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Nitrogen fixation and growth rates of *Trichodesmium* IMS-101 as a function of light intensity

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ABSTRACT: The diazotrophic cyanobacterium *Trichodesmium* is a significant contributor to marine nitrogen and carbon cycles and has been incorporated in biogeochemical ocean circulation models. To date, parameterization of light as a controlling factor for nitrogen fixation has been based on field observations, where factors other than light also affect *Trichodesmium* physiology. Here we present data on light-dependent (15 to 1100 µmol quanta m^{-2} s^{-1}) diazotrophic growth from controlled laboratory experiments and their implications for modeling approaches. We supply a simple empirical model to describe nitrogen fixation by *Trichodesmium* in batch cultures. Diazotrophic growth of axenic *Trichodesmium* IMS-101 was light saturated at 180 µmol quanta m^{-2} s^{-1} and did not vary significantly at higher photon irradiances up to 1100 µmol quanta m^{-2} s^{-1} ($\mu_{carbon\ based} \approx 0.26\ d^{-1}$). Chlorophyll *a* (chl *a*) normalized N_2 fixation rates were significantly affected by light intensity during mid-exponential growth (0.74 to 4.45 mol N fixed mol chl a^{-1} h^{-1}) over the range of photon irradiances tested. In contrast, nitrogen fixation rates normalized to the cellular carbon content were relatively unaffected by light intensity (0.42 to 0.59, averaging 0.5 mmol N mol particulate organic carbon $[POC]^{-1}$ h^{-1}). *Trichodesmium* carbon biomass can be used to estimate the nitrogen input by this diazotroph into the ocean; the maximum input rate is 350 nmol N fixed l^{-1} h^{-1}.

KEY WORDS: *Trichodesmium* · Light · Nitrogen fixation · Marine nitrogen cycle · Marine carbon cycle · Marine cyanobacteria · Diazotrophic growth

INTRODUCTION

Trichodesmium is a filamentous cyanobacterial diazotroph that can simultaneously fix nitrogen and carbon in daylight (Gallon et al. 1996, Bergman et al. 1997). This pattern has been reported for only 2 other cyanobacteria, *Lyngbya majuscula* and *Symploca* sp. (Jones 1990, Fredriksson et al. 1998). *Trichodesmium* is very abundant and is recognized as one of the most important nitrogen fixers in tropical and subtropical oligotrophic oceans (Capone et al. 1997). Current estimates of marine nitrogen fixation range between 100 and 200 Tg yr^{-1}, with *Trichodesmium* contributing 80 to 110 Tg N yr^{-1} (Gruber & Sarmiento 1997, Capone & Carpenter 1999, Karl et al. 2002).

Nitrogen fixation is an integral part of ocean biogeochemical circulation models (OBCM) and *Trichodesmium* is used as a model organism representing diazotrophic growth (Fennel et al. 2001, Hood et al. 2001, 2002, 2004). The parameterization of nitrogen fixation in OBCMs has improved during recent years as *Trichodesmium* sp. receives an increasing amount of scientific attention (LaRoche & Breitbarth 2005). While the effect of macro- and micro-nutrient availability on nitrogen fixation, carbon fixation, and growth

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of *Trichodesmium* have been characterized to a large extent, the effect of irradiance as an important forcing variable has not been fully described.

Fennel et al. (2001) and Hood et al. (2001) supplemented the lack of precise information on irradiance versus nitrogen fixation patterns by adapting parameters from photosynthesis versus irradiance (*P* vs. *E*) functions. For example, Carpenter et al. (1993) subjected field-collected *Trichodesmium erythraeum* from one sampling depth to photon irradiances up to 2500 µmol quanta m^{-2} s^{-1} of photosynthetically active radiation (PAR), equivalent to unattenuated surface irradiance, and described a *P* vs. *E* resembling nitrogen fixation pattern as a function of irradiance with indication of photoinhibition. Hood et al. (2002) based model equations for nitrogen fixation of *Trichodesmium* sp. on field observations and used a similar approach. Some estimates of areal nitrogen input derived from ship-based incubations of *Trichodesmium* colonies at different light intensities exist (Orcutt et al. 2001, Capone et al. 2005) and were recently adapted to demonstrate the great significance of *Trichodesmium* nitrogen fixation in the North Atlantic Ocean (Davis & McGillicuddy 2006). In general though, it is difficult to isolate and parameterize the effect of a single forcing variable such as light, temperature or nutrient availability on the basis of patterns observed in the natural environment. Since physiological measurements of natural populations are subject to uncertainties and show large variability, laboratory experiments are needed to isolate the effect of individual forcing variables.

Published laboratory experiments assessing diazotrophic growth of *Trichodesmium* have used growth conditions that provide between 10 and 14 h of full light intensity (~40 to 100 µmol quanta m^{-2} s^{-1}) and a dark period for the rest of the day (L:D cycle). The effect of short-term exposure (a few hours) of up to 180 µmol quanta m^{-2} s^{-1} revealed that nitrogen fixation rates increased with irradiance (Ohki & Fujita 1988, Fu & Bell 2003), which has been attributed to the increased availability of carbon metabolites from photosynthesis (Rabouille et al. 2006). However, cultures were not grown at the respective light intensities. In models describing diazotrophic growth of *Trichodesmium*, the light saturation irradiance is set at 100 µmol quanta m^{-2} s^{-1} for example, and a light inhibition parameter between 350 and 1195 µmol quanta m^{-2} s^{-1} is used (Hood et al. 2002, Rabouille et al. 2006). The saturation irradiances applied are relatively low considering that *Trichodesmium* is a surface dweller in high light sub-tropical environments. A confirmation or better determination of the irradiances saturating and inhibiting nitrogen fixation and growth is required.

Here we present results from 2 independent batch culture experiments, one using L:D cycle conditions (50 and 900 µmol quanta m^{-2} s^{-1}) and one applying a natural light cycle with peak intensities between 15 and 1100 µmol quanta m^{-2} s^{-1}, to elucidate the effects of light intensity and light regimes on diazotrophic growth of *Trichodesmium*. Further, laboratory experiments using batch cultures must take into account the fact that nitrogen fixation and carbon fixation rates are a function of cell growth phase as well as photoperiod phase. In general, nitrogen fixation rates are elevated during the exponential growth phase and are maximal at midday. Shifts in growth phase-dependent nitrogen and carbon assimilation rates are represented in elemental stoichiometry changes (Chen et al. 1998, Berman-Frank et al. 2001, Mulholland & Capone 2001). Accordingly, the experiments presented here also aim to describe a light-dependent stoichiometry of particulate organic carbon and nitrogen (POC, PON), chlorophyll *a* (chl *a*), and total protein content of cultures grown at different light intensities. Chl *a* and POC are commonly used as biomass measures in the ocean. As the chl *a* content in phytoplankton cells is acclimated to light intensity (Geider et al. 1997), we compare nitrogen fixation rates normalized either to chl *a* or POC. Finally, the results are explained in terms of a simple empirical model to describe diazotrophic growth and nitrogen input into the oligotrophic surface oceans by *Trichodesmium* as a function of light intensity.

MATERIALS AND METHODS

Growth of *Trichodesmium* cultures. The strain *Trichodesmium* IMS-101 was grown at 26°C under axenic conditions using phosphorus and iron replete YBC II media without added dissolved nitrogen (Chen et al. 1996). Axenic conditions were verified frequently by filtration of culture material onto a 0.2 µm polycarbonate membrane, Acredine Orange staining, and subsequent microscopy. Cultures were transferred during the exponential growth phase into 2 l polycarbonate bottles, using 3 replicates at each light intensity. A light- and temperature-controlled incubator (Rumed) was set up to imitate the natural solar cycle of 12 h at 0° N 90° W in January (first day of the year). The cycle was modified by setting light intensities >97% as 100%, resulting in a 2 h peak intensity. Photon irradiances of 15, 50, 180, 300, 600, 900, and 1100 µmol quanta m^{-2} s^{-1} (PAR) were created using neutral density screening and verified by measuring light penetration into a water-filled incubation bottle using a submersible 4π PAR sensor (LiCOR). In a separate experiment, cultures were

grown at a cycle of 10 h full photon irradiance (50 and 900 µmol quanta m^{-2} s^{-1} PAR), a dusk and dawn phase of 1 h each, and dark phase of 12 h. Over the course of the growth period, samples for all parameters were generally taken at 11:00 h. Maximum specific growth rates (µ) were determined by identifying the exponential growth phase in the batch cultures and applying a linear fit to the respective natural logarithm transformed POC, PON and chl *a* values. The slope of the regression represents the growth rate.

Chl *a* analysis. Chlorophyll samples were filtered on GF/F filters, stored at –20°C, and analyzed by fluorometry after bursting the cells in 90% acetone by shaking and refreezing for 24 h (modified after Welschmeyer 1994). This simple extraction method proved to be as efficient for chl *a* analysis of *Trichodesmium* as chl *a* extraction after mechanical disruption of the cells (E. Breitbarth unpubl. data).

Elemental analysis of PON and POC. Culture material was filtered on pre-combusted GF/F filters, frozen at –20°C for intermediate storage and finally dried for 48 h at 45°C. Filters were analyzed for PON and POC content on an elemental analyzer (Euro-EA, Hekatech) equipped with a chromium-oxide/cobalt-oxide oxidation reactor, a copper reduction reactor and a CHN column at an oven temperature of 45°C. Carrier gas flow (He) was set at 96 ml min^{-1}. Measurements were corrected for blank values using measurements of similarly treated filters without culture material (Ehrhard & Koeve 1999).

Nitrogen fixation measurements. Nitrogen fixation was measured using the Acetylene Reduction Assay (ARA) as described in Capone (1993) and Capone & Montoya (2001), with calculations modified after Breitbarth et al. (2004). Gas samples were analyzed on a Shimadzu GC-19B gas chromatograph equipped with a flame ionization detector and a 30 m wide-bore capillary column (0.53 mm, AluminaPlot®). This set-up, an oven temperature of 40°C, injector and detector temperature of 200°C, and a carrier gas flow (N_2) of 14.5 ml min^{-1} yielded optimal peak separation and detection limits. Three replicates for each light intensity were incubated simultaneously for 2 h in 20.2 ml headspace vials containing 19 ml of culture and 1.2 ml headspace with 0.4 ml acetylene added. ARAs were carried out every second day at peak light intensity. A ratio of C_2H_2 reduced:N_2 reduced of 4:1 was used (Montoya et al. 1996) and nitrogen fixation data were normalized to POC and chl *a* content of the cultures. The ARA is prone to methodological error and can yield variability in the data output. The coefficient of variation (CV*, corrected for bias in a small sample size of 3 replicates, Sokal & Rohlf 1995) was used to estimate the variability between triplicate measurements. Data resulting in a CV* > 25 were excluded from the analysis after identifying the source of error. Common errors include false biomass determinations (disagreement in POC/PON and chl *a* values, aggregate formation by *Trichodesmium* and thus patchy biomass distribution amongst the 3 replicate vials), leaking crimp seals, or syringe needles blocked by seal material (causing injection volumes that were too low, yielding false low ethylene values).

Photosystem response measurements. A PhytoPAM equipped with Optical Unit ED-101US/MP (Walz) was used to determine the photosynthetic quantum use efficiency by measuring the ratio of variable to maximal fluorescence (F_v/F_m) of *Trichodesmium*'s photosystem I in response to different light intensities (Kolbowski & Schreiber 1995). F_v/F_m was recorded every second day over the complete growth period of the cultures. Samples were dark-adapted for 10 min prior to the measurements.

Protein analysis. Protein samples were taken at noon and midnight on Day 16 of the natural light cycle experiment. Sample material was extracted from filtered *Trichodesmium* material by sonicating the filters in SDS/CO_3 buffer (LaRoche et al. 1993). Total protein was analyzed according to the bicinchoninic acid method using BCA protein assay reagents (Pierce) and a 96-well plate reader. The absorbance signal was calibrated against 0 to 1500 µg protein l^{-1} standards (Smith et al. 1985).

Statistical analysis. All data were analyzed by applying 1-way ANOVA models and Fisher post-hoc tests using StatView (Version 5.0.1, SAS). Time series measurements were analyzed using a repeated measures 2-way ANOVA model. The significance level was set at $p < 0.05$.

RESULTS

Growth of *Trichodesmium* cultures

Trichodesmium IMS 101 grown under a natural cycle of different light intensities possessed typical microbial batch culture growth patterns with a clear exponential and stationary phases. The lag phase was reduced as cells were transferred during the exponential growth phase.

Maximum PON and POC biomasses were reached at 300 µmol quanta m^{-2} s^{-1} and maximum chl *a* biomass was reached at 180 and 300 µmol quanta m^{-2} s^{-1} at Day 20 (Fig. 1, Appendices 1 & 2, available at: www.int-res.com/articles/suppl/m359p025_app. pdf). Cultures grown in a separate experiment under constant light for 10 h d^{-1} (L:D cycle) reached higher biomasses at 50 µmol quanta m^{-2} s^{-1} and lower

biomasses at 900 µmol quanta m^{-2} s^{-1} than cultures grown under natural light conditions during the same period of growth (Appendix 1). The L:D cycle experiment was stopped at Day 15 after PhytoPAM measurements indicated reduced cellular fluorescence; carbon- and nitrogen-specific growth also declined. The natural light cycle experiment was continued until Day 20. Final biomasses of POC and chl *a* diverged, and the high light treatments had a reduced chl *a* biomass (due to photoacclimation) and is reflected in the chl *a*:POC ratio (Fig. 2, Appendix 3, available at: www.int-res.com/articles/suppl/m359p025_app.pdf). Reduced biomasses at high light intensities are not significantly reflected in the maximum growth rates specific to each light intensity (Fig. 3), but are accounted for as an irradiance-specific maximum biomass in the model.

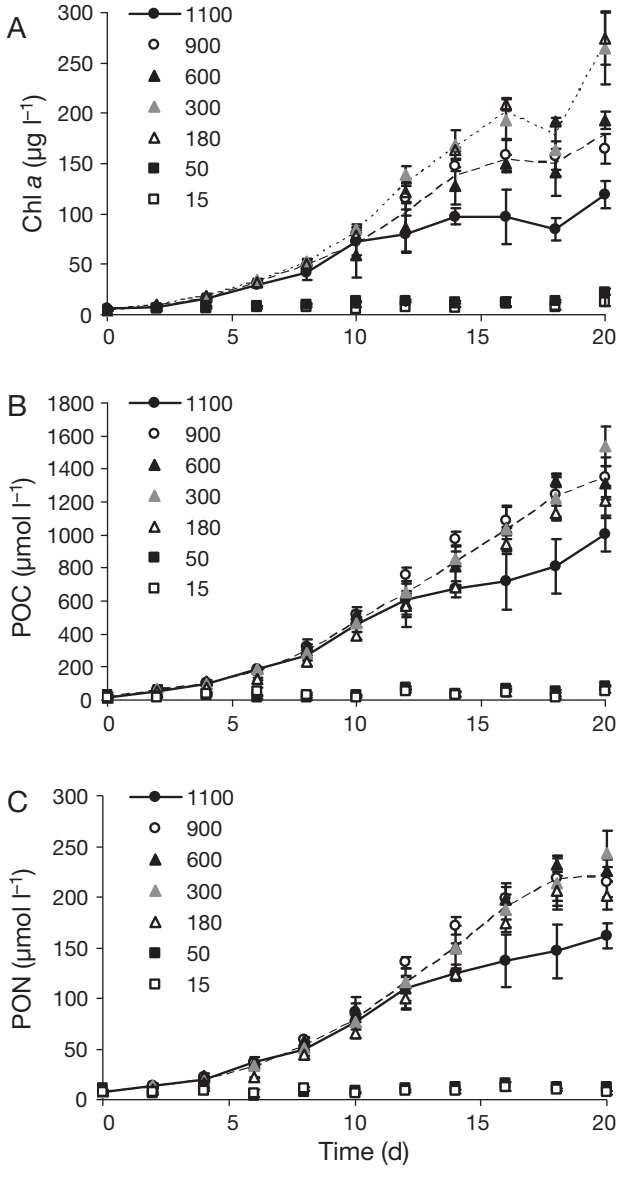

Fig. 1. (A) Chlorophyll *a* (chl *a*) concentrations, (B) carbon-specific biomass, and (C) nitrogen-specific biomass as a function of photon irradiance and growth phase of the natural light cycle experiment. (A) Mean chl *a* concentrations of the: 180 and 300 (····), 600 and 900 (- - - -), and 1100 (——) µmol quanta m^{-2} s^{-1} photosynthetically active radiation (PAR) light treatment. (B) Mean particulate organic carbon (POC) concentrations of the: 180 to 900 (- - - -) and 1100 (——) µmol quanta m^{-2} s^{-1} PAR light treatment. (C) Mean particulate organic carbon (PON) concentrations of the: 180 to 900 (- - - -) and 1100 (——) µmol quanta m^{-2} s^{-1} PAR light treatment. (A to C) Symbols show photon irradiance (µmol quanta m^{-2} s^{-1} PAR). Data points are means ± SD

The experiment conducted with the natural solar cycle yielded increasing growth rates up to 180 µmol quanta m^{-2} s^{-1}. A significant effect of light intensity was detected for carbon-, nitrogen- and chlorophyll-specific growth rates (ANOVA, $p < 0.0001$). Carbon-specific growth rates at 15 and 50 µmol quanta m^{-2} s^{-1} (μ_{POC} = 0.03 d^{-1} and 0.08 d^{-1}, respectively) differed significantly (post-hoc *F*-test, $p < 0.05$) from those from higher irradiances and from one another. Carbon specific growth at light intensities <180 µmol quanta m^{-2} s^{-1} averaged 0.26 d^{-1} and did not differ significantly. Chl *a*-specific growth rates were similar to carbon-specific growth (Fig. 3). Nitrogen-specific growth rates were lower than carbon- and chlorophyll-specific growth rates for all treatments, whereas general trends were similar. Nitrogen-specific growth rates ranged from 0.02 to 0.03 d^{-1} at 15 and 50 µmol quanta m^{-2} s^{-1}, respectively, to an average of 0.23 d^{-1} at 180 to 1100 µmol quanta m^{-2} s^{-1}.

Carbon- and nitrogen-specific growth rates under L:D cycle conditions and the simulated natural solar cycle were not statistically different at 50 µmol quanta m^{-2} s^{-1}; both rates were significantly reduced at 900 µmol quanta m^{-2} s^{-1} and they were also significantly different from one another at this irradiance level (0.19 d^{-1} carbon-specific and 0.16 d^{-1} nitrogen-specific growth rate, Fig. 3, nitrogen-specific growth rates not shown). Overall, when plotted against light intensity as a function of light attenuation at depth in oligotrophic seawater (Fig. 3), the growth rates matched the typical depth distribution of *Trichodesmium* observed in the field (Capone et al. 1997).

The energy provided by the L:D cycle treatments amounted to 1.48× the energy supplied by the natural light treatment over the course of a day. Thus, integrated over the light period, 50 and 900 µmol quanta m^{-2} s^{-1} from the constant L:D cycle provide the same photon doses as 74 and 1330 µmol quanta m^{-2} s^{-1} in the simulated natural light cycle treatment, respectively. Therefore, the reduced growth rates between 1100 (natural light cycle) and 1330 (converted from 900 µmol quanta m^{-2} s^{-1} L:D cycle) may indicate photoinhibition of diazotrophic growth.

Stoichiometry of PON, POC and chl *a*

Like growth rates, POC:PON ratios (mol:mol) were similar (overall ~5.4 to 5.6) at photon irradiances ≥180 µmol quanta m^{-2} s^{-1}. In comparison, the POC:PON ratios at 15 and 50 µmol quanta m^{-2} s^{-1} were significantly reduced and averaged 3.6 and 3.8, respectively. Values only increased at the end of the experiment to values near the other light treatments. Ratios of POC:PON from cultures grown at 180 to 1100 µmol quanta m^{-2} s^{-1} increased with growth phase from ~4.5 to 5.5 (Days 2 to 10), values slightly decreased thereafter until Day 16, recovered afterwards, and averaged 5.7 to 5.9 (Appendix 4, available at: www.int-res.com/articles/suppl/m359p025_app.pdf).

As expected, the ratio of chl *a*:POC (w:w) in *Trichodesmium* decreased with increasing light intensity. For example, chl *a*:POC at 180 µmol quanta m^{-2} s^{-1} averaged 0.018 and decreased to 0.012 at 1100 µmol quanta m^{-2} s^{-1}. At 15 and 50 µmol quanta m^{-2} s^{-1}, the ratio was highly variable, averaging at 0.028. A maximum of 0.058 was measured. For details see Fig. 2 and Appendix 3.

Total protein content

Total protein contents of the cultures were analyzed on Day 16 (Fig. 2). Like other biomass parameters, total protein content was significantly affected by the light treatment (ANOVA, $p < 0.0001$). Values ranged from 708 to 5763 µg protein l^{-1} at 50 and 300 µmol quanta m^{-2} s^{-1}, respectively. No significant differences in total protein content were detected between 15 and 50 µmol quanta m^{-2} s^{-1} , or among light intensities between 180 and 900 µmol quanta m^{-2} s^{-1}. The total protein content was significantly reduced at 15 to 50 and at 1100 µmol quanta m^{-2} s^{-1} compared to the rest of the treatments (post-hoc *F*-tests, $p < 0.05$). Nighttime measurements taken 12 h after the day-time measurements verified these trends. Differences from daytime measurements were in accord with growth rates (data not shown).

The POC:total protein ratio (weight:weight) pattern in relation to photon irradiance resembles that of the POC:PON ratio. Values ranged from 0.5 to 2.4 and were reduced in light treatments <180 µmol quanta m^{-2} s^{-1} in comparison with higher light intensities (Fig. 2). The low POC:PON ratios in the low light treatments indicate that most PON was present as pure protein.

Cellular fluorescence and photosynthetic efficiency

Overall, F_v/F_m increased from ~0.15 to ~0.28 from Day 2 to Day 10 of the growth period, regardless of the light treatment. While high light treatments in general

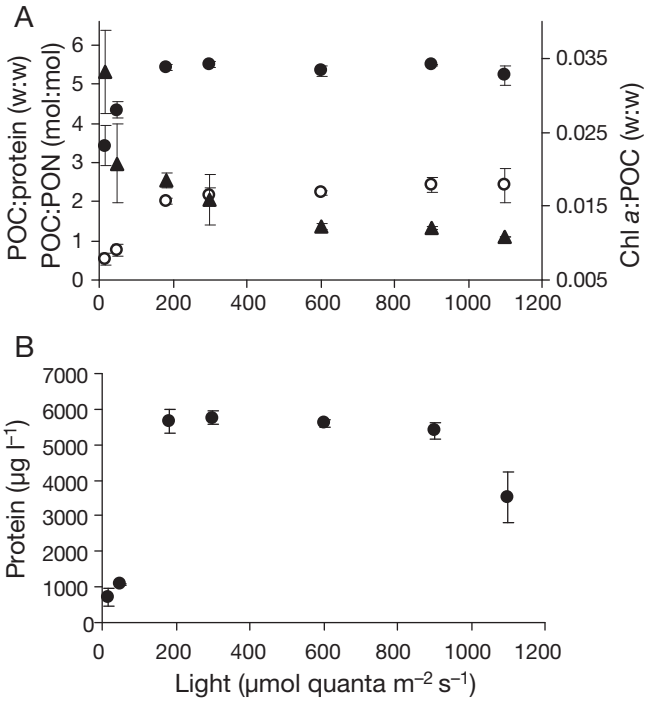

Fig. 2. (A) Overview of POC:total protein (○), POC:PON (●) and chl *a*:POC (▲) stoichiometry of *Trichodesmium* IMS-101 grown at different light intensities on Day 16 of the natural light cycle experiment. (B) Total cellular protein content on Day 16 of the natural light cycle experiment plotted against light intensity. Data points are means ± SD

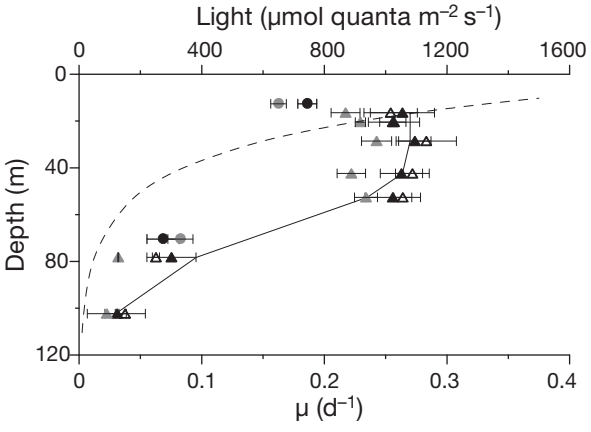

Fig. 3. Carbon- (▲), chlorophyll *a*- (Δ), and nitrogen- (▲) specific growth rates (µ, lower *x*-axis) plotted against light penetration depth using a surface photon irradiance of 2500 µmol quanta m^{-2} s^{-1} photosynthetically active radiation (PAR) and a light attenuation coefficient of 0.05. Growth rates from the light:dark (L:D) cycle experiment are plotted as circles using the same grey-scale code. Carbon-specific growth rates (—) are derived from the data based on Eq. (1). Respective light intensity at depth (----), refers to the upper *x*-axis. Data points are means ± SD

had lower F_v/F_m ratios than low light treatments, it is only possible to clearly distinguish 2 groups of responses from Day 14 onward. Photon irradiances between 600 and 1100 µmol quanta m^{-2} s^{-1} produced F_v/F_m ratios of 0.15 to 0.30, while cultures grown at lower intensities continued to yield higher F_v/F_m values, peaking between 0.39 and 0.43 on Day 18. The highest photosynthetic efficiency on average was measured at 180 µmol quanta m^{-2} s^{-1} (Fig. 4).

Nitrogen fixation measurements across light intensities and growth phase

We tested whether nitrogen fixation rates differ as a function of growth phase, light intensity and normalization to a specific biomass parameter. Nitrogen fixation rates were significantly affected by the growth phase of the culture regardless of which biomass parameter was used for normalization (repeated measures ANOVA, $p < 0.01$) (Appendices 5 & 6, available at: www.int-res.com/articles/suppl/m359p025_app. pdf). In the beginning of the growth phase, N-fixation rates were generally high, likely due to the fact that cultures were transferred from a start culture during the exponential growth phase. Fixation rates decreased until Day 8, the onset of the linear phase of exponential growth (Fig. 1), and reached a maximum at Day 12, after which they decreased again. Cultures grown at 15 and 50 µmol quanta m^{-2} s^{-1} had increased nitrogen fixation rates towards the end of the experiment (Days 18 and 20). When normalized to POC, nitrogen fixation rates did not show a significant relationship with varying photon irradiance (ANOVA, $p = 0.25$). In contrast, chl *a* normalized nitrogen fixation rates differed significantly among irradiances (ANOVA, $p < 0.01$). This was particularly evident during mid exponential growth (Days 8 to 12). At Day 12, rates ranged from 0.74 mol N_2 fixed (mol chl *a*)$^{-1}$ h^{-1} at 15 µmol quanta m^{-2} s^{-1} to 4.45 mol N_2 fixed (mol chl *a*)$^{-1}$ h^{-1} at 1100 µmol quanta m^{-2} s^{-1}; carbon-specific nitrogen fixation rates averaged 0.5 mmol N_2 fixed (mol POC)$^{-1}$ h^{-1} (0.42 to 0.59) (Fig. 5). Frequently, POC- and chl *a*-specific fixation rates at 180 µmol quanta m^{-2} s^{-1} were relatively low compared to those at 50 and 300 µmol quanta m^{-2} s^{-1}. For detailed results see Appendices 5 & 6.

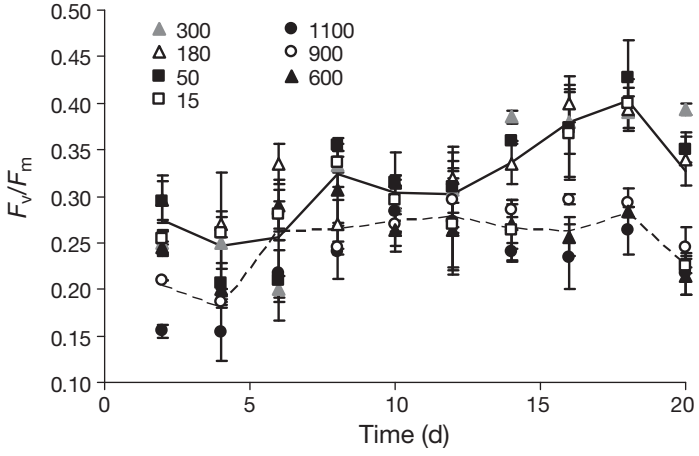

Fig. 4. Photosynthetic quantum use efficiency (ratio of variable to maximal fluorescence, F_v/F_m) of *Trichodesmium* IMS-101 as a function of growth phase and light intensity. Symbols show light treatments as µmol quanta m^{-2} s^{-1} photosynthetically active radiation (PAR) . Mean F_v/F_m values of the: 15 to 300 (——), and 600 to 1100 (- - - -) µmol quanta m^{-2} s^{-1} PAR light treatment. Data points are means ± SD

Modeling growth and nitrogen fixation by *Trichodesmium* as a function of light intensity

The data permit an empirical approach to describing new nitrogen input into seawater by *Trichodesmium* in batch culture incubations.

Specific growth rates under natural light conditions may be described by Eq. (1), (modified from Jassby & Platt 1975) using the parameters in Table 1:

$$\mu = \mu_{max} \tanh\left(\frac{\alpha I}{\mu_{max}}\right) \quad (1)$$

where μ_{max} is the maximum specific growth rate measured (0.27 d^{-1}) and α is the initial slope of the growth rate, 0.002 d^{-1} (µmol quanta m^{-2} s^{-1})$^{-1}$, plotted against photon irradiance (I in µmol quanta m^{-2} s^{-1}). Eq. (1) would be applicable only for photon irradiances up to 1100 µmol quanta m^{-2} s^{-1}, because reduced growth rates of the high light treatment in the L:D cycle experiment indicate photoinhibition. A light inhibition term is included at a later stage (see Eq. 6). It should further be noted that the maximum specific growth rate is also a function of temperature and can be corrected for different growth temperatures, if necessary (Breitbarth et al. 2007).

Trichodesmium biomass as a function of incubation time (number of days) and light intensity can be described as:

$$N_t = \frac{N_0\ K}{N_0 + (K - N_0)\ e^{-\mu\ t}} \quad (2)$$

where N_t represents the POC biomass (µmol l^{-1}) at time t (d), N_0 is the start value of the POC biomass (µmol l^{-1}), µ is the light-specific growth rate (d^{-1}) and K is the maximum POC biomass reached (µmol l^{-1}). Based on results presented above, a POC start biomass of 40 µmol l^{-1} and a maximum POC biomass of 1600 µmol l^{-1} were used.

POC-normalized nitrogen fixation rates are not a direct function of photon irradiance and thus carbon biomass can be used to approximate nitrogen fixation rates per unit volume. Data presented above demonstrate that *Trichodesmium* fixes approximately 0.5 nmol N_2 (µmol POC)$^{-1}$ h^{-1}. Thus, nitrogen fixation rate, nmol N l^{-1} h^{-1}, is directly correlated with photon irradiance-specific biomass in the batch cultures and nitrogen fixation per unit volume and can be described as:

$$N_{fix} \equiv \frac{N_t}{2} \tanh\left(\frac{\alpha_N I}{\frac{N_t}{2}}\right) \quad (3)$$

where α_N is the light absorption coefficient of POC biomass accumulation tuned to 1.6 µmol POC l^{-1} per unit light (µmol quanta m^{-2} s^{-1}).

At mid-exponential growth, observed maximum fixation rates averaged 350 nmol N l^{-1} at 300 µmol quanta m^{-2} s^{-1} (Fig. 6, Appendices 1 & 6). Growth dynamics eventually down regulate nitrogen fixation rates due to NH_4^+ exudation from cells (Mulholland & Capone 1999, Mulholland et al. 1999, 2001, Holl & Montoya 2005). Reduced fixation rates at high irradiances in the later growth phase were observed (Fig. 6b, Appendixes 5 & 6) and thus Eq. (3) would be valid only for cultures in the exponential growth phase. Mulholland et al. (2001) provide data on NH_4^+ and POC concentrations from batch cultures of *Trichodesmium* IMS-101 grown under conditions similar to those we used. We applied the NH_4^+ data from Mulholland et al. (2001) to derive a simplified relationship between POC concentration and NH_4^+ in solution:

$$NH_4^+ (\mu mol\ l^{-1}) \equiv \frac{N_t}{390} \quad (4)$$

and derived a correction factor F_t for nitrogen fixation using a type 4 Hill function. F_t is specific to an incubation time t and therefore also to the POC and the NH_4^+ concentration in solution:

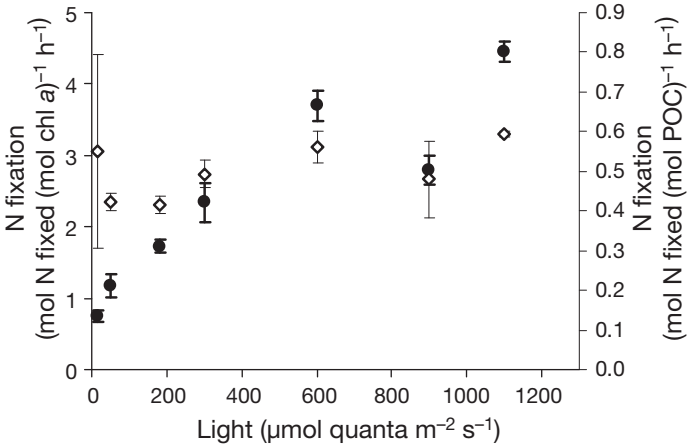

Fig. 5. Chlorophyll *a*-specific (mol N fixed (mol chl *a*)$^{-1}$ h^{-1}, ●) and carbon-specific (nmol N fixed (mmol POC)$^{-1}$, ◇) nitrogen fixation rates as a function of photon irradiance (µmol quanta m^{-2} s^{-1} photosynthetically active radiation, PAR). Measurements are from Day 12. The complete set of measurements is given in Appendices 5 & 6. Results from the experiment using a light:dark (L:D) cycle are not included here but are presented in Appendix 6. Data points are means ± SD

$$F_t = 1 + \frac{a\left(\frac{N_t}{390}\right)^b}{c^b + \left(\frac{N_t}{390}\right)^b} \quad (5)$$

where the parameters were tuned to the following values: a = 1.8, b = 8, c = 3.1.

Nitrogen fixation (N_{fix}, nmol N l^{-1} h^{-1}) is described based on Eq. (3), whereas the NH_4^+ inhibition correction factor (F_t) and a light inhibition parameter (I_{inh}) at 1200 µmol quanta m^{-2} s^{-1} are added.

$$N_{fix} = \frac{\frac{N_t}{2} \tanh\left(\frac{\alpha I}{\frac{N_t}{2}}\right)}{F_t} \times \frac{2t}{t_{max}} \times e^{\left(\frac{-I}{I_{inh}}\right)} \quad (6)$$

where t_{max} denotes the maximum incubation time in days.

Table 1. Parameters used for modeling growth and nitrogen fixation by *Trichodesmium* as a function of light intensity

Description	Symbol	Value	Units
Physical parameters			
Photon irradiance	I		µmol quanta m^{-2} s^{-1}
Light inhibition parameter	I_{inh}	1200	µmol quanta m^{-2} s^{-1}
Incubation time	t		d
Biological parameters			
Maximum growth rate	μ_{max}	0.27	d^{-1}
Light absorption coefficient for growth	α	0.002	d^{-1} (µmol quanta m^{-2} s^{-1})$^{-1}$
Start particulate organic carbon (POC) biomass	N_o	40	µmol l^{-1}
POC biomass at a given incubation time	N_t		µmol l^{-1}
Maximum POC biomass	K	1600	µmol l^{-1}
Nitrogen fixation rate	N_{fix}		nmol l^{-1} h^{-1}
Light absorption coefficient for POC accumulation	α_N	1.6	µmol l^{-1} (µmol quanta m^{-2} s^{-1})$^{-1}$
POC biomass-specific N_{fix} inhibition parameter	F_t		dimensionless

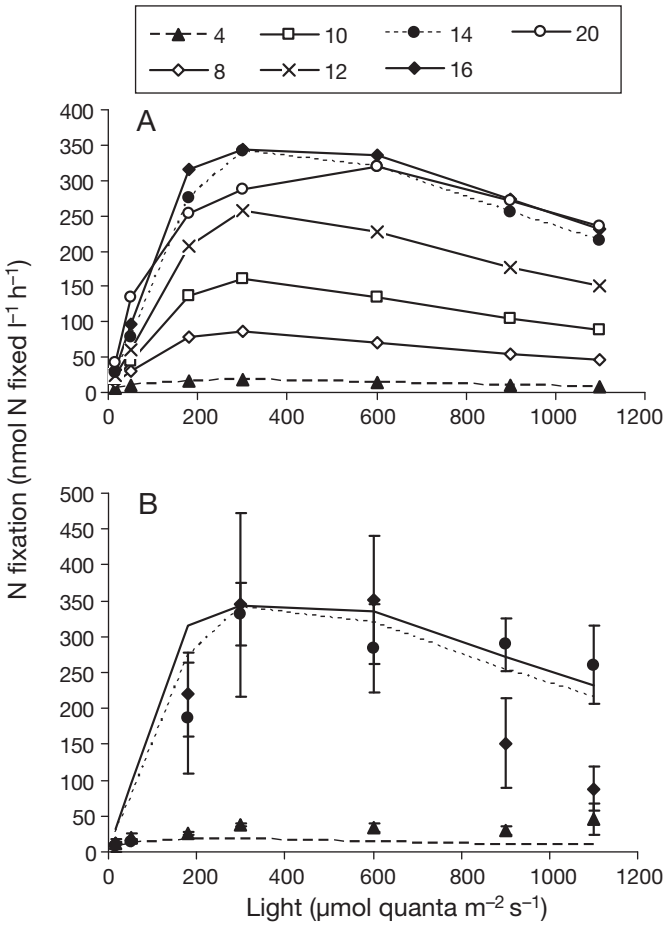

Fig. 6. (A) Modeled nitrogen fixation rates as a function of light intensity and growth phase. Key indicates days of growth. (B) Selection of measured nitrogen fixation rates on Days 4 (▲), 14 (●), and 16 (◆). Data are means ± SD. The model fit is plotted for comparison (Day 4: ----, Day 14: ·····, Day 16: ——).

Overall, the modeled nitrogen fixation rates are in reasonable agreement with the measured nitrogen fixation rates during the exponential growth phase. However, modeled results overestimate nitrogen fixation rates around the light compensation point (data here at 180 µmol quanta m^{-2} s^{-1}) and at high light intensities during the late growth phase. The model fit also yields nitrogen fixation rates that are too low at photon irradiances >180 µmol quanta m^{-2} s^{-1} during the early growth phase. The model applies photoinhibition regardless of the age of the culture, whereas this pattern may be affected by growth phase as indicated more strongly in the measured rates. Fixation rates were more reduced at high light intensities during the late growth phase than during exponential growth, where the model outcome acceptably represents the measured rates. Modeled and measured results agree well on maximum nitrogen fixation rates (~350 nmol N fixed l^{-1} h^{-1}) during mid and late exponential growth (Fig. 6). See Appendices 1 & 6 for exact values and SDs of carbon biomass and nitrogen fixation measurements.

DISCUSSION

The light regime in the euphotic zone of the open ocean is an important factor determining specific niches for photosynthetic organisms. The light energy absorbed by photosynthetic organisms is converted into ATP via photophosphorylation, which is then available for physiological processes. *Trichodesmium* sp. has a higher energy demand than other phototrophic organisms in order to maintain diazotrophic growth since nitrogen fixation requires an additional 8 e$^-$ and 16 ATP per molecule N_2 reduced to NH_4^+ in comparison to non-diazotrophic organisms that utilize dissolved NH_4^+. Additionally, the organism has to protect the nitrogenase enzyme against the photosynthetically evolved oxygen. Thus, *Trichodesmium* utilizes several energetically costly pathways to reduce intracellular oxygen concentrations down to a level allowing nitrogen fixation. High respiration rates and the photoreduction of oxygen in photosystem I (Mehler reaction) have been suggested as the main protective mechanism against oxygen damage in this non-heterocystous diazotroph (Kana 1991, Kana 1993, Carpenter & Roenneberg 1995). Overall, these energy requirements demand a light replete environment. Thus, not surprisingly, *Trichodesmium* is generally encountered in the upper portion of the euphotic zone and is well adapted to high radiation in sub-tropical/tropical oligotrophic surface waters (Carpenter & Roenneberg 1995, Capone et al. 1997). The results derived in our study support this view.

Here we elucidate the effect of different light intensities and light cycle characteristics on diazotrophic growth of *Trichodesmium* IMS-101. Growth rates increased up to photon irradiances of 180 µmol quanta m^{-2} s^{-1} and remained constant thereafter, as described by Eq. (1). Carbon-specific biomass was greatest at photon irradiances >300 µmol quanta m^{-2} s^{-1}, while photoinhibition for diazotrophic growth was indicated at high photon irradiances (>1100 µmol quanta m^{-2} s^{-1}). Chl *a* normalized nitrogen fixation rates were a function of photon irradiance, while POC normalized fixation rates were not. This effect was driven by the acclimation of chl *a* content per cell to specific light intensities (Figs. 2 & 5).

Light inhibition in *Trichodesmium* was indicated by the reduced growth rates of cultures subjected to 900 µmol quanta m^{-2} s^{-1} (L:D cycle conditions) in comparison with cultures grown using a natural solar cycle. The treatment with 900 µmol quanta m^{-2} s^{-1} for 10 h d^{-1}

plus 1 h of linear dusk and dawn phase equals a total photon flux of 36 mol m^{-2} d^{-1}. This is equivalent to the light energy of a natural solar cycle peaking at ~1330 µmol quanta m^{-2} s^{-1}. Thus, cultures grown under L:D cycle conditions receive a ~50% greater photon dose than cultures grown using a natural illumination cycle, which has to be considered in physiological experiments. Based on our findings, we conclude that photoinhibition can affect *Trichodesmium* blooms at the sea surface and the light inhibition term derived here (I_{inh}, 1200 µmol quanta m^{-2} s^{-1}; Eq. 6) closely matches the field observations of Hood et al. (2002) (1195 µmol quanta m^{-2} s^{-1}).

We therefore derive an optimum light regime for diazotrophic growth between 180 and 1100 µmol quanta m^{-2} s^{-1}. The cut-off in the increase in growth rate at a photon irradiance of 180 µmol quanta m^{-2} s^{-1} agrees reasonably well with reported light compensation points (I_c) in photosynthesis versus irradiance curves. For example, in field studies Carpenter et al. (1993) report I_c at ~150 µmol quanta m^{-2} s^{-1}, while Kana (1991) gave a value of 280 µmol quanta m^{-2} s^{-1}. Growth rates were constant above this light intensity ($\mu_{POC} \approx 0.26$ d^{-1}, Fig. 3) and nitrogen fixation rates were constant above 300 µmol quanta m^{-2} s^{-1} during mid-exponential growth (~350 nmol N fixed l^{-1} h^{-1}). Light inhibition can occur at photon irradiances above 1100 µmol quanta m^{-2} s^{-1}, which in most cases is only reached directly at the sea surface. In contrast, Carpenter & Roenneberg (1995) found that *Trichodesmium* is adapted to higher light regimes and can adjust its photosynthetic characteristics according to its position in the water column and to seasonal changes in the light regime. While they (op. cit.) detected no light inhibition of photosynthesis at 2500 µmol quanta m^{-2} s^{-1}, light inhibition of nitrogenase activity in *Trichodesmium erythraeum* at the same photon irradiance was noticed in another study (Carpenter et al. 1993). The culture organism used in our study *Trichodesmium* IMS-101 is an isolate of *T. erythraeum* (Janson et al. 1999) and was raised at the respective light intensities used for subsequent experiments. Therefore, cells were fully acclimated to the light intensities. The maximum photon irradiance of 1100 µmol quanta m^{-2} s^{-1} chosen for this experiment resembles that at 16 m depth in subtropical oligotrophic waters (based on a surface photon irradiance of 2500 µmol quanta m^{-2} s^{-1} and a light attenuation coefficient of 0.05 m^{-1}). The span of photon irradiances used in our experiments approximately reflects light regimes in a natural water column from the upper meters to the 0.5 to 1% light level at approximately 100 m depth (Fig. 3). Typically, *Trichodesmium* has a biomass maximum between 20 and 40 m depth and thus resides in a light environment of 300 to 900 µmol quanta m^{-2} s^{-1} (Capone et al. 1997, Davis & McGillicuddy 2006). This preference matches the observed light-dependent growth rate, biomass and nitrogen fixation maxima described here.

High light, >180 to 300 µmol quanta m^{-2} s^{-1}, seems to provide excess energy and growth rates saturate. It is possible though that increased gross photosynthesis or gross nitrogen fixation, and thus metabolic activity within the cell, results in higher turnover of carbon and nitrogen and thus exudation as DOC or DON, which becomes available for the growth of other phytoplankton (Glibert & Bronk 1994, Glibert & O'Neil 1999). This could affect the conversion factor of ethylene reduced:dinitrogen fixed, which can vary between e.g. 2 and 25 for other cyanobacteria under different environmental conditions (Gallon et al. 2002), and is also affected by temperature (M. Mills & E. Breitbarth unpubl. data). To date, the extent to which light intensity may also influence the ratio of gross to net nitrogen fixation and thus the ethylene produced to nitrogen-fixed conversion factor is unknown, which may have implications for oceanic nitrogen fixation estimates.

Further, the Mehler reaction consumes up to 48% of the total photosynthetic linear electron flow (Kana 1993) and thus is partly responsible for a high energetic demand with increasing photosynthetic activity at moderate irradiances, which then is not available for biomass growth. Cells have to maintain a metabolic steady-state energy budget supporting physiological maintenance processes such as O_2 scavenging or photosystem repair and growth, which according to our results is met at 180 to 300 µmol quanta m^{-2} s^{-1}; based on photosynthetic measurements this can be achieved between 150 and 280 µmol quanta m^{-2} s^{-1} (Kana 1991, Carpenter et al. 1993). Higher rates would result in luxury nitrogen incorporation, which is energetically inefficient.

Measurements of cellular fluorescence (F_v/F_m) indicate reduced photosynthetic quantum use efficiency at photon irradiances >300 µmol quanta m^{-2} s^{-1} (Fig. 4). Hence, a larger proportion of photon flux >300 µmol quanta m^{-2} s^{-1} received by the cell is not utilized, matching the growth and nitrogen fixation patterns described. Correspondingly, elemental stoichiometry suggests light limitation at <180 µmol quanta m^{-2} s^{-1}. The POC:PON ratio, as well as the POC:total protein ratio, are reduced compared to that of cultures grown at higher light levels (Fig. 2, Appendix 4). Both indicate reduced carbon incorporation into particulate matter. Photosynthetically fixed carbon is likely respired as carbohydrates at increased rates to provide energy necessary for nitrogen fixation and cellular maintenance at low light intensities. Rabouille et al. (2006) showed in a modeling approach that greater availability of metabolites from carbon fixation fuelled nitrogen

fixation at higher light intensities and while carbon assimilation increased faster than nitrogen assimilation this resulted in a POC:PON ratio increase. Increased carbohydrate storage at high light intensities for buoyancy regulation of *Trichodesmium* has also been suggested by Letelier & Karl (1998). Further, the cellular chl *a* content is adjusted to the light regime (Geider et al. 1997), making chl *a* a problematic biomass measure and normalization parameter for physiological rate measurements (Figs. 2 & 5, Appendices 3, 5 & 6). Even though chl *a* is commonly used as a biomass indicator in oceanographic studies, particulate carbon concentrations would provide a more conservative biomass measure and would be better suited to estimate nitrogen input by *Trichodesmium* into the sea. However, there are strong limitations in differentiating *Trichodesmium* POC from other living phytoplankton POC and detritus material in the field. This confines the application of POC to laboratory studies and field experiments using either hand-picked *Trichodesmium* colonies or a separate quantification of *Trichodesmium* POC from total POC in the water column by, for example, net tow profiles (Gundersen et al. 2001).

Based on our findings, we make the following recommendations for application in ocean biogeochemical circulation models:

- Nutrient replete diazotrophic growth of *Trichodesmium* as a function of light can be described based on Eq. (1). Under nutrient replete conditions, and within an optimum temperature range, a maximum *Trichodesmium* biomass of ~1500 µmol POC l^{-1} and 240 µmol PON l^{-1} is reached at 300 µmol quanta m^{-2} s^{-1} and a light inhibition term can be set at >1100 µmol quanta m^{-2} s^{-1}. A correction factor for diazotrophic growth at different temperatures can be applied based on Breitbarth et al. (2007).
- Estimation of nitrogen fixation during exponential growth is a function of *Trichodesmium* carbon biomass and can be simplified to 0.5 × POC (µmol l^{-1}) = N fixed (nmol l^{-1}).
- The maximum nitrogen fixation rate per unit volume is 350 nmol N fixed l^{-1} h^{-1} and is expressed as a function of light intensity based on Eq. (6).
- Nitrogen fixation is down regulated by exuded nitrogen sources during late exponential growth. The cut off concentrations for nitrogen fixation by NH_4^+ and NO_3^- are ~10 µM (Mulholland et al. 2001, Holl & Montoya 2005), but specific terms for a gradual NH_4^+ and NO_3^- down-regulation of nitrogen fixation need to be developed.

Our model has restrictions as follows. The bulk of nitrogen input into the surface ocean by blooming *Trichodesmium* will occur during exponential biomass build up. Our empirical model represents nitrogen input, particularly maximum rates (350 nmol N fixed l^{-1} h^{-1}), reasonably well during mid and late exponential growth in the 180 to 1100 µmol quanta m^{-2} s^{-1} photon irradiance range. This also spans the light regime in which this diazotroph is most common in the subtropic oligotrophic ocean (Capone et al. 1997, Davis & McGillicuddy 2006). However, comparing the modeling approach to the measured nitrogen fixation data (Fig. 6) reveals an apparent effect of growth phase on the light inhibition parameter. While down regulation by light inhibition was observed only at a later growth stage (higher NH_4^+ concentration), the model applies photoinhibition regardless of the age of the culture. Thus, it underestimates nitrogen fixation at high light intensities in relatively young cultures and overestimates at high light intensities during the late growth phase. The physiological basis of this requires further investigation in order to overcome this limitation of the model. The early phase in a batch culture may be representative of diazotrophic growth in seed populations of *Trichodesmium* in the field and the late exponential growth phase may apply for natural blooms that persist over longer periods of time. Therefore, considerations of the early and late exponential growth phases in modeling approaches to nitrogen fixation in natural *Trichodesmium* occurrences may be important. Further, during mid- and late exponential growth, measured nitrogen fixation rates were considerably lower than rates resulting from the model at photon irradiances <180 µmol quanta m^{-2} s^{-1}. It is possible that self shading of dense cultures caused increased light limitation at low photon irradiances. This factor was not corrected for in our calculations. Together with elemental stoichiometry data (Fig. 2, Appendix 4, Rabouille et al. 2006) the indication for a potential energy limitation of nitrogen fixation at low light intensities stresses the requirement for deriving a suitable light limitation parameter.

Surface abundance of *Trichodesmium* can be estimated using Sea-viewing Wide Field-of-view Sensor (SeaWiFS) imagery (Subramaniam et al. 2002), and Hood et al. (2002) developed a model based on SeaWiFS data describing depth integrated nitrogen fixation using surface *Trichodesmium* chl *a*. Results presented here illustrate limitations when using chl *a* as a biomass indicator to normalize physiological rate measurements over different light conditions as the chl *a*:POC ratio is acclimated to the respective light regime. However, using the chl *a*:POC relationships described here, a stoichiometric shift can be accounted for if information on irradiance and water temperature are available (Geider et al. 1997, Breitbarth et al. 2007). Second, previous measurements of nitrogen fixation as a function of light intensity were conducted on non-light-acclimated cells that were exposed to the specific light intensities for only short periods of time.

The model presented here is based on POC normalized nitrogen fixation measurements of light-acclimated axenic *Trichodesmium* cultures and therefore may help to improve depth integrated nitrogen fixation models.

In conclusion, *Trichodesmium* is well adapted to the high light regimes of the oligotrophic surface waters. Nevertheless, this diazotroph also grows at light intensities equivalent to the compensation depth in sub-tropical waters and results from this study are applicable throughout the euphotic zone. Thus, information on light regime, temperature, nutrient concentrations, and carbon biomass can be used to estimate the bulk of nitrogen input into oligotrophic waters by nitrogen fixation of *Trichodesmium*.

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