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Physiological constraints on the global distribution of *Trichodesmium* – effect of temperature on diazotrophy

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Abstract. The cyanobacterium *Trichodesmium* is an important link in the global nitrogen cycle due to its significant input of atmospheric nitrogen to the ocean. Attempts to incorporate *Trichodesmium* in ocean biogeochemical circulation models have, so far, relied on the observed correlation between temperature and *Trichodesmium* abundance. This correlation may result in part from a direct effect of temperature on *Trichodesmium* growth rates through the control of cellular biochemical processes, or indirectly through temperature influence on mixed layer depth, light and nutrient regimes. Here we present results indicating that the observed correlation of *Trichodesmium* with temperature in the field reflects primarily the direct physiological effects of temperature on diazotrophic growth of *Trichodesmium*. *Trichodesmium* IMS-101 (an isolate of *T. erythraeum*) could acclimate and grow at temperatures ranging from 20 to 34°C. Maximum growth rates (μ_{max}=0.25 day^{-1}) and maximum nitrogen fixation rates (0.13 mmol N mol POC^{-1} h^{-1}) were measured within 24 to 30°C. Combining this empirical relationship with global warming scenarios derived from state-of-the-art climate models sets a physiological constraint on the future distribution of *Trichodesmium* that could significantly affect the future nitrogen input into oligotrophic waters by this diazotroph.

1 Introduction

The diazotrophic filamentous cyanobacterium *Trichodesmium*plays a key role in the nitrogen and carbon cycles of oligotrophic oceans, contributing up to 80 Tg of fixed nitrogen yr^{-1} (Capone et al., 1997). This represents a major fraction of the total marine pelagic nitrogen fixation, currently estimated at 110 Tg yr^{-1} (Gruber and Sarmiento, 1997). Furthermore, *Trichodesmium* can account for up to 47% of the primary production in the tropical North Atlantic Ocean (Carpenter et al., 2004) and contributes to export production via nitrogen fueling of the phytoplankton community (Letelier and Karl, 1996; Karl et al., 1997). *Trichodesmium* abundance is generally limited to oligotrophic waters and its observed temperature distribution range (20°C–30°C) is also used to constrain N_2-fixation in ocean biogeochemical circulation models (OBCMs) (Fennel et al., 2001; Hood et al., 2001, 2004). The upper temperature limit is set by the current sea surface temperature (SST) maximum and not by observed physiological constraints of high temperature on *Trichodesmium* distribution. Parametrizations are based solely on field correlations and cannot differentiate between direct physiological effects of temperature on an organism from indirect effects caused by changes in the physical environment (i.e. light and nutrients) induced by temperature, and thus are of limited predictive value.

Occurrence of *Trichodesmium* at higher latitudes with water temperatures below 20°C appears to be due to drift rather then local net growth. Nitrogen fixation by *Trichodesmium* was not observed in these waters (Carpenter, 1983; Lipschultz and Owens, 1996), although diazotrophic growth at temperatures close to freezing has been reported for other cyanobacteria, i.e. *Oscillatoria* sp. (Pandey et al., 2004) or *Nostoc* sp. (Zielke et al., 2002). An upper temperature limit cannot readily be derived from field observations because the present sea surface temperatures rarely reach the observed upper tolerance limit for *Trichodesmium* (Capone et al., 1997). A few exceptions are found where blooms of

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Trichodesmium have been reported at water temperatures as high as 35°C. However, these high temperatures may have been due to intense surface heating by heat absorption of the dense *Trichodesmium* mat and probably resulted in rapid cell lysis and death (Capone et al., 1998).

While these empirical field correlations may be useful for parameterization of models, they provide no information on the direct physiological effect of temperature on the growth, nitrogen fixation, and C:N stoichiometry in *Trichodesmium*. A parameterization of models using a physiological basis for the apparent temperature control of *Trichodesmium* distribution would provide an additional predictive value.

Here we present effects of temperature on nitrogen fixation, POC:PON and Chl-*a*:POC stoichiometry, and growth for *Trichodesmium* IMS-101. We discuss the possible physiological basis for these effects relative to other factors, such as light and nutrients, also affecting the distribution of *Trichodesmium*. Based on climate model predictions of the sea surface temperature increase within this century, we point out the importance of understanding the physiological temperature limits of *Trichodesmium* growth for predicting oceanic nitrogen input by this diazotroph with OBCMs in the future.

2 Materials and methods

2.1 Growth of cultures

An axenic culture of *Trichodesmium* IMS-101 was grown at temperatures ranging between 15 and 36°C for at least three transfers (minimum of 15 generations) at each temperature, under a light:dark cycle of 12:12 h and a light intensity of 100 μmol quanta m^{-2} s^{-1} using phosphorus and iron replete YBC II media without dissolved nitrogen added (Chen et al., 1996). In order to acclimate *Trichodesmium* the cultures were transferred from the respective higher or lower temperatures where growth was detected as well as from well-growing stock cultures incubated at 25°C. Three independent attempts were made to acclimate *Trichodesmium* to grow at temperatures lower than 20°C and above 34°C without success.

2.2 Nitrogen fixation measurements

Nitrogen fixation rates were measured using the Acetylene Reduction Assay (ARA) (Capone, 1993), with calculations modified after Breitbarth et al. (2004) and a ratio of C_2H_2 reduced:N_2 reduced of 4:1 (Montoya et al., 1996). Gas samples were analyzed on a Shimadzu GC-19B gas chromatograph equipped with a flame ionization detector and a 30 m long, wide bore (0.53 mm) capillary column (AluminaPlot®, Resteck, USA). The oven temperature was set at 40°C, injector and detector temperature at 200°C, and the carrier gas flow (N_2) at 14.5 ml min^{-1}, which yielded optimal peak separation and detection limits. The effect of temperature on nitrogen fixation was determined on batch cultures that were grown at 25°C and diluted daily with fresh media to maintain a constant biomass at the maximum growth rate in order to reduce the effect of growth phase on nitrogen fixation rates. For each temperature, three replicates were incubated simultaneously for 4 h (10:00–14:00 h) during the middle of the light cycle in 20.2 ml headspace vials containing 19 ml culture and 1.2 ml headspace with 0.4 ml acetylene added. Additionally, the complete experiment was repeated three times. Nitrogen fixation rates were normalized to POC biomass.

2.3 Biomass and elemental stoichiometry

For biomass determinations, samples were filtered (GF/F, pre-combusted for elemental analysis) and stored at −20°C until further analysis.

Particulate organic nitrogen (PON) and particulate organic carbon (POC) contents of the cultures were determined after Sharp (1975) and Ehrhard and Koeve (1999). Frozen filters were dried for 48 h at 45°C and thereafter subjected to analysis using an elemental analyzer (Euro-EA, Hekatech, Germany) equipped with a chromium oxide/cobalt oxide oxidation reactor, a copper reduction reactor, and a separation column maintained at an oven temperature of 45°C. Carrier gas flow (He) was set at 96 ml min^{-1}. The data were blank corrected using measurements of identically treated filters without culture material.

The chlorophyll-*a* concentrations were determined fluorometrically based on Welschmeyer (1994) after bursting the cells in 90% Acetone by shaking and refreezing for 24 h. Results obtained from this simple extraction method were comparable to those involving mechanical disruption of the cells (data not shown).

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.

2.4 Photosystem response measurements

The photosynthetic quantum yield efficiency of the photosystem II was measured using a PhytoPAM equipped with Optical Unit ED-101US/MP (Walz, Germany) based on Kolbowski and Schreiber (1995). The ratios of variable to maximal fluorescence (F_v/F_m) of *Trichodesmium* IMS-101 in response to different incubation temperatures were recorded over the complete growth period of the cultures at the respective temperatures. Further, F_v/F_m was measured on cultures grown at 25°C after short-term exposure (4 h) to a temperature range of 14°C to 36°C. These measurements were performed on the identical samples as used for the nitrogen fixation measurements described above. Samples were dark-adapted for 10 min prior to the measurements.

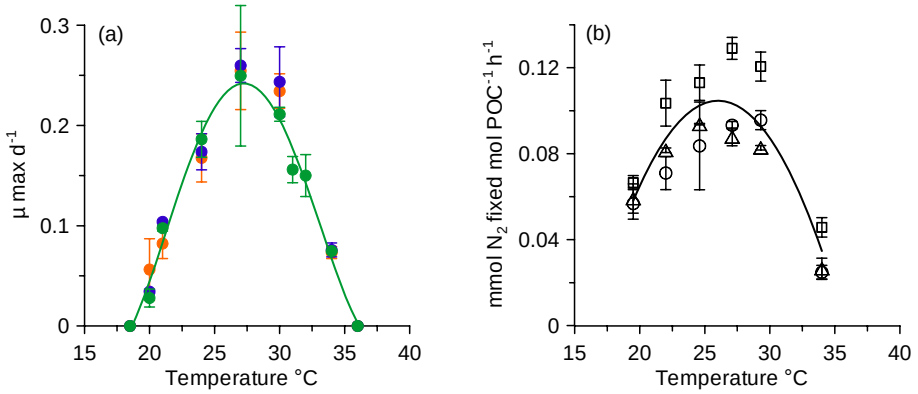

Fig. 1. **(a)** Maximum carbon (x, orange), nitrogen (x, blue) and chlorophyll$-a$ (x, green) specific growth rates (μ_{max}) as a function of temperature. The green curve gives the best fit to the chlorophyll-*a* specific growth data using the polynomial function:

$$\mu = 2.29^{-5}x^4 - 2.50^{-3}x^3 + 9.71^{-2}x^2 + 1.58x + 9.15 \quad (1)$$

where x is temperature in °C (R^2=0.98). **(b)** Carbon specific nitrogen fixation rates as a function of temperature. Different symbols denote measurements from three independently performed identical experiments. Mean values of three replicates each are plotted with error bars showing standard deviations. The curve gives the best fit using the polynomial function:

$$y = -0.001096x^2 + 0.057x - 0.637 \quad (2)$$

where x is the arithmetic mean of all measurements at the each temperature (R^2=0.97).

2.5 Sea surface temperature increase predictions

Predictions of the increase in sea surface temperature (SST) were based on two coupled atmosphere-ocean general circulation models (HadCM3 and GFDL R30). Both models predict a SST increase of up to 3°C by 2090 in our area of interest (20–30°C isotherms). The HadCM3 model run (Gordon et al., 2000) is based on the assumption that future emissions of greenhouse gases will follow the IS92a "business as usual" scenario with observed atmospheric CO_2 concentrations until 1990 and a 1% annual increase thereafter (http://www.met-office.gov.uk/research/hadleycentre/models/modeldata.html).

This prognosis is generally consistent with results from a similar experiment using the GFDL R30 climate model (Delworth et al., 2002) (http://www.gfdl.noaa.gov/~kd/ClimateDynamics/NOMADS/index.html). The SST changes predicted by the climate models over the next century are then added to current annual mean SSTs (Levitus and Boyer, 1994) and the area of various physiologically relevant temperature ranges is computed.

3 Results

3.1 Growth and nitrogen fixation

Our results demonstrate that *Trichodesmium* IMS-101 grows and fixes nitrogen at temperatures between 20–34°C (Figs. 1a, b). The cultures did not grow below 20°C or above 34°C. They could be maintained alive at 17°C for several weeks, but biomass progressively decreased. Incubations at water temperatures of 36°C resulted in cell death and lysis after two days (data not shown). Growth rates at each specific temperature did not differ significantly between chlorophyll$-a$, carbon or nitrogen specific growth, with the exception of carbon and nitrogen specific growth rates being higher than chlorophyll specific growth rates at 30°C. No differences in growth rates were detected when cultures were transferred from similar or adjacent incubation temperatures or originated from 25°C incubations. Maximum specific growth rates (μ_{max}) of the axenic *Trichodesmium* IMS-101 strain were highest in the temperature range between 24–30°C, with a peak at 27°C (μ_{max} carbon specific=0.25 day^{-1}, Fig. 1a). Growth rates were significantly reduced below and above this temperature range.

Nitrogen fixation rates were significantly affected by temperature and followed closely the relationship observed for growth rate with temperature, showing a temperature optimum between 24–30°C as well. The maximum nitrogen fixation rate of 0.13 mmol N mol POC^{-1} h^{-1} was measured at 27°C. Three individual experiments with semi-continuously growing cultures yielded a similar temperature dependence (Fig. 1b).

3.2 Elemental stoichiometry

These observations were supported by measurements of elemental stoichiometry. The cellular carbon to nitrogen ratio increased from 5.4 (mol:mol) at 20°C to a maximum of 6.8

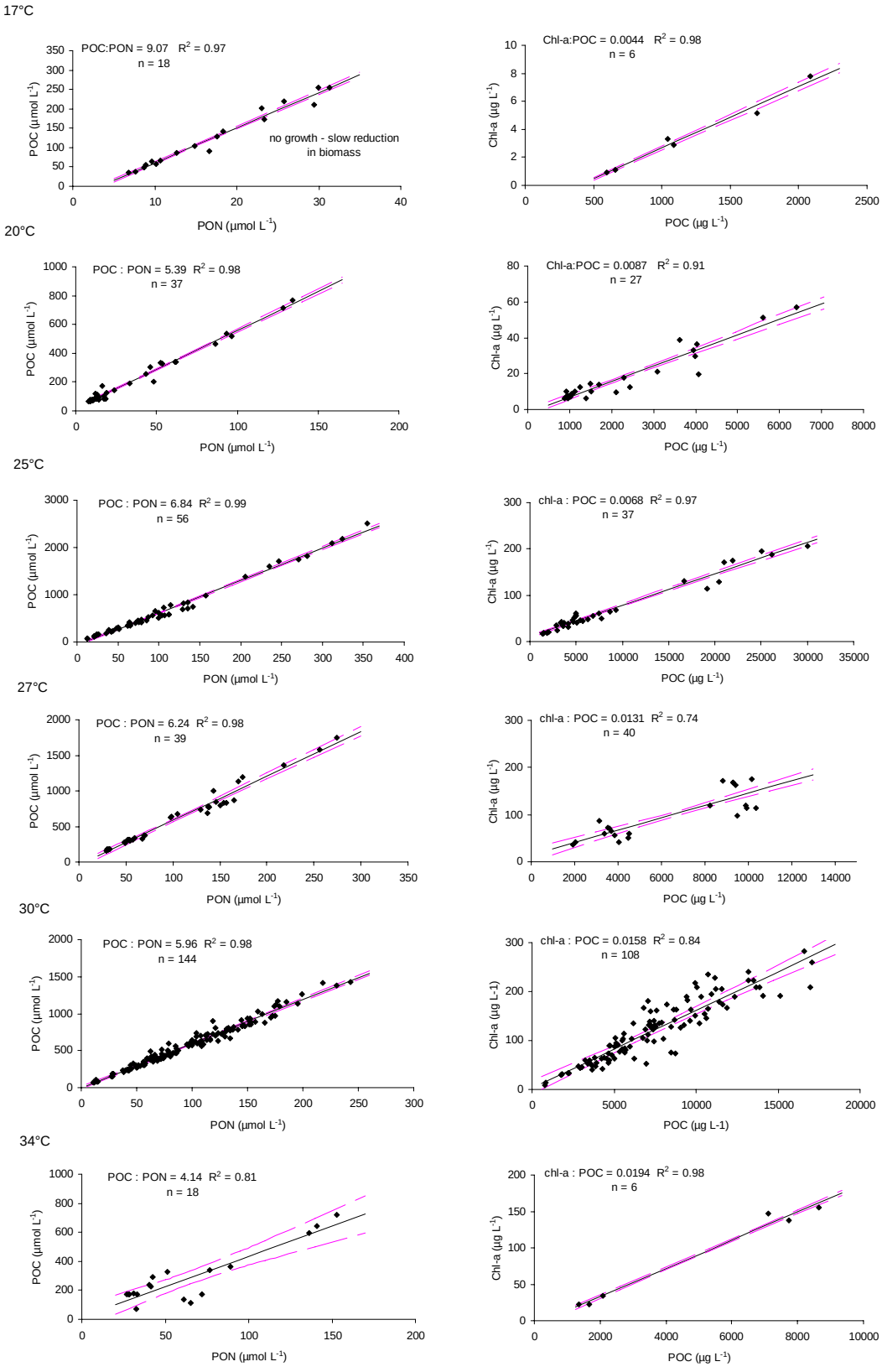

Fig. 2. Overview of POC:PON (mol:mol) and chlorophyll-*a*:POC (weight:weight) stoichiometry of *Trichodesmium* IMS-101 at different temperatures. Solid black lines are derived from linear regressions of the data at various temperatures with their respective 95% convidence intervals plotted as dashed pink lines. The regression coefficient represents the stoichiometric ratio and is included in each plot together with the coefficient of determination (R^2) and the sample size (n).

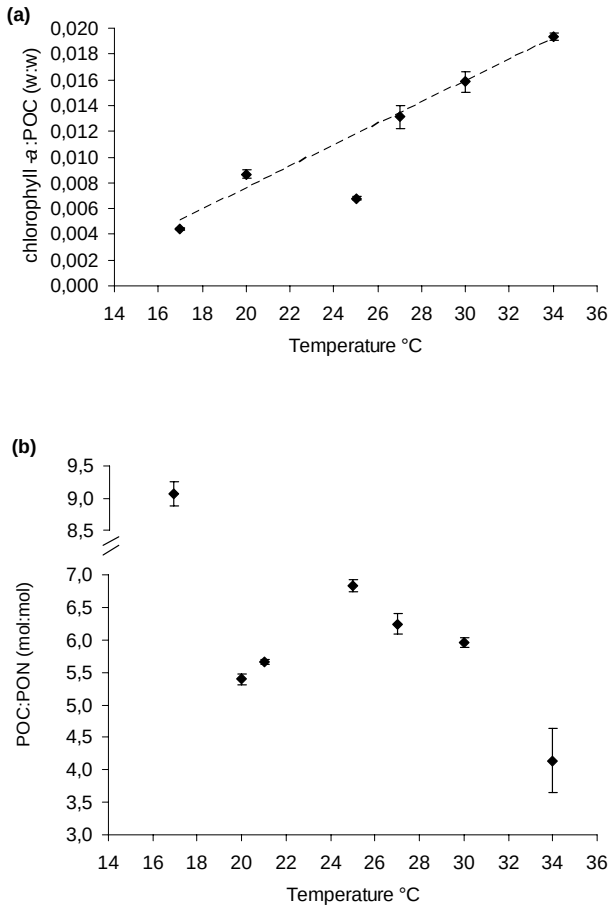

Fig. 3. Stoichiometry chlorophyll-*a*:POC (weight:weight (μg L^{-1}:μg L^{-1}), **(a)** and POC:PON (mol:mol, **(b)** of *Trichodesmium* IMS-101 as a function of growth temperature. Data points represent regression coefficients of the stoichiometric ratios at the respective temperatures and error bars denote the standard error of the regression coefficients. Please see Fig. 2 for the respective samples sizes (n) and coefficients of determination (R^2). The dashed line provides a linear fit to the data based on the regression:

$$y = 0.00084x - 0.0091, R^2 = 0.99 \quad (3)$$

where y and x are the chlorophyll-*a*:POC ratio and temperature in °C, respectively.

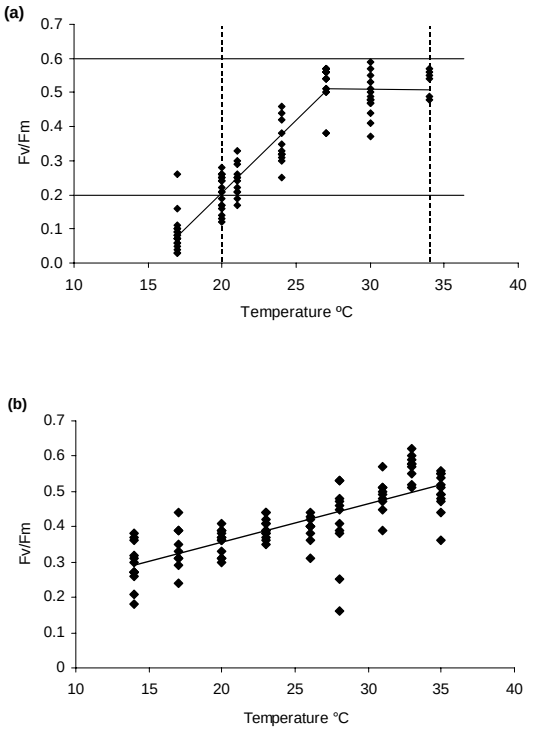

Fig. 4. (a): Photosynthetic quantum yield efficiency of only exponentially growing *Trichodesmium* IMS-101 batch cultures acclimated to various temperatures. The two solid horizontal lines indicate the theoretical minimum (0.2) and maximum (0.6) F_v/F_m values for living cyanobacteria. The two vertical dashed lines indicate the temperature tolerance range of *Trichodesmium* IMS-101 (20–34°C). **(b)**: Photosynthetic quantum yield efficiency of *Trichodesmium* IMS-101 grown at 25°C and exposed to the respective temperatures of F_v/F_m measurements for short duration (four h).

at 25°C, which is close to the Redfield ratio (6.6). At higher temperatures the POC:PON ratio decreased again to a minimum value of 4.1 at 34°C (Fig. 2). A comparatively high POC:PON stoichiometry was measured at 17°C (9.1). However, it is not clear whether or not this was an artifact of lack of growth of *Trichodesmium* at this temperature.

Further, the cellular chlorophyll-*a* to carbon ratio increased linearly from 0.0044 (g:g) at 17°C to 0.0194 at 34°C (Fig. 3a) reflecting an acclimation response of the photosynthetic apparatus to temperature (Geider et al., 1997).

The data shown in Figs. 2 and 3 are derived from measurements throughout the growth period of the batch incubations. As previously reported by Mulholland and Capone (2001), POC:PON ratios varied over the growth period and were reduced during the exponential growth phase, which is characteristic of high N_2-fixation. Nevertheless, in some of the experiments the exponential growth phase was very short, yielding very few of data points, which would make a regression analysis problematic. Furthermore, the contribution of the exponential growth phase data to the stoichiometric ratios derived from all data points of an experiment was insignificant in most cases. However, we did exclude data for cultures that were not acclimated to the incubation temperatures yet, which was the case for samples taken during the first two days of an experiment after transferring a culture to a different temperature.

3.3 Photosynthetic response

The photosynthetic quantum yield efficiency (F_v/F_m) of cultures acclimated to their growth temperature increased from below 0.10 at 17°C up to a maximum quantum-yield efficiency of 0.68 at 30°C (Fig. 4a). As there was

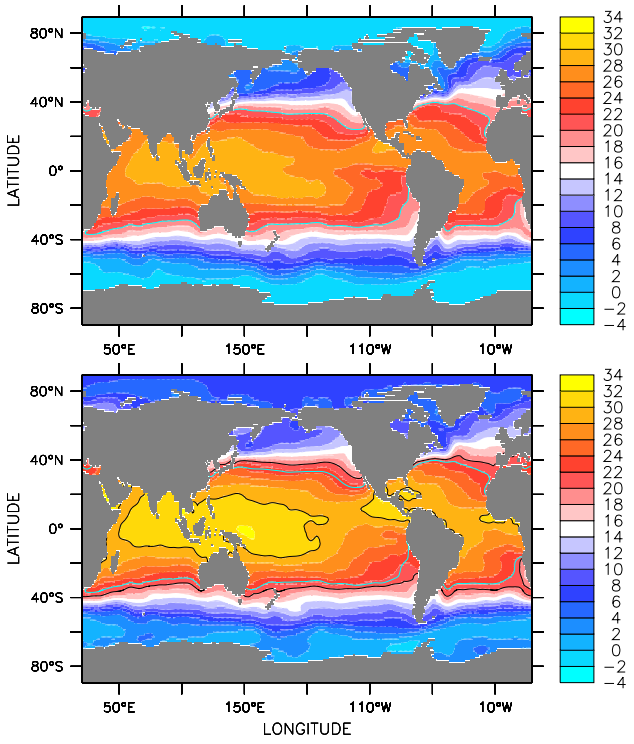

Fig. 5. The observed present-day annual mean sea surface temperature (top) in comparison to the annual mean sea surface temperature incremented by the modeled increase over the period 1990 to 2090 (bottom) based on HadCM3. In both plots, the cyan line indicates the maximum latitudinal boundary of the 20°C isotherm observed in 1990. The black lines in the bottom plot indicate the 20 and 30°C isotherms predicted for 2090.

considerable variation of (F_v/F_m) as a function of physiological differences during growth in batch cultures, only samples from the exponential growth phase were plotted in Fig. 4b. Here, the average quantum yield efficiency increased from 0.20 at the minimum feasible growth temperature (20°C) to 0.52 at 27°C and thereafter remained constant up to 34°C. Maximum values did not exceed 0.60 (Fig. 4b). In contrast, F_v/F_m measurements of *Trichodesmium* grown at 25°C and transferred to a range of temperatures (4 h incubations) were positively correlated with temperature and increased linearly from approximately 0.25 at 14°C to the maximum of 0.60 at the maximum growth temperature (34°C). This demonstrates that the photosynthetic apparatus adjusted slowly to changes in temperature. Measurements at 36°C showed reduced F_v/F_m values again (Fig. 4a).

3.4 Potential effects of predicted SST increase on *Trichodesmium* distribution

Climate models predict increases in sea surface temperature (SST) by up to 3°C by 2090 in our area of interest (20–30°C isotherms), accompanied by a poleward shift of the 20°C isotherm (Fig. 5). This will result in an 11% areal increase of *Trichodesmium's* potential geographic distribution. Moreover, maximum calculated SSTs will still be less than 34°C, which will not limit the potential distribution of *Trichodesmium* in tropical waters. Nevertheless, a decrease in the area characterized by optimum growth and N_2-fixation conditions (24–30°C) by about 16% is anticipated (Fig. 5).

4 Discussion

Temperature per se does not restrict diazotrophic growth and diazotrophs can be encountered at temperatures close to freezing (Zielke et al., 2002; Pandey et al., 2004), yet the overall distribution of *Trichodesmium* in the contemporary ocean appears well constrained by seawater temperature (∼20–30°C)(Capone et al., 1997). However, the correlation of *Trichodesmium* abundance with water temperature is generally attributed to oceanographic features associated with warm waters, such as shallow mixed layer depth, high light intensity, and oligotrophic conditions rather than a direct physiological response to temperature itself (Hood et al., 2004). Since surface water temperature and dissolved nitrate concentrations are significantly negatively correlated in the marine environment, it is not clear whether the global patterns of N_2-fixation versus water temperature are due to an inhibition of nitrogenase by low temperatures or a selection against N_2-fixers under conditions of high nitrate concentrations or both. In this work, we separated the effect of temperature from other factors (i.e. nutrients, light, and stratification) on diazotrophic growth and thus were able to demonstrate that, as suggested by Capone et al. (1997), seawater temperature sets a physiological constraint to the geographic distribution of *Trichodesmium*.

We are able to demonstrate that the strain IMS-101 of *Trichodesmium* is adapted to optimal growth at temperatures between 24 and 30°C and can tolerate water temperatures from 20 to 34°C. Analogous to our results, positive correlations of *Trichodesmium* abundance and water temperature (22–28/31°C) were also observed in field studies (Capone et al., 1997; Lin, 2002; Lugomela et al., 2002; Chen et al., 2003). However, our observation that cells can survive at 17°C for several weeks and experience a slow decrease in biomass can also explain the persistence of *Trichodesmium* transported to higher latitudes by oceanic currents (Carpenter, 1983; Lipschultz and Owens, 1996).

In contrast to our finding of an optimum temperature range between 24 and 30°C, Staal et al. (2003) described a linear increase of nitrogen fixation up to a temperature of 36°C in short-term incubations (2 min, M. Staal, personal communication). Measurements published by Staal et al. (2003) most likely reflected nitrogenase enzyme kinetics, whereas data presented here describe temperature acclimated diazotrophic growth (Fig. 1). This is based on the physiological patterns of maximum nitrogen fixation activity, highest growth rates, cellular elemental composition, and photosynthetic quantum

yield efficiency. The maximum growth rates and high nitrogen fixation rates between 24 and 30°C must be accompanied by high carbon fixation rates, which is expressed in near Redfield POC:PON stoichiometry. As an effect of temperature though, a larger fraction of fixed N_2 may be released and not incorporated into the cells when either carbon fixation is insufficient, or cells may become leakier due to increased membrane permeability at higher temperatures. The temperature acclimation of the chlorophyll-*a*:POC ratio reflects the need to reduce light absorption at low temperatures in order to equilibrate with lower enzyme activity, while this mechanism is relieved at higher temperatures. Factors such as light and nutrient regimes directly interact with temperature and will also play determinant roles. Photosynthetic organisms will acclimate to both light and temperature by adjusting the balance between light energy absorption and the rate of the dark reaction of photosynthesis, i.e. by increasing light absorption in low light and decreasing it at low temperature (Geider et al., 1997; Miskiewicz et al., 2002). The photosynthetic quantum yield efficiency clearly reflects a physiological adaptation to the temperature tolerance range of *Trichodesmium* (IMS-101). In short term incubations F_v/F_m increased linearly up to the physiological maximum temperature of 34°C. Measurements of cultures growing exponentially at the respective temperatures though reveal that the photosystem II operated at minimum efficiency at 20°C and saturated at maximum efficiencies between 27 and 34°C. Thus, the temperature tolerance range of *Trichodesmium* IMS-101 grown at 100 μmol quanta $m^{-2}s^{-1}$ is also confined by the general range of photosynthetic quantum yield efficiency for cyanobacteria (0.20–0.60, Fig. 4a). One can hypothesize that the high temperature optimum for *Trichodesmium* growth leads to a better tolerance of high light intensity, which is characteristic of tropical and subtropical regions.

While we cannot fully explain the biochemical basis for the physiological constraint to the observed temperature range, a combination of several mechanisms is likely. In *Trichodesmium*, the timing of nitrogen fixation and photosynthesis has been shown to be under the control of a circadian rhythm (Chen et al., 1998) and the temperature tolerance range may be in part set by the temperature compensation range of the circadian clock. Further, the dark reaction of photosynthesis is temperature dependent due to enzyme kinetics and membrane permeability (Falkowski and Raven, 1997). In addition, it has been shown for terrestrial plants that Rubisco activase has a lower temperature tolerance than Rubisco itself. Rubisco activase is not capable of maintaining Rubisco, the global enzyme that is essentially responsible for photosynthetic carbon acquisition, in an active form at growth temperatures outside the thermal environment to which the organism is adapted (Crafts-Brandner and Salvucci, 2000; Salvucci and Crafts-Brandner, 2004a, b). It is possible, but remains to be demonstrated, that such a mechanism also limits the photophysiology of *Trichodesmium* at the high end of the temperature tolerance range.

Overall, the mechanisms determining the optimal growth temperature in microorganisms are poorly understood but, at the most basic level, adaptions to extreme cold or heat have a genetic basis. Genomic analysis of psychrophilic bacteria for example revealed that cold-adaptation is not just a function of a specific set of proteins but also dependent on the general amino acid composition of the proteins and membrane fluidity and permeability (Methe et al., 2005). Diazotrophs in general can grow at all temperatures. In particular, *Oscillatoria*, a close relative of *Trichodesmium* is found in the Antarctic (Pandey et al., 2004). Phylogenetic analysis of the hetR gene, which is most likely involved in heterocyst and diazocyst development, revealed a high diversity level within the *Trichodesmium* clade (Mes and Stal, 2005). Thus, although the strain *Trichodesmium* IMS-101 did not adapt to growth at higher and lower temperatures in our experiments, other uncultivated strains may be capable of growing outside this temperature range.

A community shift towards other diazotrophs may also be possible. Until recently, the significance of unicellular N_2-fixers has been underestimated (Montoya et al., 2004), but latest observations suggest that these diazotrophs also thrive at the 26–30°C temperature range (Mazard et al., 2004; Falcón et al., 2005; Langlois et al., 2005). However, a few samples from temperatures below 20°C also contained *nifH* genes, indicative of the presence of diazotrophs, suggesting that some marine nitrogen fixers may also dwell in cold water (Langlois et al., 2005). Whether or not these unicellular cyanobacteria are actively fixing nitrogen, or if they can potentially fill niches for nitrogen fixers at the lower or higher temperature ranges remains to be investigated.

Acknowledging that we lack information on the physiological variability within the genus *Trichodesmium*, we suggest that future changes in SST may result in an 11% areal increase in *Trichodesmium's* potential geographic distribution due to the poleward shift of the 20°C isotherm, while the maximum calculated SSTs (34°C) will not be limiting diazotrophy of *Trichodesmium* in tropical waters. However, because of the much higher N_2-fixation rates and the growth physiology of *Trichodesmium* in the 24–30°C SST range, the effect of the 16% decrease in the area characterized by optimum growth and N_2-fixation conditions (24–30°C) is likely to outweigh the positive effect of the latitudinal increase of the total area (Fig. 5). Thus, the predicted overall increase in sea surface temperature may result in a net decrease of N_2-fixation by *Trichodesmium* by the end of this century. The effects on oceanic nitrogen cycling may be significant, taking the global importance of this diazotroph into account (Capone et al., 1997; Capone and Carpenter, 1999). As mentioned earlier, these predictions are based solely on the observed dependence of *Trichodesmium* IMS-101 growth on temperature. Additionally, our hypothesis is based on SST only and does not consider possible changes in nutrient supply and light conditions, which will also be affected

by SST increase and are, to date, more difficult to predict than changes in SST.

Current predictions of future marine nitrogen fixation diverge. In contrast to our findings, Boyd and Doney (2002) predict a future increase of N_2-fixation by 27% (from 80 to 94 Tg yr^{-1}) due to a floristic shift towards diazotrophy by *Trichodesmium* caused by combined effects of mixed layer depth (MLD), stratification, and nutrient distribution. Time series measurements near Hawaii (Karl et al., 1997) support this trend. Although SSTs in this area of the North Pacific are predicted to increase by almost 3°C (Figs. 5a and b) they will not exceed the physiologically optimal range. Nevertheless, large regions of the tropical and subtropical oceans are predicted to fall outside the optimal range. Particularly, temperatures rising above 30°C in N_2-fixation hotspots may result in significant changes of the regional nitrogen budgets. In the North Atlantic, for example, SSTs are predicted to exceed 30°C in the Caribbean Sea as well as in equatorial waters off West Africa, which are currently hotspots of N_2 fixation in a model based on field observations, MLD and light (Hood et al., 2004). Similarly high SSTs are predicted for the western Pacific and a large part of the Indian Ocean, which both are characteristic provinces for present-day *Trichodesmium* abundance (LaRoche and Breitbarth, 2005).

Whether a global SST increase in the future ocean will result in a decrease in *Trichodesmium* or lead to a community shift towards other diazotrophs rests on the physiological temperature dependence of nitrogen fixation and on the relative importance of temperature compared to other factors such as water column stability, nutrient availability and light intensity. Conversely, physical and chemical factors other than temperature may also determine the development of nitrogen fixation hotspots in the future ocean.

In conclusion, our results demonstrate that the temperature adaptation of *Trichodesmium* IMS-101 controls the geographic distribution of this species. Based on the physiological constraints of diazotrophic growth of *Trichodesmium* IMS-101, we suggest reduced fixed nitrogen input by *Trichodesmium* in response to the SST increase predicted for the end of this century. Although SSTs are expected to rise essentially everywhere, the area of surface waters with temperatures in the physiologically optimal range for growth of *Trichodesmium* will likely decline. We expect that, within the areal limits imposed by the SST, a combination of other controlling factors such as MLD, light, and nutrient regimes (including iron) will further influence the distribution of *Trichodesmium*. Considering the large fraction of N_2-fixation by *Trichodesmium* on total oceanic nitrogen input, the predicted ecophysiological changes to this diazotroph may cause significant changes in global biogeochemical cycles. Nevertheless, because little is known about temperature selection of other diazotrophs, we do not know what the overall dynamics of N_2 fixation in the future ocean will be. As N_2-fixation in currently available ocean biogeochemical circulation models is based on *Trichodesmium*, it may be necessary to adjust their parameterizations in view of the temperature-diazotrophic growth relationship presented here, and to consider taking into account other diazotrophs as well.

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