Plant Physiol. (1993) 101: 285–296

# Photoacclimation of *Prochlorococcus* sp. (Prochlorophyta) Strains Isolated from the North Atlantic and the Mediterranean Sea

**Frédéric Partensky*[1,2], Nicolas Hoepffner[3], William K. W. Li, Osvaldo Ulloa, and Daniel Vaulot**

Biological Oceanography Division, Bedford Institute of Oceanography, Dartmouth, Nova Scotia, B2Y 4A2, Canada (F.P., N.H., W.K.W.L., O.U.); and Station Biologique, Centre National de la Recherche Scientifique et Université Paris 6, B.P. 74, 29682 Roscoff Cedex, France (D.V.)

**Two Atlantic (SARG and NATL1) strains and one Mediterranean (MED) strain of *Prochlorococcus* sp., a recently discovered marine, free-living prochlorophyte, were grown over a range of "white" irradiances ($I_g$) and under low blue light to examine their photoacclimation capacity. All three strains contained divinyl (DV) chlorophylls (Chl) *a* and *b*, both distinguishable from "normal" Chls by their red-shifted blue absorption maximum, a Chl *c*-like pigment at low concentration, zeaxanthin, and α-carotene. The presence of two phaeophytin *b* peaks in acidified extracts from both Atlantic strains grown at high $I_g$ suggests that these strains also had a normal Chl *b*-like pigment. In these strains, the total Chl *b* to DV-Chl *a* molar ratio decreased from about 1 at 7.5 μmol quanta $m^{-2}$ $s^{-1}$ to 0.4 to 0.5 at 133 μmol quanta $m^{-2}$ $s^{-1}$. In contrast, the MED strain always had a low DV-Chl *b* to DV-Chl *a* molar ratio, ranging between 0.13 at low $I_g$ and 0.08 at high $I_g$. The discrepancies between the Atlantic and MED strains could result from differences either in the number of light-harvesting complexes (LHC) II per photosystem II or in the Chl *b*-binding capacity of the apoproteins constituting LHC II. Photosynthesis was saturated at approximately 5 fg C(fg Chl)$^{-1}$ $h^{-1}$ or 6 fg C $cell^{-1}$ $h^{-1}$, and growth was saturated at approximately 0.45 $d^{-1}$ for both MED and SARG strains at 18°C, but saturating irradiances differed between strains. Atlantic strains exhibited increased light-saturated rates and quantum yield for carbon fixation under blue light.**

Very small, free-living, photosynthetic prokaryotes (0.6 μm average diameter) have recently been discovered in large concentrations (typically $5 \times 10^4$–$2 \times 10^5$ cells $mL^{-1}$) in the picoplankton of subtropical oceans (Chisholm et al., 1988; Olson et al., 1990), as well as in the Mediterranean Sea (Vaulot et al., 1990). These organisms, the cultured type of which has been given the species name *Prochlorococcus marinus* Chisholm et alii (Chisholm et al., 1992), probably account for an important part of the primary production of the world ocean. They are characterized by a very specific pigmentation, which was studied first by HPLC on field samples (Chisholm et al., 1988) and then was partially confirmed by NMR studies and MS (Goericke and Repeta, 1992). *Prochlorococcus* cells contain DV derivatives of Chl *a*, *b*, and *c*. The Chl *c*-like pigment, which is spectrally similar to Mg 3,8 DVP $a_5$, is not fully characterized yet. Accessory pigments are zeaxanthin, α-carotene, and an unknown carotenoid (Goericke and Repeta, 1992). Because *Prochlorococcus* is a prokaryote containing both Chl *a*- and *b*-like pigments, it has been assigned to the Prochlorophyta (Chisholm et al., 1988). However, 16S rRNA and RNA polymerase gene sequencing has suggested a closer phylogenetic proximity between *Prochlorococcus* and the oceanic cyanobacteria *Synechococcus* than between the former and other groups, including *Prochloron* and *Prochlorothrix* (Palenik and Haselkorn, 1992; Urbach et al., 1992). Therefore, the validity of its assignment to the Prochlorophyta has recently been questioned (Urbach et al., 1992).

Although the original pigmentation of *Prochlorococcus* has been almost completely characterized, the range of variations of cell pigment content in response to changes in light irradiance and quality (i.e. WL in surface, BL at depth in oligotrophic waters) and the photosynthetic characteristics of this organism have not yet been studied in culture. Photoacclimation of natural prochlorophyte populations at depth is suggested by a large increase in their fluorescence, as measured by flow cytometry (Chisholm et al., 1988; Neveux et al., 1989; Olson et al., 1990), along with variations in their pigment content (Veldhuis and Kraay, 1990). Basic information about cellular concentrations of the major pigments and their relative ratios in pure culture is especially important to oceanographers wishing to make good estimates of the content in those pigments that *Prochlorococcus* share with other groups, such as zeaxanthin, which is also present in *Synechococcus* (Kana et al., 1988), and to evaluate the contribution of this algal group to total autotroph standing stock. It is also important to determine whether these cells can actively photosynthesize over a very large range of irradiances (1–1500 μmol quanta $m^{-2}$ $s^{-1}$), as suggested by their presence at high concentrations from the surface to the basis of the deep Chl maximum in oligotrophic oceans (Olson et al., 1990), and

[1] Supported by a postdoctoral award from the Natural Sciences and Environment Research Council (Canada).

[2] Present address: Station Biologique, Centre National de la Recherche Scientifique et Université Paris 6, B.P. 74, 29682 Roscoff cedex, France.

[3] Present address: Institute for Remote Sensing Applications, Centro Comune di Ricerca, 21020 Ispra, Italy.

* Corresponding author; fax 33–98–29–23–24.

Abbreviations: BL, blue light; DV, divinyl; DVP, divinyl-phaeoporphyrin; HL, high light; LHC, light-harvesting complexes; LL, low light; *P* versus *I*, photosynthesis versus irradiance; RC reaction center; WL, white light.

whether the ability to photosynthesize at LL results from a chromatic acclimation. These organisms are also of interest to plant physiologists, because the peculiar pigmentation of *Prochlorococcus* may provide new insights about the biosynthesis of Chl and the structure of photosystems.

The present study was designed to address some of these issues and also to compare the photophysiology of several *Prochlorococcus* isolates, which exhibited very different pigment contents, particularly in their ratios of DV-Chl *b* to DV-Chl *a*. Recent sequencing of the DNA-dependent RNA polymerase genes of two of the strains used in this study (SARG [LG] and MED [DV1]) suggest that they are very distant phylogenetically (Palenik and Haselkorn, 1992).

## MATERIALS AND METHODS

### Strain Isolation and Culture

Three unialgal strains of *Prochlorococcus* (MED, SARG, and NATL1) were grown at 18°C, a temperature at which all strains grew well, although for the SARG strain optimal temperature for growth is slightly higher (20–25°C). Cultures were maintained in 1 L of polycarbonate Nalgene flasks under a 12:12 h light:dark cycle at various growth irradiances ($I_g$), measured inside culture flasks using a 4-$\pi$ quantum-meter QSL-100 (Biospherical Instruments, San Diego, CA). Light was provided by cool-white fluorescent bulbs (Sylvania Lifeline F24T12/CW/HO, Anjou, Quebec, Canada). Several cultures were also grown in a blue Plexiglas box (Rohm and Haas 2424; bandpass = 475 ± 50 nm), which simulated light available at depth in oligotrophic waters. All cultures were acclimated to experimental light conditions at least 1 week before physiological and biochemical measurements (see below). Cultures were kept optically thin to minimize self-shading, with cell concentrations ranging between $1 \times 10^6$ and $4 \times 10^6$ cells $mL^{-1}$. Culture medium, modified from K/10(−Cu) medium (Chisholm et al., 1992), consisted of 0.2-$\mu$m Nuclepore-filtered, nutrient-poor seawater enriched with 20 $\mu$M urea, 1 $\mu$M glycerophosphate (final concentrations), and trace metals as in K/10(−Cu). Seawater and stock solutions were first autoclaved separately, then mixed, and filter sterilized through 0.2-$\mu$m Nuclepore filters.

MED and NATL1 strains were isolated using the same medium by repeated filtrations through 0.6-$\mu$m Nuclepore filters and serial dilutions. They were collected, respectively, from the Mediterranean Sea (43° 12′N, 6° 52′W) in January 1989 from a depth of 5 m (cruise No. 179 of the *R.S.S. Discovery*) and from the North Atlantic (37° 39′N, 40° 01′W) in April 1990 at 30 m (cruise No. 90–001 of the *C.S. Hudson*). SARG strain, isolated from the Sargasso Sea (28° 59′N, 64° 22′W) in May 1988 from a depth of 120 m, was obtained courtesy of Prof. S.W. Chisholm and Ms. S. Frankel (Massachusetts Institute of Technology, Cambridge, MA). Recently, clones from SARG and MED strains have been deposited under respective numbers CCMP 1375 and CCMP 1378 at the Center for the Culture of Marine Phytoplankton, Bigelow Laboratory for Ocean Sciences, West Boothbay Harbor, ME.

Physiological measurements were made during the exponential phase of cell growth. Because cultures were grown on a light:dark cycle, $^{14}C$ incubation experiments (see below) were always started at the same time of the light period (i.e. 5 h after light onset) to minimize the variations of photosynthetic parameters due to diel periodicity.

### Flow Cytometric Measurements

*Prochlorococcus* cell concentration was counted on duplicate culture samples during growth and before experiments, using an EPICS V (Coulter, Hialeah, FL) flow cytometer equipped with a Biosense flow cell and confocal lens to increase sensitivity (Olson et al., 1990; Vaulot et al., 1990). Samples were illuminated with the 488-nm line of a 5-W argon laser (Coherent, Palo Alto, CA) at 200 mW. Chl red fluorescence was collected through a 660-nm longpass filter, and right angle light scatter was used as an index of cell size. Both parameters were recorded on three-decade logarithmic scales. Specific growth rates ($\mu$) of cultures were determined from cell counts made at the time of experiment ($N_t$) and 2 d before ($N_{t-2}$):

$$\mu = \ln(N_t/N_{t-2})/2.$$

### *P* versus *I*

*P* versus *I* curves were obtained using the method of Lewis and Smith (1983). Briefly, after $NaH^{14}CO_3$ was added (about 11 $\mu$Ci/mL of culture), 1-mL aliquots were placed in 7-mL scintillation vials and incubated for 2 h in temperature-controlled (18°C) "photosynthetrons." Light was provided by two 250-W ENH tungsten-halogen lamps set below the vials and passed through neutral density filters, yielding 44 different intensities (read before each experiment using a 4-$\pi$ quantum-meter QSL-100). Incubation was terminated by acidification with 0.2 mL of 6 N HCl and shaking for 2 h, and then 5 mL of scintillation cocktail was added directly to the vials before measurement of $^{14}C$ incorporation using a Beckman LS5000CE counter (Fullerton, CA). Counts, subtracted from a reference at time zero, were corrected for quench with the H# method (Compton distribution method). Total activity was determined from five replicates after 50 $\mu$L of Protosol and 5 mL of scintillation cocktail were added to 50 $\mu$L of $^{14}C$-labeled culture. Data were normalized to DV-Chl *a* and fitted to the equation of Platt et al. (1980) using the nonlinear module of the SYSTAT software (Evanston, IL) that provided SE values for each estimated parameter:

$$P^{Chl} = P_s^{Chl}(1 - e^{-\alpha^{Chl}I/P_s^{Chl}})e^{-\beta^{Chl}I/P_s^{Chl}},$$

where $P^{Chl}$ is the carbon fixation rate per unit DV-Chl *a* (in fg C [fg Chl]$^{-1}$ h$^{-1}$), $P^{Chl}_s$ (in same units as $P^{Chl}$) is the maximum, potential, light-saturated photosynthetic rate, $I$ is the irradiance (in $\mu$mol quanta m$^{-2}$ s$^{-1}$), $\alpha^{Chl}$ is the initial slope of the $P^{Chl}$ versus $I$ curve (in fg C [fg Chl]$^{-1}$ h$^{-1}$ [$\mu$mol quanta m$^{-2}$ s$^{-1}$]$^{-1}$), and $\beta^{Chl}$ (in same units as $\alpha^{Chl}$) is a "photoinhibition parameter." From this equation, four important parameters can be derived: $P^{Chl}_m$ (in same units as $P^{Chl}$) is the maximum specific rate at light saturation, $I_m$ (in same units as $I$) is the light intensity at which photosynthesis is optimal, and $I_b$ (in same units as $I$) is an "index of photoinhibition" (Platt et al., 1980):

$$P_m^{Chl} = P_s^{Chl}[\alpha^{Chl}/(\alpha^{Chl} + \beta^{Chl})][\beta^{Chl}/(\alpha^{Chl} + \beta^{Chl})]^{\beta^{Chl}/\alpha^{Chl}},$$

$$I_m = P_s^{Chl}/\alpha^{Chl} \ln[(\alpha^{Chl} + \beta^{Chl})/\beta^{Chl}],$$

and

$$I_b = P_s^{Chl}/\beta^{Chl}.$$

The photosynthetic performance $P^{Chl}_i$ was determined by calculating $P^{Chl}$ (using the fitted curve equation) for each growth irradiance ($I_g$). The photosynthetic parameters were also expressed on a per cell basis ($\alpha^{cell}$, $P^{cell}_m$, $P^{cell}_i$, etc.) by multiplying them by the cell DV-Chl *a* content.

### In Vivo Absorption Spectra

Subsamples of each culture were filtered onto Whatman GF/F filters and immediately scanned between 380 and 750 nm using a Beckman DU-64 spectrophotometer. Because variations in wetness between samples and blank filters sometimes resulted in negative *A* values, all spectra were shifted such that $A_{750}$ was equal to zero (Bricaud and Stramski, 1990). *A* values were corrected for the so-called "$\beta$ effect" (Bricaud and Stramski, 1990) [$A_{corr.}(\lambda) = 0.31\ A(\lambda) + 0.57\ A(\lambda)^2$; N. Hoepffner, unpublished results]. Absorption coefficients (in $m^{-1}$) were then calculated as $a(\lambda) = 2.3\ A(\lambda)\ S/V$, where *S* is the clearance area of the filter and *V* is the volume of sample filtered, and converted to Chl-specific absorption coefficients [$a^*_{Chl}(\lambda)$ in $m^2$ (mg Chl)$^{-1}$] by dividing them by the DV-Chl *a* concentration determined by HPLC (see below).

Quantum yield for photosynthesis, $\phi_{CO2}$, was determined as $\phi_{CO2} = \alpha_{Chl}/a^*_{Chl}$. The spectrally weighted Chl-specific absorption cross-section, $a^*_{Chl}$, was calculated as $a^*_{Chl} = \Sigma[a^*_{Chl}(\lambda)\ I(\lambda)\ \Delta\lambda]/\Sigma[I(\lambda)\ \Delta\lambda]$, where $I(\lambda)$ is the irradiance in the spectral band $\Delta\lambda$ ($\Delta\lambda$ = 1 nm). The irradiance spectrum of the tungsten lamps of the photosynthetron was measured between 400 and 700 nm with a custom-designed spectroradiometer.

### Pigment Analyses

Filters for absorption spectra were also used for pigment analysis with a reverse-phase HPLC method. Before analysis, filters were ground in 1 mL of 100% acetone, and the pigments were extracted in the dark for several hours at 5°C. Just before the injection, samples were centrifuged and diluted with deionized water at a ratio of 1:1 (sample:water, v/v). Then, 250 $\mu$L of the extract were injected into a Beckman System Gold HPLC apparatus equipped with an Ultrasphere XL-ODS column (Beckman). The pigments were allowed to separate along a 23-min chromatographic run, which consisted of a 16-min linear gradient from 100% solvent A (methanol:0.5 M ammonium acetate, 80:20, v/v) to 100% solvent B (methanol:ethylacetate, 70:30, v/v). Aliquots of the acetone extract were acidified with 1 N HCl and analyzed as described previously to confirm the identification of the forms of Chl *a* and *b* (mono or DV; see "Results"). The pigments were monitored at 440 nm (410 nm for acidified samples) and identified by comparing their retention times and online absorption characteristics (3-nm resolution spectra) with quantitative standards (Sigma, St. Louis, MO) and plant material of known pigment composition. Extinction coefficients for DV-Chl *a* and *b* have not been definitely determined yet; therefore, concentrations of these compounds were calculated using extinction coefficients for Chl *a* and *b*. The average coefficient of variation of cell pigment concentrations were estimated from two replicate cultures to be 6%.

## RESULTS

### Pigment Content

The identity of pigments of *Prochlorococcus marinus* was recently determined on the SARG strain (Goericke and Repeta, 1992), which was therefore considered a "reference" for the pigment characterization of NATL1 and MED strains by HPLC analysis (Table I, Fig. 1). In all strains, the nonacidified extracts showed the presence of DV-Chl *a*, DV-Chl *b* (the blue absorption maxima of both were red shifted by about 10 nm compared to standard Chl *a* and *b*), a Chl *c*-like pigment, zeaxanthin, and $\alpha$-carotene. The Chl *c*-like pigment had an elution time identical with that of standard Chl $c_1$. However, its concentration was too low in MED and NATL1 strains to confirm its spectral similarity with Mg 3,8 DVP $a_5$ monomethyl ester (Goericke and Repeta, 1992). Two partially overlapping peaks were also visible between DV-Chl *b* and DV-Chl *a* (Fig. 1, A and C). The first one might correspond to DV-Chl *a* allomer, whereas the second one, which was unaffected by acidification (Fig. 1, B and D), might correspond to the "unknown carotenoid" that coeluted with DV-Chl *a* by the three-solvent chromatographic method of Goericke and Repeta. Acidification also allowed us to confirm the identification of DV-Chl *a* as the only form of Chl *a* in all strains of *Prochlorococcus*, because (a) the corresponding phaeophytin eluted at least 0.3 min after phaeophytin *a* obtained from acidified standard Chl *a* and (b) the blue maximum of its absorption spectra was red shifted by about 10 nm compared to phaeophytin *a* (data not shown). It is surprising that, in all extracts from the Atlantic strains, the acidic treatment revealed not only the expected peak due to DV-phaeophytin *b* but a second peak having the same elution time as phaeophytin *b* (Fig. 1D). Moreover, the absorption spectra of this phaeophytin *b*-like pigment showed a blue maximum comparable to that of phaeophytin *b* (within the 3-nm resolution of the system) and blue shifted by about 6 nm from that of DV-phaeophytin *b*. These results suggest that Atlantic strains are able to synthesize both DV-Chl *b* and a Chl *b*-like pigment. The presence of a pigment spectrally analogous to Chl *b* has been confirmed independently on the SARG strain grown at 63 $\mu$mol quanta $m^{-2}$ $s^{-1}$ of WL using spectrofluorimetry (J. Neveux, personal communication). If we assume that all Chl *b* molecules were altered by the acidic treatment into their corresponding phaeophytins, the ratio of DV-Chl *b* to total Chl *b* (i.e. DV-Chl *b*- plus Chl *b*-like pigment) decreased with increasing $I_g$, from nearly 100% at low $I_g$ to 40.5 and 16.8% at high $I_g$ in SARG and NATL1 strains, respectively (Table I). The phaeophytin *b*-like pigment was always below the level of detection in the MED strain, whatever the light level (Fig. 1B, Table I).

For all strains, the cell content in all chlorophyllous pigments decreased with increasing white $I_g$ (Table I). At a given

**Table I.** *Absolute concentrations of main pigments per cell, ratios of secondary pigments to DV-Chl a, and percentage of DV-Chl b to total Chl b (i.e. DV-Chl b + Chl b-like pigment) for three Prochlorococcus strains acclimated to different $I_g$ (in μmol quanta $m^{-2}$ $s^{-1}$) and light colors*

Pigment ratios are molar ratios unless specified otherwise.

| Strain | Light Color | $I_g$ | Pigment Concentration | | | | | Pigment Ratios | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | DV-Chl *a* | Total Chl *b* | Chl c-like | Zeaxanthin | α-Carotene | Total Chl *b*: DV-Chl *a* | Chl c-like: DV-Chl *a* | Chl c-like: DV-Chl *a* + *b* | Zeaxanthin: DV-Chl *a* | α-Carotene: DV-Chl *a* | DV-Chl *b*: Total Chl *b*[a] |
| | | | *fg⁻¹ cell* | | | | | | | | | | % |
| MED | Blue | 1.3 | 3.67 | 0.45 | 0.09 | 0.99 | 0.53 | 0.121 | 0.035 | 0.031 | 0.421 | 0.239 | 100.0 |
| | Blue | 3.8 | 3.32 | 0.41 | 0.08 | 0.87 | 0.47 | 0.121 | 0.035 | 0.031 | 0.411 | 0.233 | 100.0 |
| | White | 7.5 | 4.26 | 0.57 | 0.12 | 0.94 | 0.54 | 0.133 | 0.039 | 0.034 | 0.348 | 0.210 | 100.0 |
| | White | 16.0 | 1.99 | 0.21 | 0.04 | 0.76 | 0.28 | 0.103 | 0.031 | 0.028 | 0.600 | 0.238 | 100.0 |
| | White | 67.0 | 1.21 | 0.11 | 0.03 | 0.65 | 0.18 | 0.086 | 0.035 | 0.032 | 0.842 | 0.246 | 100.0 |
| | White | 133.0 | 1.36 | 0.11 | 0.04 | 0.65 | 0.18 | 0.080 | 0.041 | 0.038 | 0.749 | 0.215 | 100.0 |
| SARG | Blue | 6.0 | 1.68 | 1.28 | 0.08 | 0.53 | 0.47 | 0.754 | 0.070 | 0.040 | 0.496 | 0.469 | 95.3 |
| | White | 1.0 | 2.82 | 4.56 | 0.20 | 0.80 | 0.99 | 1.591 | 0.102 | 0.039 | 0.447 | 0.584 | 99.6 |
| | White | 7.5 | 1.98 | 2.49 | 0.12 | 0.54 | 0.60 | 1.237 | 0.086 | 0.039 | 0.426 | 0.503 | 95.5 |
| | White | 67.0 | 1.52 | 0.93 | 0.05 | 0.79 | 0.41 | 0.603 | 0.044 | 0.027 | 0.813 | 0.444 | 72.0 |
| | White | 133.0 | 1.12 | 0.59 | 0.03 | 0.83 | 0.24 | 0.522 | 0.036 | 0.024 | 1.171 | 0.364 | 40.5 |
| NATL1 | Blue | 6.0 | 3.50 | 3.11 | 0.24 | 0.78 | 0.82 | 0.875 | 0.100 | 0.053 | 0.351 | 0.391 | 86.9 |
| | White | 7.5 | 3.17 | 3.06 | 0.26 | 0.64 | 0.70 | 0.950 | 0.120 | 0.062 | 0.318 | 0.368 | 94.1 |
| | White | 67.0 | 2.22 | 1.39 | 0.11 | 0.86 | 0.43 | 0.616 | 0.072 | 0.045 | 0.607 | 0.193 | 33.5 |
| | White | 133.0 | 1.84 | 0.75 | 0.08 | 1.02 | 0.24 | 0.401 | 0.065 | 0.046 | 0.872 | 0.128 | 16.8 |

[a] Actual DV-phaeophytin *b* peak area to total phaeophytin *b* peak area ratio from acidified extracts.

$I_g$, NATL1 contained more of each pigment than SARG, but the ratios of accessory pigments to DV-Chl *a* were similar in these two strains (generally within a 100–137% range, Table I). Both Atlantic strains were characterized by remarkably high Chl *b* contents and ratios of Chl *b* to DV-Chl *a* (≥0.95 at LL), in contrast to the MED strain, for which the ratio of Chl *b* to DV-Chl *a* was always <0.14. These discrepancies result in very different slopes of DV-Chl *b* versus DV-Chl *a* plots (Fig. 2A). There is an apparent change in slope between LL- and HL-acclimated cells of the SARG strain above 66 μmol quanta $m^{-2}$ $s^{-1}$. The Chl *c*-like pigment content was low for all strains. The ratio of Chl *c*-like pigment to DV-Chl *a* was almost constant in the MED strain, but it decreased with increasing $I_g$ in the Atlantic strains. The ratio of Chl *c*-like pigment to DV-Chl *a* plus *b* was very close in the three strains and varied little with $I_g$ (range, 0.03–0.06; Table I). The two Atlantic strains showed parallel variations with regard to carotenoids. For zeaxanthin, both the absolute amount per cell and its ratio to DV-Chl *a* increased with $I_g$ (except for one high value of zeaxanthin concentration observed at 1 μmol quanta $m^{-2}$ $s^{-1}$ in the SARG strain), contrary to α-carotene. In the MED strain, when $I_g$ increased, the absolute zeaxanthin content decreased and the ratio of α-carotene to DV-Chl *a* remained constant (range, 0.20–0.24; Table I). Slopes of α-carotene versus DV-Chl *a* plots differed significantly (by a factor of 3) between Atlantic and MED strains (Fig. 2B).

At a given irradiance, cells grown under BL generally had a lower pigment content and lower proportions of auxiliary pigments with respect to DV-Chl *a* than those grown under WL (Table I), as a result of their large absorption in the blue part of the visible spectra (see below).

## Growth

All three *Prochlorococcus* strains responded to increasing $I_g$ by an increased growth rate up to a maximum of 0.4 to 0.46 $d^{-1}$, depending on the strain (Fig. 3). The MED strain grew the fastest at low $I_g$ and reached its maximum growth rate at lower $I_g$ (about 16 μmol quanta $m^{-2}$ $s^{-1}$ of WL) than the other two strains. At irradiances higher than that optimal for growth, MED and SARG strains showed a slight decrease in growth rate, indicating photoinhibition of growth. For a given irradiance, all strains grew much better under BL than WL (Fig. 3).

## Absorption Properties

Figure 4 illustrates Chl-specific absorption spectra obtained under various light conditions for GF/F-filtered cells of the three *Prochlorococcus* strains. These spectra show features similar to those obtained for suspensions of live cells of the same strains grown under low BL (A. Morel, Y.W. Hahn, F. Partensky, D. Vaulot, and H. Claustre, unpublished data). For the MED strain, the shape of absorption spectra with maxima at 444 and 672 nm is attributable mainly to DV-Chl *a*, except for a shoulder at about 480 nm due to carotenoids, and exhibited no obvious change with increasing $I_g$ (Fig. 4A) but a relative increase of the blue maximum. Large variations of the blue [$a^*_{Chl}$ (444)] and red [$a^*_{Chl}$ (672)] maxima with irradiance were inversely correlated with DV-Chl *a* content ($r$ = 0.95 and 0.86, respectively, $n$ = 6). Consequently, the spectrally averaged Chl-specific absorption cross-section, $a^*_{Chl}$, increased at increasing irradiance (Table II). Absorption spectra from the SARG and NATL1 strains were more complex because of the larger contribution of Chl *b* and, to a

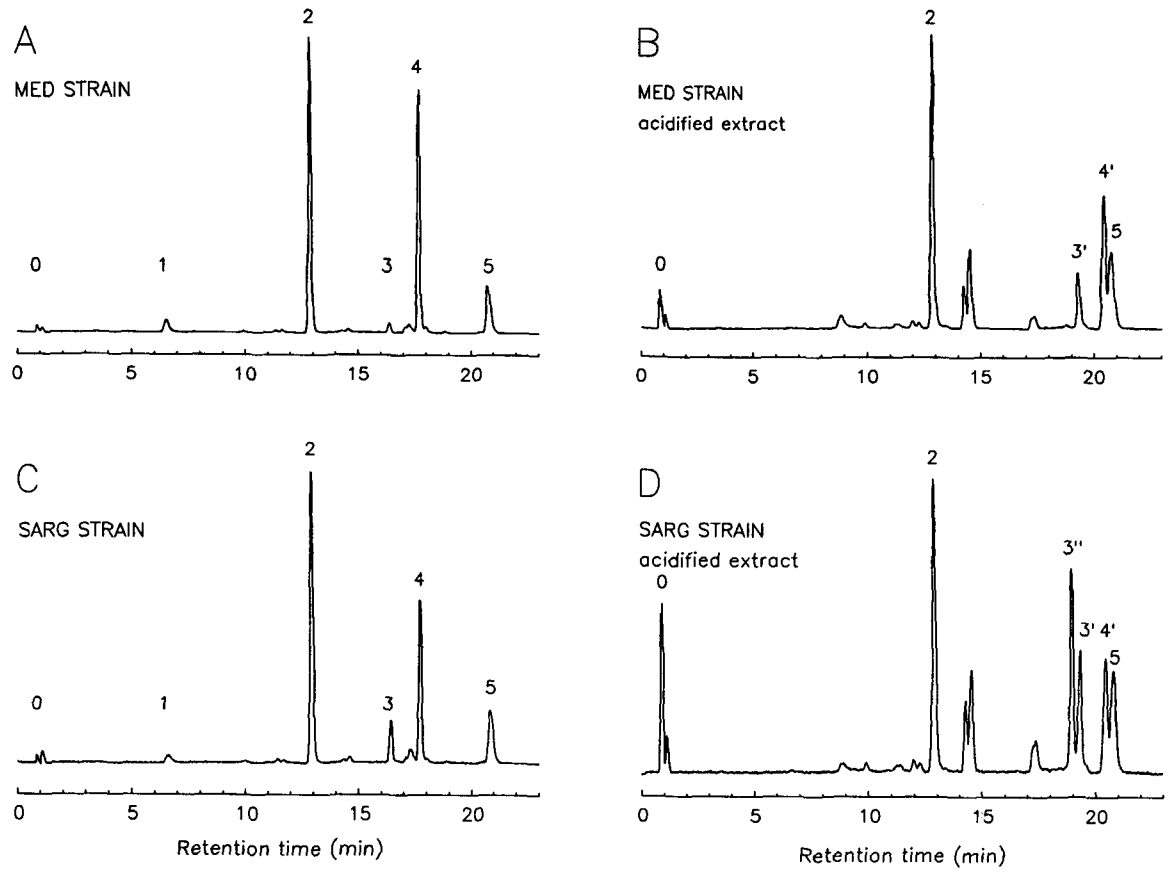


**Figure 1.** HPLC chromatograms for MED and SARG strains grown at 133 μmol quanta $m^{-2}$ $s^{-1}$. A and C, Chromatograms for nonacidified samples. B and C, Chromatograms after acidification with 0.1 N HCl. 1, Chl *c*-like pigment; 2, zeaxanthin; 3, total Chl *b* (DV-Chl *b* plus Chl *b*-like pigment); 3', DV-phaeophytin *b*; 3'', phaeophytin *b*-like pigment; 4, DV-Chl *a*; 4', DV-phaeophytin *a*; 5, α-carotene. Other minor peaks have not been firmly identified (see "Results").

lesser extent, of the Chl *c*-like pigment, which increased with decreasing $I_g$ (Fig. 4, B and C). At low $I_g$ (7.5 μmol quanta $m^{-2}$ $s^{-1}$ of WL), DV-Chl *b* (the major Chl *b* form at this light intensity, see above) produced a maximum at 476 to 478 nm, causing a shift of the DV-Chl *a* blue maximum from 444 nm toward 449 to 450 nm, and, in the case of SARG strain, a remarkable red maximum at 657 nm (Fig. 4B). An additional peak at about 600 nm probably corresponds to a mixture of Chls. In contrast to those of the MED strain, the values of the Chl-specific absorption cross-section at the blue and red peaks were not correlated with DV-Chl *a* content, because of a complex superimposition of "package effect" and a very large contribution of DV-Chl *b* (see "Discussion"). As a consequence, $a^*_{Chl}$ was high at low $I_g$ and did not increase at increasing irradiance (Table II).

### *P* versus *I*

The initial slope of *P* versus *I* curves (Fig. 5), α, showed no clear variations with irradiance when expressed per Chl ($\alpha^{Chl}$), except for a slight decrease with the SARG strain (Table II). In contrast, $\alpha^{cell}$ decreased markedly at increasing $I_g$ for all strains. At a given $I_g$, $\alpha^{Chl}$ (but not $\alpha^{cell}$) was about twice as high in SARG as in NATL1, whereas $a^*_{Chl}$ and $\phi_{CO2}$ were both larger (Table II).

Light-saturated photosynthetic rates ($P^{Chl}_m$ and $P^{cell}_m$) varied significantly between strains when grown under similar irradiances. For the MED strain, $P^{Chl}_m$ was apparently near saturation at 67 μmol quanta $m^{-2}$ $s^{-1}$, because it did not increase (or even decreased) when cells were acclimated to 133 μmol quanta $m^{-2}$ $s^{-1}$. The photosynthetic performance, $P^{Chl}_i$, was, however, significantly higher at 133 than at 67 μmol quanta $m^{-2}$ $s^{-1}$, and the ratio of $P_m$ to $P_i$, i.e. the excess photosynthetic capacity, decreased toward 1 at the highest irradiance, suggesting that cells had optimized their photosynthetic response. $P^{cell}_m$ varied little with $I_g$ for the MED strain, whereas for the Atlantic strains both $P^{Chl}_m$ and $P^{cell}_m$ increased with increasing $I_g$. The increase in $P^{Chl}_m$ was more progressive in NATL1 ($P^{Chl}_m$ was still low at 133 μmol quanta $m^{-2}$ $s^{-1}$) than in SARG, although under identical light conditions these strains had very similar $I_m$ (Table II). The ratio of $P_m$ to $P_i$ decreased asymptotically toward 1 at high $I_g$, suggesting that for both strains photosynthesis was near optimal at the highest white irradiance (Table II). After 2 h of incubation, all strains displayed strong photoinhibition of photosynthesis at the highest $I_g$ used to derive the *P* versus *I* curves, as denoted by the sharp decrease of $P^{Chl}$ and $P^{cell}$ (Fig. 5). However, for $I_g$ up to 67 μmol quanta $m^{-2}$ $s^{-1}$, the MED strain was comparatively less photoinhibited than the Atlan-

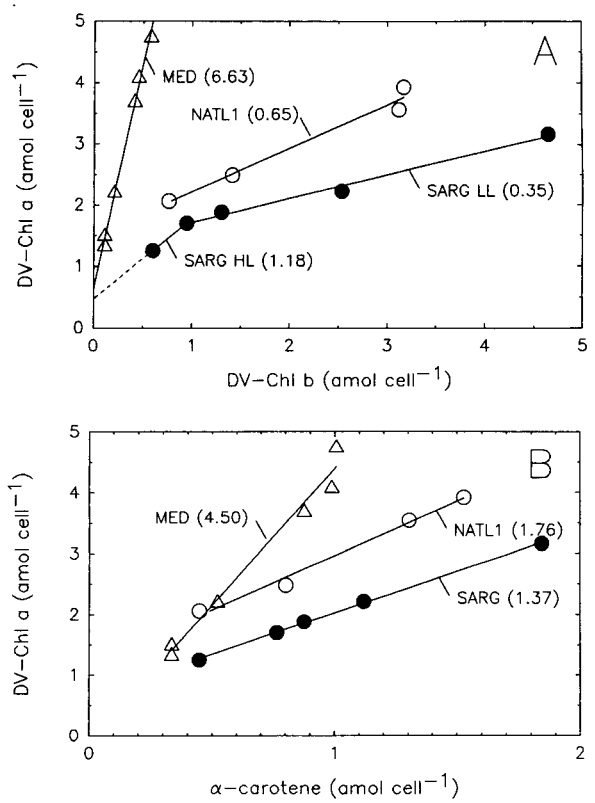


**Figure 2.** Relationship between cellular levels of DV-Chl *b* and DV-Chl *a* (A) and of α-carotene and DV-Chl *a* (B) in *Prochlorococcus* strains grown at various irradiances. Numbers in parentheses indicate the slope of the relation for each strain.

tic strains, as indicated by the lower curvature of the $P^{Chl}$ curve after $P^{Chl}{}_m$ (Fig. 5) and by higher values of $I_b$ for the former strain (Table II). It is noteworthy that for the MED strain the highest $I_b$ (and therefore the minimal photoinhibition) as well as the lowest $\alpha^{Chl}$ and $P^{cell}{}_m$ occurred at 16 μmol quanta $m^{-2}$ $s^{-1}$, at which the maximal growth rate ($\mu_{max}$) was observed (Fig. 3). For all strains, the photosynthetic quantum yield, $\phi_{CO2}$, tended to decrease at increasing $I_g$ (Table II).

BL induced no obvious effect on the photosynthetic parameters and quantum yield of MED strain, except that the values found at 3.8 μmol quanta $m^{-2}$ $s^{-1}$ of BL were intermediate between that found at 7.5 and 16 μmol quanta $m^{-2}$ $s^{-1}$ (Table II), as observed for the growth rate and pigment content. In contrast, this effect was more pronounced for Atlantic strains, which, under low BL, exhibited high $P^{Chl}{}_m$ and $P^{cell}{}_m$ values (similar or higher than that observed at 67 μmol quanta $m^{-2}$ $s^{-1}$ WL), high $\alpha^{Chl}$ and $\alpha^{cell}$, and low $I_m$ and $I_b$ (Table II and Fig. 5, C and D). Similarly, $\phi_{CO2}$ seems to increase specifically under BL, as compared to WL.

## DISCUSSION

### Specificity of *Prochlorococcus* Pigmentation

*Prochlorococcus* strains isolated from the Atlantic Ocean and the Mediterranean Sea possessed the same pigmentation, consisting of DV derivatives of Chl *a*, *b*, and *c*, α-carotene, zeaxanthin, and an unknown carotenoid, that was not completely resolved with our HPLC system. Presence in significant concentrations of a normal Chl *b*-like pigment in the Atlantic strains grown at HL is suggested by the occurrence of two phaeophytin *b* peaks in HPLC analyses of acidified samples (Fig. 1). Goericke and Repeta (1992) also noticed the possible presence of multiple phaeophytin *b* peaks after acidification but concluded that it resulted from an allomerization of this pigment. However, the SARG strain cultures used by these authors had a DV-Chl *b* to *a* ratio >1. Under such conditions, one can expect that >95% of the total Chl *b* would be DV-Chl *b*. Goericke and Repeta (1992) observed that these phaeophytins had identical visible spectra, similar to that of DV-Chl *b*. Given that (a) the two phaeophytins *b* in our extracts had absorption spectra shifted by about 6 nm, (b) the proportion of phaeophytin *b*-like pigment increased with increasing $I_g$ in acidified extracts from both SARG and NATL1 strains, and (c) only one peak, spectrally similar to DV-phaeophytin *b*, was observed in our acidified extracts from the MED strain, it appears probable that Atlantic strains may actually synthesize both forms of Chl *b*, although full confirmation awaits spectroscopic studies. It is possible that our cultures contained several populations of *Prochlorococcus* with similar flow cytometric characteristics (i.e. light scatter and red fluorescence) but different forms of Chl *b*. However, the recurrence of this phenomenon in two independent isolates is unlikely. Presence of two forms of Chl *b* in certain *Prochlorococcus* species might partly explain the apparent contradiction in the existing field data concerning pigments. In two reports, the major accessory Chl associated with DV-Chl *a*, which is the main taxonomic marker for *Prochlorococcus*, was Chl *b* (Chisholm et al., 1988; Veldhuis and Kraay, 1990), whereas it was DV-Chl *b* in another report (Neveux et al., 1989).

The occurrence of a Chl *c*-like pigment, spectrally identical with Mg 3,8 DVP $a_5$, in *Prochlorococcus* cells, raises the question of its potential role in LHCs. Alternatively, because this pigment is found at very low cell concentrations, it could simply accumulate as an intermediate of Chl biosynthesis (Goericke and Repeta, 1992). If the latter hypothesis holds true, that the ratio of Chl *c*-like pigment to DV-Chl *a* plus *b*

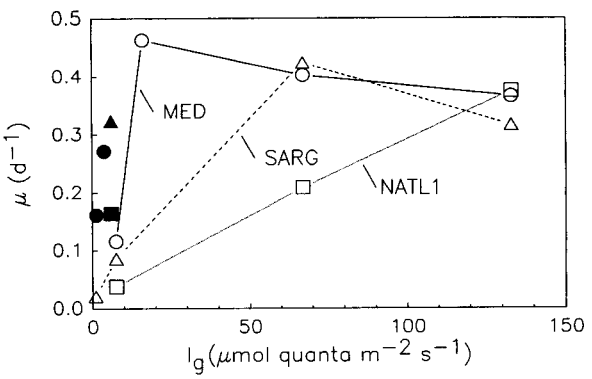


**Figure 3.** Growth rates versus $I_g$ for the three *Prochlorococcus* strains. Open symbols are for cultures grown under WL, and solid symbols are for those grown under BL.

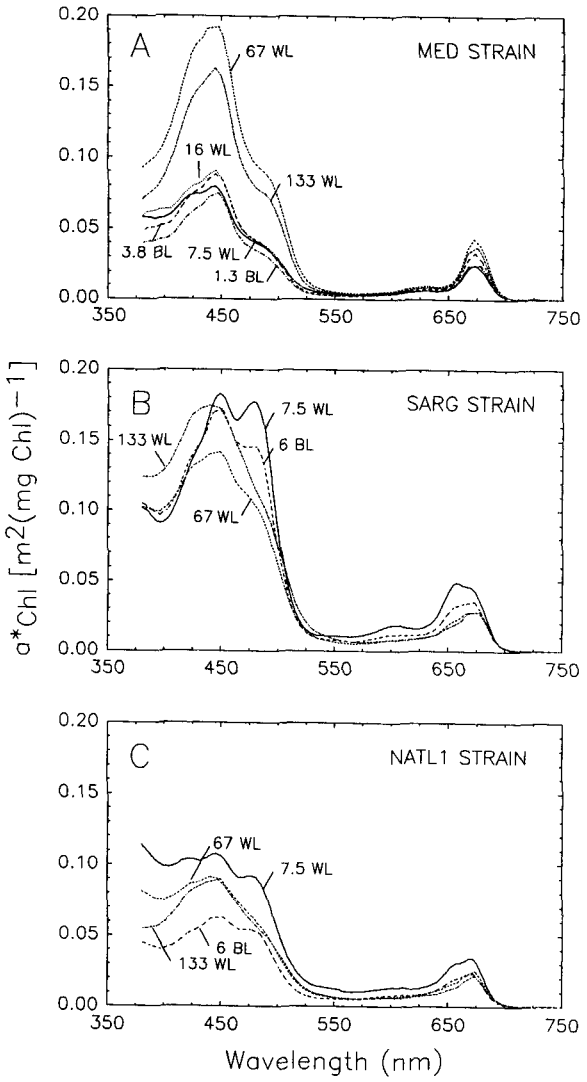


**Figure 4.** Chl-specific absorption spectra of live *Prochlorococcus* cells on GF/F filters. Strains were grown under different WL or BL (in μmol quanta $m^{-2}$ $s^{-1}$). A, MED strain; B, SARG strain; C, NATL1 strain.

remained relatively constant in all strains and at all irradiance levels, it would suggest that in this species this compound could be a precursor not only of DV-Chl *a* but also DV-Chl *b* and maybe Chl *b*. Pathways to biosynthesis of Chls are still not completely settled and might be multiple (Rüdischer and Schoch, 1988). One of the possible pathways to normal Chl *a* and *b* biosynthesis in some algae has DV-Chl *a* as a likely intermediate (Rüdischer and Schoch, 1988):

DV-protochlorophyllide → DV-Chlide *a*
→ DV-Chl *a* → Chl *a* → Chl *b*.

The DVP $a_5$ is structurally very close to DV-protochlorophyllide of higher plants, and it is, therefore, the potential precursor to DV-Chlide *a* in *Prochlorococcus*. From this scheme and our results, it can be proposed that in *Prochlorococcus* a possible biochemical pathway to the observed forms of Chl *a* and *b* is:

DVP $a_5$ → DV-Chlide *a* → DV-Chl *a*
→ DV-Chl *b* → normal Chl *b*.

These hypotheses remain highly speculative. However, it is clear that the discovery of an organism containing DV-Chl *a* instead of Chl *a* as the active form of Chl might lead to important advances in the understanding of Chl biosynthesis.

### Light-Induced Changes of Pigment Content: Implications for the Structure of PSII

All strains were capable of modifying their internal pigment concentrations in response to changes in irradiance. Atlantic strains responded to decreasing growth irradiances by (a) an increase in their DV-Chl *a* and *b* and Chl *c*-like pigment, as well as α-carotene contents, (b) an increase in their DV-Chl *b*, Chl *c*-like pigment, and α-carotene to DV-Chl *a* ratios, and (c) a decrease in their zeaxanthin content and zeaxanthin to DV-Chl *a* ratios. This is consistent with the general pattern found in microalgae containing either Chl *b* or *c* as accessory Chls (Falkowski and Owens, 1980). The MED strain showed several exceptions to this scheme, including the increase in its zeaxanthin content with decreasing $I_g$ along with the constancy of the ratios of Chl *c*-like pigment and α-carotene to DV-Chl *a*.

A remarkable trait distinguishing both Atlantic isolates from the MED strain is the high DV-Chl *b* to DV-Chl *a* ratio for the former, which can be >1 at low $I_g$, whereas the latter exhibits a ratio always <0.14. Consequently, the slope of the DV-Chl *b* versus DV-Chl *a* plots differed significantly between *Prochlorococcus* strains (Fig. 2A). Comparable plots of Chl *b* versus Chl *a* were reported by Sukenik et al. (1988) for three green algae, *Chlamydomonas reinhardtii*, *Chlorella vulgaris*, and *Dunaliella tertiolecta*. For the former two species, slopes were similar (2.28 and 2.63, respectively) and constant at all irradiances. In contrast, for *D. tertiolecta*, the slope was small (about 0.84) for high values of Chl *a* and *b* (i.e. at LL) and increased up to 5.75 for low values of Chl *a* and *b* (i.e. at HL). These authors demonstrated that the observed light-induced change in the relationship between cellular Chl *a* and *b* in this alga resulted from a change in the relative composition of LHC II. Apoproteins that predominated in the LHC II of HL-acclimated cells had a lower capacity to bind Chl *b* than those of LL-acclimated cells (Sukenik et al., 1988). If we assume that *Prochlorococcus* photosynthetic apparatus is basically similar to that of chlorophytes, and especially that Chl *b* is found mainly in LHC II (Sukenik et al., 1988), the steep slope found for the MED strain suggests that it possesses either a LHC II with an atypically low ratio of DV-Chl *b* to DV-Chl *a* (as in *Dunaliella* grown at HL) or very little LHC II. The conclusions are the inverse for both Atlantic strains, suggesting that either the ratio of LHC II to LHC I varies between strains or the LHC II pigment ratio is variable. For the SARG strain, the apparent slope change in the plots of DV-Chl *b* versus DV-Chl *a*, which might also occur for NATL1 at irradiances higher than those tested, seems to indicate that in this strain the relative apoprotein content of

**Table II.** *Parameters derived from $P^{Chl}$ versus I and $P^{cell}$ versus I curves, spectrally averaged specific absorption coefficient, and photosynthetic quantum yield for three Prochlorococcus strains acclimated to different $I_g$ and light colors*

$\alpha^{Chl}$ is expressed in fg C (fg Chl)$^{-1}$ h$^{-1}$ ($\mu$mol quanta m$^{-2}$ s$^{-1}$)$^{-1}$; $\alpha^{cell}$ is expressed in fg C cell$^{-1}$ h$^{-1}$ ($\mu$mol quanta m$^{-2}$ s$^{-1}$)$^{-1}$; $P^{Chl}_m$ and $P^{Chl}_i$ in fg C (fg Chl)$^{-1}$ h$^{-1}$; $P^{cell}_m$ and $P^{cell}_i$ in fg C cell$^{-1}$ h$^{-1}$; $I_g$, $I_m$, and $I_b$ in $\mu$mol quanta m$^{-2}$ s$^{-1}$; and $a^*_{Chl}$ in m$^2$ (mg Chl)$^{-1}$ and $\phi_{CO2}$ in mol C (mol quanta)$^{-1}$. Values for $\alpha^{Chl}$ are displayed with the SE of the estimate provided by SYSTAT. Note that some cultures for which pigments were determined in Table I are missing from this table.

| Strain | Light Color | $I_g$ | Photosynthetic Parameters | | | | | | | | | $a^*_{Chl}$ | $\phi_{CO2}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | $\alpha^{Chl}$ | $P^{Chl}_m$ | $P^{Chl}_i$ | $\alpha^{cell}$ | $P^{cell}_m$ | $P^{cell}_i$ | $P_m:P_i$ | $I_m$ | $I_b$ | | |
| MED | Blue | 3.8 | 0.036 ± 0.002 | 1.53 | NA[a] | 0.119 | 5.07 | NA | NA | 141 | 549 | 0.013 | 0.065 |
| | White | 7.5 | 0.041 ± 0.002 | 1.22 | 0.28 | 0.174 | 5.19 | 1.19 | 4.36 | 100 | 425 | 0.011 | 0.084 |
| | White | 16.0 | 0.031 ± 0.001 | 2.47 | 0.46 | 0.062 | 4.91 | 0.91 | 5.37 | 258 | 922 | 0.012 | 0.059 |
| | White | 67.0 | 0.041 ± 0.003 | 4.78 | 2.20 | 0.050 | 5.78 | 2.66 | 2.17 | 341 | 730 | 0.023 | 0.040 |
| | White | 133.0 | 0.054 ± 0.003 | 4.31 | 3.86 | 0.073 | 5.85 | 5.24 | 1.12 | 225 | 371 | 0.020 | 0.064 |
| SARG | Blue | 6.0 | 0.111 ± 0.007 | 2.68 | NA | 0.186 | 4.49 | NA | NA | 72 | 176 | 0.026 | 0.097 |
| | White | 7.5 | 0.118 ± 0.009 | 1.39 | 0.68 | 0.234 | 2.76 | 1.35 | 2.04 | 39 | 145 | 0.032 | 0.084 |
| | White | 67.0 | 0.060 ± 0.003 | 2.47 | 2.12 | 0.091 | 3.76 | 3.22 | 1.17 | 135 | 512 | 0.021 | 0.065 |
| | White | 133.0 | 0.065 ± 0.003 | 5.58 | 4.79 | 0.073 | 6.23 | 5.35 | 1.16 | 253 | 569 | 0.024 | 0.062 |
| NATL1 | Blue | 6.0 | 0.049 ± 0.003 | 1.18 | NA | 0.172 | 4.13 | NA | NA | 71 | 154 | 0.013 | 0.087 |
| | White | 67.0 | 0.037 ± 0.002 | 1.36 | 1.20 | 0.082 | 3.02 | 2.66 | 1.13 | 127 | 603 | 0.015 | 0.056 |
| | White | 133.0 | 0.032 ± 0.002 | 2.27 | 2.08 | 0.059 | 4.17 | 3.82 | 1.09 | 219 | 636 | 0.014 | 0.052 |

[a] NA, Not applicable.

LHC II may change sharply above a given irradiance (i.e. ≥ 66 $\mu$mol quanta m$^{-2}$ s$^{-1}$), as for *Dunaliella* (Sukenik et al., 1988). The *y* intercepts of the plots of DV-Chl *b* versus DV-Chl *a* are similar for MED and SARG strains and correspond to the contribution of the components of the photosynthetic apparatus containing only DV-Chl *a*, i.e. the Chl proteins of RC I (CP 1) and RC II (CP 43 and CP 47; Falkowski et al., 1989).

The good correlation observed between $\alpha$-carotene and DV-Chl *a* (Fig. 2B) suggests that the former pigment is part of the photosystems, as is $\beta$-carotene in higher plants (Siefermann-Harms, 1985) and cyanobacteria (Kana et al., 1988). Further physiological studies are needed, however, to determine whether $\alpha$-carotene has a light-harvesting function and to determine the efficiency of energy transfer between $\alpha$-carotene and DV-Chl *a*. The absence of slope changes for all three *Prochlorococcus* strains in the plots of $\alpha$-carotene versus DV-Chl *a* suggests that, as for lutein in *Dunaliella* (Sukenik et al., 1988), the molar ratio of $\alpha$-carotene to DV-Chl *a* in LHCs may not change between LL- and HL-acclimated cells. If this ratio is comparable for the two Atlantic strains, as indicated by a similar slope (1.37 and 1.76), it is certainly lower in the MED strain, which exhibited a much steeper slope (4.50).

Zeaxanthin exhibited changes with irradiance opposite to that of $\alpha$-carotene, suggesting that the regulation modes and biological roles of these two pigments are different. The increase of the zeaxanthin to DV-Chl *a* ratio with increasing $I_g$, a feature that *Prochlorococcus* shares with *Synechococcus* cyanobacteria (Kana et al., 1988), suggests that this pigment plays a photoprotective role in both prokaryotes, although not through an epoxydation-deepoxydation cycle implying the presence of two xanthophylls, as is observed commonly in higher plants and algae (Siefermann-Harms, 1985). In *Synechococcus*, the zeaxanthin concentration per cell is, however, constant with irradiance (Kana et al., 1988), an intermediate behavior between that of the Mediterranean and Atlantic *Prochlorococcus* strains. In cyanobacteria other than *Synechococcus*, zeaxanthin has been shown to play no function in energy transfer, because it is spatially separated from photosystems (Siefermann-Harms, 1985). This is also likely the same in both *Synechococcus* and *Prochlorococcus*, as suggested by the significant decrease of the apparent quantum yield for photosynthesis in the blue-green region of the visible spectrum (i.e. the region where zeaxanthin absorbs the most) for both species (Bidigare et al., 1989; F. Partensky, unpublished data).

Photoacclimation of pigment content was recently observed in natural populations of prochlorophytes from the subtropical North Atlantic (Veldhuis and Kraay, 1990). From the surface to near the base of the euphotic zone (0.3% surface irradiance), the DV-Chl *a* and total Chl *b* concentrations varied in the ranges 0.91 to 5.40 and 0.44 to 8.20 fg cell$^{-1}$, respectively (Veldhuis and Kraay, 1990). These values, corresponding to a larger irradiance range than in our experiments (approximately 1–1500 versus 1–133 $\mu$mol quanta m$^{-2}$ s$^{-1}$), correspond to the ones we observed for both Atlantic strains (1.12–3.50 and 0.6–3.11 fg cell$^{-1}$ for DV-Chl *a* and total Chl *b*, respectively), as expected. In contrast, the DV-Chl *b* concentrations that we observed in the MED strain (0.11–0.57 fg cell$^{-1}$) were almost completely outside this range. Veldhuis and Kraay (1990) also reported constant zeaxanthin concentrations with depth of 1.97 ± 0.15 fg cell$^{-1}$, i.e. about twice as large as the maximum concentration observed in cultures. It is noteworthy, however, that in the Sargasso Sea at 110 m zeaxanthin concentrations of 0.69 fg cell$^{-1}$, more consistent with our results, have been reported

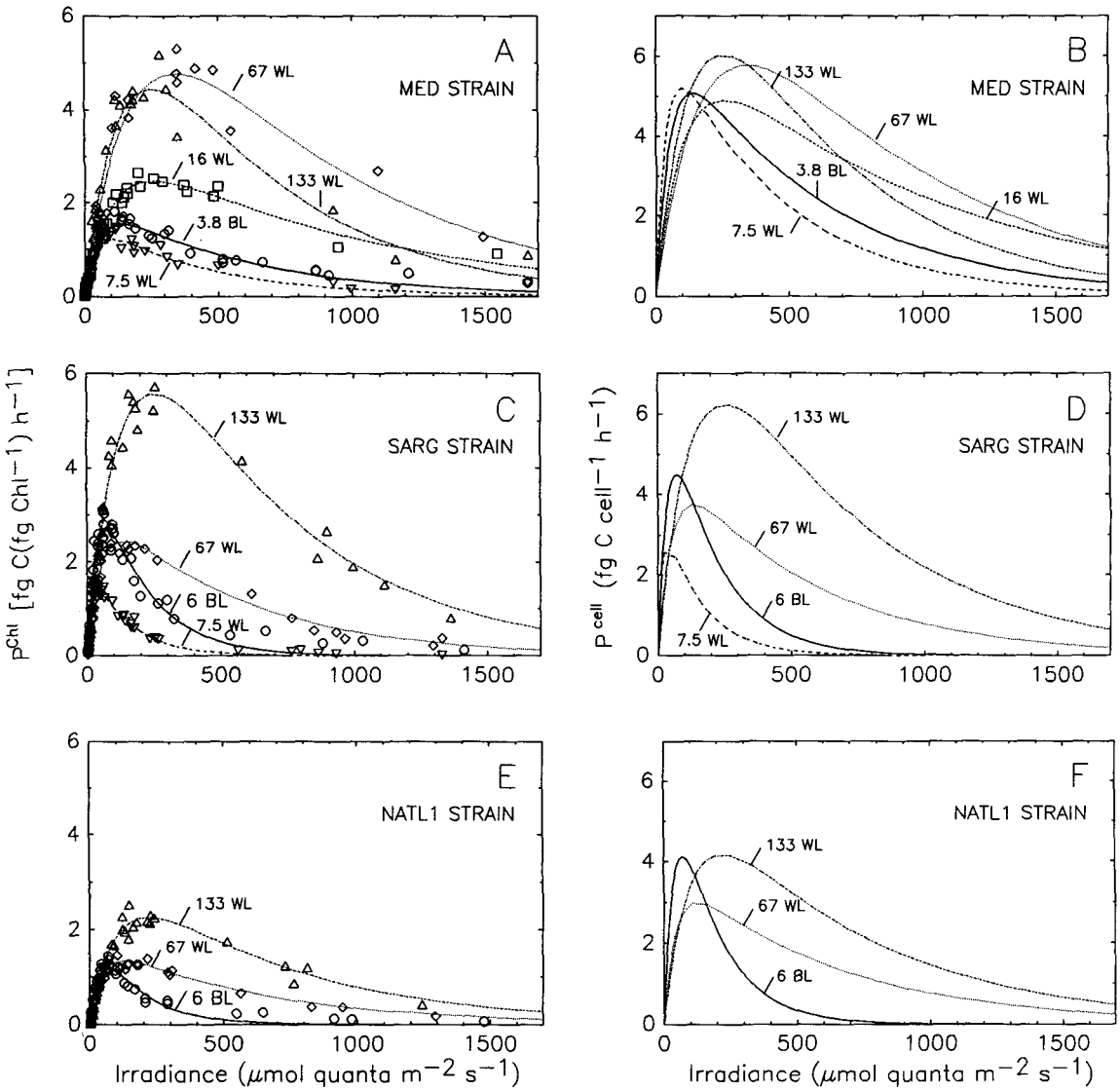


**Figure 5.** *P* versus *I* curves for *Prochlorococcus* strains grown under different WL or BL (in $\mu$mol quanta $m^{-2}$ $s^{-1}$). A and B, MED strain; C and D, SARG strain; E and F, NATL1 strain. A and C, Normalized per Chl. B and D, Normalized per cell. For readability, only fitted curves have been represented for graphs on a per cell basis (B and D).

(Chisholm et al., 1988). In the same sample, the $\alpha$-carotene concentration was 0.29 fg $cell^{-1}$ (Chisholm et al., 1988).

## Absorption Properties

Absorption spectra from intact *Prochlorococcus* cells resemble those from Chl *b*-containing eukaryotes (Chlorophyta) such as *D. tertiolecta*, which at 80 $\mu$mol quanta $m^{-2}$ $s^{-1}$ exhibits a Chl *a* to Chl *b* ratio similar to that of the SARG strain at 133 $\mu$mol quanta $m^{-2}$ $s^{-1}$ (i.e. 0.52 mol $mol^{-1}$; Soohoo et al., 1986, and this study). However, the replacement of Chl *a* by DV-Chl *a* in *Prochlorococcus* causes a 10-nm red shift of the blue maximum and a 4-nm blue shift of the red maximum. Another striking difference is that the respective ratios of the height of the blue peak to that of the red peak varied between HL- and LL-acclimated cells in the range of 2 to 5 for *Prochlorococcus* versus 1.2 to 2.5 for *Dunaliella* (Soohoo et al., 1986; Berner et al., 1989). This probably results from differences in cell size between these two genera, because absorption spectra tend to flatten out as the cell size increases (Morel and Bricaud, 1981).

The inverse relationship between pigment content and height of the blue (444 nm) peak observed in the MED strain shows the combined effect of an increase of the carotenoid to DV-Chl *a* ratio (and to a lesser extent DV-Chl *b* and Chl *c*-like pigment to DV-Chl *a* ratios) and of the package effect (Morel and Bricaud, 1986; Berner et al., 1989), whereas changes of the height of the red peak essentially result solely from the package effect. It is surprising that in Atlantic strains the Chl-specific absorption cross-sections tended to increase at LL, partly because of the very large contribution of DV-Chl *b*, which strongly counteracted the expected decrease of

$a^*_{Chl}(672)$ due to the package effect. Despite the very small size of *Prochlorococcus* cells, the latter effect does occur as a result of the large internal pigment concentrations in these cells. It has recently been estimated to produce an average 20% reduction of the absorption capacity of *Prochlorococcus* cells in the blue region compared to the same amount of pigments in solution (A. Morel, Y.W. Hahn, F. Partensky, D. Vaulot, and H. Claustre, unpublished data). Two factors probably contribute to the package effect in *Prochlorococcus* cells: (a) the degree of "transparency" of the thylakoid membranes, which may vary with the light-dependent lipid to protein ratio, and (b) the degree of stacking of thylakoid membranes (Berner et al., 1989). Thylakoids of *Prochlorococcus* are tightly packed at the periphery of cells, in contrast to those of *Synechococcus* (a species exhibiting lower package effect; Bidigare et al., 1989; A. Morel, Y.W. Hahn, F. Partensky, D. Vaulot, and H. Claustre, unpublished data), which are widely spaced (Chisholm et al., 1988). Values for specific absorption at the red peak reported in the literature for eukaryotic phytoplankton are generally lower (range, 0.011–0.026 $m^2$ [mg Chl]$^{-1}$ in Morel and Bricaud, 1986) than those measured in our cultures (0.022–0.043 $m^2$ [mg Chl]$^{-1}$). Consequently, the same is true with the spectrally weighted absorption cross-section, which ranges between 0.004 and 0.021 $m^2$ (mg Chl)$^{-1}$ in eukaryotes (Falkowski et al., 1985), as compared to 0.011 to 0.032 $m^2$ (mg Chl)$^{-1}$ in *Prochlorococcus* cultures. Although this may partly result from an approximate 24% difference in the extinction coefficients for DV-Chl *a* and Chl *a* (C.A. Rebeiz, personal communication cited in Goericke and Repeta, 1992) and possibly from uncertainty in the estimate of the "$\beta$ effect" (Bricaud and Stramski, 1990), high values for $a^*_{Chl}$ indicate that *Prochlorococcus* cells have a good capacity for harvesting light, whatever the irradiance.

The peculiar pigmentation of *Prochlorococcus* is expected to produce visible changes in absorption properties of samples collected from oceanic waters dominated by these prokaryotes, although these changes should be somewhat smoothed out by the contribution of other algal groups. For example, in the subtropical Atlantic Ocean (Sargasso Sea), where *Prochlorococcus* contribute 30 to 40% of the integrated Chl standing stock (Neveux et al., 1989), absorption spectra show, from the surface (0–60 m) to the deep (100–180 m) layers, shifts of the blue maximum from 440 to 447 nm and of the red maximum from 677.5 to 675 nm (figure 16 in Bricaud and Stramski, 1990).

### Light-Induced Changes of Photosynthesis, Quantum Yield for Carbon Fixation, and Growth

For both Atlantic strains grown under WL, an increase in $I_g$ was accompanied by large increases in both $P^{Chl}_m$ and $P^{cell}_m$ (up to 4.0- and 2.3-fold, respectively, for a 17.7-fold increase in WL) and a concomitant decrease in $\alpha^{cell}$ and $\alpha^{Chl}$ (up to 2.4- and 2-fold, respectively). This behavior appears to be quite atypical in phytoplankton, because it does not fit any of the reported models for *P* versus *I* responses (Richardson et al., 1983). A decrease in $\alpha^{Chl}$ at HL has only been observed in a diatom, *Thalassiosira pseudonana* (Cullen and Lewis, 1988), and in the prokaryote *Synechococcus* WH 7803 (Kana and Glibert, 1987). In the Atlantic strains of *Prochlorococcus*, this decrease was essentially related to the decrease of $\phi_{CO2}$ at HL, whereas $\alpha^*_{Chl}$ either decreased (SARG) or remained constant (NATL1). At a given $I_g$, the initial slope, $\alpha^{Chl}$, was about 2-fold larger in SARG than in the NATL1, as a result of both a much larger $a^*_{Chl}$ and a slightly larger $\phi_{CO2}$. The response exhibited by the MED strain was similar to that of Atlantic strains for $P^{Chl}_m$ and $\alpha^{cell}$ but not for $\alpha^{Chl}$ and $P^{cell}_m$, which were more or less constant. The small range found for $\alpha^{Chl}$ was the result of inverse variations of $\phi_{CO2}$ and $a^*_{Chl}$. This response mimicked that recently reported for *D. tertiolecta* (Sukenik et al., 1990). Moreover, for a comparable relative decrease in light intensity (700–70 $\mu$mol quanta $m^{-2}$ $s^{-1}$ for *Dunaliella*, 67–7.5 $\mu$mol quanta $m^{-2}$ $s^{-1}$ for *Prochlorococcus*), the range of variations for $P^{Chl}_m$ (3- and 4-fold decrease, respectively) and photosynthetic quantum yield (about 2.2-fold increase for both strains) were about similar for these two species, as were the relative changes in Chl *a* and *b* contents and Chl *b* to Chl *a* ratio.

The absolute values of quantum yield were also comparable, at about 0.04 (HL) and 0.08 (LL) mole of photons per mol of either oxygen (*Dunaliella*, Sukenik et al., 1990) or carbon (*Prochlorococcus*, this study). However, because for a given species the quantum yield for oxygen evolution is equal or higher than that for carbon fixation depending particularly on the nitrogen source (Bannister and Weidemann, 1984; Falkowski et al., 1985), both $\phi_{CO2}$ and $\phi_{O2}$ were higher in *Prochlorococcus* than in *Dunaliella*. An increase in quantum yield of photosynthesis at LL was reported for three other phytoplankton species belonging to different phylogenetic groups and grown in nutrient-replete conditions (Falkowski et al., 1985). If the ratio of $CO_2$ to $O_2$ given by these authors for each species is used, values of $\phi_{O2}$ (table 1 in Falkowski et al., 1985) can be converted in terms of $\phi_{CO2}$. Between 600 and 70 $\mu$mol quanta $m^{-2}$ $s^{-1}$, $\phi_{CO2}$ increased from 0.012 to 0.033 mol C (mol quanta)$^{-1}$ for the dinoflagellate *Prorocentrum micans*, from 0.018 to 0.070 mol C (mol quanta)$^{-1}$ for the chrysophyte *Isochrysis galbana*, and from 0.030 to 0.070 mol C (mol quanta)$^{-1}$ for the diatom *Thalassiosira weisflogii*. Two main conclusions can be inferred when comparing these values with those measured on *Prochlorococcus*. First, $\phi_{CO2}$ for the prokaryote are at the higher limit of those observed for the eukaryotes. Only the diatom grown at 30 $\mu$mol quanta $m^{-2}$ $s^{-1}$ reached quantum yields (0.089 mol C [mol quanta]$^{-1}$) as high as in LL-acclimated *Prochlorococcus* (0.084–0.087 mol C [mol quanta]$^{-1}$). Second, for a comparable relative change in light intensity, the range of variations of $\phi_{CO2}$ were lower in *Prochlorococcus* (e.g. 0.084–0.065 mol C [mol quanta]$^{-1}$ for the SARG strain) than in the eukaryotes, suggesting that optimal values of $\phi_{CO2}$ can be maintained over a wider range of irradiances in the prokaryote.

The MED strain showed no specific pigmentary or photosynthetic response to BL. The variations observed between WL- and BL-grown cells likely would not occur if considering irradiances weighted by spectral absorption coefficients (Morel et al., 1987). Light intensity is, therefore, more important than light quality in governing photoacclimation in this strain, as for the diatom *Chaetoceros protuberans* (Morel et al., 1987). In contrast, under BL, both Atlantic strains showed increased maximum carbon fixation rate and quantum yield for carbon fixation, the latter being close to the maximum possible $\phi_{CO2}$

of 0.1 mol C (mol quanta)$^{-1}$ (Bannister and Weidemann, 1984). This suggests a chromatic acclimation of the photosynthetic response of Atlantic strains to BL. This ability must result from either subtle structural alterations of the photosynthetic apparatus or increased activity of accessory pigments, which cannot be inferred solely from the determination of cellular pigments. Undoubtebly, increased $\phi_{CO2}$ and photosynthetic rates under BL constitute an ecological advantage for the growth of *Prochlorococcus* at depth in the open ocean.

In contrast, the strong photoinhibition exhibited by cultured strains at HL, as denoted by the strong curvature of $P$ versus $I$ curves after 2 h of incubation, and the relatively low irradiances at which both growth and photosynthesis were saturated suggest a sensitivity of *Prochlorococcus* cells to high irradiances. This observation raises the question of the ability for this prokaryote to proliferate in near-surface waters of oligotrophic areas, such as tropical Atlantic or Pacific oceans, and often to predominate over other photosynthetic groups, including *Synechococcus*, a genus that is generally found in the same ecological niche as *Prochlorococcus* (Olson et al., 1990; L. Campbell and D. Vaulot, unpublished data). $P$ versus $I$ curves obtained for cultured *Synechococcus* WH7803 (Kana and Glibert, 1987) show that this species exhibits high photosynthetic rates up to 2000 $\mu$mol quanta m$^{-2}$ s$^{-1}$ and much higher $P^{Chl}_m$ than *Prochlorococcus* (8–20 fg C [fg Chl]$^{-1}$ h$^{-1}$ versus 1.2–2.6 fg C [fg Chl]$^{-1}$ h$^{-1}$). Although *Synechococcus* WH7803 has a pigmentation and possibly photosynthetic characteristics that are not typical of cyanobacterial populations from the open ocean (Olson et al., 1988), *Synechococcus* is probably better adapted than *Prochlorococcus* for growing and photosynthesizing in near-surface waters. The predominance of the latter genus over the former would, therefore, not be the result of a better growing and photosynthesizing ability. Rather, it could result from some other physiological advantages, including a better capacity to use either organic nitrogen (e.g. amino acids, urea) or extremely low levels of inorganic nitrogen and a reduced grazing. Alternatively, it is possible that none of the *Prochlorococcus* strains maintained in culture to date is representative of surface populations.

Relationships between growth and photosynthesis apparently differed among *Prochlorococcus* strains. Their respective growth rates became light saturated at similar values (approximately 0.4–0.45 d$^{-1}$ at 18°C) but at distinct irradiances, lower than the ones saturating photosynthesis. At its optimal irradiance for growth (16 $\mu$mol quanta m$^{-2}$ s$^{-1}$), the MED strain had a large excess photosynthetic capacity ($P_m$ to $P_i$ ratio > 5), suggesting that the coupling between growth and photosynthesis may be complex. In contrast, for the Atlantic strains, the ratio of $P_m$ to $P_i$ was high at low $I_g$ (at least for the SARG strain) and approached 1 when $\mu$ approached $\mu_{max}$, a behavior similar to that observed in several other phytoplankton species (Kana and Glibert, 1987). Quantitative assessment of the coupling between growth and photosynthesis would require the knowledge of the quantum yield for growth (Falkowski et al., 1985), which was not accessible because the ratio of C to DV-Chl *a* is difficult to assess accurately in nonaxenic cultures.

## ACKNOWLEDGMENTS

We would like to thank Dr. Marlon R. Lewis for use of his photosynthetron, Drs. Erika Head and Roddy E. Warnock for technical hints and help, and Drs. Annick Bricaud, Paul G. Falkowski, André Morel, Jacques Neveux, and Shubba Sathyendranath for helpful comments on the manuscript. We are grateful to Prof. Sallie W. Chisholm and Ms. Sheila Frankel for the communication of the culture medium recipe and exchange of *Prochlorococcus* strains.

Received May 27, 1992; accepted September 21, 1992.


## LITERATURE CITED

**Bannister TT, Weidemann AD** (1984) The maximum quantum yield of phytoplankton photosynthesis in situ. J Plankton Res **6:** 275–294

**Berner T, Dubinsky Z, Wyman K, Falkowski PG** (1989) Photoadaptation and the "package effect" in *Dunaliella tertiolecta* (Chlorophyceae). J Phycol **25:** 70–78

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

**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:** 562–582

**Chisholm SW, Frankel SL, Goericke R, Olson RJ, Palenik B, Waterbury JB, West-Johnsrud L, Zettler ER** (1992) *Prochlorococcus marinus* nov. gen. nov. sp.: an oxyphototrophic marine prokaryote containing chlorophyll *a* and *b*. Arch Microbiol **157:** 297–300

**Chisholm SW, Olson RJ, Zettler ER, Goericke R, Waterbury JB, Welshmeyer NA** (1988) A novel free-living prochlorophyte abundant in the oceanic euphotic zone. Nature **334:** 340–343

**Cullen JJ, Lewis MR** (1988) The kinetics of algal photoadaptation in the context of vertical mixing. J Plankton Res **10:** 1039–1063

**Falkowski PG, Dubinsky Z, Wyman K** (1985) Growth-irradiance relationships in phytoplankton. Limnol Oceanogr **30:** 311–321

**Falkowski PG, Owens TG** (1980) Light-shade adaptation: two strategies in marine phytoplankton. Plant Physiol **66:** 592–595

**Falkowski PG, Sukenik A, Herzig R** (1989) Nitrogen limitation in *Isochrysis galbana* (haptophyceae). II. Relative abundance of chloroplast proteins. J Phycol **25:** 471–478

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

**Kana TM, Glibert PM** (1987) Effect of irradiances up to 2000 $\mu$E m$^{-2}$ s$^{-1}$ on marine *Synechococcus* WH7803. II. Photosynthetic responses and mechanisms. Deep Sea Res **34:** 497–516

**Kana TM, Glibert PM, Goericke R, Welschmeyer NA** (1988) Zeaxanthin and $\beta$-carotene in *Synechococcus* WH7803 respond differently to irradiance. Limnol Oceanogr **33:** 1623–1627

**Lewis MR, Smith JC** (1983) A small volume, short incubation-time method for measurement of photosynthesis as a function of incident irradiance. Mar Ecol Prog Ser **13:** 99–102

**Morel A, Bricaud A** (1981) Theoretical results concerning light absorption in a discrete medium, and application to specific absorption of phytoplankton. Deep Sea Res **11:** 1375–1393

**Morel A, Bricaud A** (1986) Inherent properties of algal cells including picoplankton: theoretical and experimental results. Can Bull Fish Aquat Sci **214:** 521–559

**Morel A, Lazzara L, Gostan J** (1987) Growth rate and quantum yield time response for a diatom to changing irradiances (energy and color). Limnol Oceanogr **32:** 1066–1084

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

**Olson RJ, Chisholm SW, Zettler ER, Altabet M, Dusenberry J** (1990) Spatial and temporal distributions of prochlorophyte picoplankton in the North Atlantic ocean. Deep Sea Res **37:** 1033–1051

**Olson RJ, Chisholm SW, Zettler ER, Armburst EV** (1988) Analysis of *Synechococcus* pigment types in the sea using single and dual beam flow cytometry. Deep Sea Res **35:** 425–440

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

**Platt T, Gallegos CL, Harrison WG** (1980) Photoinhibition of photosynthesis in natural assemblages of coastal marine phytoplankton. J Mar Res **38:** 687–701

**Richardson K, Beardall J, Raven JA** (1983) Adaptation of unicellular algae to irradiance: an analysis of strategies. New Phytol **93:** 157–191

**Rüdischer W, Schoch S** (1988) Chlorophylls. *In* TW Goodwin ed, Plant Pigments. Academic Press, New York, pp 1–59

**Siefermann-Harms D** (1985) Carotenoids in photosynthesis. 1. Location in photosynthetic membranes and light-harvesting function. Biochim Biophys Acta **811:** 325–355

**Soohoo JB, Kiefer DA, Collins DJ, McDermid IS** (1986) In vivo fluorescence excitation and absorption spectra of marine phytoplankton. I. Taxonomic characteristics and responses to photoadaptation. J Plankton Res **8:** 197–214

**Sukenik A, Bennet J, Falkowski P** (1988) Changes in the abundance of individual apoproteins of light-harvesting chlorophyll *a*/*b*-protein complexes of photosystem I and II with growth irradiance in the marine chlorophyte *Dunaliella tertiolecta*. Biochim Biophys Acta **932:** 206–215

**Sukenik A, Bennet J, Mortain-Bertrand A, Falkowski P** (1990) Adaptation of the photosynthetic apparatus to irradiance in *Dunaliella tertiolecta*. A kinetic study. Plant Physiol **92:** 891–898

**Urbach E, Robertson D, Chisholm SW** (1992) Multiple evolutionary origins of prochlorophytes within the cyanobacterial radiation. Nature **355:** 267–269

**Vaulot D, Partensky F, Neveux J, Mantoura R, Llewelyn C** (1990) Wintertime presence of prochlorophytes in surface waters of the North-Western Mediterranean Sea. Limnol Oceanogr **35:** 1156–1164

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