Bio-optical properties of the marine cyanobacteria *Trichodesmium* spp.

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Abstract. Bio-optical spectral properties were determined on fresh suspensions of *Trichodesmium* spp. collected in a tropical lagoon and put in seawater tanks (total chlorophyll concentrations range between 0.1 and 3.8 mg m^{-3}). The spectrum of the backscattering coefficient was a hyperbolic function with a slope of 1.2, often showing troughs at 440, 550 and 676 nm, due to absorption peaks of chlorophyll and phycoerythrin. The absorption spectrum computed with a specific beta correction for *Trichodesmium*, showed a blue to red ratio (B/R) equivalent to the one of a single colony (B/R=2), and also showed the double peak of mycosporine-like amino acids (MAA's, 330 and 360 nm). The CDOM absorption spectrum showed minor MAA peaks when cyanobacterial concentrations were above 1 mg Chl *a* m^{-3}. The chlorophyll *a*-specific backscattering and absorption coefficients at 442 nm were respectively 0.0126 m^2 (mg.chl *a*)$^{-1}$ and 0.027 m^2 (mg chl *a*)$^{-1}$. Suspensions in tanks exhibited a high backscattering ratio at 660 nm ($\tilde{b}_b = b_{bp}/b_p$). The above-water reflectance spectrum clearly showed troughs at the wavelength of the pigment absorption peaks. Datasets of *Trichodesmium* normalized absorption, backscattering and reflectance spectra will allow its detection with future hyperspectral ocean colour sensors.

Keywords: ocean optics, backscattering, absorption, reflectance, *Trichodesmium*, phytoplankton.

1 INTRODUCTION

Trichodesmium, the filamentous photosynthetic cyanobacterium, has an important role in N_2 fixation in the euphotic zone of tropical and subtropical oceans [1]. It is the major contributor to the oceanic N_2 fixation, a process which could regulate atmospheric CO_2 over geological time via the biological sequestration of CO_2 in the ocean [2]. Since ocean colour satellite data have captured their large surface blooms in the South Western Tropical Pacific (SWTP) [3-5], the challenge of optical remote sensing of *Trichodesmium* has stimulated research to

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determine their optical properties [6-8] and to quantify *Trichodesmium* from space by convenient algorithms [9-10]. Thus precise measurements of the backscattering b_b (scattering in the backward direction – Table 1) and of the absorption (a) coefficients are required, as the below-surface remote sensing reflectance (*Rrs*), is related to b_b and a through

$$R_{rs} = g_1\left(\frac{b_b}{a+b_b}\right) + g_2\left(\frac{b_b}{a+b_b}\right)^2, \qquad (1)$$

where g_1 and g_2 are constants (0.0949 and 0.0794, respectively [11].

Previous laboratory studies [7-8] on *Trichodesmium* in the Atlantic Ocean and the Caribbean Sea, indicated that their optical properties depart from those by other phytoplankton. It has 1) a low specific absorption in the blue due to a large pigment package effect of colonies, 2) a high absorption in the UV linked to mycosporin-like amino acids (MAA's), 3) a significant phycoerythrin fluorescence signal and 4) a high backscattering coefficient due to internal gas vacuoles [6-10]. These properties contrast with those of other marine phytoplankton with rather low backscattering ratios ($\tilde{b}_b$, Table 1) for which absorption [12-13] and backscattering [14-16] properties can be modelled, dependent on size and shape. Unfortunately, there is currently no published measurement using recent instrumentation for *Trichodesmium*. This paper presents a complete data set of the backscattering and absorption coefficients of freshly collected *Trichodesmium* spp. of the tropical lagoon off New Caledonia. Backscattering was measured using Hydroscat-6 instrument (HOBI Labs) [17]. Absorption of the particulate and dissolved fractions was determined with classical methods calibrated by a point-source integrating cavity absorption meter (PSICAM) [18-19]. Experimental results, after resuspending cells in seawater tanks [20, 21], are compared with preliminary results obtained during sea-cruises in the SWTP for populations of the same species of *Trichodesmium* spp. In addition, tabulated basis vectors representing the normalized absorption, backscattering and above-water remote sensing reflectance for *Trichodesmium* are provided.

Table 1. Definitions of measured optical parameters.

Symbol	Significance	Units
λ	Wavelength	nm
Chl *a*	Chlorophyll *a* concentration	mg m^{-3}
DV-chl *a*	Divinyl chlorophyll *a* concentration	mg m^{-3}
tchl *a*	Sum of chlorophyll *a* and divinyl-chlorophyll *a*	mg m^{-3}
β	Pathlength amplification correction factor	
β'	Volume Scattering Function	sr^{-1} m^{-1}
βw	Volume Scattering Function of pure water	sr^{-1} m^{-1}
$a(\lambda)$	Absorption coefficient	m^{-1}
$a_p(\lambda)$	Particulate absorption coefficient	m^{-1}
$a_d(\lambda)$	Absorption coefficient by non-pigmented matter	m^{-1}
$a_{tri}(\lambda)$	Absorption coefficient of *Trichodesmium*	m^{-1}
$a^*_p(\lambda)$	Specific absorption coefficient of particles	m^2 (mg tchl *a*)$^{-1}$
$<a_{tri}>$	Mean absorption of phytoplankton (400-700 nm)	m^{-1}
$b_{b\text{-}H6}(\lambda)$	Measured total backscattering coefficient	m^{-1}

$b_{bw}(\lambda)$	Backscattering coefficient of seawater	m^{-1}
$b_{btri}(\lambda)$	Backscattering coefficient of *Trichodesmium*	m^{-1}
$b^*_{tri}(\lambda)$	Specific backscattering coeff. of *Trichodesmium*	m^2 (mg tchl *a*)$^{-1}$
$<b_{btri}>$	Mean backscatter. of phytoplankton (400-700 nm)	m^{-1}
$b_p(\lambda)$	Scattering coefficient of particles	m^{-1}
$\tilde{b}_b$	Ratio of $b_{bp}(\lambda)$ to $b_p(\lambda)$	
$c_p(\lambda)$	Beam attenuation coefficient of particles	m^{-1}
γ	Slope of the hyperbolic function for $b_{btri}(\lambda)$	γ
$R_{rs}(\lambda)$	Remote sensing reflectance	sr^{-1}
$<R_{rs}>$	Mean remote sensing reflectance (400-700nm)	sr^{-1}
σ	Sigma-correction of Hydroscat-6 data	

2 METHODS

2.1 Experimental sampling

For two experiments in March 2004 at the laboratory of IRD Noumea (New Caledonia), 350 L tanks were filled with unfiltered seawater (reference water) freshly collected collected about 4 nautical miles beyond the coral barrier reef. Initial phytoplankton concentrations in these tanks were 0.1 and 0.5 mg chl *a* m^{-3}, respectively. Fresh *Trichodesmium erythraeum* colonies were carefully collected with nets in a nearby bay (Sainte-Marie Bay) and put in a 10 L polysulfone carboy. After passing the colonies gently through a 200 µm net, they were further concentrated by letting the buoyant colonies migrate upwards in a separating funnel. 90 mL aliquots of the concentrated suspension were added to the tanks to obtain increasing *Trichodesmium* concentrations until a maximum of 3 and 3.8 mg chl *a* m^{-3}, respectively. Assuming a constant chlorophyll *a* concentration per trichome of 99 pg [22], these maxima corresponded to trichome numbers of 300 and 380 10^3 L^{-1}. After each addition, samples from the tank were collected on GF/F filters to measure the chlorophyll concentration (0.1 to 0.5 L) and the light absorption by particles (0.25 to 0.5 L). All filters were immediately analyzed at the laboratory. The whole experiment took less than 3 hours.

For measurements of absorption by chromophoric dissolved organic matter (CDOM), 100 mL of tank water was taken and filtered under low vacuum (< 25 mm Hg) through 0.22 µm Micropore filters (diameter = 47 mm) that were previously rinsed twice with purified water (MilliQ, Millipore). Samples were kept in the dark at 4°C, in amber borosilicate bottles (DURAN, Schott), closed with Teflon caps, and analyzed within a few hours. Two additional tank experiments were performed to determine the evolution of CDOM over time: one with a moderate and un-manipulated *Trichodesmium* concentration (tchl *a* ~ 1 mg m^{-3}), and a second one with progressive additions of concentrated colonies (tchl *a* from 5 to 32 mg m^{-3}). Backscattering and absorption measurements were made first on the reference water and then after each *Trichodesmium* addition.

2.2 Particulate absorption measurements of *Trichodesmium* colonies

In vivo particulate and detrital absorption coefficients ($a_p(\lambda)$, $a_d(\lambda)$, Table 1) were measured with the Quantitative Filter Technique (QFT) as in [23] except for the correction of the pathlength amplification (see below). Optical densities (OD) were recorded from 200 to 800 nm on a single-beam Beckman DU-600 spectrophotometer, keeping maximum OD below 0.4 and subtracting the OD spectrum of a filter wetted with 0.2 µm filtered seawater (blank). A visual examination of the filters confirmed that colonies were present and homogeneously

distributed on the filters. Care was taken that a sufficient, but not too high (saturation effect) number of filaments were present on the filter and detectable in the measuring window (3 mm x 3 mm) of the spectrophotometer. The usable concentration range was equivalent to 50-450 trichomes of *Trichodesmium* per cm^2. Measurements of the absorption of filaments on the filter were taken as $a_p(\lambda)$. Liposoluble pigments in *Trichodesmium* were then extracted with methanol. Afterwards, an extraction with hot sea-water was conducted until the complete disappearance of peaks related to phycoerythrobilin (PE) absorption. In the most concentrated sample, a small residual peak remained at 510 nm. Absorption measurement after elimination of pigments was taken as $a_d(\lambda)$.

The precise determination of the absorption coefficient requires a correction for the pathlength amplification factor on the filter. Because the latter was unknown for the large size and various shapes of filaments and colonies of *Trichodesmium* spp. [6-7], we determined it by using a point-source integrating cavity absorption meter (PSICAM, [19]), and a *Trichodesmium* laboratory culture (IMS101). The amplification correction factor, ß, was calculated by a linear regression with forced intercept at the origin between absorption of colonies collected on the filter and absorption of the colony suspension put inside the PSICAM sphere (all data: OD of filter = 0.326*OD of suspension, $N = 231$, $R^2 = 0.998$). As already observed [24], the ß factor (0.326) did not show any influence on the relative amplitude of OD. The absorption coefficients of the samples $a(\lambda)$ (in m^{-1}) were then calculated from the measured optical density on filter [$OD_f(\lambda)$] following

$$a(\lambda) = 2.303\ ß\ (OD_f(\lambda) - OD_f(750)) / l, \quad (2)$$

where $OD_f(750)$ is the OD at 750 nm, and l is the sample pathlength (in m) calculated from the effective filter area (πr^2) and the filtered volume (V in m^3) following: $l = V/(\pi r^2)$. The absorption spectrum of phytoplankton, $a_{ph}(\lambda)$, was calculated as the difference between $a_p(\lambda)$ and $a_d(\lambda)$. The particulate absorption coefficient of *Trichodesmium* in tank, $a_{tri}(\lambda)$, was calculated by subtracting from $a_{ph}(\lambda)$ the values of the fresh unfiltered reference seawater measured in the tank before the *Trichodesmium* addition (Fig. 2a).

Colonies of *Trichodesmium* spp. were picked from experimental tanks or from nets during field studies in the SWTP (20°S-14°S and 160°E-180°E; April 1998 *Roger Revelle* cruise) and in Northern Australian waters (November 1999 *Maurice Ewing* cruise). Absorption spectra were measured on these colonies after gently putting them on filters saturated with filtered seawater and after placing the filter right in front of the detector. In the UV, peaks of MAA's absorption are highly dependent on handling and freezing of the filters [25] as previously reported for *Trichodesmium* [7-8] and for large dinoflagellates [26-27]. For comparison among the different communities, the *Trichodesmium* absorption spectrum ($a_{tri}(\lambda)$, m^{-1}) was normalized [28] using the mean absorption ($\langle a_{tri} \rangle$, m^{-1}, Table 1) computed between 400 and 700 nm following

$$\langle a_{tri} \rangle = \frac{1}{300} \sum_{\lambda=400}^{700} a_{tri}(\lambda) \Delta\lambda, \quad (3)$$

where $\Delta\lambda = 1$ nm.

The chlorophyll concentrations were determined by spectrofluorometry allowing the discrimination of monovinyl-Chl *a* (chl *a*) and divinyl-chl *a* (DV-chl *a*) of *Prochlorococcus* [29]. Samples were collected on 25 mm GF/F filters and extracted by grinding in 93% acetone, The chlorophyll-specific absorption coefficients of *Trichodesmium* was determined

from linear regression after plotting the data versus the total chlorophyll concentration (tchl *a* = chl *a* + DV-chl *a*) within the range of 0.1-3.8 mg tchl *a* m^{-3}.

2.3 CDOM absorption

CDOM optical densities over the wavelength range of 250-800 nm were measured in 10-cm quartz cuvettes using a dual-beam Perkin Elmer LAMBDA 20 UV/VIS spectrophotometer. Purified water (MilliQ) served as the reference medium. Samples and reference were left to stabilise at room temperature before measurement. Absorption coefficients were calculated as

$$a_{CDOM}(\lambda) = 2.303\ \mathrm{OD}(\lambda) / l_1\ , \tag{4}$$

where $a(\lambda)$ is the absorption coefficient (in m^{-1}), λ the wavelength, l_1 the geometric pathlength (0.1 m) and OD the optical density corrected for instrumental baseline offsets as in [30].

2.4 Hydroscat theory and data processing

The backscattering coefficient, $b_b(\lambda)$, was estimated using a Hydroscat-6 profiler (H6, HOBI Labs) designed to detect light scattering at 6 spectral channels, namely 440, 488, 510, 550, 620, and 676 nm, assuming a linear relationship between the scattering at 140° and $b_b(\lambda)$ [17]. Two channels (510 and 550 nm) were explicitly chosen to detect spectral modifications related to the specific pigmentation of *Trichodesmium.* The instrument was also set to measure fluorescence by chlorophyll *a* (chl *a*) and phycoerythrin (PE) with two excitation/emission couples at 442/676 and 488/550 nm, respectively. The H6 was placed horizontally, facing a black painted wooden plate at a distance of 75 cm to minimize light reflection [20, 21]. Before each measurement, water was gently stirred, bubbles carefully removed from the detector windows, and the tank carefully closed. The H6 measures scattering at a fixed angle (140°) corresponding to the minimum variation of the volume scattering function, VSF. The backscattering coefficient was estimated using the manufacturer's protocol. Briefly, the uncorrected backscattering coefficient, $b_{bu}(\lambda)$, is derived from β' at 140° [VSF(140°)] using a single conversion factor for seawater and particles as

$$b_{bu} = 2\,\pi\chi\,(\beta' - \beta_w) + b_{bw}\ , \tag{5}$$

where β_w is the scattering of pure water, and b_{bw} is the backscattering coefficient of pure water. Note that λ is omitted for clarity. χ is the coefficient of proportionality between β and b_b for particles, and while it is not constant for all particle types, it does not vary much in typical natural waters, and thus is fixed at $\chi = 1.08$. A correction (σ) is then applied to $b_{bu}(\lambda)$ to compensate for the loss of photons absorbed by the medium between the instrument and the detection volume as

$$b_{b\_H6} = \sigma . b_{bu}\ , \tag{6}$$

where σ is a function of the instrument geometry, given by

$$\sigma(K_{bb}) = k_1 \exp(k_{\exp} K_{bb})\ , \tag{7}$$

with k_1=1, $k_{exp}(\lambda)$ in each Hydroscat 6 calibration, and K_{bb} approximated from the attenuation pathlength as

$$K_{bb} = a + 0.4b \ , \tag{8}$$

where a is measured in tanks and b is estimated by

$$b = \tilde{b}_b \, . b_{bu} \ , \tag{9}$$

As no measurement of b was made in the tank, we tested the importance of the assumption made on the backscattering ratio $\tilde{b}_b$ chosen for estimating b in Equation (9). Instead of the Hobilabs default value ($\tilde{b}_b$=0.014), two representative values one for oceanic waters ($\tilde{b}_b$=0.013, [31]) and one for mineral particles ($\tilde{b}_b$=0.03), were used for the calculations. For the highest tchl a concentration in the tank (3.8 mg m^{-3}), the σ-correction enhanced the uncorrected backscattering coefficient in the blue channel (440 nm) by 3 % and 6% with the two $\tilde{b}_b$ respectively, and less than 2% in other channels, see [31]. The *Trichodesmium* backscattering coefficient, $b_{btri}(\lambda)$, was calculated by subtracting from $b_{b\text{-}H6}$ (λ) the spectrum of the fresh, unfiltered reference seawater (Fig. 3a). This was higher at all wavelengths than the spectrum measured *in situ*, with no color in the bias, as in [20]. The wavelength dependence of the b_{btri} spectrum was approximated using a hyperbolic slope γ (dimensionless, Table 1) as

$$b_{btri}(\lambda) = b_{btri}(440) \, . \, (\lambda/440)^{-\gamma} \ , \tag{10}$$

The *Trichodesmium* backscattering spectrum ($b_{btri}(\lambda)$, m^{-1}) was normalized to the average of backscattering coefficients at all 6 channels ($<b_{btri}>$, m^{-1}, Table 1).

2.6 Remote sensing reflectance

The below-surface remote sensing reflectance for *Trichodesmium* colonies was calculated as in Eq. (1) from ratios of backscattering over absorption coefficients determined in tanks (adding the contribution of backscattering and absorption of pure sea water). The above-water remote sensing reflectance R_{rs} (in units of sr^{-1}) of several dense *Trichodesmium* suspensions of unknown biomass concentration put in a Petri dish was obtained from measurements of upwelling radiance and downwelling irradiance using an Ocean Optics USB2000 spectroradiometer in the range 380-900 nm. The *Trichodesmium* reflectance spectrum ($R_{rs}(\lambda)$, sr^{-1}) was normalized to the mean remote sensing reflectance ($<R_{rs}>$, sr^{-1}, Table 1) computed as

$$\left\langle R_{rs} \right\rangle = \frac{1}{300} \sum_{\lambda=400}^{700} R_{rs}(\lambda) \Delta\lambda \ , \tag{11}$$

where $\Delta\lambda$ = 1 nm.

3 RESULTS

3.1 Particulate absorption

In vivo absorption spectra of all intact colonies (normalized to their spectral mean between 400 and 700 nm as in Eq. (3)) showed similar spectral shape (Figure 1). For all *Trichodesmium thiebautii* colonies collected during the sea cruises in the Western Tropical Pacific Ocean, the spectral variability was insignificantly low (Fig. 1a), except for green *Trichodesmium* colonies (puffs and tufts) found at 100 m in the Coral Sea. *Katagnymene* spp. exhibited slightly different spectra (Fig. 1a). The absorption spectrum of the *Trichodesmium* culture measured with a PSICAM is shown for comparison. Furthermore, no spectral variation was detected with time of day (Fig. 1b). As noted in Sec. 2.2, peaks of the mycosporine-like amino acids (MAA's) asterina-332 (absorption maximum at 330 nm) and palythene (360 nm) were variable.

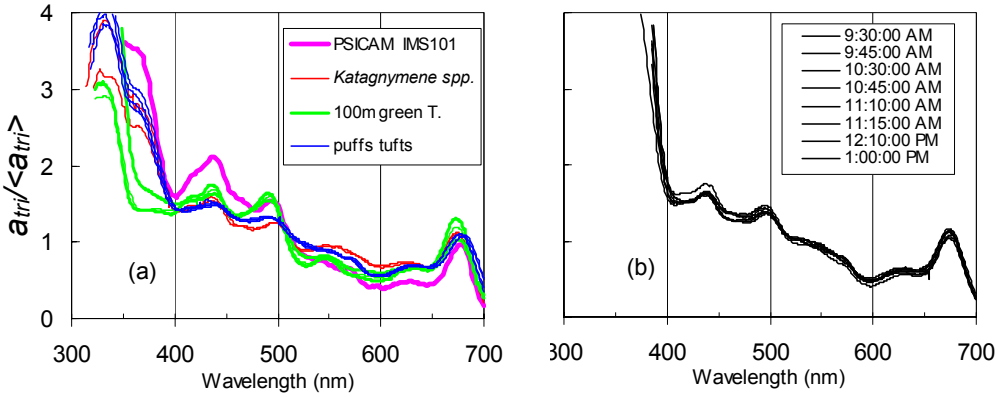

Fig. 1. Absorption spectra normalized to its spectral mean (400-700 nm) of various filamentous cyanobacteria. a) puffs and tufts of *Trichodesmium thiebautii* (Northern Australia, 16°S 146.45°E, 0m), *Katagnymene* spp. (Northern Australia, 10.31 °S 136.36°E, 20m), green *Trichodesmium* spp. in the Coral Sea (Northern Australia, 10.51°S 145.10°E, 100m) and *Trichodesmium erythraeum* culture IMS101 measured with the PSICAM. b) puffs of *Trichodesmium thiebautii* over day time (Northern Australia,13.33°S 124.30°E, 0m).

Absorption spectra of *Trichodesmium erythraeum* with increasing tchl *a* concentrations are shown in Fig. 2a. The blue to red absorption peak ratio (at and 670 nm (B/R, 440nm/670nm) decreased as chlorophyll increased from 0.13 to 3.8 mg tchl *a* m^{-3} in the tanks, linked to changes in the phytoplankton community [13, 28, 32-33]. In the initial sea-water collected to fill the tank, 44 % of the total chl *a* was divinyl-chl *a* (DV-chl *a*), a pigment associated with *Prochlorococcus* and the B/R) was greater than 4. As soon as *Trichodesmium* was dominant in the tank, and the DV-chl *a*/tchl *a* ratio was negligible, the B/R ratio reached the value determined for the *Trichodesmium* colony (1.7<B/R< 2.1, see Fig. 1).

The mean tchl *a*-specific *Trichodesmium* absorption coefficient at 442 nm, $a^*_{tri}(442)$ was 0.0278 m^2 (mg chl *a*)$^{-1}$ (R^2=0.99, *N*= 7) (Fig. 2b, Table 2). This a^*_{tri} (442) value is intermediate between the one determined on disaggregated [0.065 m^2 (mg chl *a*)$^{-1}$] and intact colonies [0.0187 m^2 (mg chl *a*)$^{-1}$] [7-8]. The lowest value is attributed to the secondary package effect in colonies. It was 23% lower at 442 nm and similar at 550 nm than the model values in [9-10] ([0.0368 m^2 mg chl *a*)$^{-1}$] and [0.0135 m^2 (mg chl *a*)$^{-1}$], respectively). Assuming a constant chlorophyll *a* concentration per trichome of 99 pg [22], this corresponds to a value for $a^*_{tri}(442)$ of $7*10^{-10}$ m^2 per trichome.

Table 2. Specific absorption and backscattering coefficients for *Trichodesmium* suspensions, in tank m^2 (mg tchl *a*)$^{-1}$.

Parameter	Wavelength (nm)						
	412	442	488	510	550	620	676
a^*_{tri}	0.0451	0.0278	0.0220	0.0171	0.0133	0.0098	0.0192
b^*_{btri}	-	0.0126	0.0115	0.0116	0.0095	0.0093	0.0077

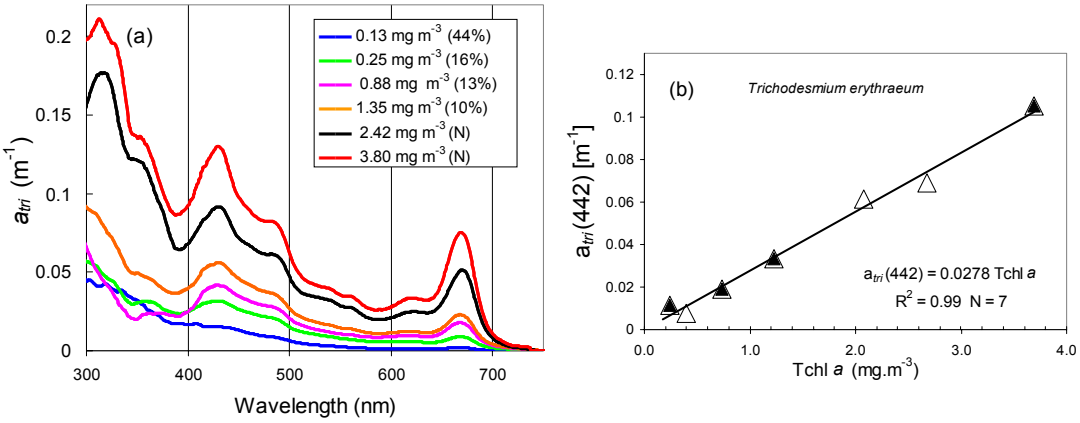

Fig. 2. *Trichodesmium erythraeum* absorption. (a) *In vivo* spectra of colonies for serial additions of colonies collected (Noumea, New Caledonia) to natural sea water in tanks during experiments on the 18th (Tank 1) and 24th March 2004 (Tank 2). The blue line corresponds to the reference water which was subtracted to get $a_{tri}(\lambda)$. The percentage of divinyl-chl *a* over tchl *a* is indicated in brackets (N, negligible). The bold black line corresponds to the reference used to calculate normalized a_{tri} (Table 3). (b) Absorption coefficient at 442 nm, $a_{tri(442)}$, *versus* the total chlorophyll concentration (Tank 1, open triangles; Tank 2, black triangles).

3.2 Backscattering and fluorescence

Trichodesmium backscattering spectra at concentrations increasing from 0.13 to 3.8 mg tchl *a* m^{-3} are shown in Figure 3a. Half of these b_{btri} spectra exhibited large troughs at 440 and 550 nm. The hyperbolic slope calculated between 440 and 676 nm for the representative b_{btri} was 1.2 (STD=0.05). In the red, values at 620 nm were equal to or higher than values at 670 nm as there was no contamination of the 676 nm backscattered signal by chlorophyll fluorescence peaking at 681 nm.

The chlorophyll-specific backscattering coefficient of *Trichodesmium* colonies at 442 nm, $b^*_{btri}(442)$, was 0.0126 m^2 (mg chl *a*)$^{-1}$ (Fig. 3b; Table 2) or $1*10^{-10}$ m^2 per trichome [22]. It was 36 % higher at 442 nm and 15 % higher at 550 nm than the model value of 0.008 m^2 (mg chl *a*)$^{-1}$ at 442 and 550 nm, and including detritus (Figure 1 in [10]). The H6-red fluorescence (fl_{676}) (in arbitrary units) was well related to *Trichodesmium* concentration (fl_{676} = 2 10^4 tchl *a*, R^2=0.87, *N*=19) and to backscattering at 442 nm (fl_{676} = 0.0186 $b_{b\text{-}H6}$, R^2=0.98, *N*=19). The H6-PE fluorescence at 555 nm was only detected at *Trichodesmium* concentrations equivalent to tchl *a* higher than 3 mg m^{-3} (or a PE concentration [22] of > 6 mg m^{-3}).

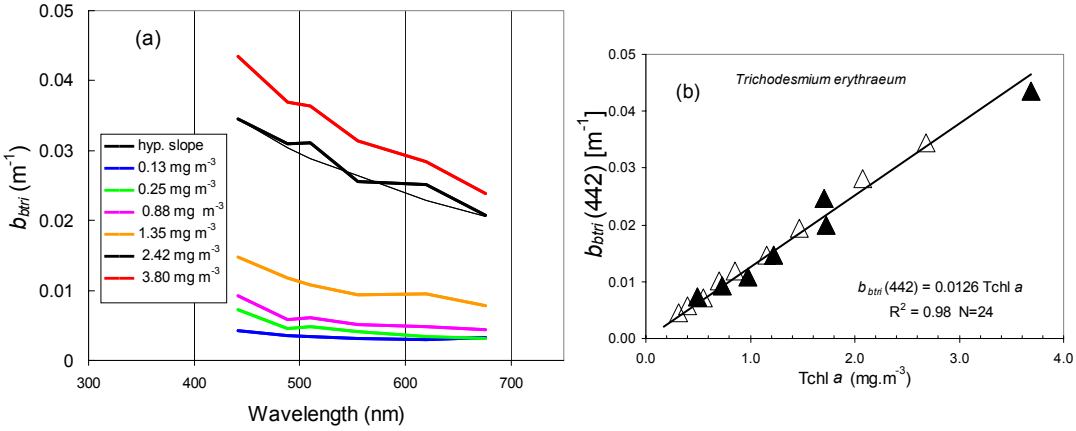

Fig. 3. *Trichodesmium erythraeum* backscattering. (a) Spectral backscattering. As in Fig. 2, the blue line corresponds to the reference water which was subtracted to get $b_{btri}(\lambda)$, the bold black line corresponds to the reference used to calculate normalized b_{btri} (Table 4) and the light black line is the hyperbolic function with $\gamma = 1.2$ (Table 3b). (b) Backscatter coefficient at 442 nm, $b_{btri(442)}$, *versus* the total chlorophyll concentration (Tank 1, white triangles, Tank 2, black triangles).

3.3 CDOM absorption

CDOM absorption spectra measured on *Trichodesmium* suspensions in tanks exhibited two distinct peaks at 330 and 360 nm attributed to the MAA's asterina and palythene [7, 34]. These MAA's are intracellular, and only a few percent were released into tank water after keeping colonies for a prolonged time (Fig. 4a). Stronger peaks were detected when tchl *a* was above 1 mg m^{-3} (Fig. 4 b,c).

3.4 Remote sensing reflectance

The above-water surface remote sensing reflectance, R_{rs} of *Trichodesmium* at the H6 channels (the values given by Eq. (1) were multiplied by 0.546 [35]) is extremely high at chlorophyll concentrations > 1 mg m^{-3} (R_{rs} = 0.02 sr^{-1} at 550 nm with tchl *a* = 3.8 $mg.m^{-3}$, Fig. 5a) as it might be expected in dense surface slicks. The CDOM absorption greatly modifies the B/R ratio of R_{rs}, by a factor of 1.5 to 2 depending on tchl *a* concentration (Fig. 5a).

Hyperspectral above-water reflectance of *Trichodesmium* suspensions measured in Petri dishes (Fig. 5b) showed a maximum at 593 nm and a red edge common to other algae [6; 36]. The difference comes from the specific suite of pigment absorption linked to the *Trichodesmium* pigmentation: at 380 nm (MAA's), 438 nm (chl a), 470 nm (carotenoids), 495 nm (phycourobilin, PU), 547 nm (phycoerythrobilin, PE), 620 nm (phycocyanin, PC) and 677 nm (chl a). Weaker peaks were observed at about 8 nm further in the spectrum (565 nm for the PE and 660 nm for the PC) that may correspond to pigment fluorescence.

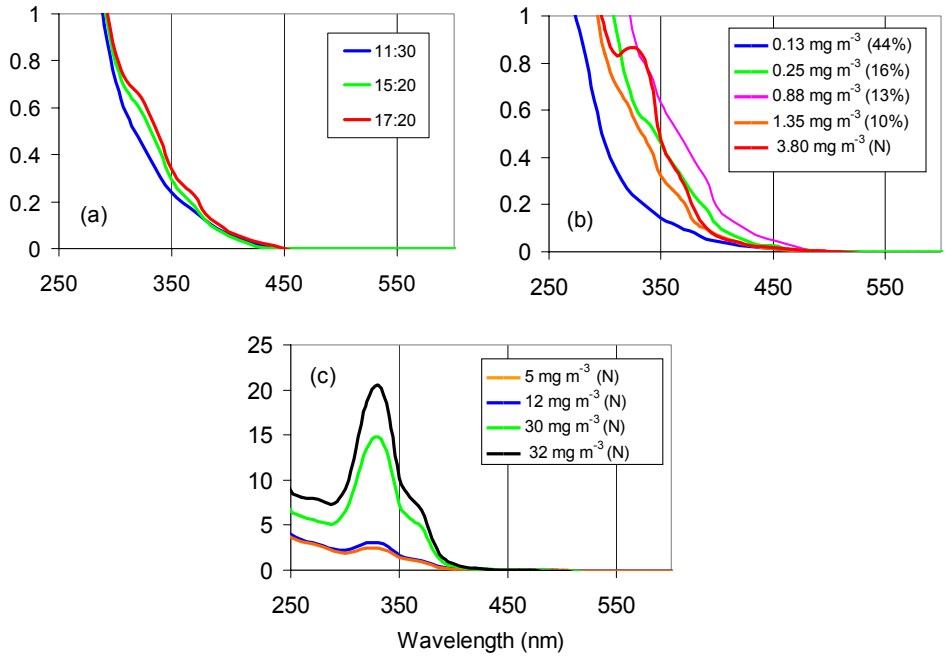

Fig 4. CDOM absorption spectra of tank water containing *Trichodesmium erythraeum* colonies (a) left un-manipulated for prolonged time in the tank (the sampling times are indicated), (b) at moderate tchl *a* concentrations in Tank 1 and 2 as in Fig. 2, (c) at high tchl *a* concentrations. The percentage of divinyl-chl *a* over total chlorophyll *a* is indicated in brackets (N, negligible).

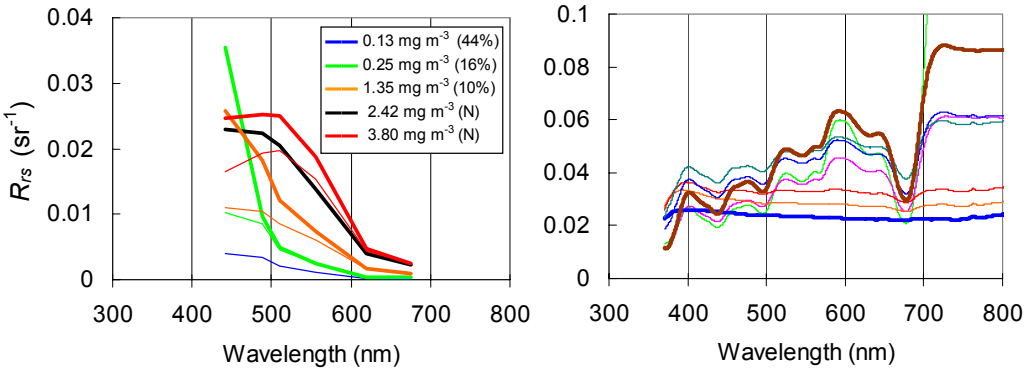

Fig. 5. Above water remote sensing reflectance of *Trichodesmium erythraeum* (a) calculated as in Eq. (1) from the Hydroscat-6 backscattering coefficient over the total particulate absorption coefficient in tank as for Fig. 2 (plus sea water) and including the measured CDOM absorption coefficient (thin line) or not (thick line), and then converted to above water *Rrs* (b) Reflectance measured with the Ocean Optics spectroradiometer with increasing concentrations of *Trichodesmium* spp. put in a Petri dish. The bold blue line corresponds to water in the Petri dish with no *Trichodesmium*, the bold brown line corresponds to the reference used to calculate normalized *Rrs* (Table 5).

Table 3. Basis vectors representing the normalized absorption for *Trichodesmium* calculated as a ratio of a_{tri} for tchl a = 2.42 mg m^{-3} divided by $<a_{tri}>$ as in Eq. (3). Wavelength (λ) in nm. Basis vector for $a^*_{tri}(\lambda)$ can be constructed by setting the value of the vector at 676 nm to 0.0192 m^2 mg^{-1} (Table 2), and scaling for the other wavelengths accordingly, as in [28].

λ	Tricho	λ	Tricho	λ	Tricho	λ	Tricho	λ	Tricho
400	1.513								
402	1.559	462	1.535	522	0.799	582	0.454	642	0.548
404	1.582	464	1.519	524	0.789	584	0.449	644	0.559
406	1.588	466	1.492	526	0.783	586	0.449	646	0.573
408	1.651	468	1.471	528	0.778	588	0.450	648	0.599
410	1.703	470	1.442	530	0.775	590	0.447	650	0.625
412	1.793	472	1.425	532	0.767	592	0.446	652	0.666
414	1.835	474	1.405	534	0.755	594	0.447	654	0.705
416	1.863	476	1.397	536	0.748	596	0.451	656	0.757
418	1.859	478	1.392	538	0.742	598	0.460	658	0.819
420	1.893	480	1.396	540	0.747	600	0.466	660	0.884
422	1.924	482	1.395	542	0.736	602	0.475	662	0.951
424	1.980	484	1.392	544	0.727	604	0.480	664	1.014
426	2.005	486	1.389	546	0.708	606	0.489	666	1.071
428	2.047	488	1.391	548	0.694	608	0.498	668	1.113
430	2.079	490	1.387	550	0.666	610	0.513	670	1.147
432	2.089	492	1.366	552	0.644	612	0.527	672	1.172
434	2.089	494	1.332	554	0.632	614	0.542	674	1.182
436	2.075	496	1.284	556	0.629	616	0.548	676	1.168
438	2.062	498	1.227	558	0.631	618	0.553	678	1.119
440	2.012	500	1.163	560	0.628	620	0.559	680	1.045
442	1.939	502	1.087	562	0.633	622	0.565	682	0.941
444	1.846	504	1.016	564	0.622	624	0.567	684	0.832
446	1.782	506	0.958	566	0.615	626	0.568	686	0.711
448	1.721	508	0.917	568	0.583	628	0.564	688	0.606
450	1.681	510	0.888	570	0.555	630	0.559	690	0.505
452	1.640	512	0.860	572	0.527	632	0.553	692	0.425
454	1.602	514	0.838	574	0.516	634	0.549	694	0.355
456	1.578	516	0.822	576	0.506	636	0.547	696	0.306
458	1.557	518	0.810	578	0.489	638	0.543	698	0.260
460	1.551	520	0.805	580	0.469	640	0.543	700	0.223

Table 4. Normalized backscattering for *Trichodesmium* calculated as a ratio of b_{btri} for tchl a = 2.42 mg m^{-3} divided by $<b_{btri}>$ as in Eq. (11). Values of $b^*_{btri}(\lambda)$ can be constructed by setting the value of the vector at 676 nm to 0.0077 m^2 mg^{-1}, and scaling for the other wavelengths accordingly.

Parameter	Wavelength (nm)					
	442	488	510	550	620	676
Norm. b_{btri}	1.231	1.104	1.112	0.915	0.897	0.740

4 DISCUSSION AND CONCLUSION

Detection and quantification of *Trichodesmium* by remote sensing assumes that chlorophyll-specific absorption and backscattering properties of this organism, in the visible channels as well as in the UV, are different from those of other phytoplankton. This implies a maximum

precision on the knowledge of the bio-optical properties of *Trichodesmium*, which is obtained by the use of new optical instruments, and measurements in mesocosms.

First, the use of a PSICAM allowed the accurate determination of ß for *Trichodesmium* spp. suspensions. ß was within the range of values determined with other methods and for different species (between 0.31 and 0.38; [37]) and was not 1 as suggested previously [7]. This result gives a full confidence in the QFT method for quantifying *Trichodesmium* absorption even in complex natural communities. Indeed, a PSICAM [18] (no filtration) or absorption and scattering- meters profilers (ac-s, WETLabs) would provide better optical density estimates at sea. These instruments should allow the discrimination in waters dominated by *Trichodesmium* from other waters using the PE and PC absorption between 500 and 650 nm. Estimating the true PE concentration as a cyanobacterial marker will be achievable with spectral decomposition methods [23, 38] and the proportion of *Trichodesmium* could be determined using its normalized absorption spectrum [28]. In the UV, the QFT method successfully detected the absorption peaks of MAA's as soon as one colony or a few filaments is present on the filter and therefore can be used to detect specifically *Trichodesmium* at least in the Pacific ocean where other species with MAA's are scarce. Again, a PSICAM [19] or a sensitive 200 cm capillary cell (Ultrapath, WPI) will provide a better assessment of the contribution of *Trichodesmium* MAA's to the total in situ CDOM absorption.

Tank measurements provided the first measurements of the *Trichodesmium* b_{bp} with the H6 VSF(140°). The b_{bp} slope for *Trichodesmium* (γ=1.2) is lower than b_{bp} slopes for cultured phytoplankton cells (γ=1.4), in [20]. It alternatively exhibited a monotonically decreasing with wavelength or troughs linked to pigment absorption. This spectral shape depends on the conversion factor which might not be neutral [39], and on the σ-correction that was applied. Experimental and model results show that for certain species with a high refraction index and high pigment content, scattering spectra exhibit troughs at the wavelengths of the chlorophyll absorption peaks [40]. Observed troughs at 550 nm would indicate the presence in front of the H6 detectors of phycoerythrin-rich *Trichodesmium* colonies. As shown in Sec. 3.2, the H6 confirmed that *Trichodesmium* colonies are backscattering 10 times more than any other phytoplankton species [15]. At 442 nm, the b^*_{btri} coefficient in tank is 36 % higher than the value used for the *Trichodesmium* bio-optical model (including detritus) [10], which is itself 6-fold higher than the b_{bp} previously measured for isolated colonies [7]. Could the tank values of b_{btri} be compared to modelled values for Case 1 waters? At 660 nm, an estimate of the backscattering ratio ($\tilde{b}_b$) was calculated from relationships between b_p and tchl *a* or Particulate Organic Carbon (POC) concentrations representative of case 1 waters [41]. When b_p(660) in the tank was calculated directly from chl *a* (as in Eq. (6) in [41]) or after conversion to POC (as in Eq. (12) in [41]), and assuming a mean ratio of POC:Chl *a* for *Trichodesmium* colonies of 200 [1]), the mean $\tilde{b}_b$ was 2.7 % or 1.7 %, respectively. Indeed, these tank $\tilde{b}_b$ values are higher than those measured in Case 1 ocean surface waters [31, 42] and are close to those of nepheloid layers with a high refraction index from minerals [43]. Under non-bloom conditions, recent findings showed that the backscattering coefficient in the oceans is mainly determined by small undefined particles (< 0.5 µm) of mineral origin [14], or colloids [44]. With *Trichodesmium* in tanks, small particles at least partly related to bacteria and other microorganisms (e.g. viruses and protozoa: the "trichosphere" [45]), or sub-micron detritus co-varying with *Trichodesmium*, could be another source of backscattering. Previous studies showed that sub-micron detritus (and minerals) could represent 31% (52 %) of b_{bp} in the continental shelf during summer [11] and a large fraction of b_{bp} in the equatorial Pacific and tropical oceans [11, 46]. Minerals and detritus would be the source of the 2-fold increase of $b_{b\text{-}H6}$ in coral reef lagoon waters compared to the tropical ocean off the barrier reef (unpubl. res.). These could be trapped within *Trichodesmium* colonies. As the Hydroscat-6 is strongly

influenced by sub-micron particles, an instrument measuring the full Volume Scattering Function at different wavelengths should be used simultaneously to distinguish between the one of *Trichodesmium* colonies and the one of their associated sub-micron material.

At sea, the influence of the trichosphere is unknown. In the SWTP, cruise data are the first determinations of $b_p(660)$ and $b_{bp}(660)$ in *Trichodesmium* rich waters (unpublished data at http://www.obs-vlfr.fr/proof/vt/op/ec/diapazon/dia.htm). Values of $\widetilde{b}_b$ in the first 10 meters at sea were between 0.6 and 1%, on average 3 times lower than for *Trichodesmium* in tanks. This could also be the result of a homogeneous vertical distribution of *Trichodesmium* in the upper layer during cruises.

Would *Trichodesmium* be easily detected at sea by remote sensing? The low B/R absorption ratios as observed in tanks at the highest *Trichodesmium* concentrations (and still higher than those of large microplankton [28]), would allow detection of the densest *Trichodesmium* populations, such as those found during summer in the lagoons and open waters of the Tropical Pacific [22, 47]. The absorption peaks of MAA's can be unambiguously associated with *Trichodesmium* at least in the SWTP since large dinoflagellates were absent [47]. Indeed, in the well-lit surface waters of tropical oceans, photobleaching may reduce absorption of CDOM in the UV (although at 313 and 333 nm MAA's peaks were detected during summer periods only [48]). The characteristic absorption spectra and H6-VSF(140°) b_{bp} signatures would be inverted from satellite ocean colour reflectance [49]. $\widetilde{b}_b$, similar to that measured in the tank, would have to be found in the first meter of *Trichodesmium* surface slicks (as in [50]) and the specific above-water R_{rs} signature may be detected in extremely calm sea conditions, with satellite sensors designed with high spatial and radiometric resolutions. If we consider the same biomass of *Trichodesmium* inside a thin slick or mixed in the first 20m of the ocean, it is still not evident that the mixed population could be seen as easily from space as a surface slick. However, the mixed population may be in a better physiological state and therefore play a more important role in C and N cycles.

The bio-optical properties of *Trichodesmium* determined here are specific enough to detect *Trichodesmium* in the surface of the ocean. This experimental knowledge will help in the analysis of absorption, backscattering, and reflectance data over the ocean and for the elaboration of dedicated algorithms for future hyperspectral sensors. The challenge is a true quantification of *Trichodesmium* biomass by ocean colour, and a true estimation of oceanic N_2 fixation and primary production [51] via climatological satellite surveys, and finally, a better knowledge of the biogeochemical role of the ocean in climate regulation [1].

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Table 5. Basis vectors representing the normalized above-water remote sensing reflectance for *Trichodesmium* calculated as a ratio of R_{rs} divided by $<R_{rs}>$ as in Eq. (12). Wavelength (λ) in nm.

λ	Tricho	λ	Tricho	λ	Tricho	λ	Tricho	λ	Tricho
400	0.736								
402	0.742	462	0.796	522	1.114	582	1.388	642	1.254
404	0.740	464	0.801	524	1.119	584	1.413	644	1.253
406	0.733	466	0.806	526	1.120	586	1.430	646	1.245
408	0.720	468	0.813	528	1.116	588	1.440	648	1.233
410	0.704	470	0.820	530	1.110	590	1.446	650	1.213
412	0.689	472	0.827	532	1.102	592	1.447	652	1.188
414	0.677	474	0.833	534	1.094	594	1.446	654	1.156
416	0.665	476	0.836	536	1.085	596	1.443	656	1.114
418	0.655	478	0.835	538	1.077	598	1.439	658	1.063
420	0.645	480	0.831	540	1.070	600	1.434	660	1.007
422	0.638	482	0.823	542	1.065	602	1.426	662	0.949
424	0.630	484	0.811	544	1.061	604	1.416	664	0.891
426	0.621	486	0.797	546	1.060	606	1.404	666	0.835
428	0.610	488	0.782	548	1.063	608	1.389	668	0.785
430	0.595	490	0.768	550	1.070	610	1.371	670	0.745
432	0.580	492	0.758	552	1.081	612	1.353	672	0.715
434	0.568	494	0.752	554	1.095	614	1.334	674	0.692
436	0.561	496	0.752	556	1.111	616	1.315	676	0.678
438	0.559	498	0.759	558	1.125	618	1.298	678	0.675
440	0.563	500	0.776	560	1.134	620	1.282	680	0.685
442	0.575	502	0.804	562	1.137	622	1.269	682	0.712
444	0.597	504	0.839	564	1.136	624	1.259	684	0.759
446	0.626	506	0.879	566	1.135	626	1.250	686	0.831
448	0.659	508	0.920	568	1.137	628	1.244	688	0.927
450	0.691	510	0.961	570	1.150	630	1.240	690	1.035
452	0.722	512	0.999	572	1.176	632	1.239	692	1.145
454	0.749	514	1.032	574	1.213	634	1.240	694	1.259
456	0.770	516	1.061	576	1.260	636	1.244	696	1.372
458	0.783	518	1.085	578	1.308	638	1.247	698	1.477
460	0.790	520	1.103	580	1.353	640	1.252	700	1.576

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