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Prochlorococcus and *Synechococcus:* A comparative study of their optical properties in relation to their size and pigmentation

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ABSTRACT

Three unialgal strains of *Prochlorococcus* and four of *Synechococcus* were grown in batch culture at low irradiances. The spectral values of light absorption, scattering and backscattering of intact cells in suspension were determined, together with cell counts, size distribution and pigment composition (via HPLC). The spectral efficiency factors Q_a, Q_b, Q_{bb} for light absorption, scattering and backscattering respectively, were derived, as well as the corresponding chlorophyll-specific coefficients a^*, b^* and b_b^*. The pigment used when normalizing is "true" chlorophyll a for *Synechococcus,* and divinyl-chlorophyll a for *Prochlorococcus*. In correspondence with small sizes (0.6 μm, on average) *Prochlorococcus* exhibits Q_b values below those of *Synechococcus* (size about 0.9 μm, on average). In contrast, Q_a is higher for *Prochlorococcus* than for *Synechococcus,* in response to high internal divinyl-chlorophyll content. In the blue part of the spectrum the probability for photons of being absorbed by a *Prochlorococcus* cell exceeds that of being scattered. Such a combination has never been found before for other algal cells, consistently more efficient as scatterers than as absorbers. The magnitude of the three efficiency Q-factors, as well as their spectral variations, can be understood and reconstructed in the frame of the Mie theory. The impact of these small organisms, dominant in oligotrophic environment, upon the optical properties of such waters are discussed on the basis of their chlorophyll-specific optical coefficients. Their absorption capabilities (per unit of chlorophyll) are not far from being maximum, to the extent that the package effect is rather reduced. With respect to scattering, *Prochlorococcus* cells have a minute signature compared to that of *Synechococcus*. This point is illustrated using vertical profiles of fluorescence, attenuation coefficient, cell number, Chl a and divinyl-Chl a concentrations, as observed in an oligotrophic tropical situation. Even if the backscattering-to-scattering ratio is, as theoretically expected, higher for *Prochlorococcus* than for all other algae (including *Synechococcus*), their light backscattering capacity definitely remains negligible.

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1. Introduction

Since their discovery in the late 70's (Waterbury *et al.,* 1979), chroococcoid marine cyanobacteria *(Synechococcus* sp.) have been found to be widely distributed in oceanic waters and the contribution of these small (typically 1 μm) prokaryotic photosynthesizing plankters to the primary production within dimly blue lighted layers has been emphasized (Murphy and Haugen, 1985; Wood, 1985; Glover *et al.,* 1986). Associated with unidentified particles below 1 μm, a chlorophyll *a* derivative was discovered with concentration exceeding that of "normal" chlorophyll *a* in oligotrophic tropical waters (Gieskes and Kraay, 1983). A few years later, using flow cytometry, extremely abundant red fluorescing small bodies (typically <0.8 μm) were identified in the same kind of waters. Because of their unusual pigmentation, they were hypothesized to be free-living marine prochlorophytes (Chisholm *et al.,* 1988; Li and Wood, 1988; Neveux *et al.,* 1989; Vaulot *et al.,* 1990). The name *Prochlorococcus marinus* (Chisholm *et al.,* 1992) was recently given to an organism of this type isolated from the Sargasso Sea, and its phylogenetic position has been clarified (Urbach *et al.,* 1992).

With changing relative proportions (Olson *et al.,* 1990a; Veldhuis and Kraay, 1990) *Prochlorococcus* and *Synechococcus* in association with picoeukaryotic algae, often appear to be dominant in oligotrophic environments, i.e. in a major part of the world ocean. Therefore, the understanding of the optical properties of many oceanic waters requires a dedicated study of the optical properties of these organisms. In addition, particular signatures, with respect to those of other algae, are expected for these procaryotes because of their minute size (in the range of the wavelength of the light) and also of their original pigmentation. Cyanobacteria have no accessory chlorophyll, but possess water-soluble phycobilin pigments and therefore peculiar absorption bands (see e.g. Prezelin and Boczar, 1986). In contrast, prochlorophytes are deprived of phycobiliproteins, but contain divinyl derivatives of chlorophyll *a* and *b*, identified as being 8-desethyl, 8-vinyl compounds (Goericke and Repeta, 1992). These pigments show a 10 nm red-shifted γ-Soret band with respect to the "normal" Chl *a* and Chl *b*. With such differing pigmentations (and also differing typical sizes), the spectral absorption of *Prochlorococcus* and *Synechococcus,* and perhaps their potential photosynthesizing performances, must differ. Not only their absorption but also their scattering efficiencies have to be determined to predict their signature inside a water body.

Information is becoming available concerning the optical properties of marine cyanobacteria *(Synechococcus* and *Synechocystis,* see e.g. Bricaud *et al.,* 1988; Perry and Porter, 1989; Stramski and Morel, 1990). Measurements, however, have been essentially restricted to some strains, typical of neritic rather than pelagic zones, and no interspecific comparisons have been carried out yet. To our knowledge, the optical properties of the newly discovered prochlorophytes have never been examined, except for some absorption measurements on intact cells retained onto GF/F

filters (Partensky *et al.,* 1993). Predictions restricted to their light scattering capabilities are possible from theoretical considerations, based on their size and plausible values for their refractive index (Morel and Ahn, 1991; Stramski and Kiefer, 1991). Concerning the absorption capabilities of tiny phytoplankters, it has often been conjectured that the "package" or "discretness" effect (Kirk, 1975; Morel and Bricaud, 1981) might be reduced, even annihilated. In such a case the efficiency in absorbing available light would be optimized, as if pigments were uniformly dispersed. This hypothesis, however, remains to be experimentally verified.

Several strains of pelagic cyanobacteria and prochlorophytes (*Synechococcus* and *Prochlorococcus*), typical of oligotrophic waters, have been recently isolated and successfully grown in batch culture (Chisholm *et al.,* 1992), allowing various optical measurements, pigment analyses and size distributions to be carried out. Contamination by heterotrophic bacteria was inevitable in these cultures and corrections for their interferences were necessary, even if not easy. The aim of this paper is to present the results of a comparative study concerning the spectral values of the Chl-specific absorption, scattering and backscattering coefficients for these two categories of picoplankters. Their optical cross sections at the level of individual cells are also derived, and these values are discussed in the light of their pigment composition determined by reverse-phase high-performance liquid chromatography (HPLC). This work is a continuation of similar studies dealing with heterotrophic organisms (Morel and Ahn, 1990; 1991) and photoautotrophic plankters of larger size (Bricaud *et al.,* 1988; Ahn *et al.,* 1992). The observations are interpreted within a physical framework (exposed in Bricaud and Morel, 1986), based on the exact Mie theory for spherical particles or on the anomalous diffraction approximation, as developed by van de Hulst (1957). The theoretical approach used here is the same as those in the earlier works to which the reader is referred for a more detailed description. The possible contributions of these organisms to the optical properties of oceanic waters, particularly in oligotrophic zones, are also discussed.

2. Materials and methods

a. Strains, culture methods and cell counting. *Prochlorococcus* strains: Three unialgal strains of *Prochlorococcus sp.* (thereafter named MED, SARG and NATL1) were used in the present study. MED strain originates from the northwestern Mediterranean Sea, whereas SARG (isolated by B. Palenik and kindly provided by S. W. Chisholm) as well as NATL1 strains were isolated from the North Atlantic Ocean (SARG is the type species *Prochlorococcus marinus*). Details on strain origin and isolation methods are given elsewhere (Partensky *et al.,* 1993). Batch cultures were grown at $17 \pm 1°C$ in 260 ml polystyrene culture flasks (Nunclon, Denmark) under a 12:12 h light:dark cycle, and a blue light irradiance of 7 ± 2 μmol quanta.m^{-2} s^{-1} (Rohm & Haas plexiglas, blue 2424). Culture medium used is a modification of "K/10" medium (Keller *et al.,* 1987, Chisholm *et al.,* 1992) with 40 μM urea as a

Table 1. Relevant informations concerning *Prochlorococcus* and *Synechococcus* strains grown in culture for pigment analyses and optical determinations.

Strains/#	D_m (μm)	"Chl a" (fg cell⁻¹) SP	HPLC	"b/a" SP	HPLC	Absorption maxima of intact cells (λ, nm)			
PROCHLOROCOCCUS									
MED (1)	0.537	1.85	1.76	0.03	0.14	445			672
(2)	0.553	1.45	—	<0.02	—	445			672
(3)	0.553	1.16	1.08	0.02	0.14	445			672
NATL1 (1)	0.644	1.21	1.00	0.82	1.20	448	480		671
(2)	0.568	0.91	0.88	0.72	1.02	448			671
(3)	0.666	2.62	—	0.65	0.86	448			671
SARG (1)	0.568	—	—	—	—	448			672
(2)	0.656	1.45	1.57	0.73	1.02	448	478		672
SYNECHOCOCCUS									
ROS04 (H)	0.966	0.90	1.18	—	—	438	495	548	678
(L)	1.074	1.03	—	—	—	439	496	546	678
MAX01 (H)	0.812	0.82	—	—	—	439	492	548	678
(L)	0.924	1.19	1.26	—	—	438	493	548	678
MAX41 (L)	0.853	1.20	1.17	—	—	438	492	544	678
DC2 (L)	1.074	1.25	—	—	—	438	496	550	678

#: (1) (2) (3): replicate cultures under the same light conditions (*Prochlorococcus*) (H) (L): cultures grown under high ($\approx$ 100 μmole quanta m⁻² s⁻¹) or low ($\approx$ 25 μmole quanta m⁻² s⁻¹) irradiances (*Synechococcus*)

D_m: modal diameter.

"Chl a": chlorophyll a content per cell for the *Synechococcus* group and divinyl-Chl a content per cell for the *Prochlorococcus* group.

"b/a": ratio of divinyl Chl b-to-divinyl Chl a (for *Prochlorococcus* only).

SP and HPLC: spectroscopic or chromatographic determinations.

nitrogen source and no copper added (S. Chisholm, pers. comm.). At the end of the incubation the cell concentrations reached 1 to 3 10⁷ cells/ml. Heterotrophic bacteria and prochlorophytes were counted with an EPICS 541 flow-cytometer optimized for sensitivity using a Biosense flow cell (all equipments from Coulter, Hialeah, FL, USA), as described in Vaulot *et al.* (1990). Prochlorophytes were discriminated from heterotrophic bacteria using side scatter vs. chlorophyll red fluorescence bi-variate histograms. After counting, samples were sent immediately (within 24 h) to Villefranche-sur-Mer for subsequent analyses. Replicate cultures under the same illumination conditions were successively prepared (numbered 1, 2 and 3 in Table 1).

Synechococcus strains: DC2 (= WH 7803 = CCMP 1334) was obtained from the Culture Collection for Marine Phytoplankton (Bigelow Laboratory, West Boothbay

Harbor, ME). ROS04 was isolated by D. Vaulot and C. Courties from coastal English Channel waters (48° 43'N, 3° 59'W, Roscoff, France). MAX01 and MAX41 were isolated by D. Vaulot and C. Courties from the Sargasso Sea during the CHLOMAX cruise (Neveux *et al.,* 1989) respectively from a surface sample taken at 26° 17'N, 63° 15'W and a 120 m-depth sample taken at 26° 18'N, 63° 26'W. Prior to optical measurements, all strains were grown in batch culture (500 ml) in f/2 media without Si (Guillard, 1975), at 17°C and under low irradiances (25 μmol quanta m^{-2} s^{-1}, white light). The two strains ROS04 and MAX01 were also grown in parallel under higher irradiances (100 μmol quanta m^{-2} s^{-1}, white light). These light conditions are denoted "L" and "H," respectively, in Table 1 and in figures.

b. Determination of the size distribution functions. The cell number density, denoted N/V, was also determined using a Coulter Counter model ZBI equipped with a 20 μm orifice. For *Prochlorococcus,* counts averaged over at least 10 determinations, agreed within $+20\%$ to -5% with the flow cytometric enumerations performed about 24 h before. The size distribution function, denoted $F_r(D)$ where D is the diameter of the equivalent sphere, was determined in relative numbers with a 100-channels analyser (Coulter channelyzer model C 1000) calibrated with 0.82 μm polystyrene beads (Coulter electronics, Inc.). Repetitive measurements demonstrated a high stability of the $F_r(D)$ functions as well as of the position of the maximum (within 0.05 μm). Each relative $F_r(D)$ function was thereafter expressed in absolute values by demanding that its integral be equal to the corresponding N/V value. Because the first channels were dominated by noise, the measured size distribution for *Prochlorococcus* was truncated at 0.40 or 0.45 μm and the missing part was extrapolated toward zero by fitting the curve to a log-normal distribution (Fig. 1a), as in Morel and Ahn (1990). In contrast, when dealing with *Synechococcus,* the size spectrum was unambiguously separated from the noise occurring below 0.45 μm so that there was no need for any extrapolation (Fig. 1b).

While heterotrophic bacteria and *Prochlorococcus* can be discriminated and separately counted by flow-cytometry, this is not possible when determining the size distribution function with the Coulter counter. Their respective sizes fall in the same range and the size spectrum does not show any particular feature that could allow a distinction to be achieved. It is therefore necessary to postulate that the shape of the $F_r(D)$ distribution as determined for the whole population is also valid for the sole *Prochlorococcus* cells. Algal number generally amounted to 70–85% of the total cell number, except for SARG (1) and (2) and for MED (1) cultures, where it was only 45–55%. According to occasional microscopic inspections (with DAPI staining), such a drawback did not occur appreciably in the *Synechococcus* cultures. The size distributions of cyanobacteria actually fell to zero without a secondary peak, or a bump within the smaller size range, that would have revealed the presence of a significant bacterioplankton population. The number density, N/V, so determined,
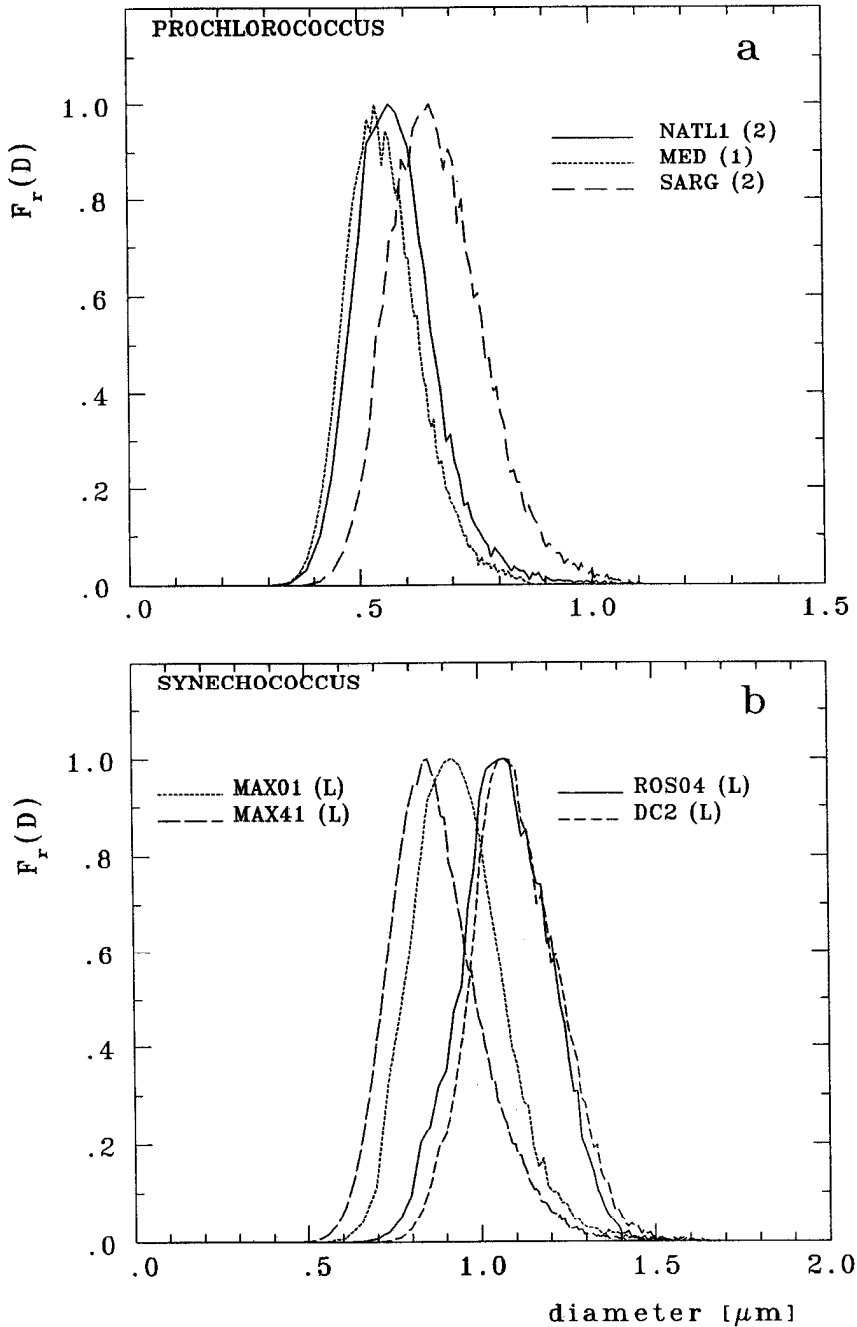
Figure 1. Relative size distribution functions (normalized to their maximum) for the various strains of *Prochlorococcus* (panel a) and *Synechococcus* (panel b), labelled as in Table 1. For clarity only one size distribution per strain is represented; the other curves, not shown, are almost identical apart from slight shifts of the maximum.

was entirely attributed to the cyanobacterial cells, without correction for heterotrophic bacteria.

c. Optical measurements. The optical measurements consisted in determining the absorption, attenuation and backscattering coefficients, $a(\lambda)$, $c(\lambda)$ and $b_b(\lambda)$ respectively, for living algae in suspension. They were carried out as previously described (Morel and Ahn, 1990, 1991 and Ahn *et al.*, 1992). The presentation of the methods is accordingly abridged and only specific details are provided. When performing the attenuation measurement using a modified (Perkin-Elmer 571) spectrophotometer, the reduced acceptance angle (0.12°) prevents most of the forward scattered radiation from entering the detector so that it can be computed (via Mie theory) that the underestimate of $c(\lambda)$ remains below 1.5%. When measuring $a(\lambda)$, the absorption coefficient, the acceptance angle is set at about 45° (optical cuvette and diffusing plate close to the entrance window of the detector), thus more than 97% of the scattered flux can reach the photomultiplier. The problem faced with almost non-absorbing cells (such as heterotrophic bacteria), as discussed in Morel and Ahn (1990), does not exist when dealing with pigmented algae. The measurement of the radiant flux backscattered by algal suspensions into an integrating sphere, provides a partial information which has to be combined with computations based on Mie theory to derive the $b_b(\lambda)$ coefficient. This rather complex procedure is detailed elsewhere (in Appendix, Ahn *et al.*, 1992).

To achieve the optical determinations, it was necessary to increase the numerical cell density of *Prochlorococcus* by eliminating part of the culture medium. By gravity filtration onto Nuclepore membrane (0.4 µm), final concentrations of about 1.5–2.7 10^8 cells ml^{-1} were reached within the remnant suspension. With *Synechococcus*, suitable densities (between 4.4 and 7.6 10^7 cells ml^{-1}) were obtained in the same way, but using 0.6 µm pore size membranes. This procedure was repeated, and the concentration again increased (by a factor of about 5), in view of performing the backscattering measurements for which higher cell concentrations are required. In some cases, the limited initial culture volume, together with the insufficient biomass concentration reached at the end of the culture experiments, did not allow the entire set of measurements to be successfully carried out.

d. Pigment determinations. The spectrophotometric method for the routine determination of the chlorophyll concentrations, and the HPLC method for a detailed analysis of the liposoluble pigment composition and concentration were simultaneously used. With the knowledge of the cell size and number, the pigment concentration within the suspension can be transformed into a (mean) content per cell (expressed as fg cell^{-1}), or an intracellular concentration, denoted C_i, and expressed as kg per m^3 of cellular material.

The standard procedure, namely a filtration onto GF/F pads, then followed by 100% acetone extraction (24 h at 5°C), was applied. However, some particular

cautions are required with *Prochlorococcus* cells, given their ability to pass through the filter; precombusted filters were preferred for their better retention and the suspensions were repeatedly filtered on the same pad. It was nevertheless necessary to count cells in the filtrate and subtract this number from that before filtration, in order to determine the exact number of cells actually collected.

Concentrations were computed from the optical densities according to Jeffrey and Humphrey (1975). It is important to note that the trichromatic equations providing "Chl *a*" and "Chl *b*" concentrations were operated in the case of *Prochlorococcus* without changing the numerical coefficients. However, the optical density usually read at 664 nm is replaced by the density recorded at the maximum, located at wavelengths ranging from 661 to 663 nm. In absence of published values of the molar extinction for the divinyl-chlorophylls, this procedure was adopted to provide consistent values (amenable to a later re-evaluation). Phaeopigments in the culture always remained at negligible levels. Entire (400–750 nm) absorption spectra of the acetone extracts have been recorded.

Before injection in the HPLC system, two pigment extract parts were mixed with one part of an ion pairing reagent (Mantoura and Llewellyn, 1983). The HPLC system consisted of a LDC CM 4000 constametric pump and a Rheodyne 7125 injection valve; the column (10 cm length, 0.46 cm internal diameter) was filled with HYPERSIL 3 μm (Société Française de Chromato Colonne). Elution conditions were as in Williams and Claustre (1991), except that flow rate was 1 ml min^{-1}. Detection was performed using a LDC spectromonitor set up at 440 nm. Peak areas were recorded and calculated using the Nelson Analytical software on a PC compatible. Identification of compounds (Fig. 2) was achieved on the basis of their UV-visible properties recorded with a Waters 991 photodiode array detector. Quantification of pigments was performed by accounting for the peak area and the response factor of the spectrophotometer detector. This factor was established either by using published extinction coefficients (Mantoura and Llewellyn, 1983), or by injection of known amounts of pigments (standards provided by R. Bidigare). For divinyl-chlorophylls, it is again assumed (as above) that molar absorption coefficients at the red maximum are identical to those of the corresponding chlorophylls leading to coefficients at the blue peak of 105.6 and 46.0 l g^{-1} cm^{-1} for divinyl-chlorophyll *a* and *b* respectively. Typical chromatograms for *Synechococcus* and *Prochlorococcus* are displayed in Figure 2, and the absorption maxima of various pigments are given in Table 2.

3. Results and discussion

a. Cell size, pigment composition and internal concentration. The modal diameters of the various strains of *Prochlorococcus* ranged from 0.54 up to 0.67 μm, and those of *Synechococcus* between 0.81 and 1.07 μm (Table 1 and Figure 1a, b). The two cyanobacteria MAX 01 and MAX 41, originating from oligotrophic waters, were
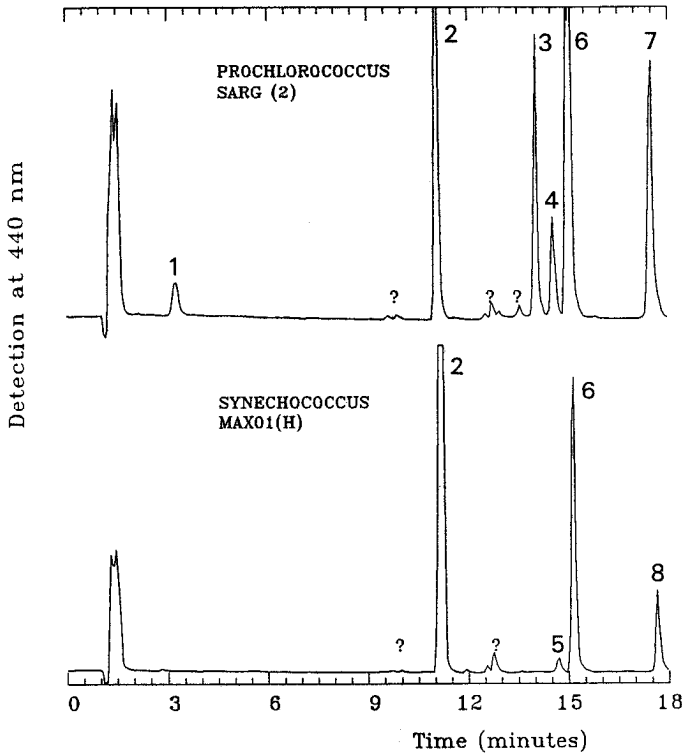
Figure 2. Typical HPLC absorbance chromatograms. These two examples are for *Prochlorococcus* (strain SARG, experiment #3) and *Synechococcus* (strain MAX01, cultivation at high irradiance). The numbers correspond to the pigments identified as follows, (1) Mg 2,4-divinylphaeoporphyrin a_5 monomethyl ester-like, (2) zeaxanthin, (3) divinyl-chlorophyll *b*, (4) unknown pigment (absorption maxima given in Table 2), (5) chlorophyll *a* allomer, (6) divinyl-chlorophyll *a* (6') chlorophyll *a*, (7) α-carotene, (8) β-carotene. Question marks are for unknown compounds.

conspicuously smaller. When grown at higher irradiance (about 100 μmol quanta $m^{-2} s^{-1}$, white light) the cyanobacteria ROS04 and MAX01 decreased in size (by 10% in modal diameter and 30% in mean volume) as well as in chlorophyll content per cell (by 15–40%, see Table 1).

For Chl *a*, divinyl-Chl *a* and divinyl-Chl *b* both spectrophotometry and HPLC provided concordant results (Table 1). The peculiarity of *Prochlorococcus* is the simultaneous presence of divinyl-Chl *a* and Chl *b* and also of a Chl *c*-like pigment. This pigment exhibits the same absorption spectrum as the Chl *c*-like pigment present in certain prasinophytes, such as *Micromonas pusilla* or *Pycnococcus provasolii* (previously known as clone Ω 48-23). It has been previously identified as being Mg, 2,4-divinylphaeoporphyrin a_5 monomethyl ester (Ricketts, 1966). For this Chl *c*-like pigment, an absorption value of 346 l g^{-1} cm^{-1} has been used (following

Table 2. Wavelengths of the maximal absorptions by the major liposoluble pigments in the HPLC solvant system.

Pigments	Goericke and Repeta	This study
PROCHLOROCOCCUS	1992	
		γ β α
divinyl-chlorophyllid *a*		441, 626, 669
Mg 2,4-divinyl-like	440, 580, 630	440, 579, 630
divinyl-chlorophyll *b*	478, 610, 658	477, 605, 656
divinyl-chlorophyll *a*	442, 620, 666	441, 619, 666
zeaxanthin	450, 478	451, 480
unknown	444, 474	446, 475
α-carotene	446, 474	446, 474
SYNECHOCOCCUS		
chlorophyll *a*		431, 617, 664
zeaxanthin		451, 480
β-carotene		452, 477

Mantoura and Llewellyn, 1983) to derive its concentration (Table 3). A minute amount of divinyl-chlorophyllide *a* (same retention time as "normal" chlorophyllide *a*, but with a blue absorption also red-shifted by 10 nm) was only detected in the MED culture (1–2% of the total porphyrin pigments).

Table 3. Pigment concentrations within the cellular material (C_i as kg m^{-3}) and pigment ratios (last column) for the various strains, as determined via HPLC. For NATL1 (3) and DC2 (L), the pigments ratios were measured, whereas, the absolute concentrations in divinyl-Chl *a* and Chl *a* were mistakenly not determined. Therefore the starred values are those derived from spectrophotometric determinations, from which the concentrations in other pigments (numbers between parentheses) are derived using the ratios.

	div. Chl *a*	div. Chl *b*	Chl *c*-like	α-carotene	zeax.	Σcar.	Σcar./ΣChl.
PROCHLOROCOCCUS							
MED (1)	16.56	2.27	0.49	2.23	5.51	8.55	0.44
MED (3)	9.84	1.40	0.27	1.12	3.00	4.52	0.39
NATL1 (1)	5.33	6.40	0.37	1.15	1.40	3.05	0.25
(2)	6.95	7.09	0.37	1.26	3.44	5.47	0.38
(3)*	11.42	(9.82)	(0.57)	(2.06)	(3.08)	(6.10)	0.28
SARG (2)	8.05	8.21	0.23	1.90	2.57	5.47	0.33
	Chl *a*			β-carotene	zeax.	Σcar.	Σcar./Chl *a*
SYNECHOCOCCUS							
ROS04 (H)	1.90			0.37	1.35	1.84	0.97
MAX01 (H)	3.44			0.45	2.45	3.04	0.88
MAX41 (L)	2.76			0.41	1.30	1.83	0.66
DC2 (L)*	1.91			(0.46)	(2.22)	(2.79)	1.46

Cyanobacteria contain β-carotene and prochlorophytes contain α-carotene, while both possess zeaxanthin, the sole pigment they have in common. An unknown carotenoid which elutes between divinyl-Chl b and divinyl-Chl a is also distinctly present in the *Prochlorococcus* strains. Two types of *Prochlorococcus* can be distinguished on the basis of their (divinyl) Chl b-to-Chl a ratios. This ratio is about 1, for the strains NATL1 and SARG, and markedly lower (<0.14) for the strain originating from the Mediterranean Sea. MED is also characterized by a low α-carotene-to-divinyl-Chl a ratio (12% instead of about 21%), even if the total carotenoid to total porphyrin ratio is higher in this strain (Table 3). In terms of intracellular composition (see C_i values in Table 3), total carotenoids are more concentrated in *Prochlorococcus* cells than in *Synechococcus* cells (by a factor 1.5 on average). The total carotenoids to total porphyrins ratio, however, is distinctly lower in the *Prochlorococcus* group (about 0.3–0.4) compared to that of the *Synechococcus* group (between 0.7 and 1.5).

Synechococcus and *Prochlorococcus* have similar cell content for Chl a and divinyl-Chl a, respectively, despite markedly different cell volumes (approximately in a ratio 4:1). As a consequence, the chlorophyll concentration within the cellular material of prochlorophytes is higher than in cyanobacteria, by a factor of about 5 in the present experiments (and more if the sum divinyl-Chl a + divinyl-Chl b is considered; see C_i values in Table 3). Since growth conditions are not identical (25 and 7 μmol quanta m^{-2} s^{-1} for cyanobacteria and Prochlorophytes, respectively), this observation cannot be generalized.

The divinyl-Chl a content per cell found in the various *Prochlorococcus* strains (Table 1) are close to those observed in culture of *Prochlorococcus marinus* grown at ≈ 25 μmol quanta m^{-2} s^{-1} (Goericke and Repeta, 1992), or to previously reported cell content for samples from the Sargasso Sea (Chisholm *et al.*, 1988). The values of Chl a-per-cell found for cyanobacteria are generally in the lower range of those reported in literature, in particular for the well documented clone DC2 (Barlow and Alberte, 1985; Kana and Glibert, 1987; Bidigare *et al.*, 1989). In other (incomplete) experiments, higher C_i values have been occasionally observed in spite of similar growth conditions. Such relatively low internal concentrations could originate from the presence of senescent cells in the present cultures that were harvested in late exponential phase in order to obtain the highest cellular densities required for optical measurements. This hypothesis is supported by the flow cytometric observation (F. Partensky, unpublished) of cells grown under similar conditions, exhibiting very low red chlorophyll fluorescence; chlorophyllide a remained undetectable supporting assurances that cultures did not contain dead cells. The heterotrophic bacterial contamination which is of minor importance cannot contribute to a lowering of the pigment-per-cell estimate by more than 10–20%. Pigments were extracted using acetone (as in Kana and Glibert, 1987). The observation of incomplete extraction, made by Stramski and Morel (1990) in the case of *Synechocystis,* and suspected to occur with *Synechococcus,* was not confirmed with these strains. On the

contrary, cold acetone is 20 to 30% more efficient than methanol and DMSO for Chl *a* (and zeaxanthin) extraction, and sonication appears to be unnecessary (H. Claustre, unpublished).

b. Spectral values of the chlorophyll-specific absorption. The absorption spectra of acetone extracts are divided by Chl *a* (or divinyl-Chl *a*) concentration to obtain a_{ac}^* (λ), the normalized (i.e. Chl-specific) spectral coefficients of all pigments extractable by acetone (Fig. 3a and 3b). By virtue of this normalization, all spectra obviously have a common value, close to 0.021 m^2 (mg Chl *a*)$^{-1}$ at 664 nm, while they differ at all other wavelengths.

The strain MED (Fig. 3a), with a low divinyl-Chl *b* content, distinctly exhibits the three Soret bands of the divinyl-Chl *a*, at 442, 622 and 664 nm. The shoulder around 480 nm is attributable to the merged influence of carotenoids (mainly α-carotene and zeaxanthin). The two other strains, with almost equal amounts of divinyl-Chl *a* and *b* (SARG and NATL1), exhibit a more complicated pattern, namely (i) the "red" peak is shifted toward 661 nm and widened by inclusion of the α-Soret band of the divinyl-Chl *b* (peaking at 656 nm), (ii) the same modification occurs in the spectral domain 605–620 nm where the β-Soret bands of both divinyl-chlorophylls are intermingled, (iii) in the blue part, the γ-Soret band of divinyl-Chl *b* (at 476 nm) is now added to the carotenoids absorption, resulting in a more efficient absorption in this domain, and also in a minute shift of the blue peak from 442 to 444 nm, (iv) in the whole spectral domain, the a_{ac}^* (λ) values are distinctly higher for these strains, for the same amount of divinyl-Chl *a*. It is worth noting that three successive cultivations of the strain NATL1 exhibited variability in pigment absorption despite identical growth conditions.

For *Synechococcus* (Fig. 3b), acetone extracts have remarkably identical absorption patterns in the red part of the spectrum, with the three typical features of Chl *a*, at 580, 620, and 664 nm. In the blue domain, beside the Chl *a* peak (at 432 nm), the variability in shape as well as the development of other maxima (at 455 and 482–485 nm) originate from the influence of carotenoids (mainly β-carotene and zeaxanthin) present in variable amounts depending on the strains. The spectra are regularly arranged according to the Σcar.:Chl *a* ratio (see Table 3), the lowest values of this ratio being for the pelagic (oligotrophic) strains (MAX 01 and MAX 41). Within a given species, high growth irradiances systematically lead to enhanced carotenoid absorption.

The Chl-specific absorption coefficients of intact cells, a^* (λ), have also been determined by dividing the absorption coefficients determined on the suspensions by the corresponding Chl *a* (or divinyl-Chl *a*) concentrations (Fig. 4a and b). The blue maximum of prochlorophytes ranges between 0.052 and 0.069 m^2 mg^{-1}. It is located at 445 nm when the divinyl-Chl *b* content is low (MED), and at 448 nm when it is high (SARG and NATL1). For the latter strains, the red peak at 672 nm is also distorted
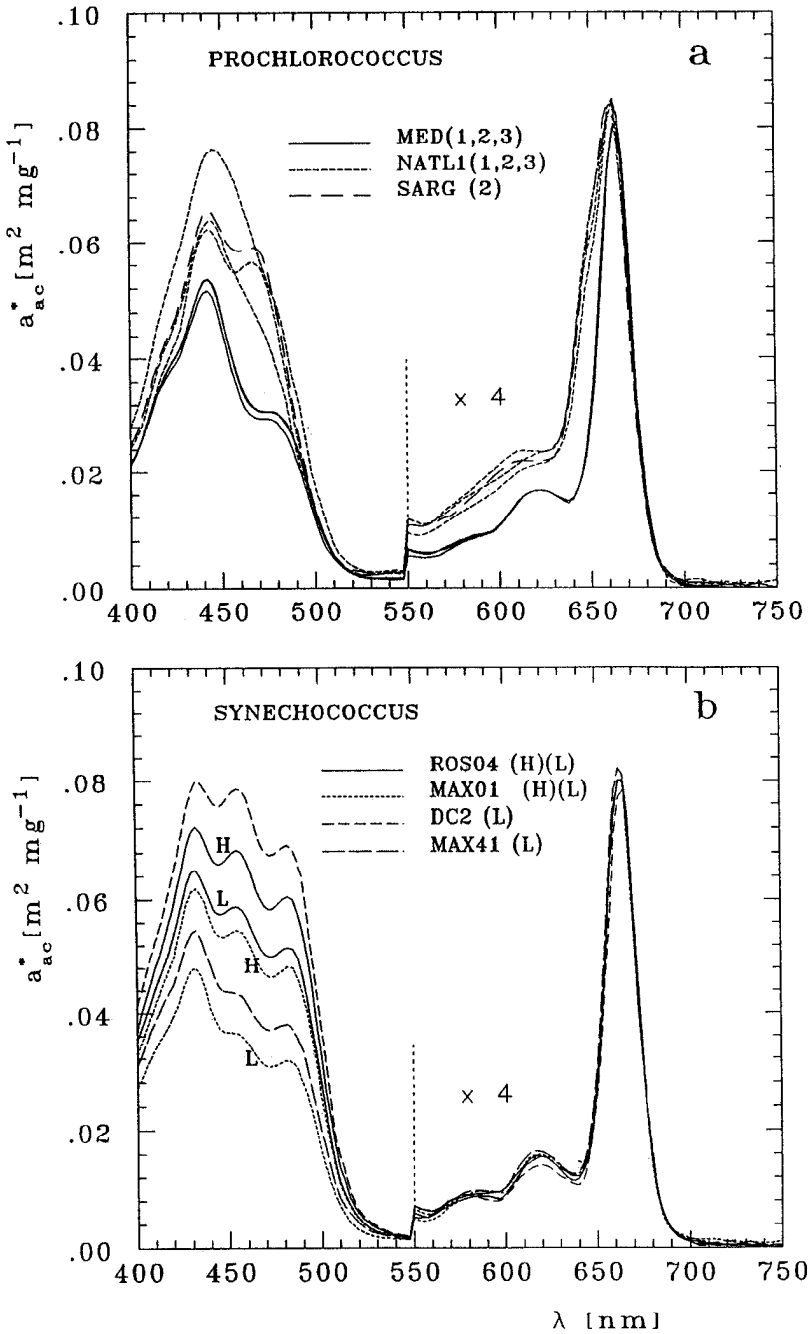
Figure 3. Absorption spectra of acetonic extracts after normalization with respect to divinyl-Chl *a* (panel a for *Prochlorococcus*) or Chl *a* (panel b for *Synechococcus*). Note the change in the ordinates scale (multiplied by 4) for λ > 550 nm.
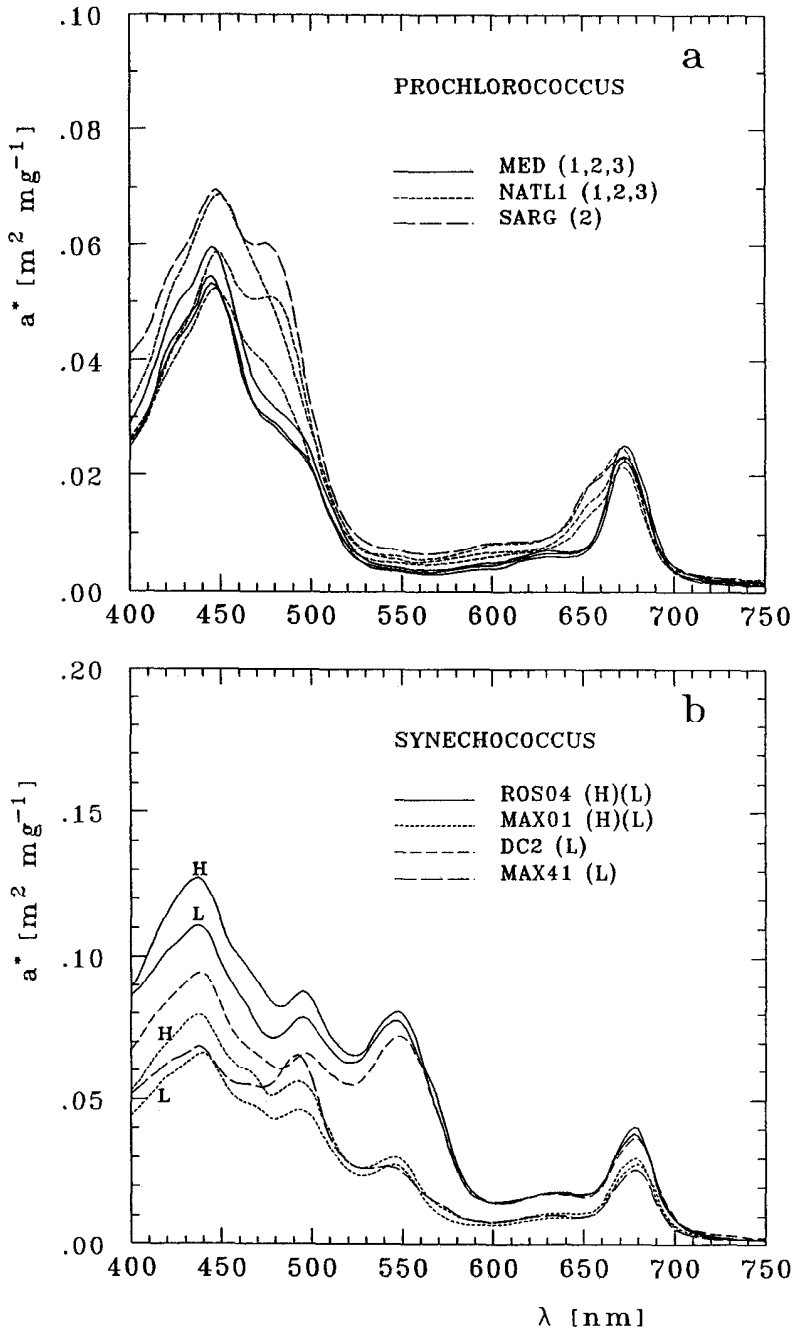
Figure 4. Chlorophyll-specific absorption spectra of intact cells for the various strains.

by the divinyl-Chl *b* absorption that induces the shoulder around 660 nm. The flat residual absorption in the 520–620 domain is very low (<0.005 m^2 mg^{-1}), so that these spectra, with only two prominent peaks, have a simple shape compared to those of many eukaryotic algae (see for instance in Ahn *et al.,* 1992). The intra-specific variability of NATL1, already noted, is also revealed by the absorption spectra of living cells. A slight overestimate of a^* (λ) near 410 nm is possible, originating from the presence of heterotrophic bacteria (see below).

Everywhere within the 400–700 nm domain, the chlorophyll specific absorption values of the *Synechococcus* strains (Fig. 4b) notably exceed those of *Prochlorococcus* (Fig. 4a); at the blue maximum (438 nm) for instance, a^* ranges from 0.065 to 0.13 m^2 mg^{-1}. These higher values are attributable to the influence of accessory pigments (carotenoids and phycobilins). Thus, with a Σcar.:Chl *a* ratio at least twice that of *Prochlorococcus,* carotenoids in *Synechococcus* reinforce their blue absorption. In the green part of the spectrum, all spectra have in common, with various intensities, the typical absorption bands of phycoerythrobilin (PEB) at 550 nm, and of phycourobilin (PUB) chromophores at 496 nm (particularly well marked for MAX41). The two strains DC2 and ROS04 exhibit higher a^* values in the 600–680 nm domain, reaching about 0.04 m^2 mg^{-1} at the red peak (678 nm). They belong to the "low PUB:PEB" type (Olson *et al.,* 1988; Bidigare *et al.,* 1989), with also a significant amount of phyocyanine (PC, absorbing in the 580–650 nm domain). The two strains isolated from Sargasso Sea (MAX01 and MAX41), less absorbing in the green-yellow parts of the spectrum, are of the "high PUB:PEB" type. With also a lower Σcar.:Chl *a* ratio, these pelagic strains have lower a^* (λ) values in the blue spectral domain, similar to those of *Prochlorococcus* (apart from the additional phycourobilin absorption).

c. Efficiency factors for absorption and scattering. These factors, denoted $\overline{Q}_a(\lambda)$ and $\overline{Q}_b(\lambda)$, are defined as the ratios of the radiant energy absorbed or scattered, respectively, by a cell, to the energy impinging onto its geometrical cross section. For a population of particles, characterized by its specific size distribution function $F(D)$, a "mean" geometrical cross section, $\overline{\sigma}_g$, can be computed as

$$\overline{\sigma}_g = (\pi/4)\, D_2^2 \tag{1}$$

where D_2, which represents the diameter of a sphere having a cross section equal to the average cross section of the entire population, is expressed according to

$$D_2^2 = (N/V)^{-1} \int_0^\infty F(D)\, D^2\, dD. \tag{2}$$

The D_2 and $\overline{\sigma}_g$ values for each strain are given in Table 4; note that Eq. 2 is equivalent to Eq. 17 a and b in Morel and Ahn (1990), where the wrong exponent -1 has to be replaced by the correct values $-\frac{1}{2}$ and $-\frac{1}{3}$, respectively. The Q-factors are derived from the optical measurements, $j(\lambda)$, where j is a or b, the absorption or scattering

Table 4. Geometrical and optical cross sections (μm^2), and optical efficiency Q-factors for the *Prochlorococcus* and *Synechococcus* strains (note that $\sigma_{a,b} = \sigma_g Q_{a,b}$). $\bar{b}_b$ and b_b^* are the backscattering ratios (dimensionless) and the Chl-specific backscattering coefficients ($m^2\ mg^{-1}$), respectively.

Strains	D_2 [μm]	σ_g [μm^2]	Q_a, Q_b (blue max.)		σ_a (blue max.)	Q_b (500 nm)	σ_b (500 nm)	$Q_{bb} \cdot 10^3$ (500 nm)	$\bar{b}_b \cdot 10^3$ (500 nm)	$b_b^* \cdot 10^3$ (blue max.)
PROCHLOROCOCCUS										
MED (1)	0.58	0.264	0.370	0.173	0.098	0.166	0.041	—	—	—
MED (2)	0.59	0.273	0.314	0.152	0.086	0.148	0.040	—	—	—
MED (3)	0.59	0.273	0.231	0.164	0.063	0.155	0.039	1.068	6.875	0.098
NATL1 (1)	0.70	0.385	0.186	0.137	0.072	0.165	0.074	—	—	—
NATL1 (2)	0.62	0.302	0.209	0.146	0.063	0.155	0.047	—	—	—
NATL1 (3)	0.73	0.419	0.327	0.211	0.137	0.218	0.091	—	—	—
SARG (1)	0.71	0.400	0.213	0.174	0.085	0.168	0.067	—	—	—
SARG (2)	0.71	0.400	0.253	0.171	0.101	0.176	0.070	0.759	4.318	0.092
SYNECHOCOCCUS										
ROS04 (H)	1.05	0.866	0.118	0.448	0.102	0.376	0.326	0.414	1.101	0.311
ROS04 (L)	1.17	1.075	0.118	0.475	0.127	0.383	0.411	0.444	1.158	0.332
MAX01 (H)	0.88	0.608	0.106	0.362	0.064	0.310	0.188	0.446	1.441	0.275
MAX01 (L)	0.99	0.770	0.104	0.361	0.080	0.307	0.236	0.638	2.078	0.384
MAX41 (L)	0.92	0.665	0.122	0.320	0.081	0.278	0.185	—	—	—
DC2 (L)	1.14	1.015	0.116	0.517	0.118	0.421	0.427	0.414	0.984	0.198

coefficients, through:

$$\overline{Q}_j = j(\lambda)[\overline{\sigma_g}(N/V)]^{-1}. \tag{3}$$

The overbar denotes that the efficiency factor represents a "mean" value to be applied to this mean cell, which is representative of the entire population and has the geometrical cross section $\overline{\sigma}_g$. The (N/V) values determined for the *Synechococcus* suspensions are directly introduced into Eq. 3. For *Prochlorococcus,* it is assumed that the heterotrophic bacteria present in the cultures have a negligible absorption compared to that of the phototrophic organisms. Therefore $\overline{Q}_a(\lambda)$ is computed using the absorption values a (λ) and the number density (N/V) corresponding only to pigmented cells. Using typical values for absorption cross sections of heterotrophic bacteria (Morel and Ahn, 1990; Stramski and Kiefer, 1990), the validity of the above assumption is easily verified; in the worst situation (50% of heterotrophic bacteria in the total cell number), the $\overline{Q}_a$ (λ) values for *Prochlorococcus* would be overestimated (due to inclusion of bacterial absorption), at the most by 10% at 400–415 nm, 5% at 445 nm, and unaffected at longer wavelengths.

Obviously, the spectral shapes of $\overline{Q}_a$ (λ) (Fig. 5) do not differ from those of $a^*(\lambda)$ (Fig. 4); their respective amplitudes, however, are deeply altered. For instance, while the a^* values for cyanobacteria were well above those for *Prochlorococcus,* the converse is true when considering the efficiency factors for absorption. On average, the $\overline{Q}_a(\lambda)$ values for *Prochlorococcus* are about twice those of *Synechococcus.* The $\overline{Q}_a$ factor depends simultaneously on the size and the internal pigment concentration that determine the absorption coefficient of the material forming the cell (see e.g. Morel and Bricaud, 1981). Therefore, within a group, the new relative disposition of the $\overline{Q}_a$ spectra is governed to a great extent by the internal pigment concentration (C_i, Table 3). Between the two groups (i.e. *Synechococcus* and *Prochlorococcus*), the decrease in size (by a factor of 1.6 on average) is largely compensated by the increase in pigment concentration that, in the end, causes over a 2-fold increase in $\overline{Q}_a$. Owing to this compensation, it turns out that the absorption cross sections ($\sigma_a = \sigma_g \overline{Q}_a$) of *Prochlorococcus* and *Synechococcus* are nearly the same (Table 4), with mean values close to 0.1 μm^2, in the blue maximum. This almost equal capacity to capture blue light, on a per cell basis, is an advantage for the smaller organism, that can achieve, in principle, a higher photosynthetic rate per unit of biomass (of carbon).

It is worth noting that, with such $\overline{Q}_a$ values (even in the blue part of the spectrum), the "package" effect remains reduced. The absorption capacity of *Prochlorococcus* cells is about 20% below that of the same amount of pigments if finely and uniformly dispersed (as it would be in "solution," with no package effect); that of *Synechococcus* is only 10% below.

For scattering measurements on non-axenic cultures, there is no way of discriminating between the effect of pigmented cells (*Prochlorococcus*) and that of almost colorless heterotrophic bacteria of the same size. Therefore (and in contrast to what
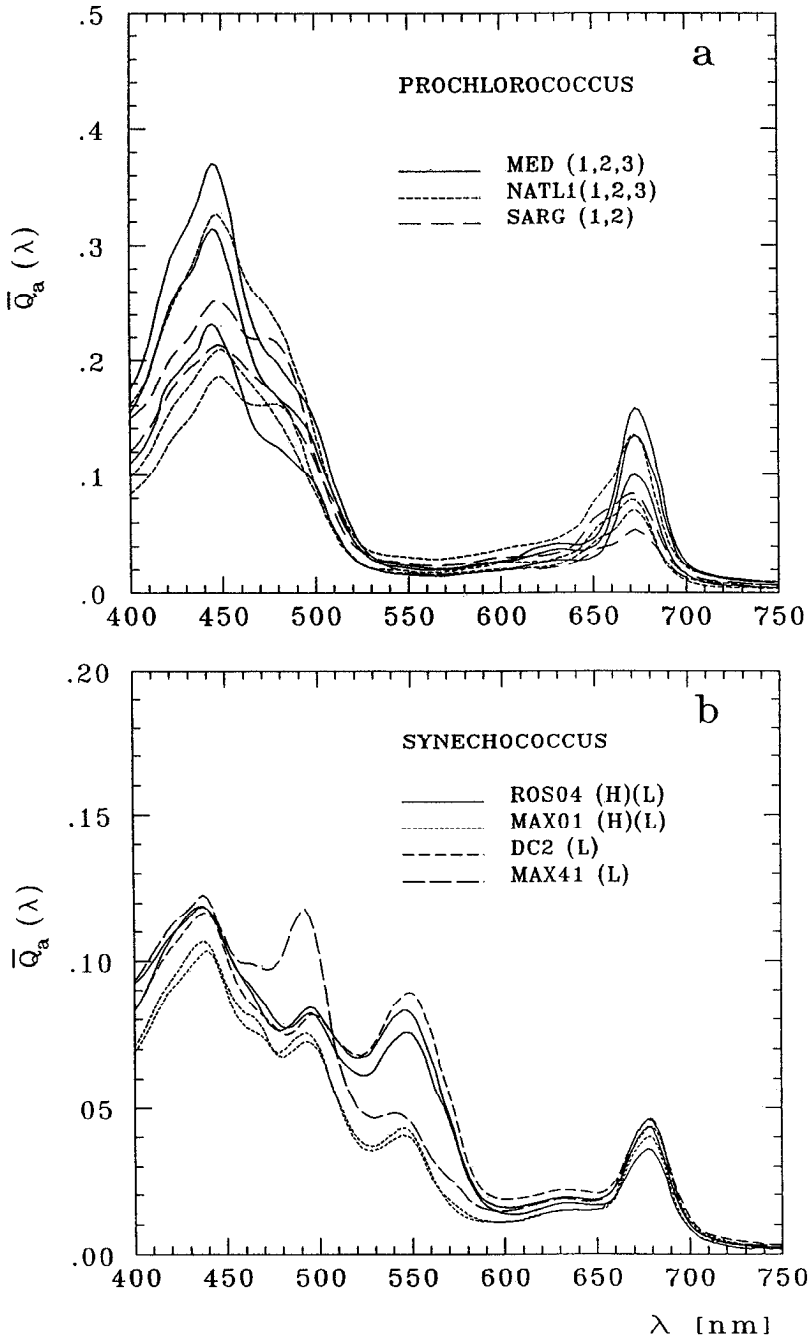
Figure 5. Spectral values of the efficiency factor for absorption for the various strains.

was made when assessing $\overline{Q}_a$), $\overline{Q}_b$ is computed by introducing into Eq. 3 the number density corresponding to the whole population of cells (both photo- and heterotrophic). The consequence of such a procedure applied to *Prochlorococcus* cultures is discussed below.

The ordering of the $\overline{Q}_b(\lambda)$ spectra (Fig. 6, see also $\overline{Q}_b$ values at 500 nm in Table 4) depends simultaneously on the mean cell size and refractive index. More precisely, m, the refractive index (relative to the index of water) is a complex number, $m = n - in'$, and it is its real part, n, which essentially determines the amplitude of $\overline{Q}_b$. Since n varies within a rather restricted range compared to size, the latter parameter is dominant in fixing $\overline{Q}_b$. For the size domain involved, $\overline{Q}_b$ is, to a good approximation, proportional to the square of the size, as confirmed by the approximate factor 2.5 which appears between the $\overline{Q}_b$ values for *Synechococcus* and for *Prochlorococcus*. The overall ascending slope toward the short wavelengths follows the λ^{-2} dependency that is theoretically expected for nonabsorbing small particles. The main absorption bands (i.e. the non-zero values of n', the imaginary part of the index) are at the origin of the various features superimposed onto the general trend (see e.g. Morel and Bricaud, 1986). After the $n'(\lambda)$ spectral values have been inferred from the $\overline{Q}_a(\lambda)$ values, this effect can be accounted for and then the features in the $\overline{Q}_b(\lambda)$ spectra accurately reconstructed via Mie theory (Ahn *et al.*, 1992). Two examples of such reconstructions are given in Figure 7.

The absorption related features have a reduced amplitude in the case of cyanobacteria by virtue of relatively low $\overline{Q}_a$ (or n') values, combined with relatively high $\overline{Q}_b$ values, imposed by the size of these organisms. The converse holds true for *Prochlorococcus*, which exhibits $\overline{Q}_b$ spectra with more pronounced undulations. They are likely underestimated, because of the inevitable inclusion in the present measurements of scattering by heterotrophs, which interfere with a featureless, regularly ascending spectrum (Morel and Ahn, 1990; Stramski and Kiefer, 1990), and therefore reduce the undulations, without changing the general slope and magnitude of $Q_b(\lambda)$. The cultures SARG (1) and (2) and MED (1), with the higher heterotroph contamination (about 50%), are indeed those showing the flatter $\overline{Q}_b$ spectra. An estimate of this "flattening" effect can be obtained by separately computing $\overline{Q}_b(\lambda)$ spectra for heterotrophic bacteria and *Prochlorococcus* cells and assuming the same size distribution for both populations. The first spectrum conforms to the theoretical λ^{-2} curvature (see e.g. Fig. 4b in Morel and Ahn, 1990); the second one is computed using Mie theory with the $n'(\lambda)$ values derived from $\overline{Q}_a(\lambda)$, which, as said above, are relevant only to pigmented cells. For the sole *Prochlorococcus* cells, this $\overline{Q}_b(\lambda)$ spectrum shows, in the blue part of the spectrum, more accentuated minima than those appearing in the curve for the actual (mixed) population (Fig. 7).

The $n'(\lambda)$ values, as being derived from the $Q_a(\lambda)$ values, exhibit patterns (not shown) like those observed in Figure 5 (a and b). If n' for *Synechococcus* is within the range found for other phytoplankters (about 4 10^{-3} and 2 10^{-3} in the blue and red
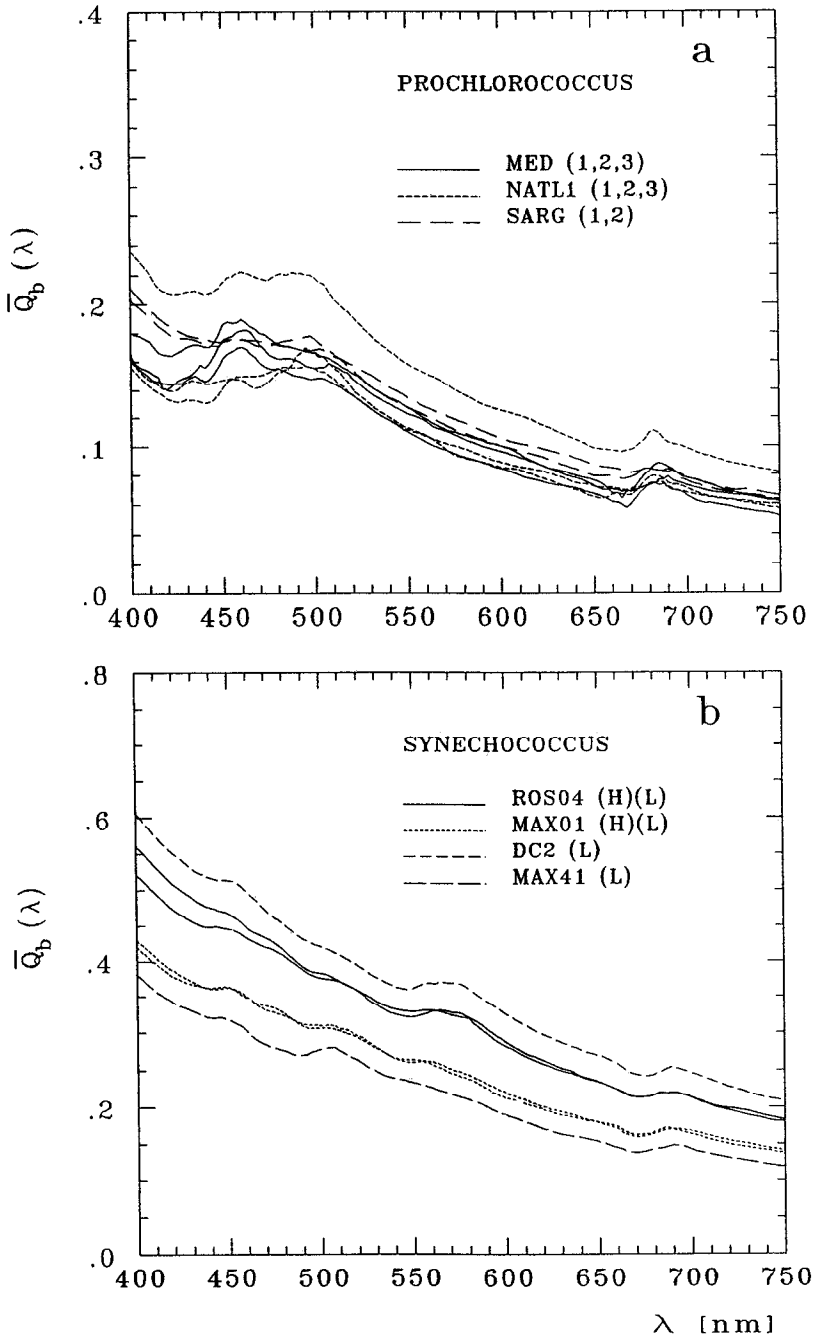
Figure 6. Spectral values of the efficiency factor for scattering for the various strains.
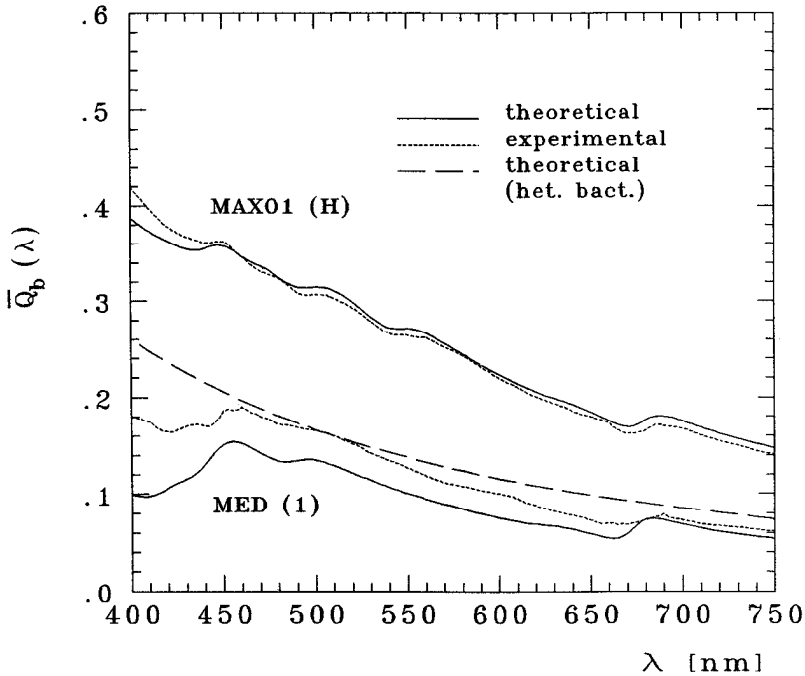
Figure 7. Comparison between experimental and theoretical values of the efficiency factor for scattering. Note that in the case of *Prochlorococcus* (MED 1), the experimental curve differs from the theoretical one, because the spurious effect of scattering by heterotrophic bacteria is eliminated, so that the theoretical spectrum is for the sole prochlorophytes; the flat dashed spectrum is for heterotrophic bacteria only (see text). For *Synechococcus* the computed spectrum, which does not account for any bacterial influence, is directly comparable (and almost coincident) with the experimental one.

maxima, respectively), the n' values for *Prochlorococcus* are exceptionally high (1 to 3 10^{-2} and 0.5 to 1 10^{-2} in the blue and red maxima, respectively), obviously as a consequence of their high intracellular pigment concentration. The values of the real part of the refractive index, n, which are derived from the theoretical reconstruction of Q_b, are in the middle of the range of values found for other species (Ahn *et al.*, 1992). For *Synechococcus* the $n(\lambda)$ spectra are rather flat with a mean value of about 1.050. For *Prochlorococcus* the $n(\lambda)$ spectra are more undulating in response to stronger absorption bands and subsequent anomalous dispersion effect; typical minimal values are around 1.038 in the 400–430 nm domain, and slightly exceed 1.05 at 500 and 690 nm.

d. Spectral values of the chlorophyll-specific scattering coefficient. Once divided by the Chl *a* (or divinyl-Chl *a*) concentration, the scattering coefficients $b(\lambda)$ determined for intact cells in suspension, are transformed into $b^*(\lambda)$, the Chl-specific scattering

coefficients (Fig. 8). If this straightforward procedure can be safely applied to *Synechococcus,* the coexistence of heterotrophic bacteria in significant amount with *Prochlorococcus,* leads to an overestimate of $b*$. This bias is eliminated by reducing $b(\lambda)$ by the appropriate factor (number of *Prochlorococcus*/total number), before performing the normalization with respect to the divinyl-Chl a concentration. As for $\overline{Q}_b(\lambda)$, the $b*(\lambda)$ spectra are admittedly slightly smoothed by the scattering interference of heterotrophs, at least in the blue domain.

Globally there is almost one order of magnitude between the $b*$ values typical of the two kinds of organisms. The magnitude of the Chl-specific coefficients and the respective arrangement of the spectra are governed by (Bricaud and Morel, 1986):

$$j*(\lambda) = \frac{3}{2}(\overline{Q}_j(\lambda))/(C_i D_{3\text{-}2}) \tag{4a}$$

where $D_{3\text{-}2}$ is defined as

$$D_{3\text{-}2} = \int_0^\infty F(D) D^3 dD / \int_0^\infty F(D) D^2 dD \tag{4b}$$

and

$$j* = a*, b* \text{ or } b_b^*$$

Q_b is 2-times smaller for *Prochlorococcus* while $C_i D_{3\text{-}2}$ is about 5-times larger, combining to create the observed 10-times difference in $b*$ between *Synechococcus* and *Prochlorococcus*. It can be verified that the respective positions of the various $b*(\lambda)$ spectra (and also of the $a*(\lambda)$ spectra) are well accounted for by Eq. 4a ($D_{3\text{-}2}$ of Eq. 4b can be replaced by D_m from Table 1 without significant deviation).

e. Backscattering properties. Unlike the absorption and scattering coefficients, b_b, the backscattering coefficient is not entirely accessible to measurement. More precisely, only a fraction of the backscattered flux can be detected with the device we used, the inevitable limitation coming from the refraction effect (see discussion in Ahn *et al.,* 1992). Therefore the measured (partial) coefficient must be multiplied by a "geometrical factor" in order to obtain b_b. The derivation of this factor requires the computation of the volume scattering function (*via* Mie theory), and for this computation the knowledge of $m = n - in'$ at all wavelengths is needed; in turn, n and n' must be derived from the other optical measurements. For that reason the backscattering determinations cumulate a variety of uncertainties, firstly those inherent to their own measurements, secondly those on Q_b and Q_a (the quantities used to derive n and n'), and thirdly those resulting from the assumption of sphericity included in Mie theory. Finally, the obtainement of theoretical values is a prerequisite for the production of "experimental" values. In summary, such "measurements" are not expected to provide more than an approximate assessment
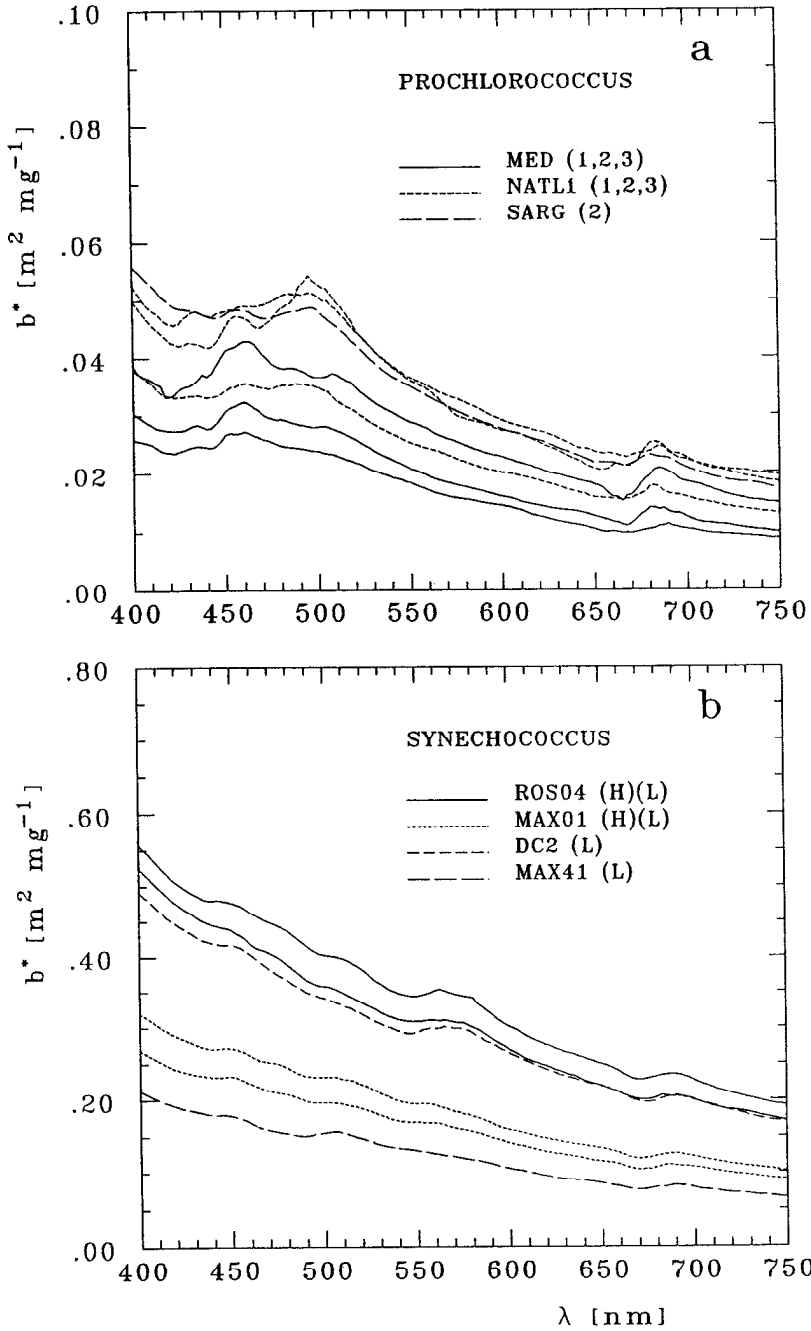
Figure 8. Chlorophyll-specific scattering spectra for the various strains.

of the absolute magnitude of the backscattering coefficient, while the spectral patterns are believed to be less affected by uncertainties.

The efficiency factor for the backscattering $\overline{Q}_{bb}(\lambda)$ is defined through Eq. 3 (with $j = b_b$). The experimental $\overline{Q}_{bb}(\lambda)$ spectra, shown in Figure 9, are scaled in such a way that they roughly coincide with the predictions from Mie computation (using the actual $F(D)$ function and the $n(\lambda) - in'(\lambda)$ values). The scaling factor (R = "experimental"-to-"theoretical") is given in each panel. Theoretical $\overline{Q}_{bb}(\lambda)$ values are generally higher than experimental ones in the case of *Synechococcus*, whereas the agreement is fairly good for *Prochlorococcus*. Because of their stronger absorption capacity, these minute organisms exhibit a featured $\overline{Q}_{bb}$ spectrum. *Synechococcus* would have a rather flat spectrum (for elastic scattering) in the absence of fluorescence emission. Actually, the "pure" backscattering process can only be observed from 400 up to 500 nm, and then beyond 770 nm, whereas the central part of the spectrum is dominated by the fluorescence of phycoerythrin (peak at about 575 nm), of phycocyanin (640 nm), and of Chl *a* (or divinyl Chl *a*) (685 – 740 nm). At 500 nm, the $\overline{Q}_{bb}$ values for *Prochlorococcus* are (as expected for small particles) distinctly above those of *Synechococcus* (see also Table 4). The backscattering ratios for *Prochlorococcus* ($\tilde{b}_b = \overline{Q}_{bb}/\overline{Q}_b$) are the highest (about 0.5%) ever observed for planktonic algae. In terms of chlorophyll-specific coefficient (Table 4 and Fig. 10), however, *Prochlorococcus* cells have rather low values, about one third of those of *Synechococcus*. Anyhow, both populations do not depart from other algae in the sense that they have an insignificant influence on the backscattering coefficient of oceanic waters (see also discussions in Morel and Ahn, 1991; and Stramski and Kiefer, 1991).

f. Ecological and bio-optical implications. According to their absorption spectra (Fig. 4b), *Synechococcus* cells are particularly well adapted to live in green waters, especially those cells with high phycoerythrobilin absorption, such as ROS04 and DC2 typical of, or at least originating from, neritic waters. *Synechococcus* strains typical of pelagic waters (MAX 01 and MAX 41), with high PUB/PEB ratio (Olson *et al.*, 1988, 1990b) and somewhat reduced green absorption capacity, partly lose this adaptation to green climate. *Prochlorococcus* cells (Fig. 4a) are definitely not well equipped for growing in green waters (either coastal waters or even Case 1 waters with moderate to high chlorophyll content). In contrast, these organisms can grow in blue low-chlorophyll waters at any depth, including the deepest levels, where the residual light is gradually centered at 465 nm. In such an environment, however, *Synechococcus* is equally well suited and takes a definite advantage over *Prochlorococcus* at intermediate depths in clear waters, (say, at mid-euphotic depth), where, aside blue light, significant amounts of green radiation (500–550 nm) still subsist. This advantage of *Synechococcus* vanishes deeper (say around and below the euphotic depth), to the extent that only blue radiation is available.
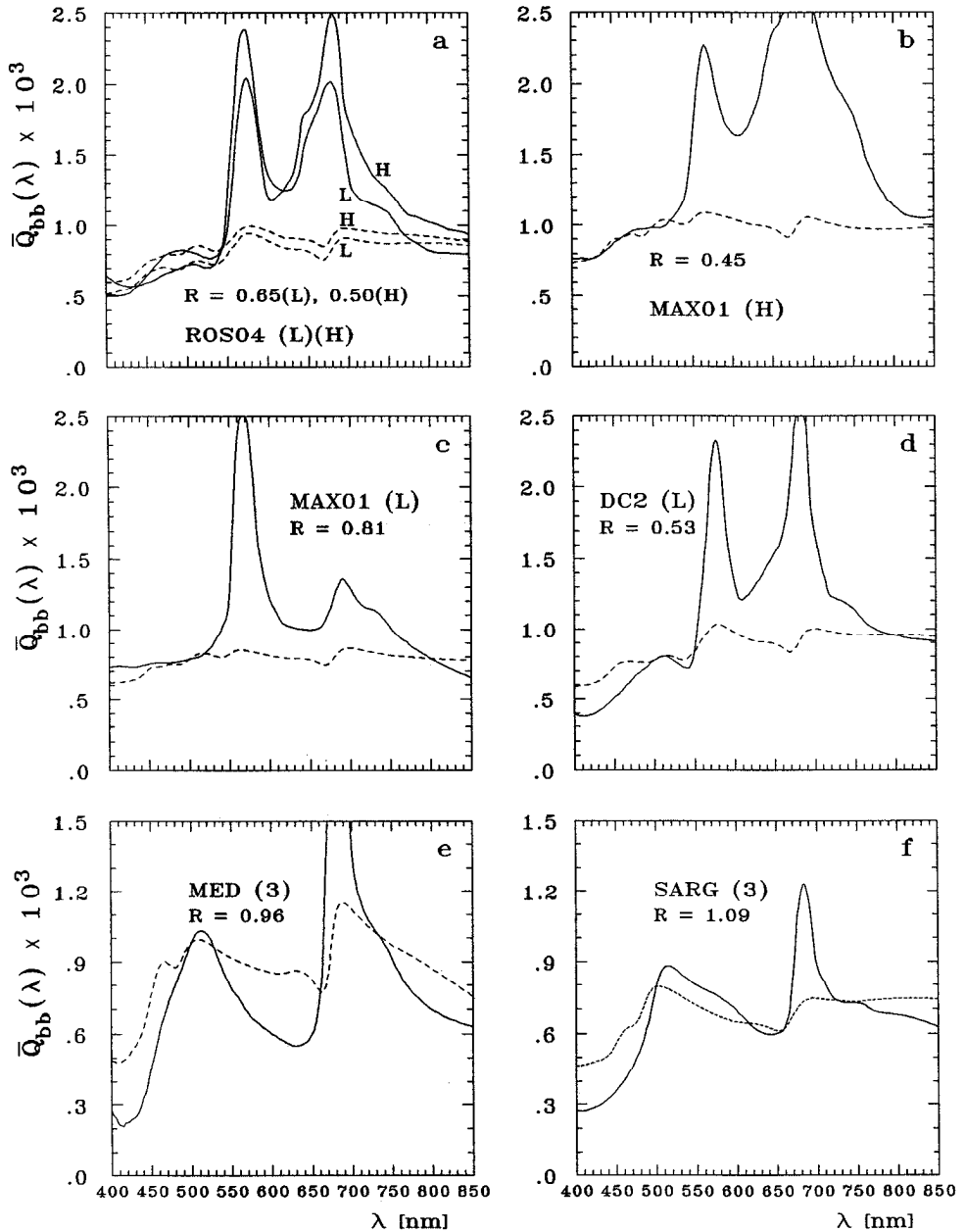
Figure 9. Spectral values of the efficiency factor for backscattering. The solid curves are for experimental determinations and the dashed curves for theoretical predictions. The experimental curves include the fluorescence emissions. The R value given in each panel is the ratio of experimental value-to-theoretical value at the wavelength 500 nm (see text).
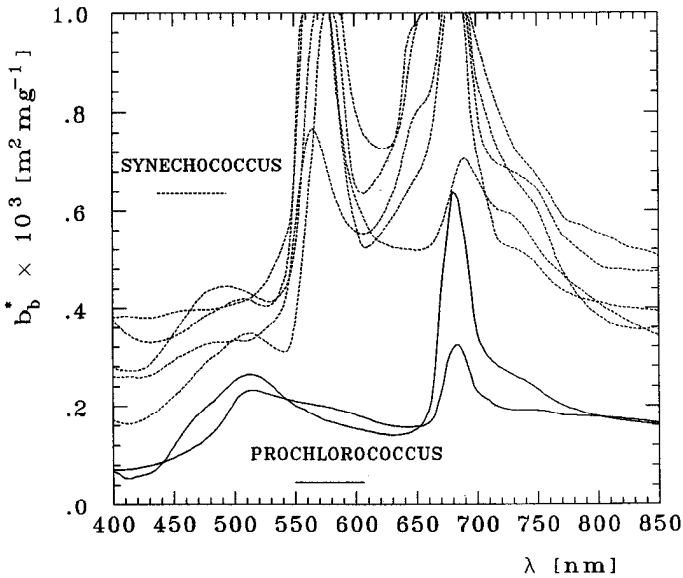
Figure 10. Spectral values of the chlorophyll-specific backscattering coefficient (note that the fluorescence emissions are included).

From the examination of the absorption cross section values (in Table 4), another inference can be drawn. Despite its smaller size, a *Prochlorococcus* cell can approximately harvest the same amount of blue radiation (400–500 nm) as a *Synechococcus* cell. This is true even for a "high-PUB-type" cyanobacterium, here well represented by the MAX41 strain, by noting that its absorption cross section (at 492 nm) is about 0.077 μm^2, comparable with those (0.05–0.09 μm^2) of the various *Prochlorococcus* strains. Under the assumption of similar yields in the photosynthetic machinery, the photosynthetic performances of a *Prochlorococcus* cell, once normalized to its biovolume or carbon content, would be much better (by a factor 4) than those of a *Synechococcus* cell (4 times bigger in volume). Their success in dominating the algal population within the deep Chl-maxima perhaps originates from such an optimized arrangement. Differences in abilities to utilize low levels of nutrients can also be invoked (Olson *et al.*, 1990a). When attempting to explain the vertical distribution and layering of the *Synechococcus* and *Prochlorococcus* populations in stable waters, insights can be gained from the consideration of the above optical properties. Nevertheless some cautions are necessary before generalization. It can be argued that, at very low irradiance level, *Synechococcus* is able to develop a more intense pigmentation than that measured in the present experiment (see Kana and Glibert, 1987), and thus can compensate for its apparent disadvantage. *Prochlorococcus*, however, has also the same, or even a more effective, capacity (see below and also Partensky *et al.*, 1993); therefore the respective absorption efficiencies per unit of
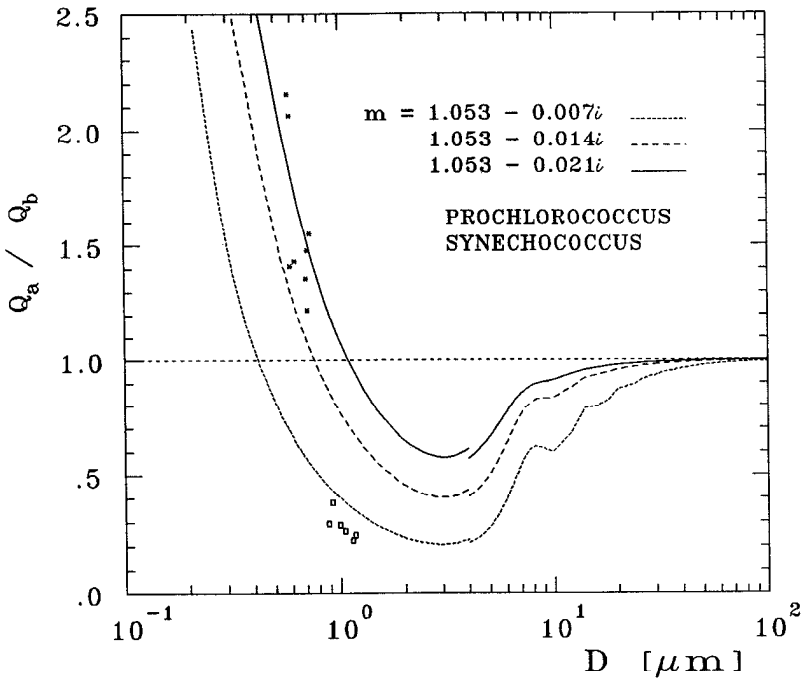
Figure 11. Ratio of efficiency factor for absorption to the efficiency factor for scattering, plotted as a function of the diameter D. The theoretical curves are computed (van de Hulst approximation for $D > 4$ μm and exact Mie solution for $D < 4$ μm) for the three values of the complex index of refraction as indicated. Note that the divergences at $D = 4$ μm is expected. Superimposed are the data for the *Prochlorococcus* and *Synechococcus* strains (see also Table 4 where are given the Q_a and Q_b values at the blue maximum).

carbon could remain in favor of the latter organisms. These speculations admittedly require further dedicated field studies.

Within the planktonic algal compartment, *Prochlorococcus* definitely forms an exceptional category from an optical viewpoint. Its minute size entails a lowered light scattering efficiency per cell (like that of a heterotrophic bacterium), whereas its relatively heavy pigmentation provides it with a high absorption capability. This conjunction places this organism in a somewhat singular domain, where the probability for photons to be absorbed by a cell can exceed that of being scattered. All phytoplankters, including cyanobacteria, are always much more efficient at scattering than capturing light. Only large cells, at the opposite end of the size scale, approach a symmetrical situation, because Q_a asymptotically increases toward unity and, consequently, Q_b decreases toward unity (*Hymenomonas elongata* is a good example of such a trend; see Ahn *et al.*, 1992). The general evolution of the Q_a-to-Q_b ratio with cell size is made explicit in Figure 11, where this ratio, at $\lambda = 450$ nm, is computed and plotted as a function of D. The complex refractive index is given the values $m =$

Table 5. Typical values of the chlorophyll-specific optical coefficient, a^*, b^* and b_b^* ($m^2 mg^{-1}$), for *Synechococcus* and *Prochlorococcus*; increase in attenuation (Δc, in m^{-1}) associated with a chlorophyll concentration of 0.5 mg m^{-3} (see text). Note that the missing b_b^* values are for those wavelengths where the fluorescence emission masks the elastic backscattering process.

λ(nm):	450	550	665
SYNECHOCOCCUS			
a^*	0.10	0.06	0.020
b^*	0.40	0.30	0.180
$b_b^*.10^3$	0.30		
Δc(m^{-1})	0.25	0.18	0.100
PROCHLOROCOCCUS			
a^*	0.06	0.005	0.015
b^*	0.04	0.030	0.015
$b_b^*.10^3$	0.09	0.021	
Δc(m^{-1})	0.05	0.018	0.015

$1.053 - in'$, with $n' = 0.007$, 0.014 and 0.021. The real part is on average representative for all strains; the lower and higher values of the imaginary part are typical of the blue absorption by *Synechococcus* and *Prochlorococcus*, respectively. The experimental values obtained for the Q_a-to-Q_b ratio are also plotted on the same graph. The particular status of the *Prochlorococcus* organisms, up to now the only algae found with Q_a distinctly above Q_b, as well as the data along the ascending branch of the curve, agree well with theoretical predictions. It can also be predicted in the case of *Synechococcus* organisms, that their Q_a values could approach their Q_b values, if, in contrast with the present experiment, their pigment content was about 4-times higher (as in Kana and Glibert, 1987), and provided that the size remains unchanged (~ 1 µm). The peculiar behavior of *Prochlorococcus* has bio-optical implications and some of the currently accepted ideas concerning the influence of phytoplankton upon the optical properties of a water body have to be reconsidered as far as such very small plankters become dominant.

Let us consider typical oligotrophic Case 1 waters with a "deep chlorophyll maximum," where the chlorophyll concentration increment above the background would be 0.5 mg m^{-3}. The increase in absorption and scattering, or cumulatively, in attenuation c ($c = a + b$), can be computed either for a "pure" *Synechococcus* population or a "pure" *Prochlorococcus* population. By adopting for the Chl-specific coefficients, a^* and b^*, mean values for each of the populations (Table 5), the increments Δa, Δb and Δc inside the deep maximum turn out to be very different according to the species. For instance, at 665 nm, where the (beam) attenuation coefficient is routinely measured, *Prochlorococcus* has a minute signature ($\Delta c = 0.015$ m^{-1}), compared to that of *Synechococcus* ($\Delta c = 0.10$ m^{-1}), essentially as a straightforward consequence of the reduced scattering efficiency of the former.
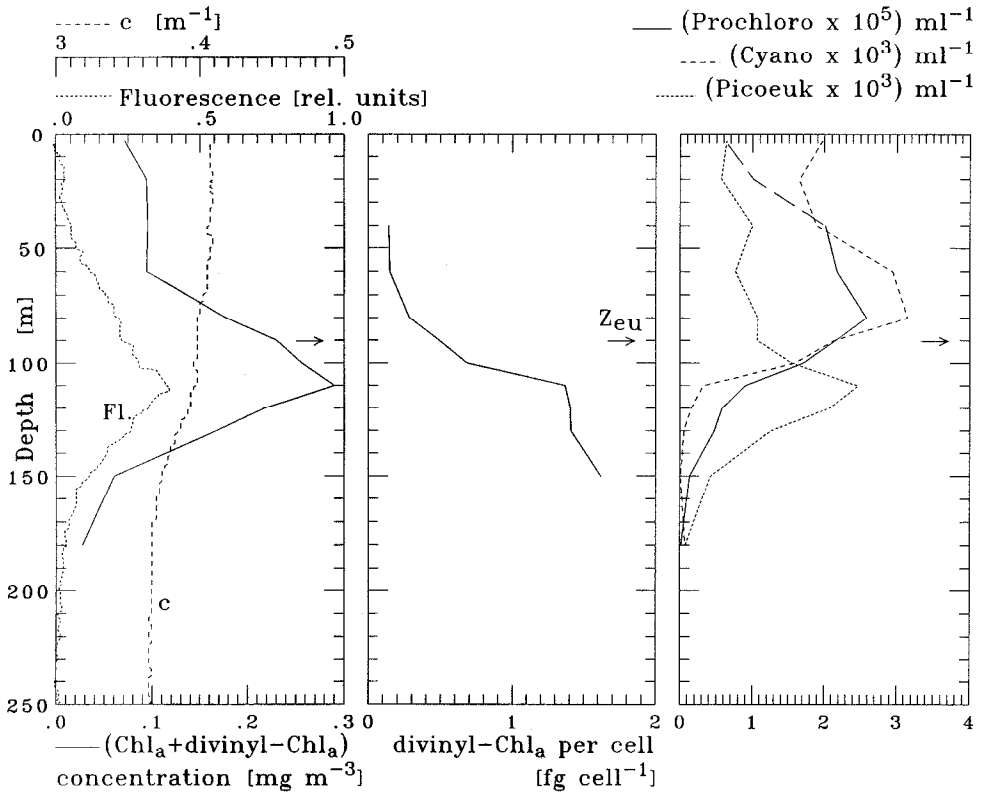
Figure 12. Vertical profiles (a) of chlorophyll fluorescence, beam attenuation (at 665 nm) and total (Chl a + divinyl-Chl a) concentration (mg m^{-3}) in the tropical Atlantic (October ·14, 1991; Station 02-05, 21N and 31W, at the oligotrophic site studied during the EUMELI-3 cruise). Vertical profiles (b) of the *Prochlorococcus* divinyl-Chl a content per cell derived from HPLC measurements and FACscan flow cytometer counting. Depth profiles (c) of numerical abundances of the three categories of algae; prochlorophytes in the surface layer (at 5 and 20 m) are admittedly underestimated (dashed part of the profile).

Therefore the attenuation vertical profiles through a chlorophyll maximum can be either almost featureless or with a maximum, according to the predominant population.

The non-correlation of the vertical structures of chlorophyll concentration and beam attenuation has already been examined by Kitchen and Zaneveld (1990). In particular they analyzed these situations encountered in the Northeastern Pacific Gyre where the chlorophyll (actually the fluorescence) maximum is in no way reflected by a maximum in the attenuation coefficient (actually a good indicator of particle concentration). A similar example (taken among more than 70 profiles typical of the oligotrophic tropical Atlantic) is provided in Figure 12a. Kitchen and Zaneveld convincingly showed that the absence of correlation has to be attributed to

an increase of the pigment content per cell with decreasing light, whereas the cell number would be essentially unchanged. Deep-living *Prochlorococcus* cells, with strongly increasing pigment content per cell, follow well this scheme (Fig. 12b); note that divinyl-Chl *b* content is also strongly increasing (not shown). Nevertheless, and in addition, a second mechanism responsible for the lack of correlation, not identified before, is the very low scattering signature of these tiny cells and therefore their reduced, almost undetectable, impact upon the attenuation coefficient. At the station shown in Figure 12, the cell concentration was maximum at about 80 m deep for *Prochlorococcus* and *Synechococcus,* and at 110 m for picoeukaryotic algae. The pigment maximum (46% is divinyl-Chl *a*), like the fluorescence maximum, was located at 110 m, i.e. below the 1% light level (occurring at about 90 m). Attenuation, with the highest values in the upper mixed layer (45 m), is steadily decreasing below and does not exhibit any particular feature at the level of the pigment maximum. Between 110 and 180 m, the decreases in divinyl-Chl *a* (associated with prochloro-phytes) and in Chl *a* (cyanobacteria and picoeukaryotes) are 0.12 and 0.14 mg m^{-3}, respectively. By assuming that picoeukaryotes and *Synechococcus,* with similar sizes, have similar optical coefficients at 665 nm, the decrease in attenuation, Δc, from 110 to 180 m can be estimated; it amounts to 0.032 m^{-1} and agrees well with the change observed in the vertical profile of the attenuation coefficient between these depths.

4. Conclusion

Two types of prokaryotic picoplankton, recognized as being abundant in open ocean and of major importance in oligotrophic ecosystems have been studied from an optical viewpoint. Their absorbing and scattering properties have been determined and related to their specific size and pigmentation. *Prochlorococcus* and *Synechococ-cus* are represented by several strains, which have been isolated and then grown in culture under low irradiances. Such experiments come up against several problems that mainly originate from the need to prepare relatively dense suspensions (of healthy cells) as required for optical determinations. In particular, heterotrophic bacteria overlapping in size with prochlorophytes cannot be separated by filtration. Corrections, based on the respective numerical counts (heterotrophic − autotrophic organisms), were nevertheless possible. The efficiency factor for absorption by prochlorophytes ($\overline{Q}_a$) is easily corrected for, whereas for $\overline{Q}_b$ (the factor for scatter-ing), the situation is more complex; the overall $\overline{Q}_b(\lambda)$ values are correctly estimated, to the extent that autotrophs and heterotrophs have the same size spectrum, even if the spectral details (the minima) admittedly are slightly smoothed. The same statement holds true for the Chl-specific spectra $b^*(\lambda)$, while $a^*(\lambda)$ spectra are free from significant bacterial interference. These drawbacks were much less crucial when dealing with *Synechococcus,* less contaminated by heterotrophic bacteria. From the various quantities derived from the present experiments, consistent with the theoretical predictions, it can be concluded that the optical properties of the two

types of small algae are reasonably well assessed and understood, so that their impact on the optical properties of open ocean oligotrophic waters is quantifiable. Full interpretation in terms of competition and photoecology is premature, however, since these species are very sensitive to radiative levels and able to drastically modify their pigment content. Further optical measurements using cells grown under the entire range of irradiance are still needed to specify their respective absorbing capacities as a function of optical depths.

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