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Bio-optical properties of the marine diazotrophic cyanobacteria *Trichodesmium* spp. I. Absorption and photosynthetic action spectra

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Abstract

The optical absorption, fluorescence excitation and emission, and photosynthetic action spectra were measured in vivo on intact colonies of *Trichodesmium* from the Caribbean Sea. The optical cross-sections were dominated by ultraviolet-A (UVA) absorption, which was a consequence of massive accumulations of mycosporinelike amino acids. The visible region of the spectrum was decomposed into several bands, among which chlorophyll *a* (Chl *a*), carotenoids, and individual phycobilipigments could be discerned. There was a clear diel periodicity in the ratio of the optical absorption cross-sections of phycourobilin (PUB) to phycoerythrobilin (PEB), increasing from around 1.7 at night to 2.1 at midmorning. The diel cycle in PUB/PEB is consistent with a reversible interconversion of the two pigments. The ratio of PUB/PEB was inversely correlated with the transfer of excitation energy to photosystem II (PSII). Light absorbed by PUB was not transferred to PSII with a high efficiency, but rather, a significant fraction was reemitted at 565 nm as fluorescence. These observations suggest that the PUBs and PEBs in *Trichodesmium* act as a dynamic biophysical energy valve that modify the rate of excitation energy delivered to PSII in response to changes in ambient light regime. The low-temperature (77 K) fluorescence emission spectra reveal an extremely weak 685-nm emission signal in relation to that at 730 nm. Based on a simple model, these data suggest that the ratio of PSI/PSII reaction centers in *Trichodesmium* is about 24 : 1. Such an extraordinary bias against PSII may help minimize damage to nitrogenase from O_2 production in PSII, but it also reduces the photosynthesis-enhanced growth and makes *Trichodesmium* virtually undetectable by chlorophyll fluorescence. The unique bio-optical properties of *Trichodesmium* can be used to develop algorithms to study its temporal and spatial distributions from remotely sensed information.

Trichodesmium spp. is a genus of nonheterocystous, marine, diazotrophic (i.e., nitrogen fixing) cyanobacteria that form extensive blooms in oligotrophic tropical and subtropical seas. These organisms possess phycobilipigments with absorption peaks centered at 495, 545, and 565 nm (Fujita and Shimura 1974). The latter two peaks correspond to PEB, while the first corresponds to PUB, which is a modified form of PEB (Sidler 1994). The combination of all three optical absorption peaks is rare among other marine cyanobacteria and is nonexistent in eucaryotes (Ong et al. 1984; MacColl and Guard-Frier 1987). In addition, *Trichodesmium* contains intracellular gas vacuoles that provide a large refractive index contrast between the colonies and the water and consequently, a high backscatter cross-section (Borstad et al. 1992). The combination of the phycobilipigments, the large backscatter cross-section, and the inherent buoyancy resulting from the intracellular gas vacuoles makes blooms of *Trichodesmium* readily observable in satellite imagery (Subramaniam and Carpenter 1994).

The absorption characteristics and pigment composition of *Trichodesmium* have been investigated numerous times (e.g., Shibata 1969; Moreth and Yentsch 1970; Shimura and Fujita 1975; Ohki et al. 1986; Haxo et al. 1987; Lewis et al. 1988; Borstad et al. 1992; Prufert-Bebout et al. 1993). A careful reading of the literature suggests inconsistencies or variations in absorption spectra that are not readily reconciled. Shibata (1969) was one of the first to describe the in vivo absorption spectra of a cyanobacterium (subsequently identified as *Trichodesmium* spp.; Haxo pers. comm.). In addition to the Chl *a* peaks in the red and Soret region and a shoulder around 625 nm, he reported peaks at 498 and 545 nm that corresponded to phycoerythrin. A strong UV absorptivity at

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332 and 362 nm was also noted and attributed to water-soluble pigments. Moreth and Yentsch (1970) developed a method for extraction of phycoerythrin from *Trichodesmium* spp. and showed that it had a single fluorescence emission peak around 565 nm when excited at 490 nm. Fujita and Shimura (1974) reported peaks in the visible portion of the spectrum at 500, 547, and 567 nm. Shimura and Fujita (1975) showed that these pigments were active in trapping light energy for photosynthesis. They noted, however, that the 500/550-nm absorption ratio was highly variable and suggested that the phycoerythrin in *Trichodesmium* was similar to *Rhodophycean* (red algal) phycoerythrin, which was subsequently found to differ from ''classical'' (i.e., lacustrine cyanobacterial) phycoerythrin by the desaturation of a single bond in the pyrrole of ring A (Sidler 1994). This modified, short-wavelength–absorbing form of phycoerythrin was named phycourobilin or CU phycoerythrin (from *C*yanobacterial phyco*U*robilin) by MacColl and Guard-Friar (1987). Other reports suggested that the amplitude of the absorption peak at 550 nm relative to that at 495 nm was higher in cultures of *Trichodesmium* isolated from the Sea of Japan (Ohki et al. 1986) but lower in natural populations (Lewis et al. 1988; Borstad et al. 1992). Because most researchers used methods similar to the opal glass technique of Shibata (1958), it is more likely that the variability in the reported absorption characteristics of the organisms is of biological origin rather than resulting from methodological differences.

In this paper, we examine the source of variability in the absorption and fluorescence characteristics of *Trichodesmium* spp. and their relationship to photosynthetic processes. While the transfer of energy from the terminal antennae (phycobilisomes) to the reaction centers (PSI and PSII) has been demonstrated in laboratory cultures, we present results that show evidence of this transfer being variable in the natural environment. We use measurements of the oxygen evolution action spectrum to show variations in the efficiencies of this energy transfer corresponding to changes in ambient light conditions. In the following paper (Subramaniam et al. in press), we examine the backscattering properties of the cells and, in conjunction with the absorption properties discussed in this paper, develop a reflectance optical model for the remote sensing of *Trichodesmium* specifically.

Materials and methods

Trichodesmium spp. colonies were collected in September 1993 and January 1995 in the Caribbean Sea (25°N, 76°W) by towing 1-m-diameter 202-μm mesh nets at ca. 1 knot for 15 min at ca. 15-m depth. Intact colonies were isolated with plastic transfer loops and rinsed with filtered seawater. Samples (typically a mixture of *T. thiebautii* and *T. erythraeum*) contained about 95–99% *Trichodesmium* colonies by volume.

For measurements of in vivo absorption spectra, approximately 200 colonies were transferred to GF/F glass-fiber filters by pouring the filtered seawater containing the colonies into a 25-mm-diameter glass-filtering tower and applying gentle vacuum. The distribution of colonies on the filter was random, albeit patchy. Absorption spectra of trichomes were measured by vortexing colonies before filtering. The vortexing action disaggregated colonies into individual trichomes without breaking the cells. *Synechococcus* (Woods Hole clone 7803), grown as semicontinuous batch culture in artificial seawater medium, enriched with f/2 nutrients at 18°C in continuous light (100 μmol quanta m^{-2} s^{-1}) was used for comparison in the fluorescence measurements.

Chlorophyll-specific absorption due to *Trichodesmium* was measured using the ''quantitative filter technique'' (Kiefer and SooHoo 1982; Mitchell 1990). The filters were frozen at −4°C and analyzed in the laboratory on a dual-beam SLM-Aminco (DW2C) spectrophotometer. This procedure did not result in cell breakage, as there was no pink coloration on the filter paper due to phycoerythrin leakage. Filters, soaked in filtered seawater and frozen at the same time as the samples, were used as blanks. Sample and blank filters were mounted side by side on a plastic slide holder (an empty 35-mm photographic slide frame) that was inserted against the diffusing plate of the beam scrambler in the spectrophotometer. This arrangement, along with the beam scrambler, enabled the photomultiplier tube detector to collect all the transmitted or forward-scattered light (i.e., light diffused by the filter and the quartz diffusing plate). Samples were scanned from 375 to 750 nm using a 3-nm slit width. The blank-filter baseline was subtracted from all measurements. Two or more readings were made on each sample by repositioning the filter (turning by 90°), and the average was used for subsequent calculations.

Chl *a* was extracted in cold methanol and quantified from absorption spectra using the equation of Porra et al. (1989). Chlorophyll-specific absorption cross-sections ($a{\cdot}\lambda$) were calculated as

$$a^*(\lambda) = 2.3 \cdot \frac{\text{Optical density}(\lambda) \cdot \text{Clearance area of filter}}{\beta \cdot \text{chlorophyll mass}} \times \left(\frac{\text{m}^2}{\text{mgChla}}\right).$$

β is the pathlength amplification factor as defined by Butler (1962). The absorption spectra were quantitatively analyzed using Peakfit® (Jandel Scientific).

To investigate the diel variability in pigment concentrations, *Trichodesmium* colonies were collected on 0.6-μm pore size Poretex filters and soaked in 1.5-ml Eppendorf tubes with 400 μl phosphate-buffered saline (PBS: 0.05 M, $NaH_2PO_4 \cdot H_2O$-$NaH_2PO_4 \cdot 2H_2O$, 7 pH). The tubes were flash frozen in liquid nitrogen, held at −4°C for 24 h, and thawed. The samples were subjected to three freeze/thaw cycles, ground with glass beads in a grinder, and centrifuged at 11,000 × *g* for 10 min. Seven hundred microliters of PBS was added to 300 μl of the supernatant, and the absorption spectrum was measured between 400 and 750 nm. The concentration of phycoerythrin was estimated as described by Wyman (1992). Chl *a* in the pellet was extracted in 1 ml MeOH at −4°C for 24 h and quantified (Porra et al. 1989).

For identification of UV-absorbing compounds, colonies were filtered onto GF/F filters, frozen in liquid nitrogen, and stored at −4°C. UV pigments were analyzed by high-pressure liquid chromatography (HPLC) as described by Dunlap

and Chalker (1986) and Karentz et al. (1991). The absorption spectrum of water-soluble compounds exuded by *Trichodesmium* was measured on cell-free filtrates; filtered seawater served as a blank for these measurements.

Fluorescence emission and excitation spectra were determined on samples flash frozen in quartz nuclear magnetic resonance spectroscopy tubes in the dark and stored at 77 K. Quantum-corrected spectra were obtained with an SLM Aminco 8000 spectrofluorometer equipped with a quartz low-temperature cuvette.

Photosynthetic oxygen evolution action spectra of *Trichodesmium* were measured at sea using a platinum-silver oxygen rate electrode and a technique similar to that described by Haxo and Blinks (1950). Light from a Xenon arc lamp with a stabilized power supply was delivered to a monochromator by a liquid light guide. A second light guide was used to deliver the monochromatic light from the monochromator to the platinum cathode. The monochromator was scanned from 300 to 750 nm at 0.5 nm s^{-1}. The entrance and exit slits were optimized to provide a 21-nm spectral resolution, and the light intensity was optimized to obtain a photosynthetic response that was linearly proportional to the photon flux density.

A drop of filtered seawater was placed on the cathode, and a colony of *Trichodesmium* was placed in this drop. Both *T. erythraeum* and *T. thiebautii* colonies were specifically identified and used in these measurements. A cellulose membrane held the colony on the cathode, and filtered seawater flowed continuously (via an airlift pump) over the sample, ensuring a constant supply of inorganic carbon. The media pumping system was enclosed in a water jacket that was connected to the ship's flow-through seawater system to maintain the media at ambient surface seawater temperatures. The current generated in the light was directly and linearly proportional to O_2 evolution. Thus, as the monochromator scanned the spectrum, wavelengths used for photosynthetic O_2 evolution produced a current that traced the O_2 evolution photosynthetic action spectrum.

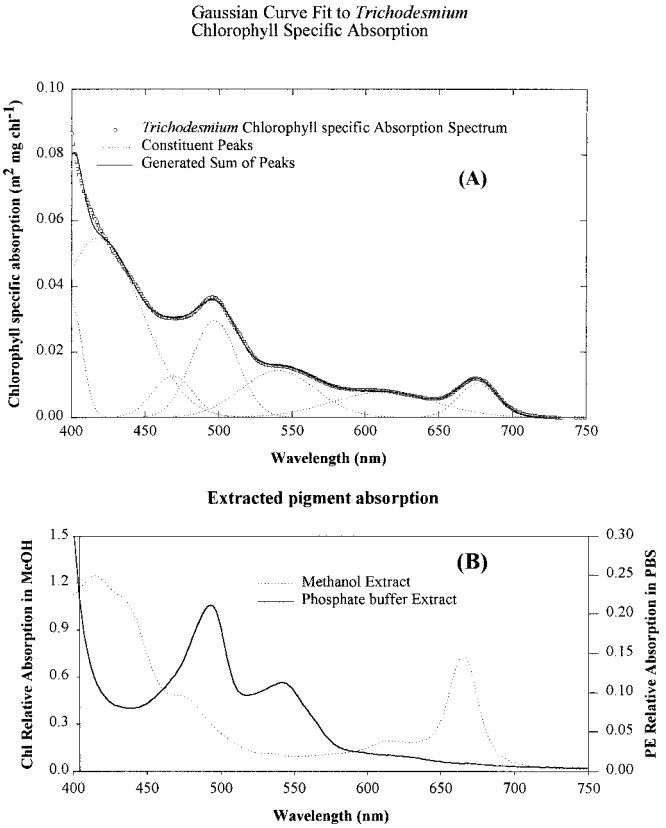

Fig. 1. (A) Gaussian curve fit to chlorophyll-specific *Trichodesmium* absorption spectrum. The constituent peaks are listed in Table 1. (B) The absorption spectra for pigments extracted in methanol and phosphate buffer. Absorption peaks due to the Soret and red bands of Chl *a;* the absorption of carotenoids is seen in the methanol extract. Absorption peaks are due to UV-absorbing compounds, and the phycobilins are seen in the phosphate-buffer extract.

Results

Absorption—The spectrally averaged chlorophyll-specific optical cross-section (i.e., for ''white'' light, 400–750 nm) *a**(bar), calculated from measurements of intact *Trichodesmium* colonies, was 0.0187 m^2 (mg Chl *a*)$^{-1}$. The optical cross-section increased approximately four times, to ca. 0.0650 m^2 (mg Chl *a*)$^{-1}$ upon disaggregation of the colonies. The increase in *a**(bar) upon disaggregation of the colonies reflects the effect of self-shading induced by the structure of the colony on the light-absorption properties of *Trichodesmium* in situ. The aggregation results in flattening of the absorption peaks. The aggregated state represents the ''true'' optical absorption cross-section that would be retrieved from remotely sensed chlorophyll-specific algorithms.

The absorption spectra of colonies of *Trichodesmium* revealed a series of peaks in the visible wavelengths (Fig. 1). Using a technique similar to that of Hoepffner and Sathyendranath (1991), the chlorophyll-specific absorption spectra were decomposed to determine the relative contribution of individual pigments to the total spectra (Fig. 1; Table 1). The spectra were decomposed in the wave number domain, assuming Gaussian distributions. There was high variability in both the amplitude and location of peak centers in the blue region (peak centered at 400 nm—column 1, Table 1) of the absorption spectrum, reflecting the variability in the presence of UV-absorbing molecules. The absorption peak at 565 nm attributed to a form of phycoerythrin (Fujita and Shimura 1974; Ohki et al. 1986; Haxo et al. 1987) was not observed in intact cells or phosphate-buffer extracts. However, the 77-K fluorescence excitation spectra for emission at 640 nm (Fig. 4A) revealed a pronounced peak around 565 nm and a small shoulder at 575 nm. The absorption spectra of methanol extracts (Fig. 1B) revealed an additional peak at ca. 475 nm due to carotenoids (Hogetsu and Watanabe 1975). The high absorbance in the in vivo absorption as well as phosphate-buffer extract below 425 nm was due to a variety of UV-absorbing pigments (*see below*).

The diel variability in pigments in *Trichodesmium* was followed in three 24-h time-course experiments, with samples taken every 3 h (i.e., a total of 24 data points). The average chlorophyll concentration, expressed per colony, was 55.5

Table 1. Peak center and area under the curve of Gaussian curve fits to the chlorophyll-specific absorption spectra of intact *Trichodesmium* colonies. Area refers to the percentage area under each Gaussian fit and can be considered indicative of the relative concentration of that pigment. The blue area refers to the Gaussian fit centered at 400 nm.

	Blue	Soret		Carotenoids		PUB		PEB		PEC		Phycocyanin		Chlorophyll	
Sample	area	Center	Area	Center	Area	Center	Area	Center	Area	Center	Area	Center	Area	Center	Area
tra1	13.8	424	50.4	466	3.6	494	18.7	546	7.4			620	2.8	675	3.3
tra2	4.7	418	43.2	469	6.4	497	17.0	541	13.5			613	10.1	676	5.1
trb1	11.4	433	22.9	463	7.1	497	23.3	544	15.2	582	1.5	629	12.6	677	6.0
trb2	9.2	431	24.8	461	5.8	496	24.6	543	13.1	571	2.8	630	13.5	677	6.2
920906	2.7	417	26.4	455	8.2	500	31.1	547	4.0	573	5.4	619	12.4	678	9.9
920907	8.0	428	34.3	470	3.8	499	16.6	545	12.4			617	17.3	679	7.6
trc1	12.5	433	29.9			495	18.1	546	13.8	586	2.2	623	13.7	675	9.7
trc2	5.7	424	34.8			495	21.4	550	8.5	574	1.4	616	17.9	675	10.3
tru1	3.6	420	36.1	459	2.2	494	23.7	543	9.7			616	15.8	676	8.9
tru2	5.2	421	32.8	459	5.1	495	24.8	546	9.4			621	12.2	677	10.4
920907	7.7	429	30.7	462	2.2	495	24.3	546	7.7	583	5.3	625	8.0	676	14.1
Mean		425	33.3	463	4.9	496	22.1	545	10.4	578	3.1	621	12.4	676	8.3
SD		5.6	7.7	4.6	2.0	1.9	4.1	2.3	3.3	5.7	1.6	5.2	4.1	1.2	2.9

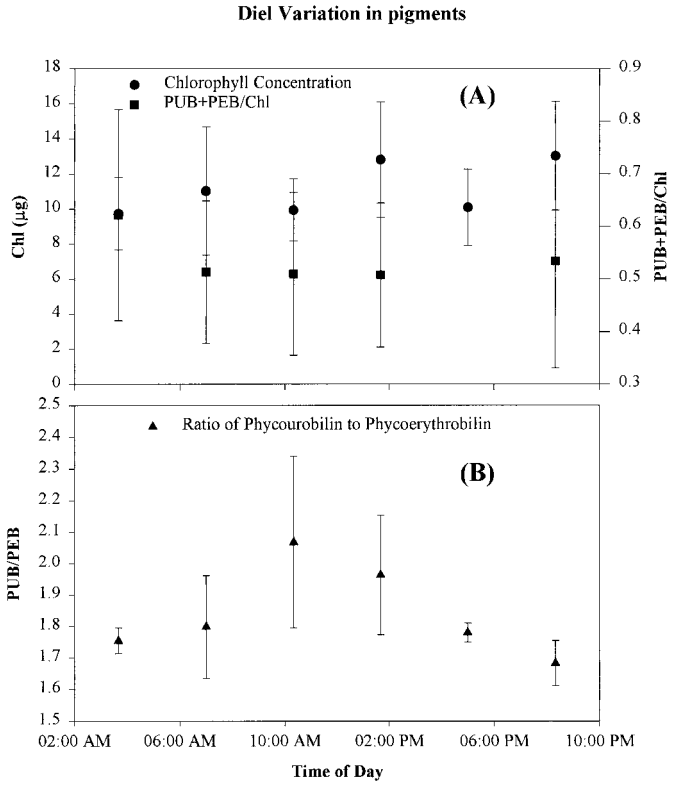

Fig. 2. Diel variation in pigments. (A) Chlorophyll concentration and chlorophyll-normalized sum of absorption peaks at 495 and 550 nm vs. time. (B) PUB/PEB variation over the same diel period.

(±6.7) ng, while phycoerythrin averaged 426 (±79.9) ng colony^{-1}. Thus, the wt:wt phycoerythrin (PE)/Chl ratio was 7.68 (±1.16). Because the samples were not collected at exactly the same time in all the diel cycles, the individual chlorophyll and phycobiliprotein measurements were grouped into six 4-h blocks and averaged. Chlorophyll biomass remained approximately constant throughout the diel period, as did the sum of PUB and PEB, estimated as the sum of peak heights at 495 and 545 nm normalized to chlorophyll (Fig. 2A). The ratio of PUB/PEB, however, showed a clear diel pattern, with a peak around noon (Fig. 2B).

UV-absorbing pigments—The UV absorption cross-section for *Trichodesmium* is among the highest known for free-living phytoplankton. The high UV absorbance is a consequence of numerous π bonds within a series of abundant small, water-soluble molecules. These so-called ''mycosporinelike amino acids'' (MAAs) were identified by HPLC. Among those identified in the chromatograms, asterina-332 (λ_{max} = 332) and shinorine (λ_{max} = 334) were the most abundant. Also found were mycosporine-glycine (λ_{max} = 310), porphyra-334 (λ_{max} = 334), and palythene (λ_{max} = 360). Two of the most abundant compounds were unidentified. There was considerable variability in the concentration of these compounds between samples, but the concentration of asterina-332 was always at least five times higher on a wt:wt basis than chlorophyll (357 ± 193 ng colony^{-1} compared to 55.5 ± 6.7 ng colony^{-1}). While some samples from 60 m

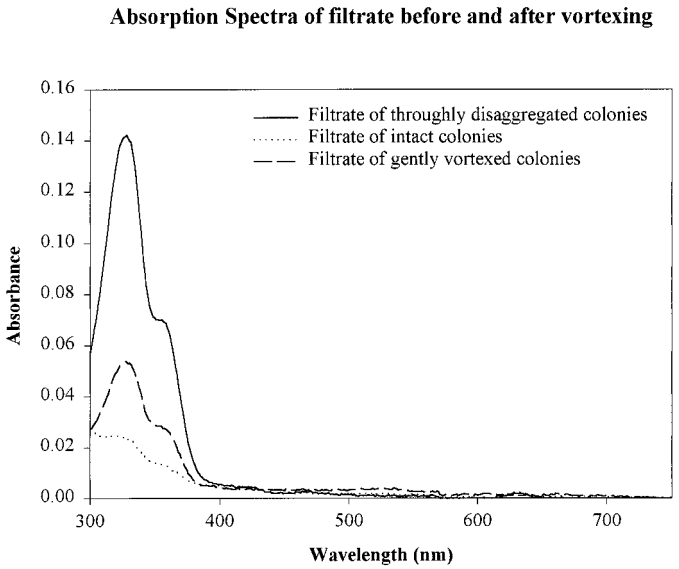

Fig. 3. Absorption spectra of filtrate before and after vortexing colonies of *Trichodesmium.* The absence of any peaks in the visible wavelengths shows that the cells did not break on vortexing, releasing water-soluble phycobilipigments. These curves suggest that a large fraction of the UV-absorbing compounds is potentially present as sheath pigments in the intracolony spaces rather than inside cells.

or deeper appeared to have lower concentrations of UV-absorbing compounds, the samples were highly variable, and there was no obvious relationship between UV absorption and such environmental factors as depth in the water column, time of day, or species. This might be partly due to loss of the UV-absorbing compounds during handling. These substances (absorption peak at 330 nm, shoulder around 365 nm in seawater—Fig. 3) are water-soluble and can be easily identified in the absorption spectrum of the filtered seawater media in which the colonies were washed prior to filtration and freezing for transport to the laboratory. The lack of any absorption in the visible (400–750 nm) attributable to water-soluble phycobilipigments indicated that there was no cell breakage. This implies that these compounds are intracellular, akin to sheath pigments commonly found in cyanobacteria. Thus, the concentration of these compounds in the colonies changed depending on the gentleness of handling. The amplitude of the absorption peak increased as the colonies were shaken and disintegrated into individual trichomes (Fig. 3).

Low-temperature fluorescence—Energy transfer from the phycobilisomes to the reaction centers in cyanobacteria can be conveniently assessed using fluorescence excitation/emission spectra. At room temperature, the fluorescence emission at 685 nm (from Chl *a* in PSII) is virtually undetectable, as is longer wavelength emission. At 77 K, photochemical processes do not effectively compete with fluorescence deexcitation pathways, and hence, fluorescence yields increase. This condition is analogous to adding DCMU (3-(3,4-dichlorophenyl)-1,1-dimethylurea) to a sample (Falkowski and Raven 1997), with the added effect of blocking photosyn-

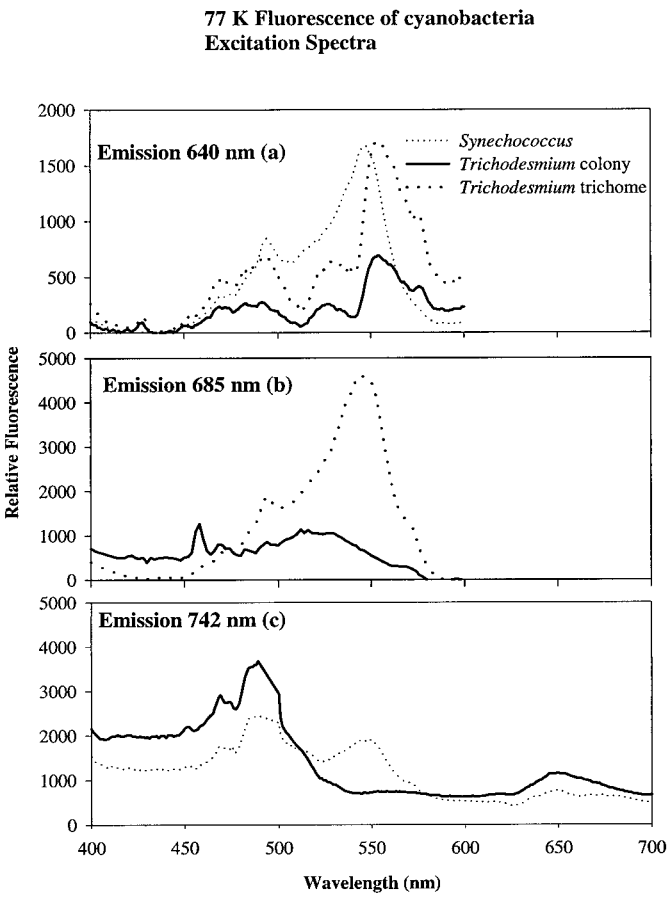

Fig. 4. 77-K fluorescence excitation spectra for *Trichodesmium* colonies, trichomes, and *Synechococcus.* (A) Emission at 640 nm due to allophycocyanin. (B) Emission at 685 nm due to PSII is shown for *Trichodesmium* (solid line) and *Synechococcus* (dotted line). (C) Emission at 742 nm due to PSI is shown for *Trichodesmium* (solid line) and *Synechococcus* (dotted line).

thetic electron transport in PSI. A scan of excitation for emission at 685 nm at 77 K in *Trichodesmium* spp. revealed no characteristic peaks, indicating a low quantum yield of fluorescence for chlorophyll in PSII (Fig. 4B). This result was surprising, in that at low temperature, PSII emission at 685 nm is usually one of the strongest signals in oxygenic photoautotrophs (Falkowski and Raven 1997). To ensure that the measurements were not an artifact, we compared the low-temperature fluorescence excitation spectrum of *Trichodesmium* to that of *Synechococcus,* where the 685-nm emission was clearly observed as a peak at 550 nm (Fig. 4B). When PUB was excited at 495 nm, the low-temperature emission spectrum of *Trichodesmium* spp. showed peaks at 560 nm due to PEB, 620 nm due to phycocyanin (PC), 640 nm due to allophycocyanin (APC), and 740 nm due to lowest singlet excited state of the special pair of Chl *a* molecules in PSI (Fig. 5). This excitation/emission series clearly demonstrates energy transfer from PUB through the longer wavelength phycobiligments, ultimately to PSI. The excitation spectra for PSI activity (emission at 742 nm) showed characteristic peaks at 495 nm for both *Synechococcus* and *Trichodesmium,* with an additional peak at 550 nm for *Syne-*

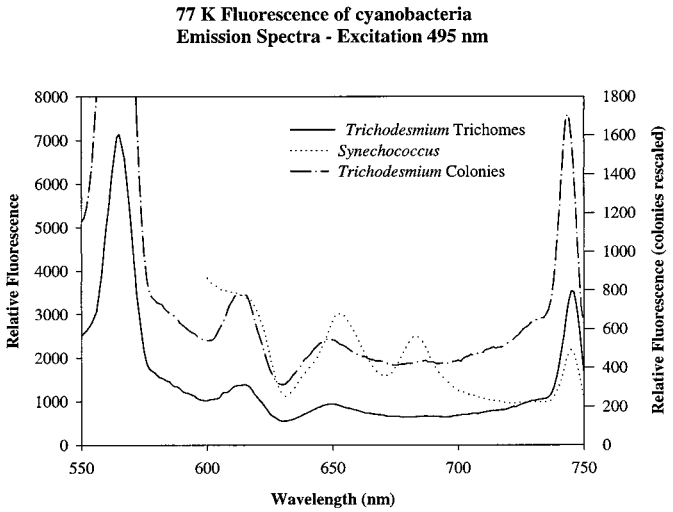

Fig. 5. 77-K fluorescence emission spectra (excitation at 495 nm) for *Trichodesmium* colonies, trichomes, and *Synechococcus.* Peaks due to PEB, PC, APC, and PSI can been seen in all three curves. *Synechococcus* has the additional peak due to PSII.

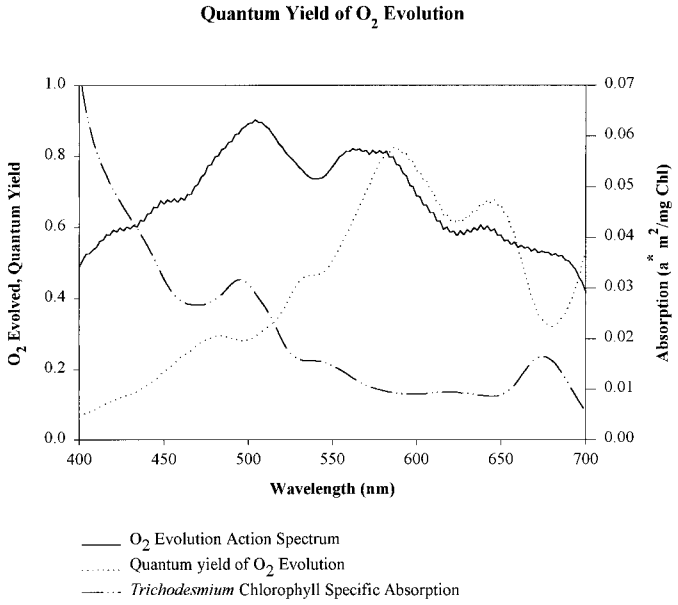

Fig. 6. Quantum yield of oxygen evolution along with the chlorophyll-specific absorption spectrum and the O_2 evolution action spectrum. Although there is oxygen evolution at 495 nm due to PUB, its quantum yield is lower than that due to PEB around 565 nm.

chococcus (Fig. 4C). In *Trichodesmium* spp., the terminal phycobilipigment, APC, was clearly energetically coupled to PEB and PUB (Fig. 4A), but the very existence of the 640-nm emission reveals imperfect energy transfer from APC to the chlorophyll proteins that serve as the core antenna for the reaction centers (Falkowski and Raven 1997).

Oxygen evolution action spectra—Action spectra for oxygen evolution reveal the extent to which specific chromophores transfer excitation energy to PSII. The initial goal of such measurements was to assess the extent to which UV absorption could promote photosynthetic processes. The quantum yield for oxygen evolution was extremely low in the UV, and it is clear that the contribution of UV-absorbing molecules to PSII is negligible. In the visible portion of the spectrum, the oxygen evolution was clearly directly coupled to phycobilisome absorption. Peaks in the action spectra were apparent at 495 and 555 nm, with a broad shoulder between 620 and 645 nm and a smaller shoulder at 440 nm (Fig. 6).

There was considerable variability in both peak heights and peak centers between samples; however, a diel pattern in the height of the peak at 495 relative to 555 nm was evident in the O_2 evolution action spectra (Fig. 7). The contribution of the 495-nm absorption to O_2 evolution action spectra was greatest just before dawn and after dusk, with a minimum around midday. The O_2 evolution action spectra for *Trichodesmium thiebautii* and *T. erythraeum* were different shapes (Fig. 8). *T. erythraeum* showed a single, broad peak around 575 nm, with little or no contribution from 495-nm absorption.

Discussion

Simple bio-optical models, especially those extended to estimate primary production, are based on chlorophyll-specific coefficients for absorption. Chlorophyll-specific coefficients have the advantage that this pigment is found in all phytoplankton, is fundamental to photosynthetic energy transfer, and is easily measurable. Normalizing to this concentration should make measurements of various parameters on different phytoplankton species comparable. However, even when the differences due to variable chlorophyll/cell

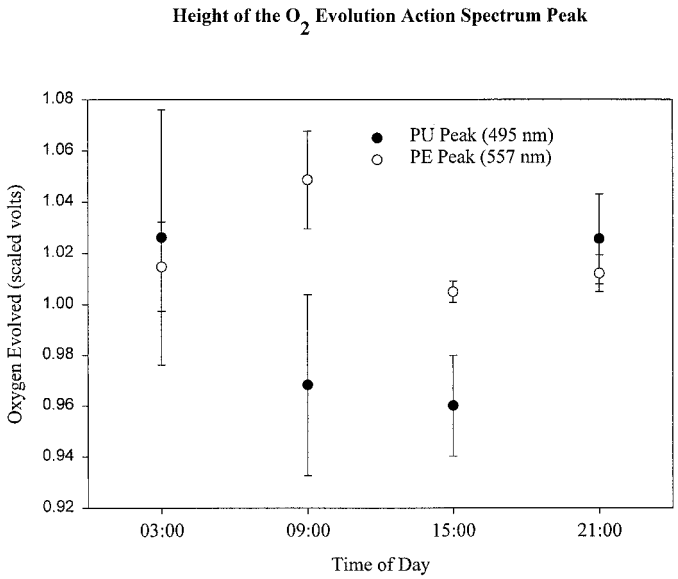

Fig. 7. Variation in height of the O_2 evolution action spectrum peaks at 495 and 555 nm over a diel period. While the PE peak is more or less constant over a diel period, there is a clear dip in the PU peak during the day, indicating that oxygen evolution is due to PU drops in high light. There is a suggestion that this is compensated for by a possible increase in oxygen evolution due to PE.

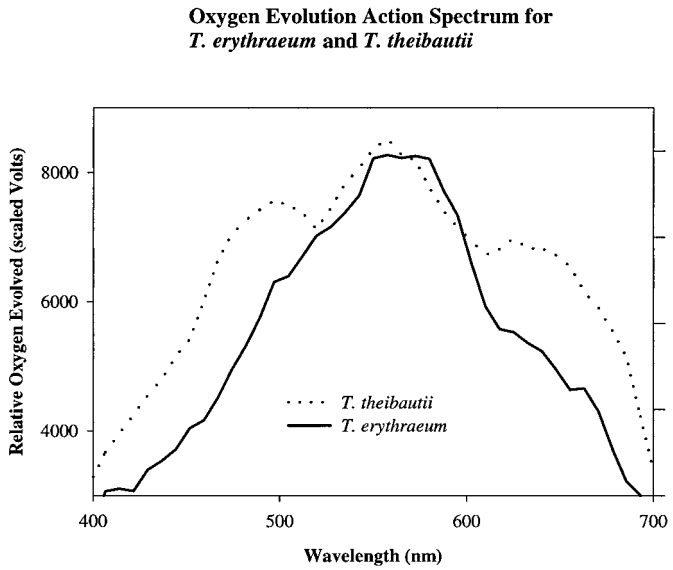

Fig. 8. Action spectra for O_2 evolution in *T. erythraeum* and *T. thiebautii.* The measurements were made on colonies from the same tow (i.e., the same time of day, depth, etc.).

are neglected, physiologically induced variability in pigment packaging and pigment plasticity make comparing chlorophyll-specific absorption of eucaryotes to cyanobacteria problematic. While chlorophyll is the major absorbing pigment in eucaryotes, directly involved in oxygen evolution, cyanobacteria have other pigments in PSII, phycobilins, in much larger concentrations than chlorophyll. These phycobilins contribute to the total absorption of light and photosynthesis by cyanobacteria. Hogetsu and Watanabe (1975) found that phycoerythrin content in *Trichodesmium* was 391% that of chlorophyll by weight (i.e., almost 4 : 1). The ratio of phycobilins to chlorophyll appears highly variable and depends on the light history, among other environmental variables. Under high light conditions, the ratio seems higher (almost 7, Subramaniam 1995), and this may explain why the chlorophyll-specific absorption at 675 nm is much higher for *Trichodesmium* than other eucaryotes (0.05 for *Trichodesmium* trichomes compared to ~0.02 for typical phytoplankton, respectively). Agusti and Phlips (1992) have measured even higher chlorophyll-specific absorption (a^*_{675} up to 0.12 m^2 [mg Chl a]$^{-1}$) for other cyanobacteria.

Shibata (1958) developed a technique of placing an opal glass diffuser behind a cuvette to measure absolute and relative values of transmittance and absorption of optically inhomogeneous biological material. By diffusing light, the opal glass method reduced the effect of inhomogeneous scattering by particles within the light path. The opal glass technique is extremely effective for optically thin suspensions of particles, but it is not directly applicable to measurements of natural populations of phytoplankton, as the concentration of cells in seawater is so low that accurate absorption measurements cannot be obtained in short pathlengths. To overcome this problem, Yentsch (1962) introduced the method of concentrating particles on a glass-fiber filter to measure their absorption. The multiple scattering by the glass-fiber filters was so large that it overwhelmed the effect of scattering by particles. However, there was a problem due to increased pathlength for particles caught in the interstices of the glass fibers. Kiefer and SooHoo (1982) proposed a pathlength amplification factor known as the β factor to correct for this. Mitchell (1990), using a regression analysis of his entire data set, derived an empirical relationship of $OD_s(\lambda) = 0.392OD_f(\lambda) + 0.655[OD_f(\lambda)]^2$. In this formulation, the correction is sensitive to the coefficients of the expansion series. Morel et al. (1993) and Moore et al. (1995) showed that β for cyanobacteria and prochlorophytes may be different from that proposed by Mitchell. Moore et al. suggested that this could be at least partly because picoplankton and prochlorophytes, being smaller than diatoms and dinoflagellates, were more likely to be caught inside the glass-fiber filter rather than forming a layer on top of it. We used the β factor from Moore et al. (1995), ($OD_s(\lambda) = 0.304OD_f(\lambda) + 0.450[OD_f(\lambda)]^2$), to derive chlorophyll-specific absorption spectra for *Synechococcus.* However, because *Trichodesmium* trichomes and colonies are large and do form a layer on top of the glass-fiber filter, no β correction was used for *Trichodesmium.*

Duysens (1956) described a so-called ''package effect'' as an optical flattening of the absorption spectrum due to heterogeneity in the distribution of algal pigments within a cell. In addition to this classical ''package effect,'' colony-forming phytoplankton can modify light absorption by aggregation. This self-shading of cells within a colony is referred to here as a ''secondary'' packaging effect. Self-shading is an important factor in modifying the absorption properties of *Trichodesmium* in situ. *Trichodesmium* spp. are usually found in colonies consisting of, on the average, 200 trichomes colony^{-1}, each trichome being made up of about 100 cells. Self-shading consequently reduces ''true'' absorption and a^*_p. From the differences in the spectrally averaged chlorophyll-specific absorption spectra for trichomes and colonies, the secondary packaging effect or self-shading was calculated to decrease the chlorophyll-specific absorption by a factor of almost four [a^*(bar) = 0.065 vs. 0.018 m^2(mg Chl a)$^{-1}$, respectively].

The magnitude of the secondary packaging effect is controlled by the size and shape of the colonies. *Trichodesmium* colonies are typically cylindrical (tuft shaped) or radial (puff shaped) in form (*see* figure 1 in Borstad et al. 1992). Cylindrical colonies have a higher proportion of self-shaded cells than radial colonies. The absorption measurements presented here were made for a mixture of cylindrical and radial colonies. Therefore, to the extent that a single, average chlorophyll-specific optical absorption cross-section is accepted for remote-sensing purposes, our results suggest that the chlorophyll biomass of *Trichodesmium* will be underestimated by approximately fourfold as a consequence of self-shading within the colonies. This underestimate could be easily corrected if independently one could determine that the chlorophyll within a pixel was primarily *Trichodesmium,* and the appropriate chlorophyll-specific optical cross-section was applied.

Fluorescence—Low-temperature fluorescence spectra of samples obtained in the evening revealed that light absorbed at 495 nm by PUB is transferred to PSI via an energy cas-

cade within the phycobilisomes. This energy transfer is directed via resonance transfer from lower to higher wavelengths and includes PEB, which absorbs at 555 nm; phycocyanin, which absorbs at 620 nm; and allophycocyanin, which absorbs at 640 nm. A small fraction of the energy is then directed to PSII and a larger fraction to PSI reaction centers through the terminal emitters of the phycobilisomes. The excitation spectra for emission at 645 nm associated with allophycocyanin in the periphery of PSII showed the peaks associated with PUB and PEB clearly. These results strongly suggest that the number of functional PSII reaction centers in *Trichodesmium* colonies is small relative to the absorbing chromophores. These results also suggest that these organisms have an extremely low PSII : PSI ratio (*see below*).

Oxygen evolution action spectra—Shimura and Fujita (1975) and others (reviewed by MacColl and Guard-Friar 1987) have advanced the teleological argument that marine cyanobacteria have PUB and PEB because they are usually found at great depths, where most of the available light is blue and overlaps the absorption bands of these chromophores. The pigmentation of *Trichodesmium* presents an apparent paradox. Although *Trichodesmium* spp. can vertically migrate (Villareal and Carpenter 1990; Romans et al. 1994), they are usually found in the upper 15 m of the water column, often with a population maximum at the surface. *Trichodesmium* contains a higher concentration of PUB than PEB (i.e., the cells absorb shorter wavelengths of light), and the ratio of PUB to PEB increases at midday under high light conditions (Fig. 2B).

An alternative explanation for the role of PUB and PEB was advanced by Wyman et al. (1985), who suggested that the pigment-protein complexes might serve as a nitrogen reserve. *Trichodesmium* exhibits a very strong diel signal in its N_2 fixation (Capone et al. 1990), and if the phycobiliproteins were indeed a nitrogen reserve, there should be a strong diel signal in their concentration as well. The sum total of PUB and PEB does not vary over the diel period, however (Fig. 2A), and hence, it would appear unlikely that either pigment protein complex serves as a significant nitrogen storage product in *Trichodesmium.*

We propose a different model that involves an interconversion between PUB and PEB (Fig. 9). When *Trichodesmium* is exposed to high light, the overreduction of PSII phenomenologically leads to a decrease in PEB and hence, the effective PSII cross-section. The decrease in PEB is stoichiometrically coupled to an increase in PUB. While light absorbed by PUB at 495 nm can contribute to O_2 evolution, the quantum yield is low. A large fraction is emitted as fluorescence at 565 nm (as noted by Wyman et al. 1985), indicating a reduction in energy transferred from PUB to PSII reaction centers. O_2 evolution driven by PEB absorption around 550 nm remains largely unaffected, indicating that energy absorbed by PEB is efficiently transferred to PSII. Thus, even as the absorption by PUB increased compared to that by PEB (Fig. 2B), the oxygen evolution action spectra exhibited the opposite behavior (i.e., the height of the 495-nm peak decreased compared to that at 555 nm; Fig. 7). Under low light conditions, however, such as might be found

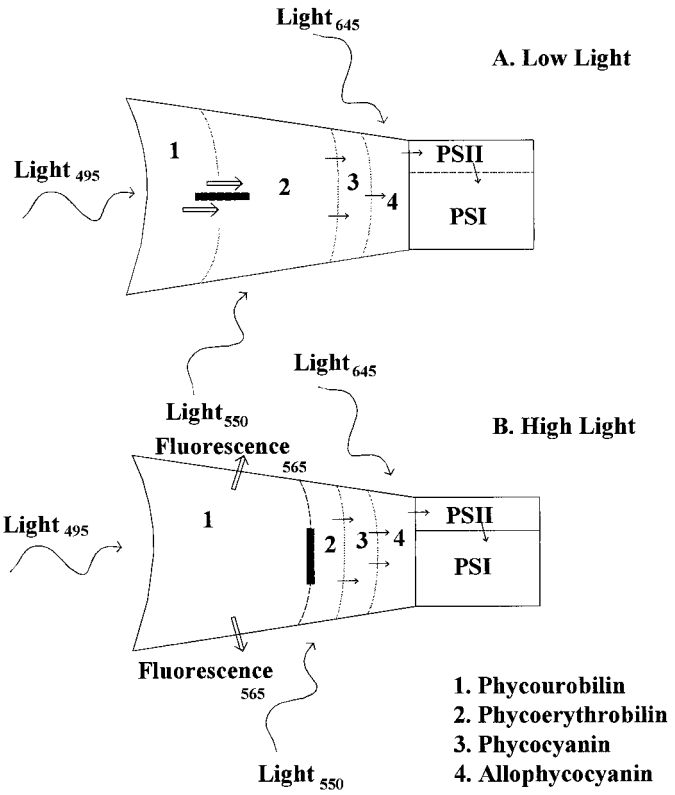

Fig. 9. A model for photosynthetic energy transfer from the phycobilisomes to the photosystems in *Trichodesmium.* (A) Under low light conditions, the biophysical energy valve is open, and energy absorbed by PUB at 495 nm is transferred to PSII through PEB, phycocyanin, and allophycocyanin. (B) Under high light conditions, the biophysical valve closes, and energy absorbed by PUB at 495 nm does not reach PSII. Instead, it is released as fluorescence. Energy absorbed at 550 nm by PEB is transferred to PSII through phycocyanin and allophycocyanin.

at greater depths or early or late in the photoperiod, PUB transfers energy to PEB and on to PSII with high efficiency. In this case, both PUB and PEB were active in O_2 evolution (Fig. 7).

The increase in PUB noted under high light conditions suggested that this pigment may also serve as a photoprotectant by reemitting absorbed light as fluorescence at 565 nm, as observed by Wyman et al. (1985). The interconversion between PUB and PEB could occur within a phycobilisome by the reversible saturation of a single double bond on ring A (Sidler 1994). The changes in pigment content under varying light conditions explain the discrepancies reported for the absorption spectra of *Trichodesmium* grown in culture, under relatively low irradiance conditions (Ohki et al. 1986; Haxo et al. 1987; Prufert-Bebout et al. 1993), compared with the absorption spectra of natural populations of *Trichodesmium* sampled from high irradiance conditions (Shimura and Fujita 1975; Lewis et al. 1988; Borstad et al. 1992) *see Fig. 1*). The reversible interconversion of PEB to PUB provides a dynamic biophysical valve for delivery of excitation energy to PSII. This valve is adjusted in response to light intensity.

In all photosynthetic diazotrophic organisms, N_2 fixation

requires a large input of ATP. In heterocystic organisms, the ATP is generated via cyclic electron flow around PSI. This photosystem is sequestered in the heterocyst, and in the process of differentiation of that specialized cell, PSII is lost. The loss of PSII facilitates a photosynthetic generation of energy with a concomitant evolution of O_2. Hence, organisms with heterocysts are able to maintain simultaneous O_2 evolution and N_2 fixation by spatial segregation. In unicellular diazotrophic cyanobacteria such as those described by Mitsui and Cao (1988), N_2 is temporally segregated from O_2 evolution via a circadian rhythm, in which N_2 fixation occurs at night, and electron and ATP requirements are generated via the respiratory electron transport process at the expense of photosynthetic products.

In contrast to these two strategies, *Trichodesmium,* which is an obligate diazotroph, fixes N_2 during the photoperiod in the same cells that evolve O_2. As in all diazotrophs, its nitrogenase is irreversibly damaged by O_2. The cellular basis by which the concurrent fixation of N_2 and evolution of O_2 is facilitated within the same cell remains a mystery (Carpenter 1983; Carpenter et al. 1990). The fluorescence emission spectra for *Trichodesmium* presented here reveal an extremely small luminosity for bands that correspond to PSII. Assuming to a first order, the relative fluorescence intensity at 77 K is conserved in cyanobacteria (the amino acid sequences of the major emitters, namely CP43 and CP47, are highly conserved in all cyanobacteria), we may infer the relative ratio of PSII to PSI by comparing the fluorescence emission spectra of *Trichodesmium* with that of *Synechococcus,* whose stoichiometry of the two reaction centers has been determined. Assuming an average PSI to PSII ratio of 4 to 1 in *Synechococcus* (Kawamura et al. 1979), corresponding to the emission at 742 and 683, we infer a PSI to PSII ratio of 24 : 1. These results suggest that a large fraction of the excitation energy absorbed by *Trichodesmium* is used to drive PSI, presumably to facilitate cyclic electron transport and generate ATP required for N_2 fixation. The consequences of this unusual stoichiometry of the reaction centers imply an extremely low quantum yield for O_2 evolution and hence, photoautotrophic growth.

The variability in absorption properties of the phycobilins reflects an evolutionarily selected pathway that reversibly regulates the distribution of excitation energy to PSII. From the data presented, it is not possible to detect the extent to which the changes in absorption of the phycobilins result in the redirection of the absorbed energy to PSI. Nonetheless, the diurnal cycle is complimentary to changes in PUB to PEB ratios, and the subsequent change in quantum yield of O_2 evolution represents a biophysical change in energy transfer that is a consequence of chemical change within the chromophore. This interconversion is reminiscent of the xanthophyll cycle seen in eucaryotic algae and higher plants, in which reversible deepoxidation of xanthophylls is used to regulate the energy transfer from the Soret bands of Chl *a* to reaction centers. While it is premature to suggest anything other than parallel evolution in the biophysical energy valve, it distinctly differs from the evolutionarily ancient protein phosphorylation mechanisms associated with the so-called ''state transitions'' (Fujita et al. 1994). In the latter, energy from phycobiliproteins is reversibly coupled to one or the other photosystems by phosphorylation of the protein, without any change in the chromophore structure. The phenomenon we report, in contrast, suggests a change in the chromophore structure. Whether such a change is accompanied by an alteration of the phosphorylation status of the protein scaffold remains to be determined.

We propose, as a working hypothesis, that this energetic valve evolved in *Trichodesmium* and was selected to support simultaneous N_2 fixation and O_2 evolution. *Trichodesmium,* unlike nondiazotrophic marine cyanobacteria such as *Synechococcus,* possesses gas vacuoles that enhance buoyancy. Consequently, a large fraction of the *Trichodesmium* population can be found in the upper few meters of the ocean, where the visible and UV irradiance are relatively high. In contrast to most nondiazotrophic cyanobacteria, these organisms have to downregulate the energy supply to PSII to prevent damage to those reaction centers. The interconversion between PUB/PEB is one mechanism by which this downregulation is achieved. A second is the synthesis and accumulation of UV and visible pigments that do not contribute to overall light harvesting. For the shorter wavelengths, UVB protection is presumably afforded by the high optical cross-section (10 times higher than pigments in the visible) contributed by the identified MAAs. For the visible wavelengths, there is an overall reduction in the ratio of absorption of phycobilin to chlorophyll in comparison with unicellular cyanobacteria, which are commonly found deeper in the oceans. The third phenomenon is the formation of carotenoids, such as β-carotene, which absorb light but do not transfer energy to PSII. Finally, on a community level, the organisms aggregate in chains to form ''trichomes'' and subsequently, colonies. The macroorganizational structure of essentially filamentous algae leads to a self-shading effect that reduces the overall optical absorption cross-section of chlorophyll by at least a factor of four in comparison to that of the individual filaments.

In conclusion, our results clearly demonstrate an extraordinary degree of plasticity in the optical properties in *Trichodesmium* that appears to vary on a diel basis. The extent to which this variability is a consequence of a true circadian rhythm or is a response to changes in spectral irradiance remains to be elucidated. Nonetheless, the mechanism underlying the changes in optical properties appears to be unique to this cyanobacteria and provides half the information required for the development of an algorithm that can potentially retrieve these organisms from remotely sensed information. The absolute value of spectral backscatter coefficients provides the other half of the critical elements required to derive an algorithm. In the following paper, we analyze the scattering properties of *Trichodesmium* and derive an optical model for remote sensing.

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