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Diel variations in *Prochlorococcus* optical properties

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Abstract

Prochlorococcus is an important component of phototrophic biomass in oligotrophic areas. In such systems, diel variations in optical properties have been reported. In order to better understand these natural variations, the optical (absorption, attenuation, and scattering) and biochemical (carbon and pigment) properties of an axenic clone of *Prochlorococcus*, PCC 9511, grown in a turbidostat set under light conditions that simulated those found near the ocean surface, were monitored every 2 h for several consecutive days. All optical parameters showed pronounced diel patterns except the divinyl–chlorophyll *a* (Dv–Chl *a*) specific absorption coefficient at 676 nm, $a^*(676)$, which remained stable (0.019 m^2 mg Dv–Chl a^{-1}). The diel oscillations of the Dv–Chl *a* specific absorption coefficient at 440 nm, $a^*(440)$ (43% increase between sunrise and sunset), were essentially governed by variations in the zeaxanthin to Dv–Chl *a* ratio (52% increase of this ratio between sunrise and sunset). The scattering cross section at 555 nm, $\sigma_b(555)$, showed oscillations with the largest amplitude (182% increase between sunrise and sunset). Finally, the carbon-specific attenuation coefficient at 660 nm, $c_c^*(660)$ (1.04 m^2 gC^{-1}) is less than half that of the other algal groups. This estimation, together with the diel fluctuations in $c_c^*(660)$ highlighted in the present study, is discussed in the context of using in situ measurements of optical properties to infer biogeochemical stocks (vegetal or detrital biomass) or processes (primary production).

Increasing attention is paid to variations in the optical properties of the upper ocean at the diel scale. There are several possible explanations for this growing interest. First, the diel scale is fundamental for phytoplankton (Sournia 1974; Prézelin 1992), which, together with heterotrophic organisms and detritus, largely contribute to the optical properties of open-ocean case 1 waters. Generally, these variations are ignored in the interpretation of optical data sets. They could, however, explain a significant part of the observed variability in these data (Stramski and Reynolds 1993). Second, with the development of new observational platforms, in particular buoys equipped with optical sensors, it is becoming easier to document diel variations in optical properties. Indeed, these new platforms allow one to obtain quasicontinuous data sets from which variations over a scale continuum, including the diel scale, can be exemplified and studied (Stramska and Dickey 1992; Dickey et al. 1998; Kinkade et al. 1999). Finally, some optical measurements permit the nonintrusive quantification of optically significant constituents such as the particulate (and/or colored dissolved) matter. Therefore, it is tempting to use (small) variations in optical properties for inferring biogeochemical processes (e.g., Siegel et al. 1989; Walsh et al. 1995; Claustre et al. 1999) at the diel scale. The prerequisite for such inversions is that measurements have sufficient accuracy to detect small changes and that they can be conducted at high frequency, two conditions which are about to be met with new types of optical platforms (Dickey et al. 1998).

Diel variations in the particle attenuation coefficient [c_p (m^{-1}), equivalent, at 660 nm, to the particle scattering coefficient b_p (m^{-1}); Loisel and Morel 1998] have been documented using conventional transmissiometers in various open-ocean waters such as the Pacific (Siegel et al. 1989; Walsh et al. 1995; Claustre et al. 1999) and the Indian (Kinkade et al. 1999) Oceans. The increase in c_p observed during the day is generally balanced by a decrease of similar amplitude at night (Siegel et al. 1989; Claustre et al. 1999). Thus, in most cases, the net change over the course of a day is nil. This daily increase in c_p has sometimes been used as a proxy of carbon fixation (e.g., Siegel et al. 1989; Claustre et al. 1999). Such an optically based estimation of primary production relies upon (1) the accurate estimation of the carbon-specific attenuation coefficient (c_c^*, in m^2 g C^{-1}), which allows the conversion of attenuation measurements into their carbon content equivalents and upon (2) the critical assumption that c_c^* remains constant (or weakly variable) over the course of the day (Stramski and Reynolds 1993; Cullen and Lewis 1995), an assumption that, in some cases, can be challenged (Stramski et al. 1995).

In contrast to variations in scattering or attenuation coefficients, diel variations in the particle absorption coefficient (a_p, in m^{-1}), if any, have never been documented in situ. Indeed, even in the phytoplankton pigments blue and red absorption bands, the absorption coefficient of particulate

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material remains about one order of magnitude lower than the particle scattering or attenuation coefficients. Accurate particulate absorption measurements in open-ocean waters require concentrating particles and performing an in vitro analysis (e.g., Bricaud and Stramski 1990). Such a protocol precludes any time series with high temporal resolution to be recorded, from which diel variation in a_p could be documented and analyzed. Nevertheless, the recent development of in situ absorption meters like the AC9 from WET Labs Inc. might open the possibility to monitor variations in the spectral absorption coefficient at high frequency (e.g., Claustre et al. 2000), including at the diel scale.

In open-ocean oligotrophic areas, and especially in subtropical gyres, which are characterized by their clarity and stability, observations of a diel cycle for c_p seems to be the rule (e.g., Siegel et al. 1989; Claustre et al. 1999). In such waters, phytoplankton communities are dominated by photosynthetic picoplankton, which can be roughly subdivided in three main groups: *Prochlorococcus*, *Synechococcus*, and picoeukaryotes (e.g., Campbell and Vaulot 1993; Claustre and Marty 1995; Claustre et al. 1999). Among these three algal groups, the diel cycle in optical properties has been analyzed in detail on laboratory cultures of *Synechococcus* (Stramski et al. 1995) and of two picoeukaryotes: *Nannochloris* sp. (DuRand and Olson 1998) and *Micromonas pusilla* (DuRand et al. 2000). Within the intertropical oceanic belt, *Prochlorococcus* is often numerically the most abundant organism (Partensky et al. 1999). This single genus accounts for one-third to more than half of the total chlorophyll biomass (e.g., Goericke and Welschmeyer 1993*b;* Claustre and Marty 1995; Claustre et al. 1999). It is therefore timely to evaluate the capacity of *Prochlorococcus* to shape optical properties of the upper oceanic layers as well as in driving their diel variations.

Recently, a *Prochlorococcus* strain, PCC 9511, was made axenic (Rippka et al. 2000) and successfully grown under continuous culture conditions (Bruyant et al. 2001). This culture setup allows control of growth conditions and the physiological state, which impacts the biochemical composition and, ultimately, the optical properties of these cells (e.g., Stramski 1999). With this optimized culture system, the objective of the present study is triple. First, we investigate the optical properties (spectral absorption, attenuation, and scattering) of *Prochlorococcus.* Such properties have been already documented in the past, especially for absorption (Morel et al. 1993; Moore et al. 1995; Bricaud et al. 1999), but measurements were always conducted in nonaxenic batch cultures, which might be critical for attenuation and/or scattering measurements. The second main objective of this study is to quantify and analyze variations in *Prochlorococcus* optical properties, when cells are subjected to variations in irradiances that mimic those experienced by natural surface populations over 1 d. Finally, the results obtained on these cultures are extrapolated to the natural habitat by assessing the relative contribution of *Prochlorococcus* cells to the variations in optical properties of natural waters at the diel scale.

Materials and Methods

Culture conditions—Characteristics of the axenic strain *Prochlorococcus marinus* subsp. *pastoris* PCC 9511 used in this study as well as the recipe of the culture medium (PCR-S11) have been previously described (Rippka et al. 2000). Details on the turbidostat setup and illumination systems have been given elsewhere (Bruyant et al. 2001). Briefly, two replicate 11-liter turbidostat cultures of PCC 9511 were grown in 20-liter polycarbonate flasks, partially immersed in a thermoregulated bath at 21 ± 1°C set under a 12 h/12 h light/dark cycle (from 0600 to 1800). Such a design can be called a cyclostat (sensu Chisholm and Costello 1980). During the photoperiod, cells were illuminated by two symmetrical computer-controlled banks of light bulbs (OSRAM Dulux®L 55W daylight) providing a modulated irradiance varying in a sinusoidal way from 0 to 970 μmol quanta m^{-2} s^{-1} (*see results*). Over a full day, the irradiance received on average by the cells was ~300 μmol quanta m^{-2} s^{-1}. Nutrients were never limiting. After 15 d of acclimation to these conditions, the two turbidostat cultures were sampled during four consecutive photocycles. During the night, sampling was made under low green illumination, which is not absorbed by *Prochlorococcus* cells (Morel et al. 1993). The two turbidostats were not sampled at the same frequency. Turbidostat 1 was sampled every 2 h (except at 0200, a time of minimum metabolic activity) for optics, flow cytometry, pigments, and particulate carbon measurements (the data from this study were essentially acquired on this turbidostat). Turbidostat 2 was sampled every 4 h for RNA and cytometric measurements.

Cell concentration and cell cycle were analyzed in both turbidostats using a FacSort flow cytometer (Becton Dickinson). The red fluorescence of PCC 9511 cells was generally too low under the high irradiance used in this experiment to completely resolve the cells from background noise. Immediately after sampling, cells were fixed for 15 min with 1% paraformaldehyde and 0.05% glutaraldehyde, then stained using the DNA dye SYBR-Green I before counting them on the basis of their green DNA fluorescence, as detailed elsewhere (Marie et al. 2000). Duplicates of each sample were snap-frozen in liquid nitrogen and reanalyzed later on. They were also used for analysis of DNA distribution from which the percentages of cells in the different phases of the cell cycle were computed (Marie et al. 2000). Cell density in the cyclostat was adjusted by modifying the flow rate of fresh medium into the culture after measuring cell concentration, generally at the time of dark-to-light transition (~0600 h).

The cell density evolved similarly in both replicate turbidostats (Fig. 1A), although the range of variation was greater in turbidostat 1 than in turbidostat 2. The observed temporal variability in cell density essentially resulted from the combination of highly synchronized division with variations in sampling volumes that modified the overall culture volume and, thus, the dilution rates (ratio of flow rate by the culture volume). As a consequence, the stabilization of PCC 9511 at a constant biomass level was uneasy. Nevertheless, although significant, the recorded variations in cell density

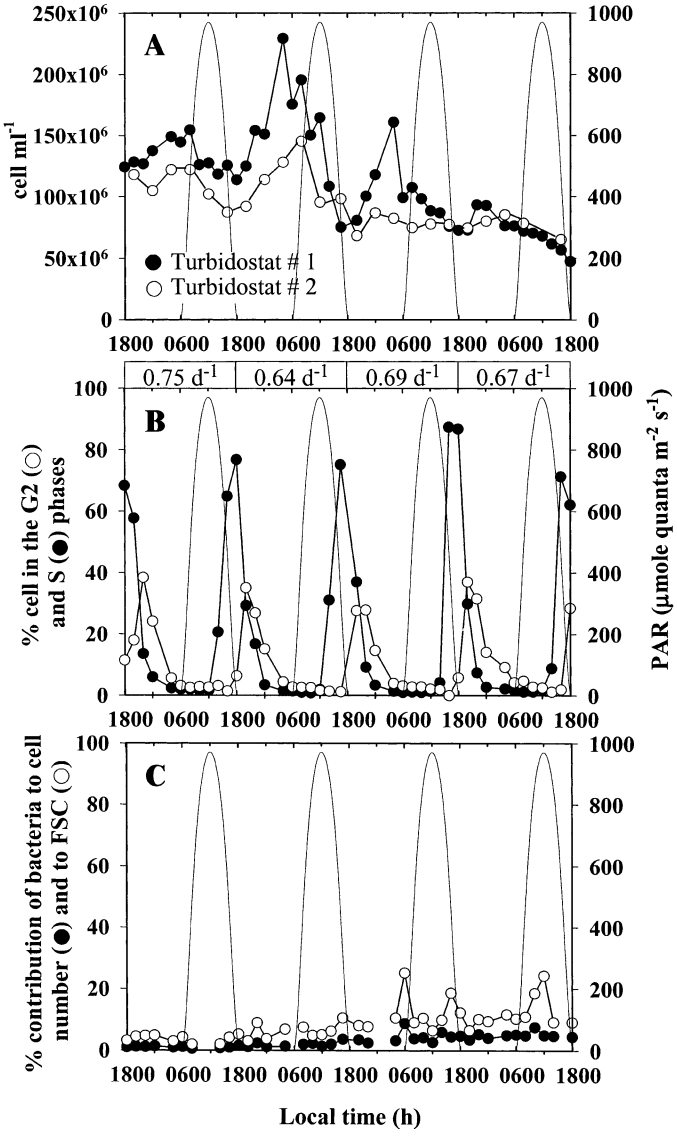

Fig. 1. Characterization of *Prochlorococcus* PCC 9511 cultures. (A) cell density in both replicate turbidostats. (B) DNA synthesis cycle in turbidostat 1. Growth rates are calculated over a 24-h interval (starting at 1800) according to Carpenter and Chang (1988), using a value of 4 h for time duration between peaks of S and G2 phases, value reported by Jacquet et al. (2001) from highly resolved temporal data. This value can be considered as maximal so that growth rates reported here likely correspond to a lower limit of the actual growth rate. (C) Estimation of the percentage contribution of bacteria to total cell (bacteria + *Prochlorococcus*) density and scattering in turbidostat 1. Note that PAR is plotted as a continuous (sinusoidal) line.

for turbidostat 1 (Fig. 1A) do not reflect variations in physiological state. Indeed, PCC 9511 DNA synthesis cycle was extremely reproducible over the four replicate days in turbidostat 1 (Fig. 1B). Subsequently, growth rates, derived from these DNA synthesis cycle data using the equation of Carpenter and Chang (1988), were also highly reproducible. The average growth rate determined here for PCC 9511 (μ = 0.69 ± 0.04 d^{-1}) is very close to the maximum growth rate determined under continuous irradiance for MED4 clone (μmax = 0.63 ± 0.06 d^{-1}, Moore et al. 1995). Similarly, observations based on gene expression measurements confirm that the *Prochlorococcus* cells were also physiologically in steady state throughout the 4-d experiment for replicate turbidostat 2 (Garczarek et al. 2001; Holtzendorff et al. 2001). Thus, the present results not only suggest that PCC 95511 physiological state was stable over the four cycles, but also that cells were growing under nutrient-replete conditions associated with optimal irradiances. Therefore, although optical and biochemical measurements were not replicated for both turbidostats, the reproducibility of these measurements over 4 d for turbidostat 1 appears sufficient to appropriately address the objectives of this study.

During the preconditioning phase (15 d), the culture was kept axenic. During the experiment, a slight bacterial contamination was detected. Using cytometric determination of bacteria numerical density, the bacterial contribution to the total (bacteria + PCC 9511) cell density was estimated. This contribution slightly increases, on average, from cycle to cycle (1%, 2%, 4.2%, 4.9%, for cycles 1, 2, 3, and 4, respectively) (Fig. 1C). If the forward side scatter (FSC) measured by cytometry is taken as a proxy of the scattering cross section of bacteria and PCC 9511 cells (e.g., DuRand and Olson 1998), the contribution of bacteria to scattering by prokaryotic biomass can also be estimated (Fig. 1C). This contribution gradually increases with time (3.7%, 6.3%, 11.7%, 12%, on average, for cycles 1, 2, 3, and 4). It will be shown later that the effect of bacterial contamination on bulk optical properties and their diel variations is not significant. This means that bacterial influence on optical measurements is not an issue in the present study.

Pigments and carbon—Triplicate 15-ml culture samples were filtered onto GF/F glass fiber filters and immediately stored in liquid nitrogen. High-performance liquid chromatography (HPLC) analysis was performed within 1 month of collection according to a slight modification of the protocol of Vidussi et al. (1996) described in Hooker et al. (2001). By use of these analytical conditions, and for an average cell density of ~10^8 cell ml^{-1}, (Fig. 1), the pigment detection limit was 1.6 μg Dv–Chl *a* L^{-1}, 4.5 μg Dv–Chl *b* L^{-1}, 0.6 μg zeaxanthin L^{-1}, and 0.7 μg α carotene L^{-1}.

For carbon measurements, triplicate 50-ml culture samples were filtered onto GF/F filters previously cleaned in soxlet with dichloromethane. After filtration, samples were stored at 50°C awaiting analysis performed within 3 months after collection. Samples were analyzed using a LECO 900 carbon analyzer with ethylenediaminetetraacetic acid (EDTA) as a standard. Valid data are available for the first two and half cycles only.

Optical measurements—Spectral attenuation $c(\lambda)$ and absorption $a(\lambda)$ coefficients were measured in a 1-cm quartz cuvette using a dual-beam spectrophotometer (Perkin-Elmer Lambda 19) equipped with an integrating sphere and applying the optical setup described in Stramski et al. (2002). All measurements were performed in triplicate with a 1-nm resolution over the 190–800-nm spectral range and referenced against culture medium filtered onto a 0.2-μm syringe filter

Table 1. Biochemical and optical characteristics in *Prochlorococcus* PCC 9511 grown in turbidostat under a daily light cycle.

	Average ±1 SD	Min–Max†	Dawn value ±1 SD‡
Dv-Chl *a* (fg cell^{-1})	0.39±0.06	0.24–0.50	—
Zeaxanthin (fg cell^{-1})	0.51±0.09	0.28–0.69	0.37±0.08 (73%)
C (fg cell^{-1})	27±6	17–38	19±4 (101%)
C : Dv–Chl *a* (g : g)	70±13	54–94	56±2 (93%)
Zeaxanthin : Dv–Chl *a* (g : g)	1.31±0.20	0.94–1.74	1.04±0.07 (52%)
a^* (440) (m^2 mg Dv–Chl *a*$^{-1}$)	0.0858±0.0119	0.0686–0.1131	0.0735±0.0058 (43%)
a^* (676) (m^2 mg Dv–Chl *a*$^{-1}$)	0.0186±0.0023	0.0143–0.0256	—
b^* (555) (m^2 mg Dv–Chl *a*$^{-1}$)	0.1139±0.0345	0.0590–0.2039	0.0682±0.0081 (154%)
σ_b (555) (10^{-14} m^2 cell^{-1})	4.48±1.53	1.94–7.69	2.40±0.46 (182%)
σ_c (660) (10^{-14} m^2 cell^{-1})	3.18±1.08	1.40–5.38	1.70±0.26 (184%)
c_c^* (660) (m^2 g C^{-1})	1.04±0.15	0.77–1.32	0.81±0.05 (58%)

† Range of observed values.
‡ The number in parentheses refers to the percentage of increase in this parameter from dawn to dusk.

(Nalgene). The replicate spectra were subsequently averaged. The scattering coefficient, $b(\lambda)$, was calculated as the difference between $c(\lambda)$ and $a(\lambda)$. The absorption $[\sigma_a(\lambda)]$, scattering $[\sigma_b(\lambda)]$, and attenuation $[\sigma_c(\lambda)]$ cross sections were calculated by normalizing $a(\lambda)$, $b(\lambda)$, and $c(\lambda)$ by cell density. These coefficients have dimension of m^2 cell^{-1}. The Dv–Chl *a* specific coefficients for absorption $[a^*(\lambda)]$ and scattering $[b^*(\lambda)]$ were calculated by normalizing $a(\lambda)$ and $b(\lambda)$ to the Dv–Chl *a* concentration and have dimension of m^2 (mg Dv–Chl *a*)$^{-1}$. The carbon-specific attenuation coefficient, $c^*(\lambda)$ (in m^2 gC^{-1}), is obtained by normalizing $c(\lambda)$ by the carbon concentration.

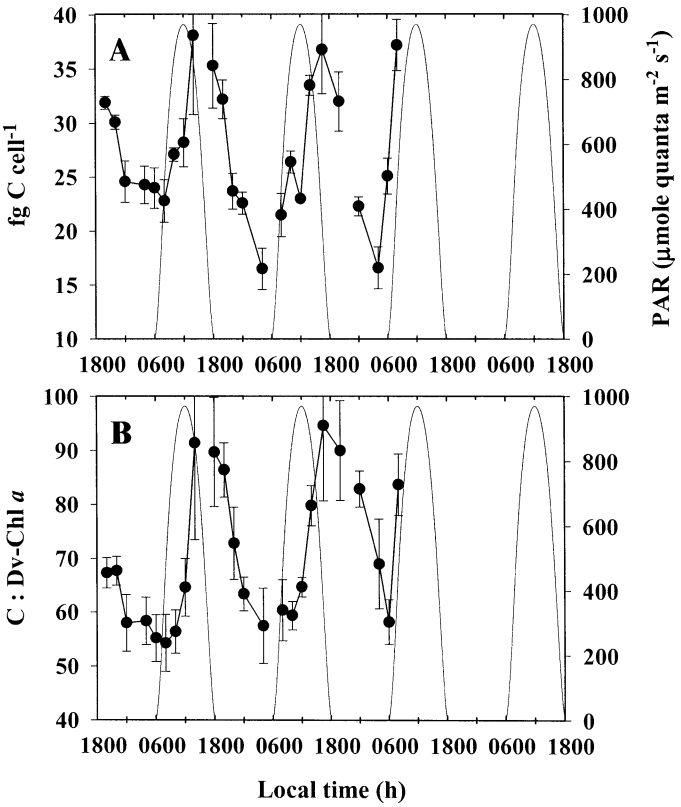

Fig. 2. Temporal variations of biochemical characteristics of *Prochlorococcus* PCC 9511 cells grown in a cyclostat. (A) Carbon cellular concentration. (B) (C) Dv–Chl *a* ratios.

Results and Discussion

Biomass and biochemical ratios—The pigmentation of PCC 9511 grown at such high irradiance was very simple: besides the main pigments zeaxanthin and Dv–Chl *a*, only α carotene was detected. Conversely to previous studies on MED4 (Moore et al. 1995; Cailliau et al. 1996; Bricaud et al. 1999), a closely related but not identical clone, the Dv–Chl *b*/Dv–Chl *a* ratio was, on average, below 0.06, which is an indication of adaptation to very high irradiances (Moore et al. 1995).

The average cellular Dv–Chl *a* content (0.39 ± 0.06 fg cell^{-1}) (Table 1) was significantly lower than the values reported by Cailliau et al. (1996) (1.5 fg Dv–Chl *a* cell^{-1}) for MED4 grown at a constant PAR (photosynthetically available radiation) of 56.7 μmol quanta m^{-2} s^{-1}, but this value agrees with the large range of values reported by Morel et al. (1993) (~0.15–1.7 fg cell^{-1}) or Gibb et al. (2001) (0.22–1.83 fg cell^{-1}) for natural *Prochlorococcus* populations. The cellular zeaxanthin (0.51 ± 0.09 fg cell^{-1}) was nearly two times lower than that reported by Cailliau et al. (1996) and Moore et al. (1995) (1 fg zeaxanthin cell^{-1}). The same observation applies for carbon content (27 ± 6 fg C cell^{-1}, Table 1) when compared to the results of Cailliau et al. (1996) (49 fg C cell^{-1}). These low cellular contents likely result from adaptation to high irradiances (e.g., reduction of the pigment content) and also possibly of a reduction of cell size affecting the overall pool of organic material. Indeed Bricaud et al. (1999) have shown that cell size of MED4 grown under high light is smaller than size of cells grown under low light.

Over the first two cycles investigated, the daily fluctuations in cellular carbon were regular. The C content increased during the day and decreased during the night (Fig. 2) by a factor of ~2 (Table 1). Similarly, the C to Dv–Chl *a* ratio increased during the day and decreased just after the light-to-dark transition. These values are within the range reported for eukaryotic algae (e.g., Geider 1987).

The cellular content of zeaxanthin (Fig. 3) always de-

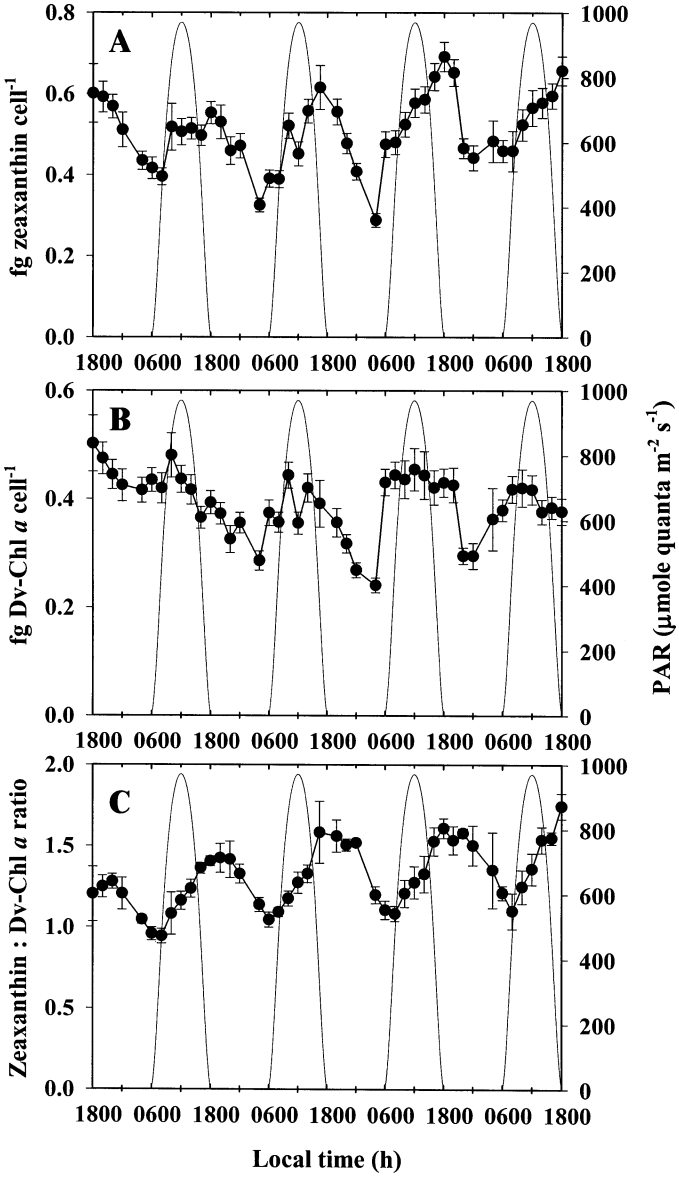

Fig. 3. Temporal variations of the pigment content of *Prochlorococcus* PCC 9511 cells grown in a cyclostat. (A) Zeaxanthin cell content. (B) Dv–Chl *a* cell content. (C) Zeaxanthin: Dv–Chl *a* ratio.

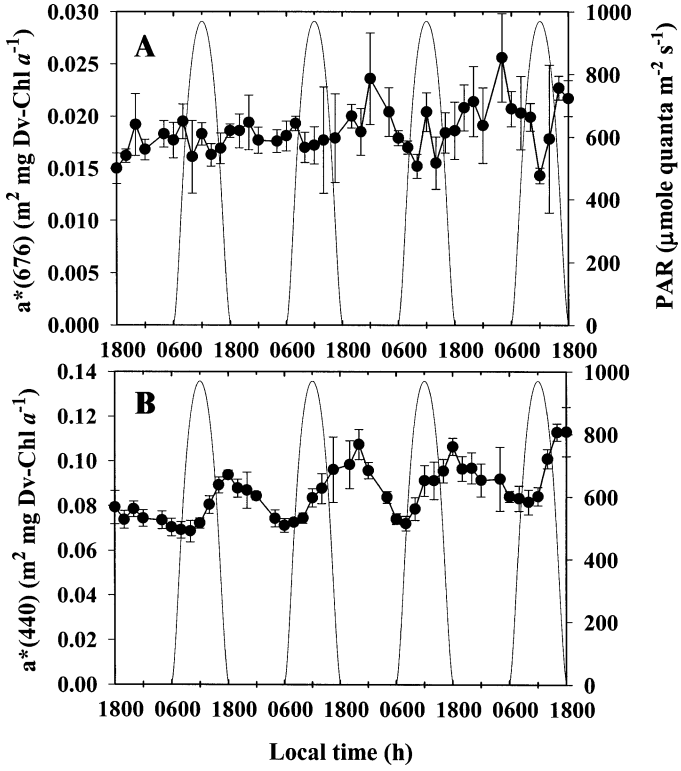

Fig. 4. Temporal variations of the absorption properties of *Prochlorococcus* PCC 9511 grown in a cyclostat. (A) Dv–Chl *a* specific absorption coefficient at 676 nm. (B) Dv–Chl *a* specific absorption coefficient at 440 nm.

creased at night. During the diel period, this content increased stepwise (cycle 1) or regularly (cycles 2, 3, and 4). The cellular content in Dv–Chl *a* decreased during the day and the early part of the night (except for the third cycle where it remains stable) and increased rapidly over the few hours preceding sunrise. The zeaxanthin to Dv–Chl *a* ratio (hereafter named ZDR) exhibited a very regular oscillation with time; it increased during the day and decreased at night. The average value of ZDR is 1.3 ± 0.2, which is in agreement with the one that can be found for MED4 at PAR ~ 300 µmol quanta m^{-2} s^{-1} (Moore et al. 1995; their figure 8B).

Besides the fairly regular daily oscillations in ZDR, a slight increase of the average value of ZDR over the four experiment days is seen in Fig. 3. This trend is estimated to ~6% per day, which has to be compared to the ~50% variations (between minima and maxima) associated to the diel changes. Given that ZDR can, to some extent, be considered as an index of photoprotection, this trend may result from a slight change in irradiance within the culture vessel due to the progressive decrease in cell density with time (Fig. 1).

Absorption—The Dv–Chl *a* specific absorption coefficient at 676 nm (i.e., in the red absorption band of Dv–Chl *a*), *a**(676), did not show any daily variations (Fig. 4). Rather, *a**(676) fluctuated around a central value of 0.019 ± 0.002 m^2 (mg Dv–Chl *a*)$^{-1}$ (Table 1), which is very close to the maximum theoretical value of this coefficient for pure Chl *a* [0.021 m^2 (mg Dv–Chl *a*)$^{-1}$, Bricaud et al. 1983]. This result means that pigment packaging had small or no effect on absorption at this wavelength and that Dv–Chl *b* did not contribute significantly to absorption at 676 nm.

By contrast, in the blue part of the spectrum, there was a very clear diel cycle in the Dv–Chl *a* specific absorption coefficient [e.g., *a**(440), Fig. 4]. *a**(440) consistently increased during the daily period (by a 35 to 55% factor according to the cycle considered) and decreased at night. Variations in the Chl-specific absorption of natural phytoplankton populations are governed by the ''package'' effect and/or the contribution of accessory pigments (Bricaud et al. 1995). It is likely that the package effect, which essentially depends upon the size and the internal pigment concentration (Morel and Bricaud 1981), was minimal in our

study, even at this wavelength. Indeed, previous work has shown that, as a result of its tiny size, the absorption capacity in the blue domain of the MED4 clone is only reduced by less than 20% (Morel et al. 1993) or even 10% (Bricaud et al. 1999), comparatively to the absorption capacity of the same amount of pigments, if they were in solution. Furthermore, the low cellular Dv–Chl *a* content reported here as a result of photoacclimation (*see above*) would also result in low packaging. It is thus expected that pigment packaging, if any, was even lower in our study than the (already weak) package effect reported in the studies of Morel et al. (1993) and Bricaud et al. (1999). Thus, the relatively large diel oscillations in $a^*(440)$ were most probably related to changes in the contribution by accessory pigments to absorption.

The diel variations in $a^*(440)$ (Fig. 4) were in phase with those in ZDR (Fig. 3), an observation that supports the control of specific absorption of *Prochlorococcus* by accessory pigment dynamics. This influence of accessory pigments can be quantified by partitioning the specific absorption $a^*(440)$ into a photosynthetic [$a^*_{PS}(440)$] and a nonphotosynthetic [$a^*_{NPS}(440)$] contribution, in a similar way as performed by Babin et al. (1996) or Allali et al. (1997). In the present case, due to the very simple pigmentation of *Prochlorococcus*, the nonphotosynthetic pool was restricted to zeaxanthin, while both Dv–Chl *a* and α carotene were the only contributors to the photosynthetic pool [the excellent correlation between Dv–Chl *a* and α carotene pools ($r^2 = 0.99$; $n = 44$) is a strong indication that α carotene is a photosynthetic pigment]. On the basis of the concentration and of the specific absorption coefficient of these pigments at 440 nm [0.027 m^2 (mg Dv–Chl *a*)$^{-1}$; 0.039 m^2 (mg zeaxanthin)$^{-1}$; 0.043 m^2 (mg α carotene)$^{-1}$], the partial contribution (in percentage) of $a^*_{PS}(440)$ and $a^*_{NPS}(440)$ can be estimated (Fig. 5a). On average, the percentage of $a^*_{PS}(440)$ is only 40.8 $\pm$ 3.4%, which means that more than half of absorbed photons were not directed toward photosynthesis. The percentage of $a^*_{PS}(440)$ was maximal at sunrise (45–48%), when ZDR was minimal, and minimum at sunset (35–38%), when ZDR was maximal.

These partial absorption contributions, when multiplied by $a^*(440)$, allow the determination of $a^*_{PS}(440)$ and $a^*_{NPS}(440)$, respectively (Fig. 5B). Contrary to $a^*_{NPS}(440)$, $a^*_{PS}(440)$ did not present any daily variations. Daily variations in *Prochlorococcus* $a^*(440)$ were thus exclusively governed by variations in $a^*_{NPS}(440)$. In other words, because $a^*_{NPS}(440)$ was here equivalent to Dv–Chl *a* specific absorption by zeaxanthin, variations in $a^*(440)$ were only driven by the relative variations in cell contents of zeaxanthin and Dv–Chl *a*.

The potential of *Prochlorococcus* for absorbing, per unit Dv–Chl *a*, energy usable for photosynthesis remained constant throughout the day [$a^*(676)$ and $a^*_{PS}(440)$ kept unchanged]. *Prochlorococcus* grown under high irradiance (as in the upper layer of the surface ocean) was therefore likely unable to adjust its absorption capacity to light change at the daily scale (1) because this capacity was already nearly maximal (close to the theoretical maximum, *see above*) as the package effect was minimal and (2) because *Prochlorococcus* lacked a significant amount of accessory photosynthetic pigments (Dv–Chl*b* and α carotene remained minor in any case).

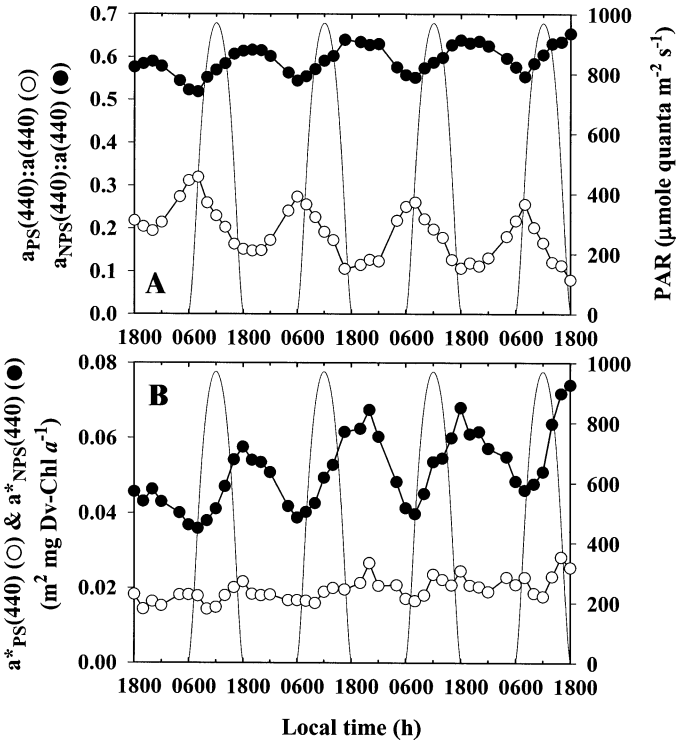

Fig. 5. Temporal variations of the absorption properties of *Prochlorococcus* PCC 9511 grown in a cyclostat. (A) Relative proportion of photosynthetic and nonphotosynthetic absorption. (B) Dv–Chl *a* specific absorption coefficient at 440 nm for photosynthetic and nonphotosynthetic pigments.

Besides pigment absorption bands in the blue (zeaxanthin and Dv–Chl *a*) and in the red (Dv–Chl *a* only) part of the spectrum (Fig. 6A), *Prochlorococcus* exhibited a pronounced absorption band in the UV domain, between 250 and 270 nm. This band exhibited very clear diel oscillations, either on a per cell or on a per Dv–Chl *a* basis (Fig. 7). The experimental setup of the culture (neon tubes, polycarbonate vessel within a glass aquarium) prevented *Prochlorococcus* biochemical properties from being influenced by UV radiation, so that the enhanced absorption in the 250–270-nm range was not a response to UV stress. We believe that this UV absorption band might characterize the plastoquinone pool (Falkowski and Raven 1997, their figure 9.12) or the presence of antioxidant compounds (Dunlap and Shick 1998), whose function is to counteract the presence of various damaging O_2 species as a result of excess excitation energy (O_2 reduction into O_2^- or H_2O_2 through PS I, singlet oxygen). It is worth noting that this UV absorption feature is typical of cyanobacteria that lack of xanthophyll cycle, the function of which in eukaryotic phytoplankton is, at least partly, to avoid the formation of this O_2 destructive species (photooxidation) (Demmig-Adams and Adams 1993).

Scattering—General variations: The spectral scattering cross section (Fig. 6B) regularly increased with decreasing wavelength, with an average $\lambda^{-2.1}$ dependency (for all the spectra), consistent with theoretical expectations for nonabsorbing particles of very small size (Morel and Bricaud

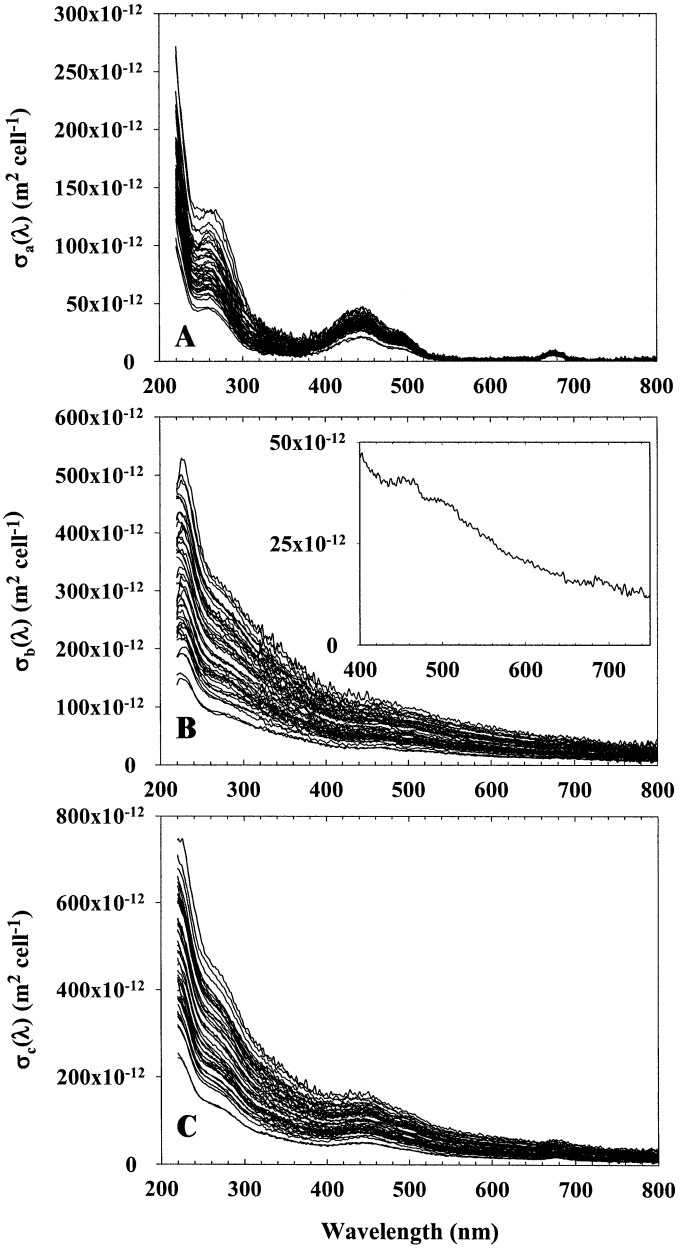

Fig. 6. Optical cross section spectra (220–800 nm) for *Prochlorococcus* PCC 9511 grown in a cyclostat. (A) Absorption cross section. (B) Scattering cross section. The inset presents a representative spectrum, which is detailed in the visible domain. (C) Attenuation cross section. 48 spectra are presented in each panel.

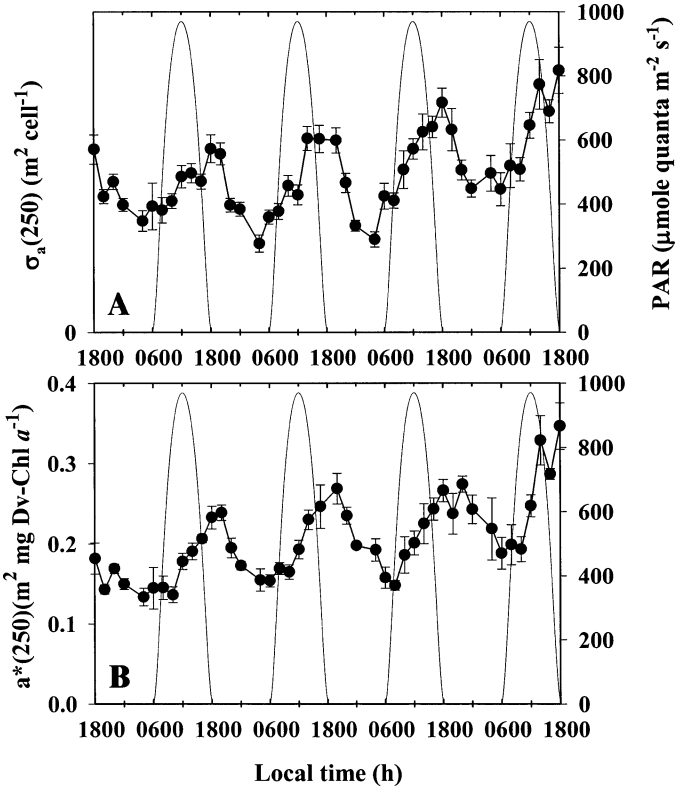

Fig. 7. Temporal variations of the absorption properties of *Prochlorococcus* PCC 9511 grown in a cyclostat. (A) Absorption cross section at 250 nm. (B) Dv–Chl *a* specific absorption coefficient at 250 nm.

1986). Superposed to this general trend, some slight spectral irregularities could be noticed in the vicinity of the pigment absorption bands (when enhancing the scale of Fig. 6B); these spectral irregularities were much less pronounced than those reported by Morel et al. (1993) for the MED4 strain grown at 7 μmol quanta m^{-2} s^{-1}. Interestingly, absorption bands due to pigments were apparent in the spectral attenuation cross section (Fig. 6C), a feature seldom observed for phytoplankton.

The Dv–Chl *a* specific scattering coefficient at 555 nm, $b^*(555)$, had an average value of 0.114 m^2 (mg Dv–Chl $a)^{-1}$, ~5 times greater than the value reported for MED4 by Morel et al. (1993), while the average scattering cross section, $\sigma_b(555)$, was nearly identical for both studies (0.04×10^{-14} μm^2 $cell^{-1}$; Table 1). The higher $b^*(555)$ reported here essentially resulted from low cellular Dv–Chl *a* content as a consequence of PCC9511 adaptation to very high irradiances.

The cellular scattering cross section, $\sigma_b(555)$, as well as in the Dv–Chl *a* specific scattering cross section, $b^*(555)$, showed very similar trends (Fig. 8). Both quantities regularly increased during the daily period and decreased at night. These oscillations were in phase with oscillations of FSC of PCC9511 while FSC of bacteria did not present any diel trend (data not shown), which confirms that observed diel variations were exclusively due to *Prochlorococcus.* Interestingly, $\sigma_b(555)$ and $b^*(555)$ variations were remarkably phased with diel changes in the attenuation coefficient, as reported by Claustre et al. (1999) in the euphotic layer of the South Pacific Gyre, where *Prochlorococcus* contributed to 60% of the attenuation by algal material. The amplitude of variation in $\sigma_b(555)$ or in $b^*(555)$ spanned a factor of ~3, which was much higher than the amplitude of variation in $a^*(440)$ (~1.5, Fig. 4). Therefore, compared to absorption, scattering properties of *Prochlorococcus* are much more influenced by diel variations in irradiance, corroborating a previous report on the diatom *Thalassiosira pseudonana* (Stramski and Reynolds 1993). As a consequence, the single

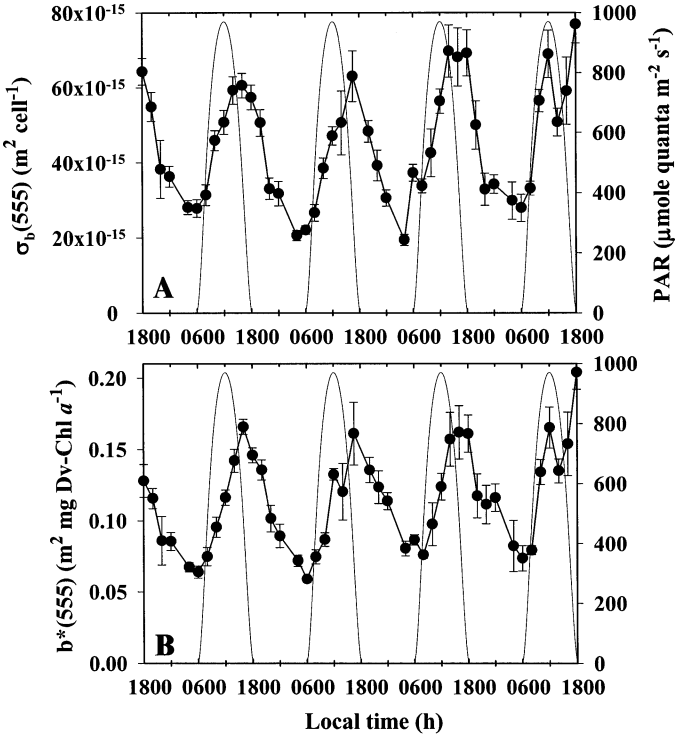

Fig. 8. Temporal variations of the scattering properties of *Prochlorococcus* PCC 9511 grown in a cyclostat. (A) Scattering cross section at 555 nm. (B) Dv–Chl *a* specific scattering coefficient at 555 nm.

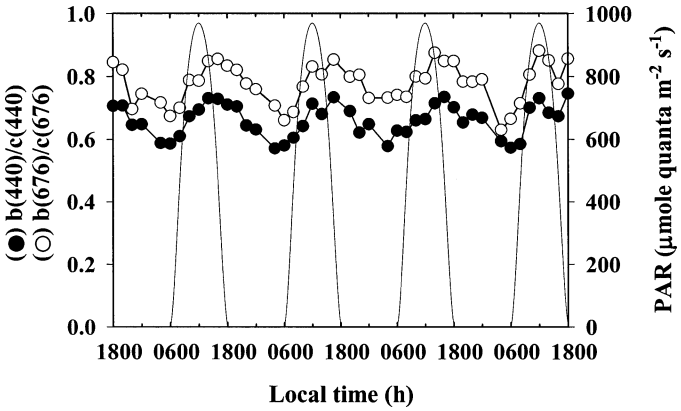

Fig. 9. Temporal variations in $b(440)/c(440)$ and $b(676)/c(676)$ ratios of *Prochlorococcus* PCC 9511 grown in a cyclostat.

scattering albedo (scattering-to-attenuation ratio) $\omega(\lambda)$ (Fig. 9 for $\lambda = 440, 676$ nm) and the scattering-to-absorption ratio (data not shown) exhibited very reproducible temporal trends similar to that of $\sigma_b(555)$ or $b^*(555)$. For example, over the four cycles, $\omega(440)$ varied between very stable limits, from 0.58 ± 0.01 (at sunrise) to 0.74 ± 0.01 (at sunset).

Biogeochemical implications: In the open ocean, and especially in subtropical gyres, the predominating phytoplanktonic species (*Prochlorococcus*, *Synechococcus*, and picoeukaryotes) are characterized by their very small size (Chisholm 1992). In these oligotrophic areas, very clear daily oscillations have been reported for the attenuation coefficient (e.g., Siegel et al. 1989; Claustre et al. 1999), as well as for individual cell properties (Vaulot et al. 1995; Vaulot and Marie 1999; DuRand and Olson 1996). Because subtropical gyres represent a very significant part of the world ocean, it is essential to address in detail the diel variations in the optical properties of their representative phytoplankton populations, for at least two reasons: (1) for evaluating the potential bias in using optical data sets when the ''diel effect'' (whose magnitude is linked to sampling time) is not taken into consideration (Stramski and Reynolds 1993) and (2) for the accurate retrieval of carbon standing stocks and even primary production from attenuation measurements (e.g., Siegel et al. 1989; Cullen and Lewis 1995; Claustre et al. 1999).

The present study of the diel optical properties in *Prochlorococcus* complements similar previous studies performed on other phytoplankton taxa a priori representative of oligotrophic conditions, namely *Synechococcus* sp. (Stramski

Table 2. Comparison of optical and biochemical properties of phytoplankton a priori representative of oligotrophic conditions and subjected to diel variations in irradiances. The first numbers refer to the dawn value; the number in parentheses refers to the percentage of increase in this parameter from dawn to dusk.

	Prochlorococcus PCC9511 (this study)	*Synechococcus* sp. WH8103 (Stramski et al. 1995)†	*Nannochloris* sp. (DuRand and Olson 1998)
Max. PAR (μmole quanta m^{-2} s^{-1})	970	300	1,500
Light type	Artificial, sinusoidal	Natural	Natural
Cell size (μm)	—	1.13 (17%)	2.67 (14%)
Cell volume (μm^3)	—	0.76 (60%)	9.97 (48%)
C (fg $cell^{-1}$)	19 (101%)‡	205 (36%)	~2,000 (~100%)
σ_c (660) (10^{-14} m^2 $cell^{-1}$)	1.7 (184%)§	55 (72%)	~600 (133%)
$c_c^*(660)$ (m^2 gC^{-1})	0.81 (58%)‡	2.5 (32%)	3.0 (17%)

† Two cycles are analyzed in the study of Stramski et al. (1995). However, we use here only results of the second cycle, for which light intensity was higher than for the first one.
‡ Average of the first two cycles.
§ Average of the four cycles.

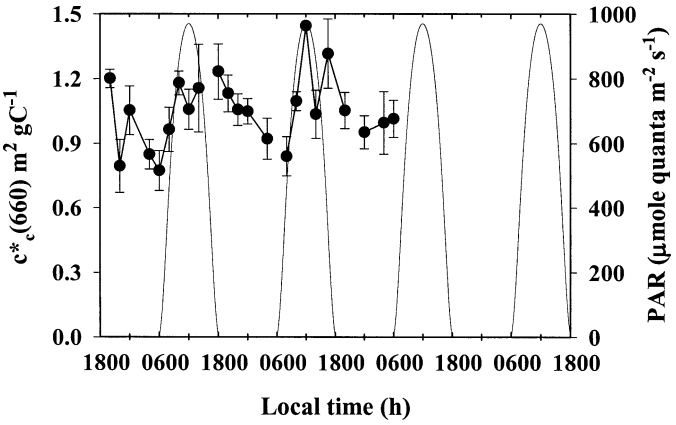

Fig. 10. Temporal variations in the carbon-specific attenuation coefficient at 660 nm of *Prochlorococcus* PCC 9511 grown in a cyclostat.

et al. 1995) and *Nannochloris* sp. (DuRand and Olson 1998). Table 2 summarizes, for some important cellular and optical properties, the differences between these three phytoplankters. Their carbon content spans over two orders of magnitude (~20, 200, 2,000 fg C cell^{-1}, for *Prochlorococcus, Synechococcus, Nannochloris*, respectively). The range of variation in the attenuation cross section at 660 nm (the wavelength at which most in situ measurements are done), $\sigma_c(660)$, is even greater (0.017, 0.55, 6 μm^2 cell^{-1} for *Prochlorococcus, Synechococcus, Nannochloris,* respectively). The increase in $\sigma_c(660)$ over the daily period is always greater than 70% of the morning value and even reaches ~180% for *Prochlorococcus* (this study). This result clearly illustrates that attenuation/scattering properties of the upper ocean might be very sensitive to the diel cycle, in particular (but not exclusively) if *Prochlorococcus* significantly contributes to the biomass and to the attenuation coefficient. Claustre et al. (1999) have estimated the contribution of *Prochlorococcus* attenuation to be 8% and 12% of particle attenuation in the euphotic zone of subequatorial (5°S, 150°W) and subtropical (16°, 150°W) stations of the South Pacific, respectively. In the equatorial Pacific, this contribution is in the range ~5–15% (DuRand and Olson 1996; Chung et al. 1998).

Accurate determinations of carbon stocks from attenuation measurements (generally at 660 nm) require that appropriate conversion factors are used. The carbon-specific attenuation coefficient $c_c^*(660)$, in m^2 g C^{-1}, is the parameter that is generally chosen for that purpose (e.g., Siegel et al. 1989; Cullen et al. 1992). Thanks to the low biomass of bacteria in the culture, *Prochlorococcus* $c_c^*(660)$ was here determined for the first time (Fig. 10). The average value, ~1 m^2 g C^{-1}, is ~3 times lower than estimates for *Synechococcus* and *Nannochloris* and even more if compared to the diatom *Thalassiosira pseudonana* (3.8 m^2 g C^{-1}) (Stramski and Reynolds 1993). The value of $c_c^*(660)$ for *Prochlorococcus* is also lower than the corresponding estimation for particulate matter in the equatorial and subequatorial Pacific (2 m^2 g C^{-1}; Claustre et al. 1999) and lower still than similar estimations in the North Pacific (3.9 m^2 gC^{-1}; Siegel et al. 1989) or the North Atlantic (3.9–5.6 m^2 g C^{-1}; Walsh et al. 1995).

The actual attenuation capabilities of the living carbon of *Prochlorococcus* are at least three times lower than for other phytoplankton and two times lower than for bulk particles. It is also a confirmation of the previous study of Morel et al. (1993) demonstrating that, as a consequence of its low scattering efficiency $Q_b(\lambda)$ (always <0.2) resulting from its tiny size, *Prochlorococcus* has a lower effect than other phytoplankton on the particle attenuation coefficient c_p. Therefore, even if *Prochlorococcus* is numerically the most abundant photosynthetic organism in the ocean (Partensky et al. 1999) and if its Dv–Chl *a* biomass often represents one-third or more of the total Chl *a* biomass (Goericke and Welschmeyer 1993*b*; Claustre and Marty 1995; Gibb et al. 2001), its impact on attenuation is expected to remain low.

Interestingly, the estimation of *Prochlorococcus* $c_c^*(660)$ allows us to estimate the carbon-specific attenuation for nonalgal material (heterotrophs, but mostly detritus), an important and unknown pool that might contribute to 50%–80% of the particle attenuation (DuRand and Olson 1996; Chung et al. 1998; Claustre et al. 1999). For that calculation, we use the value of $c_c^*(660)$ for particulate matter (2 m^2 g C^{-1}) obtained for a particle assemblage constituted by 61% nonalgal material, 23% picoeukaryotes, 8% *Synechococcus,* and 8% *Prochlorococcus* (Claustre et al. 1999). The $c_c^*(660)$ values reported on Table 2 are assigned to these phytoplankton (*Nannochloris* is assumed to be representative of picoeukaryotes) so that $c_c^*(660)$ for nonalgal material is estimated to ~1.5 m^2 g C^{-1}. This rather low value (comparatively to other phytoplankton larger than *Prochlorococcus,* Table 2) is an indication that the nonalgal pool contributing to attenuation is likely dominated by small particles, with a low scattering efficiency.

Applying a generic $c_c^*(660)$ value to get carbon measurement from $c(660)$ in any trophic situation is not valid. Indeed, the differences recorded in $c_c^*(660)$ for various phytoplankton species and nonalgal material imply that $c_c^*(660)$ for bulk particulate material is dependent upon the proportion of algal and nonalgal material as well as on the composition of the vegetal assemblage. This observation explains, at least partly, regional differences between the low estimation of $c^*(660)$ (2 m^2 g C^{-1}) for the South Pacific (Claustre et al. 1999) compared to the higher $c_c^*(660)$ values reported for the North Pacific (Siegel et al. 1989) or North Atlantic (Walsh et al. 1995). Thus, (at least a few) carbon measurements on discrete samples are always necessary to establish regional $c(660)$ versus carbon relationships for the purpose of inverting $c(660)$ measurements into carbon concentration equivalents. Additionally, information on the composition of the particle assemblage, including phytoplankton, is needed for resolving regional variations in such relationships.

DuRand and Olson (1996) have shown that daily change in the attenuation by algal material accounts for most of the daily change recorded in $c(660)$ (in other words, attenuation by nonalgal material does not vary at the daily scale). Table 2 shows that, although $\sigma_c(660)$ for representative phytoplankton species exhibits a considerable variation at the daily scale (~70–180% of the morning value), $c_c^*(660)$ varia-

tions remain restricted to a much narrower range (<58%) (*see also Fig. 10*). A prerequisite in using daily increase in $c(660)$ as an estimation of primary production is the temporal stability of $c_c^*(660)$, or at least its weak variability (Stramski and Reynolds 1993; Cullen and Lewis 1995). The daily variability in $c_c^*(660)$ reported here is significant enough to prevent accurate primary production estimation from $c(660)$ changes. In situations where only optical measurements are available (e.g., moored sensors), a constant value of $c_c^*(660)$, determined for particulate matter, can only be used as a first approximation to estimate primary production.

The measurement of optical properties at the cell level (e.g., forward scattering as measured by flow cytometry) allows us to infer, from theoretical calculation, cell attenuation cross sections (DuRand and Olson 1996). Because the variability of $c_c^*(660)$ among various phytoplankters is now partly documented (*see Table 1*), the diel variations in cell cross section might be converted into taxon-specific primary production. Knowing carbon fixation rate at the level of phytoplankton group is an important issue for marine biogeochemistry. To date, promising techniques for estimating taxon-specific primary production like pigment labeling (e.g., Goericke and Welschmeyer 1993*a*,*b*; Cailliau et al. 1996; Goericke 1998) or cell sorting on ^{14}C labeled phytoplankton (e.g., Li 1993), have failed to become of general use. It is possible that flow cytometry supported by optical modeling and completed by some discrete measurements of POC in the ocean might be an alternative for the future.

References

ALLALI, K., A. BRICAUD, AND H. CLAUSTRE. 1997. Spatial variations in the chlorophyll-specific absorption coefficients of phytoplankton and photosynthetically active pigments in the equatorial Pacific. J. Geophys. Res. **102:** 12,413–12,423.

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

BRICAUD, A., M. BABIN, A. MOREL, AND H. CLAUSTRE. 1995. Variability in the chlorophyll-specific absorption coefficients of natural phytoplankton: Analysis and parameterization. J. Geophys. Res. **100:** 13,321–13,332.

———, A. MOREL, AND L. PRIEUR. 1983. Optical efficiency factors of some phytoplankters. Limnol. Oceanogr. **28:** 816–832.

———, AND D. STRAMSKI. 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.

———, AND OTHERS. 1999. Divinyl chlorophyll *a*-specific absorption coefficients and absorption efficiency factors for Prochlorococcus marinus: Kinetics of photoacclimation. Mar. Ecol. Prog. Ser. **188:** 21–32.

BRUYANT, F., AND OTHERS. 2001. An axenic cyclostat of Prochlorococcus PCC 9511 with a simulator of natural light regimes. J. Appl. Phycol. **13:** 135–142.

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

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

CARPENTER, E. J., AND J. CHANG. 1988. Species-specific phytoplankton growth rates via diel DNA synthesis cycles. I. concept and methods. Mar. Ecol. Prog. Ser. **43:** 105–111.

CHISHOLM, S. W. 1992. Phytoplankton size, p. 213–247. *In* P. G. Falkowski and A. D. Woodhead [eds.], Primary productivity and biogeochemical cycles in the sea. Plenum Press.

———, AND J. C. COSTELLO. 1980. Influence of environmental factors and population composition on the timing of cell division in *Thalassiosira fluviatilis* (Bacillariophyceae) grown on light-dark cycles. J. Phycology **16:** 375–383.

CHUNG, S. P., W. D. GARDNER, M. R. LANDRY, M. J. RICHARDSON, AND I. D. WALSH. 1998. Beam attenuation by microorganisms and detrital particles in the equatorial Pacific. J. Geophys. Res. **103:** 12669–12681.

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

———, AND OTHERS. 1999. Variability in particle attenuation and chlorophyll fluorescence in the Tropical Pacific: Scales, patterns, and biogeochemical implications. J. Geophys. Res. **104:** 3401–3422.

———, AND OTHERS. 2000. Continuous monitoring of surface optical properties across a geostrophic front: Biogeochemical inferences. Limnol. Oceanogr. **45:** 309–321.

CULLEN, J. J., AND M. R. LEWIS. 1995. Biological processes and optical measurements near the sea surface: Some issues relevant to remote sensing. J. Geophys. Res. **100:** 13,255–13,266.

———, ———, C. O. DAVIS, AND R. T. BARBER. 1992. Photosynthetic characteristics and estimated growth rates indicate grazing is the proximate control of primary production in the Equatorial Pacific. J. Geophys. Res. **97:** 639–654.

DEMMIG-ADAMS, B., AND W. W. ADAMS III. 1993. The xantophyll cycle, p. 206–246. *In* A. Young and G. Britton [eds.], Carotenoids in photosynthesis. Chapman & Hall.

DICKEY, T., AND OTHERS. 1998. Initial results from the Bermuda Testbed Mooring program. Deep-Sea Res. I **45:** 771–794.

DUNLAP, W. C., AND J. M. SHICK. 1998. Ultraviolet radiation-absorbing mycosporine-like amino acids in coral reef organisms: A biochemical and environmental perspective. J. Phycol. **34:** 418–430.

DURAND, M. D., R. E. GREEN, H. M. SOSIK, AND R. J. OLSON. 2000. Diel variations in optical properties of *Micromonas pusilla*, a prasinophyte. Ocean Opt. **XV:** 1–11.

———, AND R. J. OLSON. 1996. Contributions of phytoplankton light scattering and cell concentration changes to diel variations in beam attenuation in the Equatorial Pacific from flow cytometric measurements of pico-, ultra- and nanoplankton. Deep-Sea Res. II **43:** 891–906.

———, AND ———. 1998. Diel patterns in optical properties of the chlorophyte *Nannochloris* sp.: Relating individual-cell to bulk measurements. Limnol. Oceanogr. **43:** 1107–1118.

FALKOWSKI, P. G., AND J. A. RAVEN. 1997. Aquatic photosynthesis. Blackwell Science.

GARCZAREK, L., AND OTHERS. 2001. Differential expression of antenna and core genes in *Prochlorococcus* PCC 9511 (Oxyphotobacteria) grown under a modulated light-dark cycle. Environ. Microbiol. **3:** 168–175.

GEIDER, R. J. 1987. Light and temperature dependence of the carbon to chlorophyll *a* ratio in microalgae and cyanobacteria: Implications for physiology and growth of phytoplankton. New Phycol. **106:** 1–34.

GIBB, S. W., D. G. CUMMINGS, X. IRIGOIEN, R. G. BARLOW, R.

FAUZI, AND C. MANTOURA. 2001. Phytoplankton pigment chemotaxonomy of the northeastern Atlantic. Deep-Sea Res. II **48:** 795–823.

GOERICKE, R. 1998. Response of phytoplankton community structure and taxon-specific growth rates to seasonally varying physical forcing in the Sargasso Sea off Bermuda. Limnol. Oceanogr. **43:** 921–935.

———, AND N. A. WELSCHMEYER. 1993*a*. The carotenoid-labeling method: Measuring specific rates of carotenoid synthesis in natural phytoplankton communities. Mar. Ecol. Prog. Ser. **98:** 157–171.

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

HOLTZENDORFF, J., AND OTHERS. 2001. Diel expression of cell cycle-related genes in synchronized cultures of *Prochlorococcus* sp. strain PCC 9511. J. Bacteriol. **183:** 915–920.

HOOKER, S., AND OTHERS. 2001. The first SeaWiFS HPLC analysis Round-Robin experiment (SeaHARRE-1). NASA Technical Memorandum 2000-206892 **14:** 1–42.

JACQUET, S., F. PARTENSKY, D. MARIE, R. CASOTTI, AND D. VAULOT. 2001. Cell cycle regulation by light in Prochlorococcus strains. Appl. Environ. Microbiol. **67:** 782–790.

KINKADE, C. S., J. MARRA, T. D. DICKEY, C. LANGDON, D. E. SIGURDSON, AND R. WELLER. 1999. Diel bio-optical variability observed from moored sensors in the Arabian Sea. Deep-Sea Res. II **46:** 1813–1831.

LI, W. K. W. 1993. Estimation of primary production by flow cytometry. ICES Mar. Sci. Symp. **197:** 79–91.

LOISEL, H., AND A. MOREL. 1998. Light scattering and chlorophyll concentration in case 1 waters: A reexamination. Limnol. Oceanogr. **43:** 847–858.

MARIE, D., N. SIMON, L. GUILLOU, F. PARTENSKY, AND D. VAULOT. 2000. DNA, RNA analysis of phytoplankton by flow cytometry., p. 11.12.11–11.12.14. *In* J. W. Sons [ed.], Current Protocols in Cytometry. Int. Soc. Anal. Cytol.

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

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

———, AND A. BRICAUD. 1981. Theoretical results concerning light absorption in a discrete medium, and application to specific absorption of phytoplankton. Deep-Sea Res. **28:** 1375–1393.

———, AND ———. 1986. Inherent optical properties of algal cells, including picoplankton. Theoretical and experimental results. Can. J. Fish. Aquat. Sci. **214:** 551–559.

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

PREZELIN, B. B. 1992. Diel periodicity in phytoplankton productivity. Hydrobiologia **238:** 1–35.

RIPPKA, R., AND OTHERS. 2000. *Prochlorococcus marinus* Chisholm et al. 1992 subsp. pastoris subsp. nov. strain PCC 9511, the first axenic chlorophyll a2/b2-containing cyanobacterium (Oxyphotobacteria). Int. J. Syst. Bacteriol. **50:** 1833–1847.

SIEGEL, D. A., T. D. DICKEY, L. WASHBURN, M. K. HAMILTON, AND B. G. MITCHELL. 1989. Optical determination of particulate abundance and production variations in the oligotrophic ocean. Deep-Sea Res. **36:** 211–222.

SOURNIA, A. 1974. Circadian periodicities in natural population of marine phytoplankton. Adv. Mar. Biol. **12:** 325–389.

STRAMSKA, M., AND T. D. DICKEY. 1992. Variability of bio-optical properties of the upper ocean associated with diel cycles in phytoplankton population. J. Geophys. Res. **97:** 17,873–17,878.

STRAMSKI, D. 1999. Refractive index of planktonic cells as a measure of cellular carbon and chlorophyll *a* content. Deep-Sea Res. I **46:** 335–351.

———, AND R. A. REYNOLDS. 1993. Diel variations in the optical properties of a marine diatom. Limnol. Oceanogr. **38:** 1347–1364.

———, A. SCIANDRA, AND H. CLAUSTRE. 2002. Effect of temperature, nitrogen, and light limitation on the optical properties of the marine diatom *Thalassiosira pseudonana.* Limnol. Oceanogr. **47:** 392–403.

———, A. SHALAPYONOK, AND R. A. REYNOLDS. 1995. Optical characterization of the oceanic unicellular cyanobacterium *Synechococcus* grown under a day-night cycle in natural irradiance. J. Geophys. Res. **100:** 13,295–13,307.

VAULOT, D., AND D. MARIE. 1999. Diel variability of photosynthetic picoplankton in the equatorial Pacific. J. Geophys. Res. **104:** 3297–3310.

———, ———, R. J. OLSON, AND S. W. CHISHOLM. 1995. Growth of *Prochlorococcus,* a photosynthetic prokaryote, in the Equatorial Pacific Ocean. Science **268:** 1480–1482.

VIDUSSI, F., H. CLAUSTRE, J. BUSTILLOS-GUZMAN, C. CAILLIAU, AND J. C. MARTY. 1996. Determination of chlorophylls and carotenoids of marine phytoplankton: Separation of chlorophyll *a* from divinyl-chlorophyll *a* and zeaxanthin from lutein. J. Plankton Res. **18:** 2377–2382.

WALSH, I. D., S. P. CHUNG, M. J. RICHARDSON, AND W. D. GARDNER. 1995. The diel cycle in the integrated particle load in the Equatorial Pacific: A comparison with primary production. Deep-Sea Res. **42:** 465–477.

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