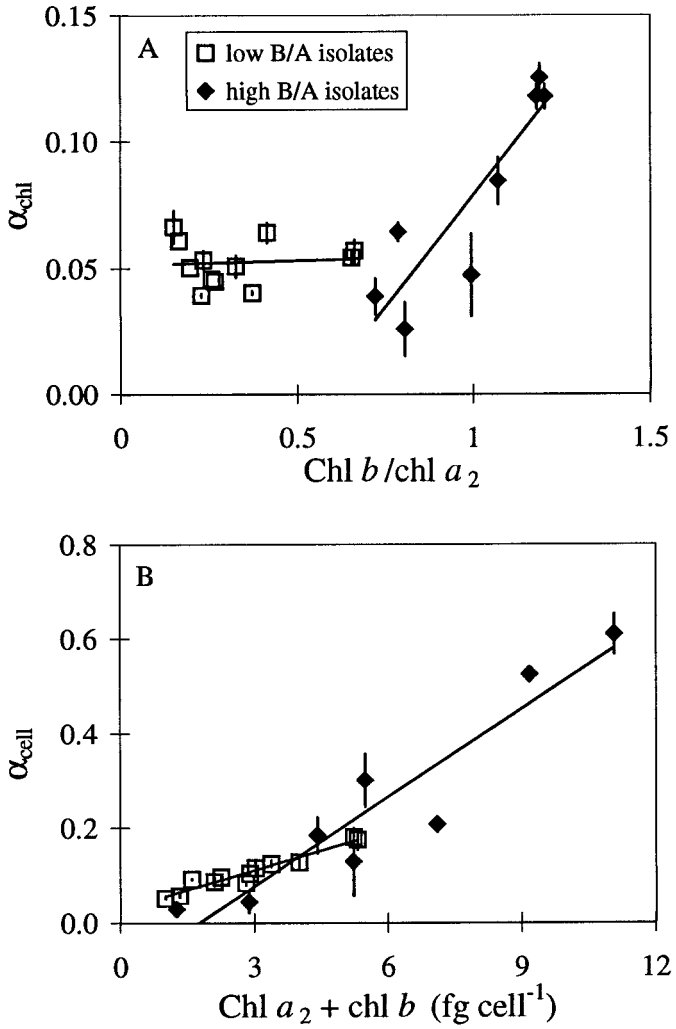

Fig. 6. (A) α_{chla} as a function of chl b/a_2 ratio. (B) α_{cell} as a function of total cellular chlorophyll. High B/A (closed diamonds) and low B/A isolates (open squares). Values are means (±1 SE) of duplicate cultures. Error is smaller than symbol size for data without error bars.

MIT9312 is surprising, and we have no explanation for it at the moment.

When P_{max} was normalized to cell (P^{cell}_{max}), the overall range of values was much greater (1.8–12 fg C cell^{-1} h^{-1}) (Fig. 5D), and growth irradiance did not have a pronounced effect on the values. As observed for α_{cell}, the two high B/A isolates MIT9303 and MIT9313 exhibited the highest light-saturated maximum photosynthetic rates under both growth irradiances examined (Fig. 5D), approaching values reported for *Synechococcus* (Wood 1985; Glibert et al. 1986; Waterbury et al. 1986; Kana and Glibert 1987*b*; Shimada et al. 1996). Increases in P^{cell}_{max} were correlated with increases in total cellular chlorophyll content (Chl $a_2 + b_1 + b_2$) for the high B/A isolates ($r^2 = 0.91$) but not the low B/A isolates (data not shown).

Photoinhibition of photosynthesis at high light intensities was a consistent feature for all of the isolates regardless of growth irradiance (Fig. 4). The irradiance at which photoinhibition is first apparent (I_b) was generally lower in the high B/A isolates, consistent with the relative differences in photoinhibition of growth response between the two groups (Fig. 1A,B). Because of the high initial slope of the P-I curves and low P^{chla}_{max}, the irradiance at which photosynthesis saturated (I_k) was very low (18–87 μmol Q m^{-2} s^{-1}) and was lowest, on average, for the high B/A *Prochlorococcus*. Also, as expected, I_k increased for cultures grown under higher irradiances (Fig. 5E).

The maximum quantum yield of photosynthesis (ϕ_m) of all isolates grown at low light was relatively high (0.063–0.107 mol C mol Q^{-1}) (Fig. 5F), approaching the theoretical maximum of 0.125 mol C mol Q^{-1}. Similarly high values of ϕ_m (up to 0.10 mol C mol Q^{-1}) have been reported previously for this group (Partensky et al. 1993), *Synechococcus* (Iturriaga and Mitchell 1986; Bidigare et al. 1989), and for some eukaryotic species, such as *P. tricornutum* (Geider et al. 1985) and the prymnesiophyte *Chrysochromulina polylepis* (Johnsen et al. 1992). The value of ϕ_m decreased by at least half (1.9- to 4.2-fold) when the high B/A isolates were grown at higher light intensities, whereas the low B/A isolates showed only a minor response. These patterns were strongly correlated with changes in α_{chla} ($r^2 = 0.96$). Although decreases in ϕ_m at high growth irradiances have been reported previously for *Prochlorococcus* (Partensky et al. 1993), *Dunaliella tertiolecta* (Sukenik et al. 1990), and *P. tricornutum* (Geider et al. 1985), decreases of the magnitude we observed have only been reported for natural phytoplankton assemblages. The latter have been attributed to nitrogen limitation and/or high light absorption by nonphotosynthetic pigments (Cleveland et al. 1989; Kolber et al. 1990; Babin et al. 1996; Sosik 1996). Absorption of light by zeaxanthin, the major nonphotosynthetic pigment in *Prochlorococcus*, may be responsible for some of the changes in ϕ_m we observed in the high B/A cells. Unfortunately, we did not measure this pigment for the cultures used in the P-I experiments.

Ecological implications of Prochlorococcus *ecotypes*—It has been hypothesized that *Prochlorococcus* ecotypes partition the water column with respect to depth, high B/A ecotypes (previously called low light adapted) primarily occupying the deep euphotic zone and low B/A ecotypes (previously called high light adapted) extending to the surface waters (Campbell and Vaulot 1993; Goericke and Repeta 1993; Moore et al. 1998). Indeed, the range of Chl b/a_2 ratios measured in the field is broader than that of any one isolate, and the ratios found in the surface and deep euphotic zone (Goericke and Repeta 1993; Claustre and Marty 1995) are consistent with the characteristic Chl b/a_2 ratios and light-dependent growth responses of low B/A and high B/A *Prochlorococcus*, respectively. Some preliminary phylogenetic analyses of natural populations (Ferris and Palenik 1998; West and Scanlan pers. comm.) also are consistent with the vertical partitioning hypothesis, but multiple *Prochlorococcus* ecotypes (Moore et al. 1998) and phylotypes (Palenik 1994; Ferris and Palenik 1998; Urbach and Chisholm 1998) have been observed in single water samples, reflecting the dynamic nature of the ocean mixed layer. It is clear that vertical distributions of ecotypes cannot easily be

interpreted without a detailed understanding of the relative scales of mixing and growth rates of the different ecotypes and the past mixing history of the water column. More importantly, environmental factors other than light undoubtedly are important in shaping these patterns.

Multiple ecotypes are well known in *Synechococcus,* which is a close relative of *Prochlorococcus* and cooccurs with it in the open ocean. Like *Prochlorococcus, Synechococcus* ecotypes differ in their photosynthetic accessory pigment composition (Waterbury et al. 1986; Waterbury and Rippka 1989) and appear to be distributed based on their relative light-harvesting abilities (Alberte et al. 1984; Glover et al. 1986; Olson et al. 1988, 1990*b*). Genetically distinct ecotypes also have been reported for the marine diatom *Skeletonema costatum* (Gallagher et al. 1984; Gallagher and Alberte 1985), which differ in their cellular photoadaptive features and their seasonal pattern of abundance.

Conclusions

All of the *Prochlorococcus* isolates we analyzed are very efficient at growth and photosynthesis under low light conditions relative to most other phytoplankton groups and as such, should be considered low light adapted. There are distinct differences among the isolates, however, which cluster into two groups based on their growth, photosynthesis, and pigment responses to changing light intensities. One of the groups, which is comprised of previously labeled high light-adapted ecotypes, grows optimally at light intensities that totally photoinhibit the remaining isolates (which were previously designated low light-adapted ecotypes). We propose that these groups be called low and high B/A ecotypes, respectively, because the Chl b/a_2 ratios are a primary differentiating feature between the two groups, and this terminology would be in keeping with that used for ecotypes of marine *Synechococcus.* Furthermore, since all *Prochlorococcus* isolates are low light adapted relative to other phytoplankton species as a whole, this new terminology will avoid confusion when comparing them with other species.

The low B/A isolates have very uniform physiological characteristics and form a well-supported cluster in molecular phylogenies constructed using 16S rDNA sequences. Although the high B/A isolates are distinctly separate from this cluster, they do not themselves form a distinct phylogenetic cluster (Moore et al. 1998; Rocap et al. 1998; Urbach et al. 1998), which is consistent with the diversity seen in several of the physiological parameters within this group. It is not clear how the physiological differences between the high B/A isolates are reflected in their ecology. However, because they all share characteristically high chl b/a_2 ratios and because high chl b/a_2 ratios are found primarily in deep euphotic zone waters, we have grouped them together as high B/A ecotypes until further information warrants a new designation. We suspect that as more isolates are collected and examined physiologically and phylogenetically, additional ecotypes may emerge, and it is possible that some of these groups will be designated as distinct species of *Prochlorococcus.* Analyses customarily used to define species, such as %G + C content (Priest and Austin 1993) and DNA-DNA hybridization (Stackebrandt and Goebel 1994), have awaited the availability of axenic cultures of *Prochlorococcus,* which have only recently been obtained.

The presence of multiple ecotypes of *Prochlorococcus* in a given environment should allow them to thrive over a much broader range of environments than would be possible for a physiologically and genetically homogeneous population. This undoubtedly contributes to their ecological success throughout the marine environment.

References

ALBERTE, R. S., A. M. WOOD, T. A. KURSAR, AND R. R. L. GUILLARD. 1984. Novel phycoerythrins in marine *Synechococcus* spp. Plant Physiol. **75:** 732–739.

BABIN, M., A. MOREL, H. CLAUSTRE, A. BRICAUD, Z. KOLBER, AND P. G. FALKOWSKI. 1996. Nitrogen- and irradiance-dependent variations of the maximum quantum yield of carbon fixation in eutrophic, mesotrophic and oligotrophic marine systems. Deep-Sea Res. **43:** 1241–1272.

BANNISTER, T. R. 1979. Quantitative description of steady-state nutrient-saturated algal growth, including adaptation. Limnol. Oceanogr. **24:** 76–96.

BIDIGARE, R. R., O. SCHOFIELD, AND B. B. PREZELIN. 1989. Influence of zeaxanthin on quantum yield of photosynthesis of *Synechococcus* clone WH7803 (DC2). Mar. Ecol. Prog. Ser. **56:** 177–188.

BINDER, B. J., S. W. CHISHOLM, R. J. OLSON, S. L. FRANKEL, AND A. Z. WORDEN. 1996. Dynamics of pico-phytoplankton, ultraphytoplankton, and bacteria in the central equatorial Pacific. Deep-Sea Res. **43:** 907–931.

BLANCHOT, J., AND M. RODIER. 1996. Picophytoplankton abundance and biomass in the western tropical Pacific Ocean during the 1992 El Nino year: Results from flow cytometry. Deep-Sea Res. **43:** 877–895.

BRAND, L. E., AND R. R. L. GUILLARD. 1981. The effects of continuous light and light intensity on the reproduction rates of twenty-two species of marine phytoplankton. J. Exp. Mar. Biol. Ecol. **50:** 119–132.

CAMPBELL, L., H. LIU, H. A. NOLLA, AND D. VAULOT. 1997. Annual variability of phytoplankton and bacteria in the subtropical North Pacific Ocean at Station ALOHA during the 1991–1994 ENSO event. Deep-Sea Res. **44:** 167–192.

———, AND D. VAULOT. 1993. Photosynthetic picoplankton community structure in the subtropical North Pacific Ocean near Hawaii (station ALOHA). Deep-Sea Res. **40:** 2043–2060.

CHISHOLM, S. W., AND OTHERS. 1992. *Prochlorococcus marinus* nov. gen. nov. sp.: An oxyphototrophic marine prokaryote containing divinyl chlorophyll *a* and *b.* Arch. Microbiol. **157:** 297–300.

———, 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 J-C. MARTY. 1995. Specific phytoplankton biomasses and their relation to primary production in the tropical North Atlantic. Deep-Sea Res. **42:** 1475–1493.

CLEVELAND, J. S., M. J. PERRY, D. A. KIEFER, AND M. C. TALBOT. 1989. Maximal quantum yield of photosynthesis in the northwestern Sargasso Sea. J. Mar. Res. **47:** 869–886.

FERRIS, M. J., AND B. PALENIK. 1998. Niche adaptation in ocean cyanobacteria. Nature **396:** 226–228.

GALLAGHER, J. C., AND R. S. ALBERTE. 1985. Photosynthetic and cellular photoadaptive characteristics of three ecotypes of the

marine diatom *Skeletonema costatum* (Grev.) Cleve. J. Exp. Mar. Biol. Ecol. **94:** 233–250.

———, A. M. WOOD, AND R. S. ALBERTE. 1984. Ecotypic differentiation on the marine diatom *Skeletonema costatum:* Influence of light intensity on the photosynthetic apparatus. Mar. Biol. **82:** 121–134.

GEIDER, R. J., B. A. OSBORNE, AND J. A. RAVEN. 1985. Light dependence of growth and photosynthesis in *Phaeodactylum tricornutum* (Bacillariophyceae). J. Phycol. **21:** 609–619.

GLIBERT, P. M., T. M. KANA, R. J. OLSON, D. L. KIRCHMAN, AND R. S. ALBERTE. 1986. Clonal comparison of growth and photosynthetic responses to nitrogen availability in marine *Synechococcus* spp. J. Exp. Mar. Biol. Ecol. **101:** 199–208.

GLOVER, H. E., M. D. KELLER, AND R. R. L. GUILLARD. 1986. Light quality and oceanic ultraphytoplankton. Nature **319:** 142–143.

GOERICKE, R., AND D. J. REPETA. 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.

———, AND N. A. WELSCHMEYER. 1993. The marine prochlorophyte *Prochlorococcus* contributes significantly to phytoplankton biomass and primary production in the Sargasso Sea. Deep-Sea Res. **40:** 2283–2294.

HESS, W. R. 1999. Structure, expression and phylogeny of a gene cluster coding for phycobiliproteins in *Prochlorococcus marinus. In* A. W. D. Larkum and L. Charpy [eds.], Marine cyanobacteria. In press.

———, F. PARTENSKY, G. W. M. VAN DER STAAY, J. M. GARCIA-FERNANDEZ, T. BORNER, AND D. VAULOT. 1996. Coexistence of phycoerythrin and a chlorophyll *a/b* antenna in a marine prokaryote. Proc. Natl. Acad. Sci. USA **93:** 11126–11130.

IRIARTE, A., AND D. A. PURDIE. 1993. Photosynthesis and growth response of the oceanic picoplankter *Pycnococcus provasolii* Guillard (clone Ω48–23) (Chlorophyta) to variations in irradiance, photoperiod and temperature. J. Exp. Mar. Biol. Ecol. **168:** 239–257.

ITURRIAGA, R., AND B. G. MITCHELL. 1986. Chroococcoid cyanobacteria: A significant component of the food web dynamics of the open ocean. Mar. Ecol. Prog. Ser. **28:** 291–297.

JEFFREY, S. W., AND G. F. HUMPHREY. 1975. New spectrophotometric equations for determining chlorophyll *a, b,* c_1 and c_2 in higher plants, algae, and natural phytoplankton. Biochem. Physiol. Pflanz. **168:** 191–194.

JOHNSEN, G., E. SAKSHAUG, AND M. VERNET. 1992. Pigment composition, spectral characterization and photosynthetic parameters in *Chrysochormulina polylepis.* Mar. Ecol. Prog. Ser. **83:** 241–249.

JOHNSON, Z., AND OTHERS. 1999. Energetics and growth kinetics of a deep *Prochlorococcus* spp. population in the Arabian Sea. Deep-Sea Res. In press.

KANA, T. M., AND P. M. GLIBERT. 1987*a*. Effect of irradiances up to 2000 μE m^{-2} s^{-1} on marine *Synechococcus* WH7803. I. Growth, pigmentation, and cell composition. Deep-Sea Res. **34:** 479–496.

———, AND ———. 1987*b*. Effect of irradiances up to 2000 μE m^{-2} s^{-1} on marine *Synechococcus* WH7803. II. Photosynthetic responses and mechanisms. Deep-Sea Res. **34:** 497–516.

KOLBER, Z., K. WYMAN, AND P. G. FALKOWSKI. 1990. Natural variability in photosynthetic energy conversion efficiency: A field study in the Gulf of Maine. Limnol. Oceanogr. **35:** 72–79.

LANDRY, M. R., J. KIRSHTEIN, AND J. CONSTANTINOU. 1995. A refined dilution technique for measuring the community grazing impact of microzooplankton, with experimental tests in the central equatorial Pacific. Mar. Ecol. Prog. Ser. **120:** 53–63.

LANGDON, C. 1988. On the causes of interspecific differences in the growth-irradiance relationship for phytoplankton. II. A general review. J. Plankton Res. **10:** 1291–1312.

LINDELL, D., AND A. F. POST. 1995. Ultraphytoplankton succession is triggered by deep winter mixing in the Gulf of Aqaba (Eilat), Red Sea. Limnol. Oceanogr. **40:** 1130–1141.

LIU, H., H. A. NOLLA, AND L. CAMPBELL. 1997. *Prochlorococcus* growth rate and contribution to primary production in the equatorial and subtropical North Pacific Ocean. Aquat. Microb. Ecol. **12:** 39–47.

MASKE, H., AND H. HAARDT. 1987. Quantitative *in vivo* absorption spectra of phytoplankton: Detrital absorption and comparison with fluorescence excitation spectra. Limnol. Oceanogr. **32:** 620–633.

MCMANUS, G. B., AND R. DAWSON. 1994. Phytoplankton pigments in the deep chlorophyll maximum of the Caribbean Sea and the western tropical Atlantic Ocean. Mar. Ecol. Prog. Ser. **113:** 199–206.

MILLIGAN, A. J., AND E. M. COSPER. 1997. Growth and photosynthesis of the ''brown tide'' microalga *Aureococcus anophagefferens* in subsaturating constant and fluctuating irradiance. Mar. Ecol. Prog. Ser. **153:** 67–75.

MITCHELL, B. G., AND D. A. KIEFER. 1988. Chlorophyll *a* specific absorption and fluorescence excitation spectra for light-limited phytoplankton. Deep-Sea Res. **35:** 639–663.

MOORE, L. R. 1997. Physiological ecology of *Prochlorococcus:* A comparison of isolates from diverse oceanographic regimes. Ph.D. thesis, MIT.

———, R. GOERICKE, AND S. W. CHISHOLM. 1995. Comparative physiology of *Synechococcus* and *Prochlorococcus:* Influence of light and temperature on growth, pigments, fluorescence and absorptive properties. Mar. Ecol. Prog. Ser. **116:** 259–275.

———, G. ROCAP, AND S. W. CHISHOLM. 1998. Physiology and molecular phylogeny of coexisting *Prochlorococcus* ecotypes. Nature **393:** 464–467.

MOREL, A., Y-H. AHN, F. PARTENSKY, D. VAULOT, AND H. CLAUSTRE. 1993. *Prochlorococcus* and *Synechococcus:* A comparative study of their optical properties in relation to their size and pigmentation. J. Mar. Res. **51:** 617–649.

NELSON, D. M., C. F. D'ELIA, AND R. R. L. GUILLARD. 1979. Growth and competition of the marine diatoms *Phaeodactylum tricornutum* and *Thalassiosira pseudonana.* II. Light limitation. Mar. Biol. **50:** 313–318.

OLSON, R. J., S. W. CHISHOLM, E. R. ZETTLER, M. A. ALTABET, AND J. A. DUSENBERRY. 1990*a.* Spatial and temporal distributions of prochlorophyte picoplankton in the North Atlantic Ocean. Deep-Sea Res. **37:** 1033–1051.

———, ———, ———, AND E. V. ARMBRUST. 1988. Analysis of *Synechococcus* pigment types in the sea using single and dual beam flow cytometry. Deep-Sea Res. **35:** 425–440.

———, ———, ———, AND ———. 1990*b.* Pigments, size, and distribution of *Synechococcus* in the North Atlantic and Pacific Oceans. Limnol. Oceanogr. **35:** 45–58.

———, E. R. ZETTLER, S. W. CHISHOLM, AND J. DUSENBERRY. 1991. Advances in oceanography through flow cytometry, p. 351–399. *In* S. Demers and M. Lewis [eds.], Particle analysis in oceanography. Springer.

PALENIK, B. 1994. Cyanobacterial community structure as seen from RNA polymerase gene sequence analysis. Appl. Environ. Microbiol. **60:** 3212–3219.

———, AND R. HASELKORN. 1992. Multiple evolutionary origins of prochlorophytes, the chlorophyll *b*-containing prokaryotes. Nature **355:** 265–267.

PARTENSKY, F., J. BLANCHOT, F. LANTOINE, J. NEVEUX, AND D. MARIE. 1996. Vertical structure of picophytoplankton at different trophic sites of the subtropical northeastern Atlantic Ocean. Deep-Sea Res. **43:** 1191–1213.

———, N. HOEPFFNER, W. K. W. LI, O. ULLOA, AND D. VAULOT. 1993. Photoacclimation of *Prochlorococcus* sp. (Prochlorophy-

ta) strains isolated from the north Atlantic and the Mediterranean Sea. Plant Physiol. **101:** 285–296.

———, J. La Roche, K. Wyman, and P. G. Falkowski. 1997. The divinyl-chlorophyll *a*/b-protein complexes of two strains of the oxyphototrophic marine prokaryote *Prochlorococcus*—characterization and response to changes in growth irradiance. Photosynth. Res. **51:** 209–222.

Pinevich, A. V., S. G. Averina, and N. V. Velichko. 1997. Another view on the role of photosynthetic pigments in taxonomy of oxygenic-phototrophic bacteria: Proposed rejection of the order *Prochlorales* Florenzano, Balloni, and Materassi 1986 (emend. Burger-Wiersma, Stal, and Mur 1989), the family *Prochloraceae* Florenzano, Balloni, and Materassi 1986, and the family *Prochlorotrichaceae* Burger-Wiersma, Stal, and Mur 1989. Int. J. Syst. Bacteriol. **47:** 1264–1267.

Platt, T., C. L. Gallegos, and W. G. Harrison. 1980. Photoinhibition of photosynthesis in natural assemblages of marine phytoplankton. J. Mar. Res. **38:** 687–701.

Priest, F., and B. Austin. 1993. Modern bacterial taxonomy. Chapman and Hall.

Raps, S., K. Wyman, H. W. Siegelman, and P. G. Falkowski. 1983. Adaptation of the cyanobacterium *Microcystis aeruginosa* to light intensity. Plant Physiol. **72:** 829–832.

Rocap, G., L. R. Moore, and S. W. Chisholm. 1999. Molecular phylogeny of *Prochlorococcus* ecotypes. *In* A. W. E. Larkum and L. Charpy [eds.], Marine cyanobacteria. In press.

Shalapyonok, A., R. J. Olson, and L. S. Shalapyonok. 1998. Ultradian growth in *Prochlorococcus* spp. Appl. Environ. Microbiol. **64:** 1066–1069.

Shimada, A., T. Maruyama, and S. Miyachi. 1996. Vertical distributions and photosynthetic action spectra of two oceanic picophytoplankters, *Prochlorococcus marinus* and *Synechococcus* sp. Mar. Biol. **127:** 15–23.

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

Sosik, H. M. 1996. Bio-optical modeling of primary production: Consequences of variability in quantum yield and specific absorption. Mar. Ecol. Prog. Ser. **143:** 225–238.

Stackebrandt, E., and B. M. Goebel. 1994. Taxonomic note: A place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. Int. J. Syst. Bacteriol. **44:** 846–849.

Sukenik, A., J. Bennett, A. Mortain-Bertrand, and P. G. Falkowski. 1990. Adaptation of the photosynthetic apparatus to irradiance in *Dunaliella tertiolecta*. Plant Physiol. **92:** 891–898.

Ting, C. S., G. Rocap, J. King, and S. W. Chisholm. 1999. Characterization of phycoerythrin genes in the chlorophyll a_2/b_2-containing prokaryote *Prochlorococcus* sp. MIT9303. 11th International Photosynthesis Congress. In press.

Urbach, E., and S. W. Chisholm. 1998. Genetic diversity in uncultured *Prochlorococcus* populations flow cytometrically sorted from the Sargasso Sea and the Gulf Stream. Limnol. Oceanogr. **43:** 1615–1630.

———, D. Robertson, and S. W. Chisholm. 1992. Multiple evolutionary origins of prochlorophytes within the cyanobacterial radiation. Nature **355:** 267–270.

———, D. J. Scanlan, D. L. Distel, J. B. Waterbury, and S. W. Chisholm. 1998. Rapid diversification of marine picophytoplankton with dissimilar light-harvesting structures inferred from sequences of *Prochlorococcus* and *Synechococcus* (Cyanobacteria). J. Mol. Evol. **46:** 188–201.

Vaulot, D. 1989. cytopc: Processing software for flow cytometric data. Signal Noise **2:** 8.

———, D. Marie, R. J. Olson, and S. W. Chisholm. 1995. Growth of *Prochlorococcus*, a photosynthetic prokaryote, in the equatorial Pacific Ocean. Science **268:** 1480–1482.

Veldhuis, M. J. W., and G. W. Kraay. 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.

———, G. W. Kraay, J. D. L. Van Bleijswijk, and M. A. Baars. 1997. Seasonal and spatial variability in phytoplankton biomass, productivity and growth in the northwestern Indian Ocean: The southwest and northeast monsoon, 1992–1993. Deep-Sea Res. **44:** 425–449.

Waterbury, J. B., and R. Rippka. 1989. Subsection I. Order Chroococcales Wettstein 1924 (Emend. Rippka et al. 1979), p. 1728–1738. In Bergey's manual of determinative bacteriology. Willliams and Wilkins.

———, 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.

Wood, A. M. 1985. Adaptation of photosynthetic apparatus of marine ultraphytoplankton to natural light fields. Nature **316:** 253–255.

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