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Ecophysiology of the marine cyanobacterium *Trichodesmium*

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Der Dekan

A *Trichodesmium* bloom off the northern Coast of Australia. Photo: NASA

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ABSTRACT

The aim of this thesis is to improve the mechanistic understanding of nitrogen fixation by the marine non-heterocystous cyanobacterium *Trichodesmium*. This diazotroph is a major contributor to the marine nitrogen cycle and thus is important for parameterizations for nitrogen fixation in Ocean Biogeochemical Circulation Models. A wide-ranging amount of information on ecophysiological characteristics of *Trichodesmium* has been published and is summarized in a literature review (chapter I). This review stresses that *Trichodesmium* abundance in the open ocean can be limited by the nutrients iron and/or phosphorus and is constrained by the physical parameters temperature and light. Nevertheless, chapter I also identifies that the effects of temperature and light availability on diazotrophic growth of *Trichodesmium* were not fully understood and required further investigation. Laboratory results (chapter III) demonstrate that *Trichodesmium* N_2 fixation is confined to water temperatures of 20 – 34 °C with an optimum range of 24 – 30 °C. These findings are of particular interest with respect to global warming, considering the important role of *Trichodesmium* in the marine nitrogen cycle. Combining these data with climate models (HadCM3 and GFDL R30) chapter III predicts a future decline in the fixed nitrogen input by *Trichodesmium* that could significantly affect marine nitrogen cycling within this century. Chapters IV and V address the role of light for diazotrophic growth of *Trichodesmium*. Results show that *Trichodesmium* is well adapted to the light regimes throughout the euphotic zone of tropical and subtropical oceans. The carbon specific growth rate increases up to an irradiance of 180 µmol quanta m^{-2} s^{-1}, and is constant (0.26 d^{-1}) thereafter up to 1100 µmol quanta m^{-2} s^{-1}, where light inhibition sets in. The maximum nitrogen fixation rate measured was 350 nmol N_2 fixed l^{-1} h^{-1}. Chapter IV further provides a simple numerical model to describe nitrogen input into seawater by *Trichodesmium* as a function of light intensity. This outcome is complemented by a conceptual model of nitrogen and carbon fixation of *Trichodesmium* presented in Chapter V. Additionally, a reassessment of a commonly applied method to measure nitrogen fixation (Acetylene Reduction Assay) was conducted (chapter II). This method was improved by providing newly derived ethylene gas solubility coefficients that are required to accurately calculate nitrogen fixation rates. These were previously unavailable from published literature and thus the presented publication contributes to a standardization of nitrogen fixation measurements. Further methodological approaches to assess nitrogen fixation and release of fixed nitrogen by diazotrophs are provided in an outlook for future work in chapter V.

ZUSAMMENFASSUNG

Ziel dieser Arbeit ist die Untersuchung der Stickstofffixierung zu Grunde liegender Mechanismen am Beispiel des marinen Cyanobacteriums *Trichodesmium.* Die N_2-Fixierungsleistung dieses Diazotrophen stellt einen maßgeblichen Beitrag zum marinen Stickstoffkreislauf dar und wird in vielen Fällen als Grundlage der in marinen biogeochemischen Zirkulationsmodellen verwendeten N_2-Fixierungsraten eingesetzt. Veröffentlichungen zu ökophysiologischen Studien und Charakteristika von *Trichodesmium* sind weitreichend verfügbar und in einem Literatur Review (Kapitel I) zusammengefasst. In der Literatur sind vor allem die Nährstoffe Eisen und Phosphat sowie die physikalischen Parameter Temperatur und Licht als limitierende Faktoren der Abundanz von *Trichodesmium* bekannt. Die Rolle von Temperatur und Licht als regulierende Faktoren für diazotrophes Wachstum ist jedoch noch nicht hinreichend beschrieben und bedarf genauerer Betrachtung. In eigenen Studien zu diesem Thema (Kapitel III) wird gezeigt, dass sich das Wachstum von *Trichodesmium* auf einen Temperaturbereich von 20 – 34 °C, mit einem Optimum zwischen 24 – 30 °C, beschränkt. In Hinblick auf globale Klimaerwärmung und die zentrale Rolle von *Trichodesmium* im marinen Stickstoffkreislauf sind diese Ergebnisse von großer Bedeutung. Unter Verwendung dieser Daten in Kombination mit verschiedenen Klimamodellen (HadCM3 und GFDL R30), lässt sich eine Abnahme des Stickstoffeintrages durch *Trichodesmium* innerhalb dieses Jahrhunderts prognostizieren, welches den marinen Stickstoffkreislauf maßgeblich beeinflussen könnte. Der Einfluss von Licht auf diazotrophes Wachstum von *Trichodesmium* wird in den Kapiteln IV und V behandelt. Die Ergebnisse zeigen, dass *Trichodesmium* gut an die Lichtbedingungen der euphotische Zone in tropischen und subtropischen Ozeanen angepasst ist. Bei Lichtintensitäten von 15-180 µmol Quanten m^{-2} s^{-1} ist ein Anstieg der kohlenstoffspezifischen Wachstumsrate auf ein bis zu einer Intensität von 1100 µmol Quanten m^{-2} s^{-1} konstantes Maximum von 0.26 d^{-1} zu erkennen. Erst bei Lichtintensitäten von mehr als 1100 µmol Quanten m^{-2} s^{-1} setzt eine Lichtinhibition ein. Die in diesen Versuchen gemessene maximale N_2-Fixierungsrate beträgt 350 nmol N_2 l^{-1} h^{-1}. Des Weiteren wird in Kapitel IV ein einfaches numerisches Modell zur Berechnung des N_2-Eintrages in Seewasser in Abhängigkeit von der Lichtintensität durch *Trichodesmium* vorgestellt. In Kapitel V wird dieses durch ein Konzeptmodell zur Stickstoff und Kohlenstofffixierung in *Trichodesmium* ergänzt. Ferner wurde in der vorliegenden Arbeit eine häufig verwendete Methode zur Bestimmung von N_2-Fixierungsraten (Acetylene Reduction Assay) optimiert (Kapitel II). Zur korrekten Berechnung der N_2-Fixierung sind Ethylen Gaslöslichkeitskoeffizienten notwendig, welche zuvor in der Literatur nicht angegeben wurden. Diese wurden semi-empirisch ermittelt und zur Standardisierung der N_2-fixierungsmessungen eingesetzt. Kapitel V beschreibt die Notwendigkeit weiterer methodischer Ansätze zur Untersuchung von N_2-Fixierung und N-Abgabe durch Diazotrophe als Ausblick für zukünftige Arbeiten.

INTRODUCTION

PREFACE

This doctoral thesis was conducted in the context of the European Union project IRONAGES (Iron Resources and Oceanic Nutrients Advancement of Global Environment Simulations). The goal of IRONAGES was to develop new Ocean Biogeochemical Circulation Models (OBCM's) for budgeting and exchanges of carbon dioxide (CO_2) and Dimethylsulfide (DMS). In the models co-limitation by 4 nutrients of 5 major taxonomic classes of phytoplankton, DMS pathways, global iron cycling and chemical forms of iron and iron supply into surface waters were implemented. Iron limits phytoplankton productivity in 40% of the oceans, and can be a co-limiting nutrient in the remaining 60% of surface waters. In contrast to the paradigm that a single factor is limiting phytoplankton blooms, co-limitations by light, and the nutrients N, P, Si and Fe are accounted for. The task of this thesis is to provide data on growth and export production of nitrogen fixing cyanobacteria (Diazotrophs, namely *Trichodesmium* sp.) as one out of three functional groups (Diatoms, Diazotrophs, Nano- and Picoplankton), other than DMS(P) producers that are considered to represent the 'biological pump' in OBCM's. The new OBCM's developed during IRONAGES are targeted to improve climate change scenarios, most notably climatic feedbacks on oceanic biogeochemistry.

INTRODUCTION

Cyanobacteria are nearly ubiquitous organisms, which can be encountered in almost any ecosystem. They are visible to the bare eye in form of dark coloring of old stone structures, surface slicks on lakes and in the ocean or as a thin black slippery crust in the rocky splash zone of the sea. The presence in melt water ponds of Antarctic ice sheets as well as in hot springs demonstrates wide ranging adaptations of cyanobacteria to their environment. Cyanobacteria form lichens with fungi, symbiosis with unicellular algae and are present in root nodules of some higher plants. Further, cyanobacteria are a major component of the microbial communities forming stromatolite reefs. Cyanobacteria are economically important by naturally fertilizing rice fields with fixed nitrogen based on a symbiosis with a water fern (*Azolla*) that also grows in the rice ponds.

Evolutionary aspects

Cyanobacteria are of fundamental significance to the history of the biosphere. The primordial atmosphere was mainly composed of H_2O, N_2, CO_2 and CH_4, but contained no elemental oxygen. NH_3 was present as a gas and sulfides were present in form of H_2S and FeS. The average surface temperature on earth was estimated at above 100°C, thus water was not present in liquid form. As the planet gradually cooled down, energy provided by UV, lightning and thermal energy from volcanic activity might have caused important biochemical molecules such as amino acids and sugars to form from the gases present in the atmosphere. Polymerization of those to polypeptides and polynucleotides eventually set the stage for the origin of life. The first geological records of living organisms are 3.5 billion year old stromatolite structures found in Australia. Ancient cyanobacteria in these stromatolites still had to be thermophilic and some are still present as descendent species in modern hot springs. Beginning approximately 3.8 billion years ago, the evolution of oxygenic photosynthesis in the proterozoic ocean, first attributed to anaerobic bacteria and approximately 100-200 million years later to cyanobacteria, very gradually oxygenated the ancient neutral to reducing environment and lead to the transition into the contemporary atmospheric composition of mainly N_2, O_2 and CO_2 (Falkowski and Raven 1997; Madigan et al. 2000). In the primordial ocean ammonia was present at high concentrations and thus no deficiency of inorganically bound nitrogen for cellular growth would select for the evolution of nitrogen fixing enzymes such as nitrogenase. It is likely that ancestral forms of nitrogenase functioned to detoxify cyanides and other chemicals from the reducing atmosphere in the cells. As combined nitrogen was progressively exhausted from the atmosphere and nitrogenase is unselective to triple bound compounds such as acetylene, cyanine and N_2, this form of a detoxase likely evolved into nitrogenase. In parallel, NH_3 was oxidized to nitrite and nitrate (Figure 1). Today, many forms of the nitrogenase enzyme (encoded by *nifD*, *nifK*, *nifE* and *nifN* genes) exist throughout several bacterial kingdoms and archaea. Nevertheless, the enzyme originates from a common ancestor in the oxygen free proterozoic ocean (Fani et al. 2000). Nitrogenase is irreversibly inhibited by oxygen, but the photosynthesizing cyanobacteria increased the oxygen concentration in the atmosphere (Figure 1). Therefore, nitrogenase in modern day organisms is either confined to anaerobic bacteria and archaea or to aerobes that evolved physiological strategies to allow for nitrogen fixation and oxygenic photosynthesis within the same organism. These include oxygen protective sheaths (heterocysts, heterocystous cyanobacteria, i.e. *Aphanizomenon* in the Baltic Sea) or temporal and spatial separation of these two contradicting processes including oxygen-detoxifying mechanisms within the cells (Berman-Frank et al. 2001b; Berman-Frank et al. 2003).

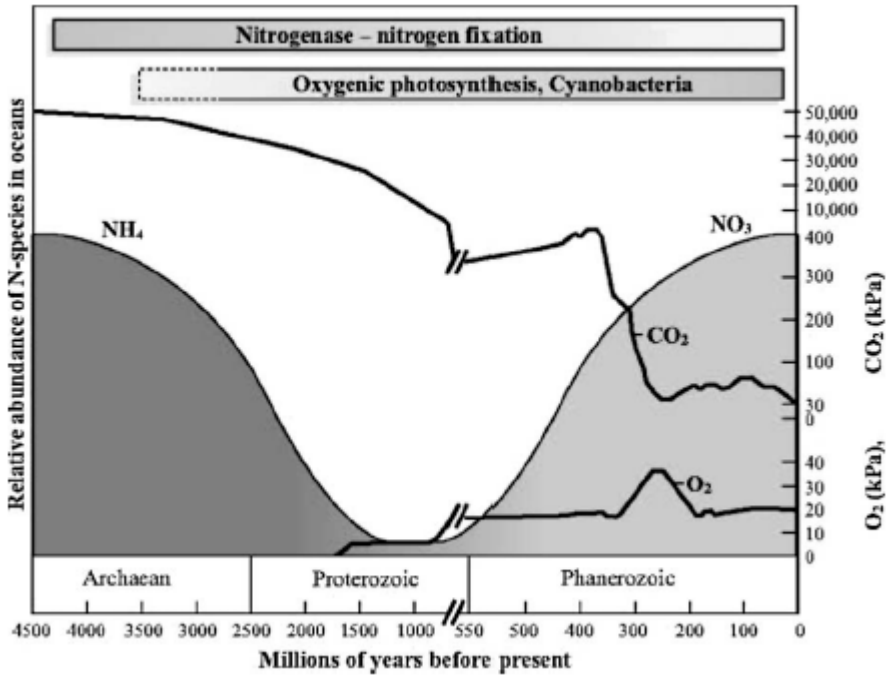

Figure 1: Evolution of the biogeochemical cycles of oxygen, CO_2, nitrogen (ammonia and nitrate), and the corresponding evolution of the metabolic pathways of oxygenic photosynthesis and N_2 fixation (presence of nitrogenase) in cyanobacteria. Dashed segment for oxygenic photosynthesis indicates the debated origins of this process in cyanobacteria (Berman-Frank et al. 2003).

The marine Nitrogen Cycle

Marine nitrogen fixation provides NH_4^+, which next to NO_3^- and NO_2^- is assimilated by bacteria and phytoplankton and synthesized into amino acids and proteins. Organic nitrogen can be released as NH_4^+. Ammonia that is not assimilated into organic material can be oxidized via nitrification into nitrite and further into nitrate. This nitrate can be another nitrogen source to the euphotic zone by diffusion and advection from below the nitracline. Alternatively, nitrate can be reduced back to ammonia (nitrate reduction). During nitrification hydroxylamine (NH_2OH) can be formed as an intermediate product. Ammonia can also be oxidized with nitrate via hydroxylamine and hydrazine (N_2H_4) to elemental nitrogen or N_2O under anoxic conditions (anamox reaction). Nitrate is reduced via denitrification in several steps into elemental nitrogen. Intermediate products are NO and N_2O (nitrous oxide). This requires anoxic or suboxic conditions and nitrate instead of oxygen acts as an electron acceptor. Assimilatory nitrate reduction refers to biological uptake into organic material via nitrite, using energy. Further, atmospheric deposition of NH_4^+, NO_3^- and dissolved organic

nitrogen (DON) can be a source of fixed nitrogen to the euphotic zone. Of the gases that are exchanged with the atmosphere (NO, N_2O, N_2 and NH_3), N_2O is known as a potent greenhouse gas (Figures 2 + 3). The biologically driven nitrogen cycle has been altered anthropogenically to a large extent. Nitrogen oxidation into nitrate via the Haber-Bosch process for fertilizer production introduced large amounts of nitrate into lakes and oceans and thus unbalanced many coastal ecosystems.

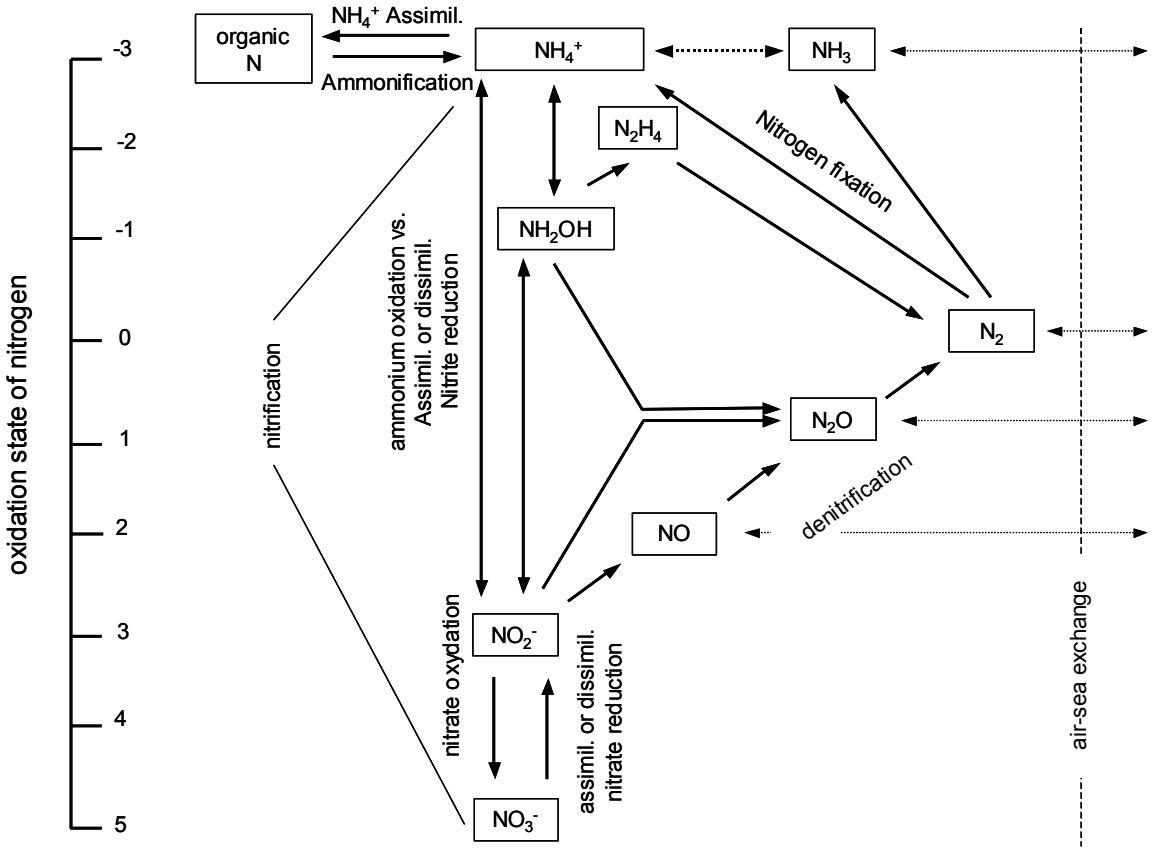

Figure 2: The marine N-cycle modified after Capone, 1991.

The role of *Trichodesmium* in the marine Nitrogen and Carbon Cycle

The cyanobacterium *Trichodesmium* is a critical link in the marine nitrogen cycle of the contemporary ocean. This diazotroph has been recognized as one of the major contributors to oceanic nitrogen fixation (Capone et al. 1997) and further as an important primary producer in tropic and sub-tropic oligotrophic oceans (Carpenter et al. 2004). In contrast to the vertical diffusion or mixing of NO_3 through the stable pycnocline, nitrogen fixation provides a source of new nitrogen that enters from the atmosphere rather then from below. Thus, *Trichodesmium* also significantly contributes to export production via inputs of fixed nitrogen, which fuels the phytoplankton community.

Large imbalances in the budget of nitrogen have recently suggested that the importance of N_2 fixation has been grossly underestimated (Gruber and Sarmiento 1997; Michaels et al. 1996). In particular, data from the Hawaiian Ocean Time Series (HOT) suggest that significant amounts of nitrogen fixation stimulated by climatic variations such as the El Nino Southern Oscillation (ENSO) can supply nitrogen input to normally nitrogen-limited oceanic areas, shifting the ecosystem from nitrogen limitation towards phosphate limitation (Karl et al. 1997). It has also been suggested that variations in Fe inputs may be instrumental in modulating the importance of new production driven by N_2 fixation in oligotrophic environments (Berman-Frank et al. 2001a; Karl et al. 1997). Recently, Montoya et al. (2004) and Zehr et al. (2001) suggested that unicellular nitrogen fixers in addition to the filamentous cyanobacterium *Trichodesmium* may be of great significance to the marine nitrogen cycle as well. Their contribution to the marine nitrogen budget has not been determined yet and to date no information on distribution and ecophysiological characteristics exist.

Currently total marine nitrogen fixation is estimated as 110 Tg y^{-1} based on the N* parameter (Gruber and Sarmiento 1997). It should be noted, that Gruber and Sarmiento (1997) derived N* based on an N:P ratio of 125 for blooming *Trichodesmium* (Capone and Carpenter 1999; Karl et al. 1991). Thus, even though Capone and Carpenter (1999) calculated nitrogen fixation by *Trichodesmium* of 80 Tg y^{-1} based on species abundance data, Gruber and Sarmiento's (1997) estimation of total marine nitrogen fixation is partly based on physiological patterns of this diazotroph as well.

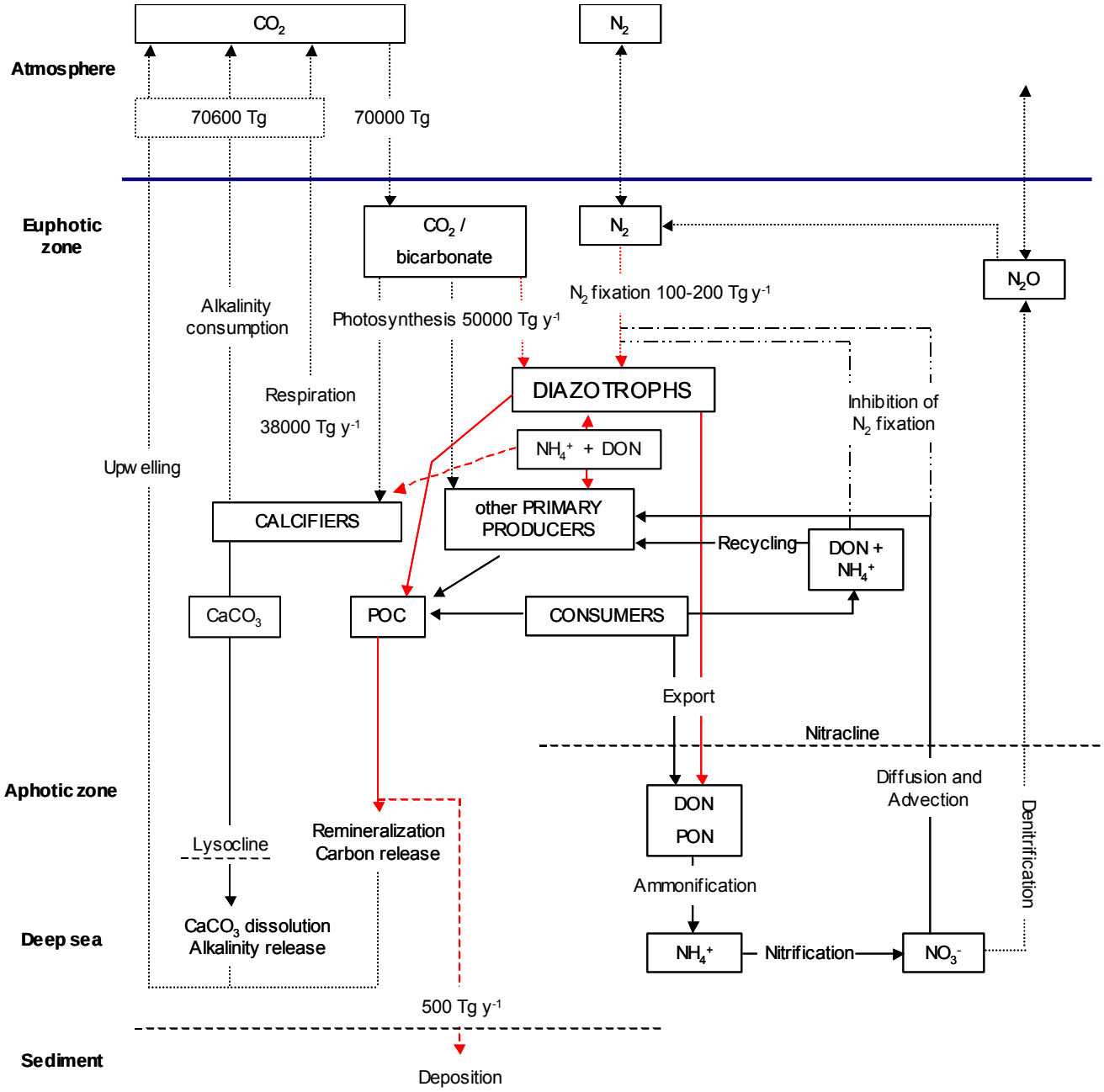

Figure 3: The marine carbon and nitrogen cycle. Note that the carbon cycle does not budget. The 1100 Tg C y^{-1} brought into the oceans by riverine influx are not shown in the figure. Atmospheric deposition of NH_4^+, NO_3^- and DON is not shown in the nitrogen cycle, but may be of great importance. Further, details of denitrification or the anamox reaction are not shown in the nitrogen cycle, but can be seen in Figure 2. These processes take place in sub- or anoxic waters as well as in sediments. Nitrogen fixation is inhibited by NH_4^+ and NO_3^- (see also chapters I and IV). High concentrations of $NH4^+$ and NO_3^- can reduce or completely alleviate the role of diazotrophy in providing inorganic nitrogen to the euphotic zone. Is this not the case, diazotrophs, namely *Trichodesmium* in the oligotrophic sub-tropical oceans, fuel other primary producers with ammonia and DON, thus contribute to PON and POC export themselves and indirectly. Calcifiers are potentially fuelled by diazotrophs. Planktonic calcifiers are not described to co-occur with *Trichodesmium*. In contrast, calcifying coral-reef organisms do co-occur with *Trichodesmium* and account for approximately 50% of the global calcium carbonate production. Carbon and nitrogen cycle pathways that are directly influenced by diazotrophy are highlighted in red.

Clearly these estimates are subject to uncertainties. Hansell et al. (2004) derive a nitrogen input of 0.045 mol N m^{-2} year into the North Atlantic Ocean based on abundance of excess nitrate, which is only 62% of the estimate provided by Gruber and Sarmiento (1997). The estimates by Hansell et al. (2004) are in accordance with marine nitrogen fixation measurements, which in return are largely represented by data on nitrogen fixation by *Trichodesmium*. Matching the estimates by Hansell et al. (2004), Hood et al. (2004) provide an assessment of the contemporary distribution and nitrogen fixation of *Trichodesmium* in the Atlantic Ocean by a numerical model based on field observations of *Trichodesmium*, mixed layer depth (MLD) and light.

On an organism level, *Trichodesmium* clearly differs from other conspicuous nitrogen fixers in that it does not possess heterocysts, but yet simultaneously fixes nitrogen and carbon during daytime. This is an apparent paradox and functional adaptations to this are discussed in the chapters I and V. *Trichodesmium* shares this distinctive feature with three other non-heterocystous diazotrophs grouped in the order Oscillatoriales: *Symploca sp.* (Fredriksson et al. 1998), *Lyngbya majuscula* and *Katagnymene sp.*. It should be noted that descriptions of the nitrogen and carbon fixation pattern of *Lyngbya majuscula* are controversial (Jones 1990; Lundgren et al. 2003). Further, *Katagnymene sp.* has also been grouped as one genus with *Trichodesmium* (Lundgren et al. 2001). Of those genera, *Trichodesmium* species are described to be the most abundant dinitrogen fixing cyanobacteria in tropical and subtropical waters and their contribution to marine nitrogen fixation is estimated at 80 Tg N_2 fixed $year^{-1}$ of a total of 110 Tg $year^{-1}$ (Capone et al. 1997; Gruber and Sarmiento 1997). Next to the simultaneous activity of the photosynthetic enzymes and nitrogenase in an aerobic environment, *Trichodesmium* is characterized by two other important ecophysiological aspects. This diazotroph carries out vertical migrations in the water column and a significant proportion (up to 50%) of the nitrogen fixed is extracellularly released as dissolved amino acids and NH_4^+ (Glibert and O'Neil 1999; Letelier and Karl 1998; Mulholland and Capone 2000). Detailed information on the biology and ecophysiology of *Trichodesmium* is provided in Chapter I.

AIM OF THIS THESIS

This thesis aims to contribute to the mechanistic understanding of diazotrophic growth by *Trichodesmium*. Chapter I reviews published literature and identifies gaps of knowledge required for parameterization of OBCMs. Particular tasks identified were the effect of the abiotic factors temperature and light on growth and nitrogen fixation by *Trichodesmium*.

These are addressed in laboratory studies within this project (Chapter III + IV). Prior to this, potential sources of methodological error were identified in a commonly applied nitrogen fixation measurement technique, the Acetyle Reduction Assay (ARA). A reevaluation of this method was carried out and synthesized in chapter II.

Nitrogen fixation measurement techniques

A number of methods are at hand to research nitrogen fixation processes. Most commonly, nitrogen fixation is measured using the acetylene reduction assay (ARA), ^{15}N stable isotope analysis or to some extend laser photoacoustic detection (Capone and Montoya 2001; Montoya et al. 1996; Zuckermann et al. 1997). ARA has been the method of choice in numerous marine studies for the past 30 years, mainly due to its low cost and ease of use. Generally this method involves analyzing a gaseous phase that is in equilibrium with the liquid phase of interest. As a substrate, acetylene (C_2H_2) blocks the reduction of dinitrogen by the nitrogen fixing enzyme nitrogenase, and is instead reduced to ethylene (C_2H_4). Ethylene is detected easily and with high sensitivity using gas chromatography. As for any gas, the solubility of ethylene in aqueous solution depends on the solutions temperature, pressure and salinity. Even though numerous studies have been conducted applying the ARA, gas solubility coefficients for ethylene in seawater were not available from the literature. In order to assess the effect of temperature on nitrogen fixation of the marine cyanobacterium *Trichodesmium* (Chapter III), the ARA method was reevaluated. Gas solubility coefficients of ethylene (here Bunsen coefficients) were determined and corrections were made to gas solubility calculations applied in the ARA (Chapter II).

Marine Nitrogen Fixation and Global Warming

It is of great interest how biogeochemical cycles are affected by global change processes. Published perspectives of future marine nitrogen fixation diverge largely. While a potential decrease of nitrogen input by *Trichodesmium* during this century is suggested due to reducing iron fluxes into the ocean (Berman-Frank et al. 2001a), Boyd and Doney (2002) predict a future increase of marine nitrogen fixation by 27% (from 80 to 94 Tg yr^{-1}) at low and mid latitudes. Analogous to these findings, Cox et al. (2000) further predict a decrease in ocean productivity as a feedback mechanism due to global warming based on the same (HadCM3) model used in Chapter III. While increased stratification results in decreased upwelling at low latitudes and thus lower primary productivity of non-diazotrophs (Bopp et al.

2001), this mechanism would favor diazotrophic growth. This in return though would not yield increased primary productivity if these diazotrophs were growing outside their temperature optima, regardless of nutrient conditions. Cox et al. (2000) argue that (in terrestrial systems) increasing temperature does not necessarily yield increasing productivity, and thus CO_2 uptake, due to increasing respiration rates. This might be well applicable to marine phytoplankton, which could increase carbon uptake with increasing pCO_2 in seawater (Riebesell et al. 1993) without necessarily resulting in increased growth rates and thus incorporation of carbon in particulate matter. Nevertheless, temperature and pCO_2 rise might affect species succession and thus marine biogeochemistry (Rost et al. 2003; Tortell et al. 2000). In order to isolate the single effect of temperature on diazotrophy, the relationships between temperature and growth and nitrogen fixation by *Trichodesmium* were assessed and incorporated into global warming scenarios from state-of-the-art climate models (Chapter III).

Co-limitation of iron, phosphorus and light on diazotrophic growth

The possibility that Fe limits N_2 fixation in the ocean has been recently considered on the basis of theoretical arguments and it has been speculated that differences in Fe supplies to the oceans and thereby nitrogen fixation fluxes may account for glacial/interglacial changes in atmospheric CO_2 (Raven and Falkowski 1999). The theoretical iron requirement for photosynthetic diazotrophs is higher than in other phytoplankton. Additional Fe is required for the Fe-Mo subunit of nitrogenase and thus initially the iron requirement was calculated to be 100 times higher than for phytoplankton growing on nitrate (Raven 1988). This has been recently revised and derived to be only 3-4 times higher (Sanudo-Wilhelmy et al. 2001) in close agreement with Kustka et al. (2002) (5 times higher). The high iron requirements of *Trichodesmium* led to the assumption that nitrogen fixation could be iron limited in oligotrophic oceans. As aforementioned, Berman-Frank et al. (2001a) derives future iron limitations from laboratory results on iron limited *Trichodesmium* growth. In contrast, Sanudo-Wilhelmy et al. (2001) concludes phosphorus and not iron limitation for diazotrophy in the sub-tropical Atlantic Ocean. Interestingly, *Trichodesmium* can bloom where PO_4 concentrations are very low and it is therefore likely that *Trichodesmium* relies heavily on DOP to meet its P requirements (Sanudo-Wilhelmy et al. 2001; Wu et al. 2000) (see chapter I). Nevertheless, in agreement with Berman-Frank et al. (2001a), Mills et al. (2004) clearly stress the importance of aeolian dust input and thus iron supply for nitrogen fixation in the eastern subtropical North Atlantic and conclude an iron-phosphorus co-limitation. An open question to date is how atmospheric deposited iron in surface waters is taken up by

phytoplankton. *Trichodesmium* can scavenge Fe from a variety of siderophores, but it is unclear whether *Trichodesmium* itself can produce siderophores (Rueter 1988). Siderophore 'piracy' from associated heterotrophic bacteria (Hutchins et al. 1999) may play an essential role in the Fe chemistry in *Trichodesmium* colonies. Further details are discussed in chapter I.

Assessing the photosynthetic characteristics of *Trichodesmium* grown at different Fe concentrations showed that this diazotroph possesses reduced photosynthetic efficiency and lowered light compensation points under conditions of iron limitation (E. Breitbarth, unpubl. data). This suggests an inverse co-limitation of iron and light. If *Trichodesmium* is iron limited, the patterns in reduced light compensation points and maximum electron transport rates indicate that the photosynthetic apparatus could not process high light levels efficiently. Photosynthesis at high light levels, as common in tropic and sub-tropic oceans, is bound to iron replete conditions. Diazotrophically growing cyanobacteria require additional energy (16 ATP per N_2 molecule reduced) compared to NH_4^+ assimilating phototrophs. This energy is provided by the photosystem. Thus light limitation for diazotrophic cyanobacteria may occur at higher light levels compared to other phototrophs. Hood et al. (2002) measured nitrogen fixation by *Trichodesmium* as a function of light intensity in the tropical Atlantic Ocean and included the parameter to model nitrogen fixation. While the model proved to be insensitive to forcing by surface irradiance, field measurements showed a wide scatter of maximum nitrogen fixation rates (0.64 – 10 nmol N µg Chl-*a*$^{-1}$ h^{-1}), light inhibition (266 – 3519 µmol quanta m^{-2} s^{-1}) and the light limited slope of nitrogen fixation (0.003 – 0.092 nmol N µg Chl-*a*$^{-1}$ h^{-1} (µmol quanta m^{-2} s^{-1})$^{-1}$). To better understand the basic mechanistics, the direct effect of light intensity on diazotrophic growth is assessed using *Trichodesmium* cultures that were grown under iron replete conditions (chapters IV+V). Photoautotrophs adjust the photosynthetic apparatus to ambient light regimes via acclimation of light harvesting pigments. As a general response, the relative amount of chlorophyll-*a* per carbon biomass is reduced with increasing light intensities (Geider et al. 1997). This may result in a bias of physiological rate measurements if these are normalized to chlorophyll biomass, as modeled by Hood et al. (2002). Chapter IV addresses this by comparing chlorophyll-*a* and carbon normalization of nitrogen fixation rates of *Trichodesmium* at different light intensities. The chapter aims to improve the parameterization of light for nitrogen fixation throughout the euphotic zone and provides a three-step model to describe diazotrophic growth of *Trichodesmium* as a function of light intensity.

Chapter V provides an outlook for perspective future work based on new questions raised by the work presented in chapters II-IV. The thesis concludes with a synthesis of published

data and own results to a conceptual model of nitrogen and carbon fixation in *Trichodesmium* with particular respect to energy limitation in different light regimes.

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CHAPTERS

List of chapters

This doctoral thesis is based on the following publications/manuscripts:

I. LaRoche, J. and E. Breitbarth. 2004. Importance of the diazotrophs as a source of new nitrogen in the ocean. *Journal of Sea Research* 53: 67-91.

II. Breitbarth, E., M.M. Mills, G. Friedrichs and J. LaRoche. 2004. The Bunsen gas solubility coefficient of ethylene as a function of temperature and salinity and its importance for nitrogen fixation assays. *Limnology and Oceanography: Methods* 2: 282-288.

III. E. Breitbarth, A. Oschlies, and J. LaRoche. 2004. Global warming may decrease nitrogen fixation by *Trichodesmium*. to be submitted to *Geophysical Research Letters*

IV. Breitbarth, E., I. Peeken, J. Wohlers, J. Kläs and J. LaRoche. 2004. Nitrogen fixation and growth rates of *Trichodesmium* IMS-101 as a function of light intensity. to be submitted to *Limnology and Oceanography*

V. Synthesis and perspective future work

Statement on my contribution to the publications/manuscripts

publication I:
This work originated from the IRONAGES work plan and is designed to serve as a reference tool for biogeochemical modelers. As a basis for this review literature references and data collections from over 200 publications were compiled and summarized in tables and graphical form by me. The manuscript was co-written by J. LaRoche and myself.

publication II:
The concept for this paper was derived by M.M. Mills and myself. The paper was written by myself. G. Friedrichs contributed in writing, expertise and scientific advice. J. LaRoche provided scientific advice.

manuscript III:
Laboratory experiments were planned, conducted and analyzed by me. The paper was written by me. A. Oschlies provided the model analysis and edited the writing. J. LaRoche edited the manuscript and provided scientific advice.

manuscript IV:
The experiments were planned and conducted by me in cooperation with I. Peeken, J. Klaes and J. Wohlers contributed to the data collection. The manuscript was written by me. J. LaRoche and I. Peeken edited the manuscript and provided scientific advice.

chapter V:
This chapter contains data from experiments that were planned and conducted by me in cooperation with I. Peeken, J. Klaes and J. Wohlers.

Importance of the diazotrophs as a source of new nitrogen in the ocean

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Importance of the diazotrophs as a source of new nitrogen in the ocean

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Abstract

Nitrogen fixation is one of the important biochemical pathways that play a role in controlling the oceanic nitrogen inventory. Here we review nitrogen fixation in the ocean, with a particular emphasis on *Trichodesmium*, one of the dominant marine diazotrophs. Distribution data for diazotrophs are scarce, except in specific regions where *Trichodesmium* is known to bloom. Although some regions are clearly under-sampled, *Trichodesmium* can generally be found in tropical regions where temperature is at least 20 °C, except in the North Atlantic, where drift to higher latitudes is possible via the Gulf Stream. Likewise, biomass estimates are problematic because of the colony-forming habit of this organism. *Trichodesmium* grows slowly with reported maximum growth rates of approximately 0.14 d^{-1}. Studies of the photosynthetic physiology indicate that *Trichodesmium* can tolerate high light intensity with I_k and I_c values of ~300 and ~140 μmole photons m^{-2} s^{-1}, respectively. Review of the elemental composition of *Trichodesmium* indicates that the C:N molar ratio of 6.3:1 does not depart significantly from the predicted Redfield stoichiometry of 6.6:1. Overall, measured N:P ratios from the field and the laboratory were around 50, a significant departure from the Redfield stoichiometry of 16:1. Whether this indicates phosphorus limitation is not clear at present. The iron requirements of diazotrophs in general and of *Trichodesmium* in particular have been the subject of debate, but some recent laboratory studies have converged on Fe:C (μmole:mole) of approximately 50 at 70% of the maximum growth rates (μ_{max}) to 250 at μ_{max} for this species. There is a noticeable lack of information on growth rate as a function of phosphorus and fixed nitrogen sources. Although *Trichodesmium* is a non-heterocystous cyanobacterium, carbon and nitrogen fixation co-occur during the light period, indicating that light energy is required for both of these processes. This is likely to be achieved through cellular differentiation of the trichomes and a tight control of the temporal expression of many biochemical pathways. A summary table presents a set of values for the initial parameterisation of parameters relevant to the incorporation of nitrogen fixation in biological and biogeochemical models.

Keywords: Marine diazotrophs; *Trichodesmium*; Biogeochemical modelling; Iron

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1. Introduction

1.1. Role of nitrogen fixation in the N cycle

Gruber and Sarmiento (1997) calculated pelagic nitrogen fixation to be 110 Tg N y^{-1}, which is 47.5% of the total nitrogen sources (231 Tg N y^{-1}) in the global marine nitrogen budget. Next to riverine input (33%), atmospheric deposition (13%) and benthic nitrogen fixation (6.5%) account for a large fraction of the nitrogen sources to the oceans. However, atmospheric and riverine inputs disproportionately affect the coastal zone and continental shelf. Thus, pelagic nitrogen fixation is the dominant source of new nitrogen to the open oligotrophic ocean. In contrast, denitrification is the major sink (86% of 204 Tg N y^{-1}) with pelagic denitrification accounting for 80 Tg N y^{-1} (39%). The loss to sedimentation is estimated at 12% and N_2O formation is responsible for 2% of the nitrogen sinks from the ocean. Denitrification, which releases N_2 to the atmosphere, and biological nitrogen fixation, which fixes N_2 gas, are thus the two opposing biochemical pathways balancing the oceanic nitrogen cycle. In the ocean, a change in the rate of either of these pathways will result in a change in the global oceanic nitrogen inventory. All of these figures are subject to considerable uncertainty, particularly N_2 fixation. For example, recent estimates of nitrogen fixation using geochemical methods (Hansell et al., 2004) were only 15% of those calculated by Gruber and Sarmiento (1997) for the North Atlantic.

The contribution of fixed nitrogen to the marine environment has been at the centre of a long-term debate concerning whether nitrogen or phosphorus is the nutrient ultimately limiting oceanic production (Falkowski, 1997; Falkowski et al., 1998; Tyrell, 1999). Geologists have argued that over geological time scales, phosphorus rather than nitrogen must be limiting, any deficiency in nitrogen being compensated by fixation of dinitrogen gas. Conversely, biologists have argued that evidence for significant nitrogen fixation in oligotrophic areas has not been documented and that environmental factors regulating nitrogen fixation are not well understood. Dissolved phosphorus and energy supplies are important, but trace elements may also play a role. It has recently been established through in situ Fe enrichment experiments that Fe limits primary production in HNLC regions (Falkowski, 1997; Boyd et al., 2000). It also appears that Fe plays an important role in shaping the community structure in other marine environments (Hutchins et al., 2001) or for particular algal groups (e.g. red tides) (Lenes et al., 2001). It has recently been hypothesised that low Fe concentrations throughout the world oceans, and particularly in warm oligotrophic waters, may limit the abundance of diazotrophs (Falkowski, 1997). The debate as to whether dissolved phosphorus (Sanudo-Wilhelmy et al., 2001) or iron supply (Wu et al., 2000) to the euphotic zone controls diazotroph growth awaits field experimental evidence and additional measurements of the optimal elemental composition of marine diazotrophs.

1.2. Nitrogen fixation

Biological nitrogen fixation is a biochemical process that is confined to a limited number of prokaryotes. It plays an indispensable role in the global nitrogen cycle by providing fixed nitrogen. Bacteria performing this function are present in virtually all ecosystems. Diazotrophs are found in all environments ranging from *Azotobacter* species in aerobic soils, *Rhizobium* in nodules of legume roots to *Trichodesmium* in the surface layer of the oceans.

The nitrogenase complex catalyses biological nitrogen fixation, the conversion of N_2 to NH_4^+, as represented by:

$$N_2 + 8H^+ + 8e^- + 16ATP = 2NH_3 + H_2 + 16ADP + 16Pi$$

where ATP, ADP and Pi represent adenosine triphosphate, adenosine diphosphate and inorganic phosphorus, respectively. Although thermodynamics favours the reduction of N_2 to NH_3, the high activation energy required to break the triple bond is responsible for the high ATP requirements. The nitrogenase enzyme complex catalyses the reaction. The electrons are obtained from reduced ferredoxin while various ATP-generating processes are involved in providing the energy necessary for the reaction. Nitrogenase functions under anaerobic conditions and is deactivated by high levels of oxygen. Thus the high energy demands for nitrogen fixation together with the need to reduce intracellular O_2 levels set some constraints on their light and temperature requirements, and ultimately on the growth rates of diazotrophs.

Table 1
Summarised physiological parameters of Trichodesmium species (for references see text and http://www.nioz.nl/projects/ironages)

Parameter	Value mean (range)	Unit
Photosynthesis and growth		
Optimal specific rate of photosynthesis k_{max}	26 (6.3–91)	mg O_2 mg Chl-a^{-1} h^{-1}
Photosynthetic efficiency α	0.09 (0.01–0.27)	
Light adaptation parameter I_k	296 (142–687)	μmol photon m^{-2} s^{-1}
Light compensation I_c	143 (59–280)	μmol photon m^{-2} s^{-1}
Photoinhibition index β	small up to 2000	μmol photon m^{-2} s^{-1}
Specific rate of maintenance metabolism	30	% of gross photosynthesis
Maximum specific growth rate μ	0.14	d^{-1}.
Temperature range for growth	20–34	°C
Nutrient limitation/uptake		
K_s/V_{max} NO_3	39/not detected	mmol m^{-3}/h^{-1}
K_s/V_{max} NH_4	0.26/13	mmol m^{-3}/h^{-1}
K_s/V_{max} PO_4	0.0004/0.29	mmol m^{-3}/d^{-1}
K_s/V_{max} SO_4	not applicable	
Elemental composition		
C : N	6.3 (4.7–7.3)	mol:mol
N : P	51.3 (4.8–150)	mol:mol
Chl-a: C	96.5–320	μmol:mol
Fe : C	7.1–500	μmol:mol
(req. for diazotrophic vs. NH_4 replete growth)	(38–48 vs. 8)	
Loss terms		
Sinking rate	buoyant	
Cell lysis	virus and apoptosis	
Grazing rate	0.14–2.75	mg C m^{-2} d^{-1}
Carbon fixation		
Carbon fixation rate biomass^{-1}*	up to 4.5	μg C μg Chl-a^{-1} h^{-1}
Estimated total annual carbon fixation rate**	0.41	Gt y^{-1}
Nitrogen fixation		
Total annual nitrogen fixation rate	0.065	Gt y^{-1}
Nitrogen fixation rate volume^{-1}***	40 (0.0071–711)	μmol N l^{-1} d^{-1}
Nitrogen fixation rate biomass^{-1}****	0.1–10	mol N mol Chl-a^{-1} h^{-1}
Photoinhibition	813 (266–3519)	μmol photon m^{-2} s^{-1}
Inhibition by NO_3	70%	At 10 μM NO_3

* Function of groth phase and daytime (Berman-Frank et al., 2001b; Mulholland and Capone, 2001).

** Based on annual N-fixation rate and C : N = 6.3.

*** Own laboratory N-fix measurement on exponentially growing batch culture (0.06 μmol Chl-a l^{-1}), mass balance in agreement with PN increase in cultures (literature range in parentheses).

**** Represents range in possible agreement with mass balance, highest measurements reported are 40 and 2242 mol N mol Chl-a^{-1} h^{-1} in the laboratory and in the field, respectively.

1.3. Goals

Although recent studies demonstrated that the diversity of marine diazotrophs is greater than previously thought (Zehr et al., 1998, 2000; Zehr and Ward, 2002; Montoya et al., 2004), this review will focus primarily on *Trichodesmium*. This filamentous non-heterocystous species is considered the most important diazotrophs globally, and is physiologically well characterised. Several reviews on *Trichodesmium* have been published within the past ten years (e.g. Gallon et al., 1996; Bergman et al.,

1997; Bergman, 1999; Mulholland and Capone, 2000). These reviews mainly focus on specific morphological or physiological aspects of diazotrophic growth of *Trichodesmium*. In the present work we summarise data from both laboratory and field studies performed with *Trichodesmium* in order to provide (a) a general overview of current knowledge of the biology of *Trichodesmium,* (b) a compilation and evaluation of data relevant to the parameterisation of oceanic biogeochemical models. Initial estimates of parameters needed for physiologically based phytoplankton growth models are summarised (Table 1), emphasising important biochemical pathways and other factors that can influence nitrogen fixation. Additionally, we provide conversion factors for biomass units generally applied in physiological rate measurements (Table 2). More detailed information can be found in a set of Appendices (http://www.nioz.nl/projects/ironages) organised by type of parameters measured, including range, median, mean and standard deviation of each parameter listed whenever possible (Table 3; http://www.nioz.nl/projects/ironages). We also point out where discrepancies are found and whether the experimental techniques or conditions for measurements differed greatly from the rest of similar data.

Finally, we indicate where further knowledge is required and propose a list of processes that should be considered for incorporation into ecological and biogeochemical models but for which data are missing.

Table 2
Cells, trichome and colony sizes and elemental contents (for details and references see http://www.nioz.nl/projects/ironages)

Parameter	Data	Units
Cell, trichome, colony measures		
cell volume	540–1690	μm^3
cell diameter	5–21	μm
trichome width	8–10	μm
colony diameter	0.5–1000	mm
cells trichome^{-1}	~ 100 (6–340)	Cells
cells colony^{-1}	29800 (+/−7800)	″
trichomes colony^{-1}	~ 100 (7.5–372)	Trichomes
Elemental content		
C trichome^{-1}	4.2–4.8	nmol C trichome^{-1}
C colony^{-1}	0.81–0.92	μmol C colony^{-1}
N cell^{-1}	3.6–70.4	pmol N cell^{-1}
N trichome^{-1}	0.69	nmol N trichome^{-1}
N colony^{-1}	7.1–172.8	nmol N colony^{-1}
Protein N colony^{-1}	97 (51–204)	nmol col^{-1}
P trichome^{-1}	16.5	pmol P trichome^{-1}
P colony^{-1}	3.9–15.5	nmol P colony^{-1}
Chl-a cell^{-1}	1.18–1.73	fmol Chl-a cell^{-1}
Chl-a colony^{-1}	89.5 (42.2–109.7)	fmol Ch-a colony^{-1}

Table 3
Overview of Appendices

Appendix	Parameters measured in *Trichodesmium* sp.
I	cell density and biomass in the field
II a	cells, trichome and colony sizes and conversion factors
II b	elemental content
III a	carbon turnover times
III b	growth rates-field data
III c	growth rates-lab data
IV	photosynthetic characteristics
V a	nitrogen fixation rates-field data
V b	nitrogen fixation rates-lab data
VI a	elemental composition-field data
VI b	elemental composition-lab data
VII	cellular Fe stoichiometry and iron uptake rates
VIII	nutrient uptake rates

The appendices are available at: www.nioz.nl/projects/ironages.

2. Diversity of marine diazotrophs

2.1. Overall diversity of diazotrophs

Historically, marine diazotrophs have not been considered to be either diverse or abundant, with *Trichodesmium*, a genus of filamentous cyanobacteria without heterocysts, as the only globally significant oceanic photosynthetic diazotroph (Capone et al., 1997). Although the prevalence of *Trichodesmium* in the Sargasso Sea has long been acknowledged (Dugdale et al., 1964; Goering et al., 1966; Dugdale and Goering, 1967), unequivocal evidence that this filamentous cyanobacterial species rather than the associated heterotrophic bacteria was the diazotroph came years later (Bergman and Carpenter, 1991; Ohki and Fujita, 1988). Diatoms containing cyanobacterial endosymbionts (Janson et al., 1999b) have also been observed to form intense blooms, but the frequency of these blooms is poorly characterised.

Recent work using molecular biological techniques has revealed a much higher diversity in the *nifH* gene,

encoding for the iron protein component of the nitrogenase enzyme (Zehr et al., 1998, 2000, 2001), from nucleic acids isolated from the marine environment. Perhaps of greatest importance so far, is the identification of a group of larger unicellular cyanobacteria (3–10 micron in size) phylogenetically related to *Cyanothece* (ATCC 51142) or *Synechocystis* (WH8501). Nitrogen fixation by these unicellular species may contribute significantly to the global nitrogen cycle (Montoya et al., 2004). However, the limited information on their overall global distribution and their physiology (Mitsui et al., 1986) prevents the incorporation of these organisms into global biogeochemical models. In addition to cyanobacteria, several other groups of marine heterotrophic bacteria also harbor *nifH* genes (Zehr et al., 1998). The role of these organisms in the oceanic nitrogen cycle is not understood at present.

2.2. Diversity of the genus Trichodesmium

This genus was first described in 1830 by Ehrenberg (Ehrenberg, 1830) and since then has been shown to be widely distributed in many tropical and subtropical oceans (Capone et al., 1997). Although there are potentially other important diazotrophs in the ocean, *Trichodesmium* is unique in its capacity to form visible blooms, often dense enough to locally modify the environment. *Trichodesmium* is the only marine photosynthetic diazotroph species that has been experimentally manipulated in the laboratory and in the field and for which there is significant quantitative information. Its genome has been sequenced by the Joint Genome Institute, U.S. Department of Energy and the resulting database will provide invaluable information concerning the biochemical pathways involved in regulating nitrogenase activity, photosynthesis, nutrient (N, P, and Fe) uptake, and growth.

Several species of *Trichodesmium* have been reported as well as several morphotypes within a given species. Two cultured isolates are available: *Trichodesmium* NIBB1067 from the Pacific Ocean and IMS 101 from the Atlantic Ocean. Molecular phylogenetic data showed that both of the cultured strains are isolates of *Trichodesmium erythraeum* (Ben-Porath and Carpenter, 1993). A recent study (Janson et al., 1999a) comparing morphological characteristics of *Trichodesmium* species and DNA sequence information (Janson et al., 1995) shows clustering of *T. contortum* and *T. tenue* and clustering between *T. thiebautii* and *T. hildebrandtii*. Another filamentous diazotroph, *Katagnymene* sp. forms a monophyletic group within the *Trichodesmium* genus (Lundgren et al., 2001), based on the sequence similarity of the *nifH* and *hetR* genes, and despite the wide phenotypic variation observed within this proposed single genus. The strategy of nitrogen fixation under aerobic conditions, i.e. the spatial and temporal segregation of nitrogen fixation and photosynthesis is conserved throughout this group (Lundgren et al., 2001), supporting the molecular phylogenetic data.

3. Autoecology of *Trichodesmium*

3.1. Observed distribution

Accurate global distribution maps of *Trichodesmium* do not exist yet because of the difficulty in sampling this species. The diazotrophic growth of *Trichodesmium* is generally confined to waters warmer than 20°C of oligotrophic provinces and this has been an important factor in generating theoretical distribution limits for this species. Thus, distribution maps of *Trichodesmium* are more often based on physiological criteria such as temperature tolerance range, nutrient regime preferences and iron requirements (Capone et al., 1997; Berman-Frank et al., 2001a) than on field observations. With a few exceptions (Wille, 1904; Lipschultz and Owens, 1996; Tyrrell et al., 2003), distribution and abundance of *Trichodesmium* have been reported primarily for regions where this species can form blooms, biasing to some extent our view of the global distribution of *Trichodesmium*. Fig. 1 shows a distribution map of *Trichodesmium* that combines temperature-based theoretical distributions and field observations. Detailed assessments of the *Trichodesmium* distribution has been hindered by the lack of appropriate techniques. *Trichodesmium*'s buoyancy prevents its enumeration using traditional Utermohl techniques. In fact there is very little agreement in general between *Trichodesmium* counting techniques (Chang, 2000; Tyrrell et al., 2003), and the presence of

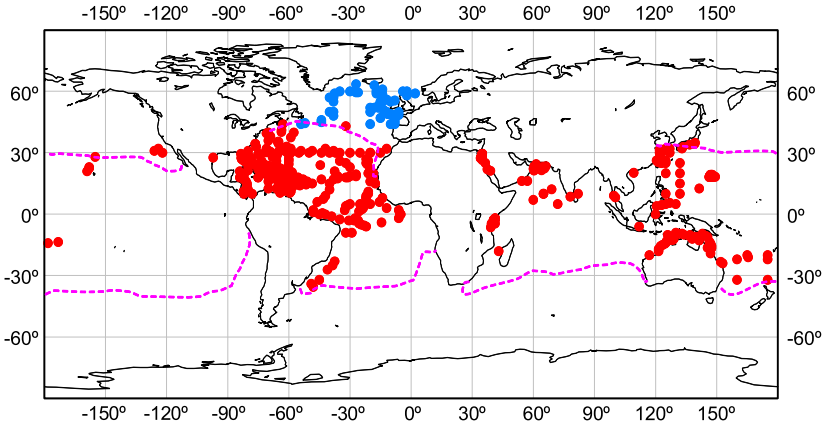

Fig. 1. Global distribution of *Trichodesmium* based on field studies as reported in peer reviewed literature. The distribution of the data points illustrates occurrences, but not abundances of *Trichodesmium.* Sampling points where no *Trichodesmium* was encountered are not shown. Red dots show sampling points where either physiological rate measurements were done or where nitrogen fixation by *Trichodesmium*. is likely because the organism was encountered within oligotrophic waters with surface temperatures (SST)>20°C. The 20°C isotherm of the annual mean SST (Levitus Atlas) is shown by the pink dotted line. Blue dots indicate *Trichodesmium* that drifted to higher latitudes within the Gulf Stream and are unlikely to fix nitrogen due to unfeasible abiotic factors (Lipschultz and Owens, 1996) Note that most cruises were set out to specifically work in regions where *Trichodesmium* is known to occur (i.e. Caribbean and Sargasso Sea). Thus, a wider distribution throughout the tropical and subtropical oligotrophic oceans is likely (i.e. Indian Ocean and South Pacific), but not represented in this map due to under-sampling of these regions.

trichomes and multiple colony morphotypes necessitates the use of conversion factors in order to compare cell densities (Table 2). Despite the sampling difficulties, the observed distribution patterns of *Trichodesmium* remain within the temperature limits except for a region in the north Atlantic that probably reflects passive drift with the Gulf Stream (Fig. 1). *Trichodesmium* inhabits oligotrophic tropical and subtropical regions and is found in high abundance in western boundary currents, in tropical portions of gyres and ocean margin seas. Sightings of *Trichodesmium* blooms in tropical and subtropical seas have often been made by sailors because when abundant, this organisms is readily identified by its sawdust appearance and reddish colour in surface waters of calm seas (e.g. in the Red Sea).

On the East African coast, *T. thiebautii* is a permanent member of the plankton, together with a frequently present symbiotic *Richelia intracellularis-Rhizosolenia* association (Villareal, 1991). *Trichodesmium* is responsible for the red tides in these areas which occur mainly during the dry season (Aleem, 1980). Most of the reports of *Trichodesmium* blooms are from tropical coastal areas. For example, surface blooms were abundant throughout the Gulf of Mexico in 1995 (Biddanda and Benner, 1997). In certain areas, *Trichodesmium* abundance follows a seasonal pattern as is the case in the coastal waters of Tanzania (East Africa) where *Trichodesmium* blooms dominate during the northern Monsoon period (Bryceson and Fay, 1981), which lasts from November to March. *Trichodesmium* also showed seasonal variability in coastal areas of tropical northern Australia (Burford et al., 1995,) where it bloomed in the wet season (i.e. during the light northeast to northwest monsoon when water temperature is generally higher and winds are also lighter). The most extended blooms are during periods of low or no winds. The largest bloom of *Trichodesmium* reported so far was off the coast of New Caledonia in the South Pacific and had an estimated coverage of 90 000 km^2 (Dupouy et al., 1988). Other important areas are shelf waters of north and northwest Australia, Great Barrier Reef, west and east coast of India, east coast of Africa, Madagascar during the Northeast Monsoon, Gulf of Thailand, south-western South Pacific Ocean, Caribbean Sea and Gulf of Mexico (Carpenter and Capone, 1991). The southwest coast of Florida (about 75 km off

shore) has a record of *Trichodesmium* blooms dating back to the 1950s (Lenes et al., 2001). It is clear that blooms of *Trichodesmium* can either be coastal or oceanic, and in some region the blooms are seasonal (e.g. Indian Ocean) (Bergman, 2001). In addition to the distribution and abundance data of *Trichodesmium* reported from trans-meridional cruises in the Atlantic Ocean, seasonal distribution data are available for time-series sites such as HOTS and BATS (Karl and Michaels, 1996).

Satellite remote sensing of *Trichodesmium* abundance holds some promises in providing a global picture of *Trichodesmium* distribution, at least for the documentation of bloom occurrence and magnitude. The identification of *Trichodesmium* in SeaWifs images is possible at a concentration greater than 0.5 mg chl-a m^{-3} (Subramaniam and Carpenter, 1994; Subramaniam et al., 2002; Borstad et al., 1991). *Trichodesmium* is well suited to detection by satellite because its absorption and reflectance characteristics give it a relatively unique spectral signature. Its buoyancy results in high cell densities in surface waters and a shallow subsurface maximum, also facilitating detection from space. Incorporating the strong reflectance of *Trichodesmium* relative to other cyanobacteria makes it possible to differentiate between unicellular cyanobacteria and *Trichodesmium*.

3.2. Physical factors influencing distribution

In general, conditions favourable for *Trichodesmium* growth are a stable water column, with an upper mixed layer around 100 m, low nutrients, very clear water and deep light penetration (Capone et al., 1997). The ranges and optimum temperature, salinity and light for *Trichodesmium* growth provide an empirical framework upon which to base the distribution of *Trichodesmium* in models.

Anecdotal information suggests that nitrogen fixation by this cyanobacterium is limited to water temperatures above 20°C and that its presence in waters with lower temperature is due to drift rather than net growth (Lipschultz and Owens, 1996). Temperature is often reported to have a significant effect on nitrogen fixation rates but there are no laboratory data documenting the long-term physiological effect of low temperature on growth, nitrogen fixation and photosynthesis in *Trichodesmium*. The observed correlation of *Trichodesmium* with high temperatures in the field does not necessarily signify a direct relationship between growth and temperature. Variables that tend to co-vary with temperature including low nutrients, higher light or increased stratification of the water column, may provide the underlying mechanism that accounts for the observed co-variability of *Trichodesmium* with temperature. For example, models based on mixed layer depth and light were sufficient to reproduce the distribution of *Trichodesmium* in the Atlantic Ocean (Hood et al., 2004). *Trichodesmium* can grow well at a temperature of 28 °C (Mulholland et al., 1999b) but can tolerate growth temperatures ranging between 20 and 34 °C (Eike Breitbarth, pers. obs.). Field studies report active blooms of *Trichodesmium* at temperatures as high as 35 °C in the Arabian Sea (Capone et al., 1998). These authors concluded that a temperature increase from 30.5 to 35 °C during an 8-d period was partially due to the formation of the bloom. Through a positive feedback, the accumulation of *Trichodesmium* colonies during calm conditions of minimal mixing led to heat absorption, increased stability and further warming of the water column. It has been speculated that the biologically mediated heating of the surface layer might indeed influence the air-sea heat exchange (Sathyendranath et al., 1991).

In *Trichodesmium*, both photosynthesis and nitrogen fixation as well as the genes encoding for the components of these systems exhibit a circadian rhythm of approximately 24 h, when entrained by a light-dark cycle (Chen et al., 1998, 1999). The period of this circadian rhythm after transfer to continuous light is temperature compensated, with very little effect on the length of the period at temperatures ranging from 24 to 28 °C but the periodicity breaks down completely above 30°C. At 31°C, nitrogenase activity was not detectable under continuous light while the light-dark controls behaved normally (Chen et al., 1998). If the observed circadian rhythms of photosynthesis and nitrogen fixation are an essential requirement for diazotrophic growth of this non-heterocystous filamentous cyanobacteria, then the temperature dependence may be linked to the lower and upper limit for temperature compensation of the circadian rhythm (Chen et al., 1998).

Short-term nitrogenase activity in *Trichodesmium* increases linearly between 15 and 35°C (Staal et al., 2003). As there are no apparent restrictions for the nitrogenase enzyme to function over a wide temperature range (Staal et al., 2003), the effect of temperature on *Trichodesmium* growth and other physiological processes definitely require further investigation, especially in laboratory cultures. Work on tropical and Antarctic strains of *Gloeocapsa* sp. showed that the nitrogenase in the Antarctic strain had a temperature optimum at 20°C, 10 degrees lower than the tropical strain (Pandey et al., 2000). However higher temperature should be more favourable for nitrogen fixation because of the inverse relationship between temperature and the solubility of O_2 in seawater coupled with higher respiration rates at higher temperatures. The temperature optimum and tolerance range in *Trichodesmium* are likely to be related to the overall physiology of this organism rather than to a specific requirement of the nitrogenase enzyme. This is discussed in more detail in Section 5.1.

In the laboratory, *Trichodesmium* can tolerate salinities ranging from 22 to 43, with an optimum for growth and nitrogen fixation between 33 and 37 (Fu and Bell, 2003a). *Trichodesmium* is most often found in waters with a salinity around 35, but has also been regularly cited in waters with a salinity ranging from 27 to 36, for example in the Red Sea and in coastal regions around the Great Barrier Reef. However, as for temperature, the field observations do not necessarily reflect physiological requirements for a high salinity but may be confounded with other variables correlated with high salinity, for example, nutrients. Eastern Caribbean waters of lower salinity, highly influenced by the Orinoco River discharge, were devoid of *Trichodesmium* colonies, probably due to an excess of dissolved reactive nitrogen (Navarro et al., 2000). The light requirements of *Trichodesmium* are included in a general discussion of the photosynthetic apparatus in Section 5.1. and the effect of light on nitrogen fixation is described in Section 5.3.

3.3. Biological factors influencing distribution

Trichodesmium species often form surface blooms. This capability can be attributed both to the presence of gas vacuoles and to a photosynthetic apparatus that can tolerate high light (see Sections 5.1. and 5.3.). The gas vacuoles account for perhaps 5–15% of the total cell protein and exhibit some of the strongest turgor pressure observed in cyanobacteria (Walsby, 1991). It is possible that the large floating velocities (0.1 to 3 mm s^{-1}) observed in *Trichodesmium* enable the colonies to stay in the euphotic zone, and when mixed down, allows them to rapidly float back to the surface. However, highest *Trichodesmium* densities are often at a depth of 20–40 m, implying boyancy regulation in this species. Carbohydrate ballasting is the most likely mechanism for boyancy regulation and the observed daily cycles of sinking and rising (Villareal and Carpenter, 1990). These vertical excursions have been proposed to serve as a mechanism for phosphorus mining at the nutricline, but this hypothesis is not supported by simple model calculations that take into account the natural buoyancy, the sinking velocity as a function of carbohydrate loading, the high respiration rate of *Trichodesmium*, and the depth of the nutricline in oligotrophic regions (100–180 m) . The measured carbohydrate content of *Trichodesmium* in the laboratory and in the field can support vertical excursions of approximately 50 m. Thus it is not clear at this point whether the vertical excursions due to carbohydrate loading are simply a result of the uncoupling between nitogen fixation and photosynthesis, or whether they are of true adaptive value for *Trichodesmium* (Villareal and Carpenter, 1990; Letelier and Karl, 1998).

3.4. Trichome and colony morphology

Trichodesmium can be present in the water column as single trichome or as colonies. There is evidence that trichome morphology differs between species of *Trichodesmium*, but that alone is not sufficient for species identification. Likewise, the colonies are characterised into two forms: puffs or spherical aggregates of trichomes and tuft, a fusiform aggregate (Paerl and Bebout, 1988; Paerl et al., 1994). Recent work suggests that there is very little phylogenetic diversity between the spherical and fusiform aggregates of a single species (Janson et al., 1999a). The ecological significance of the two different types of colonies is not clear. Many factors, for example high salinity (Fu and Bell, 2003a), appear to enhance colony formation, but the underlying reason for the change from isolated trichomes to colony has not been

elucidated. Whether or not the *Trichodesmium* are present as single trichomes or as colony may affect the physiological characteristics but it is not clear if colony type is also important. For example, light absorption cross section (Lewis et al., 1988) should be different between single trichomes, fusiform or spherical colonies, and size of colonies may be limited by self-shading (Carpenter et al., 1991). Colony shape may affect the extent of O_2-depleted microzones measured in colonies of *Trichodesmium* (Paerl and Bebout, 1988). It was once believed that the internal sections of the colonies carried out little photosynthesis, thereby providing spatial segregation of CO_2 and nitrogen fixation (Paerl, 1994), and therefore suggesting that only the colonial form could fix nitrogen. Recent and more detailed immunocytological work has to a large extent refuted this hypothesis (Lin et al., 1998). It is now known that single trichomes can also actively fix nitrogen. Most of the recent work points to some degree of cell differentiation along the trichome of *Trichodesmium* (Lin et al., 1998; El-Shehawy et al., 2003). Immuno-labelling indicates that about 15% of the diazocytes, cells containing nitrogenase, seems to be adjacent to each other and concentrated in the lighter region of the trichome (Lin et al., 1998). Unlike heterocysts, the diazocytes are consecutive, contain the PSII pigment phycoerythrin and lack thick cell walls. The early hypothesis of cellular segregation of the photosynthetic and nitrogen fixation processes remains equivocal even with immunocytological studies (Siddiqui et al., 1992b; Lin et al., 1998).

3.5. Associated organisms

Several organisms are associated with *Trichodesmium* blooms or communities, including bacteria and fungi, as well as other phytoplankton species such as the dinoflagellate *Peredinium trochoideum* and diatoms of the genus *Nitzschia*. Colonies can harbour large populations of ciliates and flagellates as well as hydroids (*Pelagiana trichodesmiae*) (Borstad and Borstad, 1977; O'Neil and Roman, 1991; Sheridan et al., 2002).

3.6. Grazers

Out of several pelagic copepods only the harpacticoid copepod species *Macrosetella gracilis* Dana 1848 can graze *Trichodesmium* (O'Neil and Roman, 1994). The biology of *Macrosetella* has been studied in detail (Huys and Boettger-Schnack, 1994) but only information relevant to grazing is presented here. The cyanobacterial filaments serve both as food source for adults and as substrate for the non-pelagic juveniles (Roman, 1978). Studies of the vertical migration and population structure of *Macrosetella* in the Red Sea suggest a very tight dependence on the occurrence of *Trichodesmium* blooms for successful reproduction (Boettger-Schnack and Schnack, 1989). Reproduction of this copepod species occurs only during blooms of *Trichodesmium* but the mature individuals appear to have a resting stock at depth ready to reproduce again during blooms. *Macrosetella* sp. can graze 90–126% of their body weight per day as *Trichodesmium* biomass (Roman, 1978), particularly the non-toxic *T. erythraeum*. Although the copepod can consume 100% of the newly fixed nitrogen each day, 48% of its body nitrogen can be released again each day via excretion (O'Neil et al., 1996). Reported grazing rates range between 0.14–2.75 mgC m^{-2} d^{-1} and 0.03–0.06 mgN m^{-2} d^{-1} (Roman, 1978).

3.7. Ecology, toxicity and economics

It has been suggested that nitrogen fixation by *Trichodesmium* or *Rhizosolenia-Richelia* in the subtropical North Pacific gyre can fuel surface phytoplankton blooms over several months (Wilson, 2003). In this region, blooms consisting primarily of diazotroph species appear to last for only a few weeks, but are often followed by a succession of diatoms.

Similar effects have been observed in tropical coastal regions that have suffered increased discharge of phosphorus. There, *Trichodesmium* may contribute to the general eutrophication problem by adding newly fixed nitrogen to the water, promoting the growth of other bloom-forming species such as *Nitzschia pungens* and *Gymnodinium* sp. (Lenes et al., 2001). In both coastal and oceanic regions, the fuelling of secondary blooms has been attributed to the release of nitrogen upon the demise of the *Trichodesmium* bloom. Increased nutrient concentrations on coral reefs promote growth of micro-and macro algae and thus can result in a benthic community structure shift from coral to macro algae and filter feeders (Bell, 1991; Lapointe, 1997; Lapointe et al., 1997; Gast et al., 1999).

Trichodesmium has become common on the list of toxic algae (Chen and Gu, 1993). Toxin has been reported more often in *T. thibautii* (Carpenter et al., 1991; Hawser and Codd, 1991) but recently also in *T. erythraeum* (Endean et al., 1993). These authors provide a comparison of *T. erythreum* and *T. thibautii* in the field and conclude that the higher abundance of *T. thibautii* may be due to the higher nitrogen fixation rates and in part to the presence of a neurotoxin which would be absent in *T. erythraeum*. Blooms of *Trichodesmium* have been reported to cause extensive damage to coastal aquaculture initiatives in tropical regions (Suvapepun, 1991; Negri et al., 2004). Problems associated with *Trichodesmium* blooms may be due to toxin production, anoxia or starvation of cultured organisms due to gill clogging.

4. Growth physiology of *Trichodesmium*

4.1. Growth rate

The maximum specific growth rate for *Trichodesmium* is low compared to other phytoplankton species, ranging between 0.12 and 0.16 d^{-1} under optimal growth conditions in the laboratory (Prufert-Bebout et al., 1993; Mulholland and Capone, 1999; Berman-Frank et al., 2001a). Growth rate estimates are highest in *T. thibautii*, perhaps due to higher nitrogen fixation rates in this species (Ohki and Fujita, 1982; Prufert-Bebout et al., 1993). Estimated division rates in the field, based on turnover of C, N or ATP, are highly variable ranging between 0.006 and 0.88 d^{-1}, but overall confirmed that *Trichodesmium* grows slowly.

4.2. Photosynthesis and oxygen uptake

Although numerous studies have reported photosynthesis rates, only four studies so far (Lewis et al., 1988; Kana, 1993; Roenneberg and Carpenter, 1993; Villareal, 1995) can be used to characterise the relationship of photosynthesis as a function of photon flux density (Platt and Silvert, 1981). Half-saturation parameters for photosynthesis (I_k) range between 142 and 687 μmol photons m^{-2} s^{-1}, with a median of 300 μmol photons m^{-2} s^{-1} (Kana, 1993; Carpenter and Roenneberg, 1995; Villareal, 1995) (http://www.nioz.nl/projects/ironages Appendix 4). However, in some of these studies, saturation of photosynthesis by photon flux density was not achieved even at the maximum photon flux densities tested (i.e. at 1600 μmol photons m^{-2} s-1, Kana, 1993), making photosynthetic parameters difficult to calculate. *Trichodesmium* is able to tolerate very high photon flux density without apparent signs of photoinhibition. The photon flux density at which photoinhibition sets in may be higher in colonies than in filaments (Lewis et al., 1988). The light compensation point (I_c) is much higher in *Trichodesmium* (59–280 μmol photons m^{-2} s^{-1}) than in other phytoplankton (typically<10 μmol photons m^{-2} s^{-1}), and showed diel variation with a maximum around 300 μmol photons m^{-2} s^{-1} at midday and a minimum of around 100 μmol photons m^{-2} s^{-1} at dusk and dawn. This diurnal trend in I_c was attributed to the light-dependent reduction of O_2 via the Mehler reaction (Kana, 1993; Carpenter and Roenneberg, 1995). Although dark respiration is usually low in cyanobacteria grown in the light, *Trichodesmium* seems to be an exception, with high basal dark respiration rates of 0.18 μmol O_2 (μg Chl-a)$^{-1}$ h^{-1} (Kana, 1993; Carpenter and Roenneberg, 1995; Letelier and Karl, 1998; Berman-Frank et al., 2001b). As a result of these high dark respiration rates, oxygen is consumed at low light intensity. Together, high dark respiration rates and O_2 uptake in the light result in photosynthetic quotients (PQ, moles of O_2 evolved: moles of CO_2 fixed) for *Trichodesmium* that range between 0.46 and 0.61 and are low compared to the characteristic 1.2 found in other eukaryotic phytoplankton (Carpenter and Roenneberg, 1995).

The major pigments of *Trichodesmium* include phycourobilin (PUB) and phycoerythrobilin (PEB) as well as Chl-a, and phycobilisomes are the major light-harvesting antennae of PSII, as for other cyanobacteria. Under nutrient-replete growth, the ratio of PSI to PSII of 1.3 (Berman-Frank et al., 2001a) is high compared to higher plants, but is within that observed for other cyanobacteria. This has been suggested as evidence for a low quantum yield for O_2 evolution and for the importance of high cyclic electron transport activity in *Trichodesmium* (Subramaniam et al., 1999; Berman-Frank et al., 2001a). There appears to be an interconversion between the PUB and PEB pigments to regulate the amount of light received by PSII. According to

detailed absorption spectra, it seems that PUB and light absorption by PSII would be at their minimum during the middle of the light period, at a time when O_2 evolution might most hinder nitrogen fixation. However, the high PSI:PSII ratio found in cyanobacteria in general can also be explained in terms of the relative light-harvesting antennae serving each photosystem. The phycobilisomes, associated with PSII, have a large absorption cross-section, while the Chl-a containing PSI antenna has a small absorption cross section. The high PSI:PSII ratios may serve to balance electron flow between PSII and PSI (Falkowski and Raven, 1997). *Trichodesmium* possesses mycosporin-like amino acids to protect against UV irradiation (Subramaniam et al., 1999).

4.3. Nitrogen fixation

Estimates of nitrogen fixation and the relative contribution from *Trichodesmium* have been steadily increasing over the years (Carpenter and Romans, 1991; Capone and Carpenter, 1982). Comparison of published nitrogen fixation rates is difficult, partly because of differences in methodology and partly because poorly defined biomass units, such as colonies and trichomes, make normalisation of these rates to a common biomass unit difficult. Whenever possible, nitrogen fixation rates were normalised to mol N fixed mol Chl-a^{-1} h^{-1} allowing the comparison of data from 26 studies. In these studies, nitrogen fixation rates varied between 0.006 mol N fixed mol Chl-a^{-1} h^{-1} and 2242 mol N fixed mol Chl-a^{-1} h^{-1}, with most of the data ranging between 0.1 and 50 mol N fixed mol Chl-a^{-1} h^{-1} (Fig. 2). As the discrepancies are undoubtedly in part caused by methodology, a brief discussion of the currently utilised methods is warranted.

Most commonly, nitrogen fixation is measured using the acetylene reduction assay (ARA). Alternatively enrichment of seawater samples with $^{15}N_2$ gas followed with stable isotope analysis has been used in field studies (Montoya et al., 1996; Gallon et al., 2002). New methods such as extend laser photoacoustic detection have been developed but are not yet generally available for field studies (Zuckermann et al., 1997; Capone and Montoya, 2001). In general ARA is preferred due to its high sensitivity and ease of use. Detailed method descriptions can be found in Capone (1993), Montoya

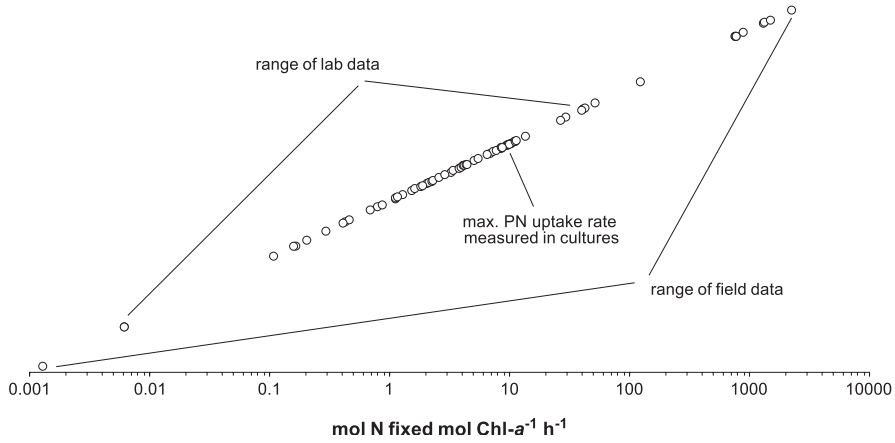

Fig. 2. Overview of field and laboratory measurements of nitrogen fixation by *Trichodesmium* species. Data represent a compilation of 25 publications and selected references are indicated in the figure. Nitrogen fixation rates are normalised to mol N fixed mol Chl-a^{-1} h^{-1}. Conversions between C_2H_4 and N_2 reduction are based on a 4:1 ratio unless a different factor is given in the original publication. Conversions of other biomass estimates used in the original publications were done according to Table 2, with the exception that a ratio of 10 000 cells colony^{-1} was used. Note that biomass conversions can yield inaccurate nitrogen fixation rates as biomass ratios such as Chl-a colony^{-1} are subject to large uncertainties. Exceptionally high nitrogen fixation rates can be a product of such an artifact. Maximum nitrogen fixation rates and maximum particulate nitrogen (PN) increase from controlled laboratory experiments are indicated. Reported laboratory measurements of nitrogen fixation range from 6.1–3 to 40 mol Chl-a^{-1} h^{-1}. Data of field measurements are distributed over the total range of the illustration.

et al. (1996), Capone and Montoya (2001) and Breitbarth et al. (2004). Theoretically, the reduction of acetylene to ethylene can be converted to nitrogen reduction through electron equivalents, assuming that nitrogenase affinity for N_2 gas and acetylene are the same. A theoretical value for acetylene:dinitrogen of 3:1 is based on the fact that 2 e^- are needed to reduce C_2H_2 to C_2H_4 and 6 e^- are needed to reduce N_2 to NH_4^+. It has been argued that a ratio of 4:1 might be more appropriate, since hydrogenase activity of nitrogenase produces 1 mole H_2 per mole of N_2 fixed, which requires two additional electrons. However, the ratio of 3:1 should be valid when the ARA is conducted at C_2H_2 concentration saturating for nitrogenase which blocked hydrogenase activity (Stewart et al., 1968; Montoya et al., 1996). In contrast, the ^{15}N stable isotope method measures new nitrogen fixed into the particulate organic nitrogen. As a significant and variable fraction of the nitrogen fixed by diazotrophs can be directly released into the water as dissolved nitrogen (Glibert and Bronk, 1994), it is likely that this method will result at times in an underestimate of the true nitrogen fixation rates (see Section 4.8.). In fact direct comparison of both the ARA and the stable isotope method rarely yield the theoretical C_2H_2 to N_2 ratio of 3, with values ranging from 1.4:1 to 20:1. Variation in this ratio depends on the physiological state of the population under study, but this is not well understood at present (Gallon et al., 2002).

In *Trichodesmium*, nitrogenase activity occurs concurrently with photosynthesis, with a narrow peak around the middle of the light period of a light/dark cycle, during which a reduction in net oxygen evolution is observed (Berman-Frank et al., 2001b). Diel periodicity in nitrogenase activity was initially observed in field samples (Saino and Hattori, 1978; Capone et al., 1990; Wyman et al., 1996) both by immunological measurements of nitrogenase enzyme and by acetylene reduction, but laboratory experiments confirmed the presence a circadian rhythm in nitrogenase activity (Chen et al., 1996). The daily increase in nitrogenase enzyme requires new protein synthesis. There is an endogenous cycle for the synthesis, activity and degradation of nitrogenase in *Trichodesmium*, set by illumination patterns (Chen et al., 1998). Cellular clock controlled rhythms are likely to be important in allowing this non-heterocystous filamentous cyanobacteria to photosynthesise and fix nitrogen during the light period. The strong diurnal cycle in nitrogen fixation observed in *Trichodesmium* necessitates that the time of day and length of the incubation be carefully considered when extrapolating nitrogen fixation measurements to daily rates.

In laboratory experiments, nitrogen fixation rates rarely balance the net increase in particulate nitrogen biomass over the entire growth curve. In the few studies that attempted a mass balance comparison, the agreement between N_2 fixation rate measurements and particulate nitrogen accumulation was poor (Mulholland and Capone, 2001). Nitrogen fixation only accounted for 14–30% of PN growth and explained 8 and 60% of particulate N accumulation (Prufert-Bebout et al., 1993; Chen et al., 1996). While seawater-based medium used by Prufert-Bebout et al. (1993) may have contained additional N sources, mass balance was also not possible in studies carried out using N-free media, although N_2 fixation could account for a larger fraction of the PON.

4.4. Nutrient uptake

Trichodesmium can grow on nitrate, ammonium and urea as sole sources of nitrogen (Mulholland et al., 1999a), but the potential for the utilisation of other organic nitrogen substrate is not known. There are very few measurements of uptake kinetics for reactive nitrogen sources and the available measurements are derived from cultures grown in nitrogen-free media or from environmental samples from oligotrophic waters. In constrast to early studies, recent studies of nitrogen uptake kinetics using unialgal cultures found low K_s for NH_4 uptake and maximum uptake rates of 0.26 μM and of 13 h^{-1} for *Trichodesmium* NIBB 1067, respectively (Mulholland and Capone, 1999; Mulholland et al., 1999a). In addition, *Trichodesmium* could utilise organic nitrogen sources such as urea (K_s=6.95 μM, V_{max}=19 h^{-1}), and glutamate (K_s=1.34 μM, V_{max}=15 h^{-1}) (Mulholland and Capone, 1999). Assuming that only 10–15% of the *Trichodesmium* cells contain nitrogenase and therefore are capable of fixing N_2 gas, most of the nitrogen uptake from the adjacent cells devoid of nitrogenase should be in the form of recycled nitrogen, for example NH_4, urea or glutamate released from the nitrogen fixing cells (Mulholland et al.,

1999a). This can account for the high NH_4 uptake rates found in cultures (Mulholland et al., 1999a). In the natural environment, however, other phytoplankton cells may also use the released NH_4 and ambient sources of dissolved inorganic and organic nitrogen could also be used by *Trichodesmium* to supplement nitrogen fixation. NO_3 uptake is low in *Trichodesmium* grown in N-free medium and the K_s of nearly 40 μM suggests that nitrate is a poor source of nitrogen for *Trichodesmium* (Mulholland et al., 1999a). However, *Trichodesmium* possesses an operon with the full complement of the genes for nitrate utilisation (Wang et al., 2000). The *napA* gene from *Trichodesmium*, encoding a protein involved in NO_3 transport, could complement a bacterial mutant deficient in a NO_3 transporter gene. Based on the published data for half saturation constants, *Trichodesmium* preference for various nitrogen could be ordered as NH_4>glutamate>urea>NO_3. The position of N_2 gas in this series is problematic: On the one hand, one would assume that given the high energetic cost associated with N_2 fixation, any fixed nitrogen sources would be preferred to the N_2 gas. However, only NH_4 and urea additions completely repressed the expression of nitrogenase. Finally, there are no kinetic studies of nitrogen fixation as a function of partial pressure of N_2 gas. As this is the major component of our atmosphere, one can probably safely assume that N_2 gas is always saturating and that this biochemical process is energy-rather than substrate-limited.

Of the macronutrients, both dissolved nitrogen and phosphorus can be important for *Trichodesmium*. Phosphorus uptake kinetic parameters are available only from the very early work on *Trichodesmium*. Half-saturation constant (K_s) for dissolved inorganic PO_4 (DIP) was initially measured to be as high as 9.0 μM PO_4 (McCarthy and Carpenter, 1979), suggesting that *Trichodesmium* is not well adjusted for uptake of DIP at very low concentrations. This value appears unrealistic considering that natural DIP levels do not usually exceed 200 nM in regions where *Trichodesmium* blooms (Wu et al., 2000; Sanudo-Wilhelmy et al., 2001) and in the eastern Tropical Atlantic, small additions of 200 nM DIP, added together with 2 nM Fe, were sufficient to stimulate nitrogen fixation in natural populations of diazotrophs dominated by *Trichodesmium* (Mills et al., 2004). Nitrogen fixation rates in cultures grown on DIP as a sole source of phosphorus became saturated at a concentration of 1.2 μM (Fu and Bell, 2003b) and decreased again at concentration above 6.5 μM, casting further doubt on the validity of a high K_s value for DIP uptake by *Trichodesmium*.

Trichodesmium grows as well on glycerophosphate as on DIP based culture media (Stihl et al., 2001). It is therefore likely that *Trichodesmium* relies also on dissolved organic phosphorus to meet its P requirements. The uptake of DOP in the field can be very high with measured values of 170–300 nM P Chl-a^{-1} h^{-1} (McCarthy and Carpenter, 1979). In laboratory culture experiments, alkaline phosphatase activity (APA) varied as a function of DIP supply and appeared to be an indicator of P limitation in *Trichodesmium*. Low APA was found in cultures grown with DIP, while high APA was detected in P-limited cultures or cultures grown on glycerophosphate (Stihl et al., 2001). *Trichodesmium* from coastal waters, where sufficient DIP was present, hydrolysed p-nitrophenylphosphate (PNPP) slowly at rates of 0.2–0.5 μmol PNPP μg Chl-a^{-1} h^{-1}. In contrast, P-limited cultures and cultures grown with glycerophosphate had APA of 4 and 7 μmol PNPP μg Chl-a^{-1} h^{-1}, respectively. Measurements in this study were carried out at optimum temperature (37°C) and saturating substrate concentration for enzyme activity and thus may have yielded maximum PNPP turnover rates. Others have corroborated the repression and stimulation of APA by DIP and DOP, respectively (Mulholland et al., 2002) but much lower rates (0.03–0.24 μmol μg Chl-a^{-1} h^{-1}) have been measured at in situ temperature, using 4-methylumbellyferyl phosphate (MUF-P). In oligotrophic regions, concentrations of DOP are higher than DIP implying that DOP could contribute significantly to the P demand of *Trichodesmium*. More laboratory studies are needed in order to obtain P uptake kinetic parameters from *Trichodesmium* grown under controlled conditions for both organic and inorganic P.

4.5. Elemental composition

As for other phytoplankton groups (Geider and La Roche, 2002), the C:N ratio of *Trichodesmium* biomass deviates only slightly from the Redfield stoichiometry of 6.6 with a mean value of 6.3 and a minimum and maximum values of 4.71 and 7.32,

respectively (http://www.nioz.nl/projects/ironages Appendix 6). Some of the daily variation observed in particulate C:N ratio may be due to changes in C respiration or excretion, leading to a decrease in PC:PN of ~30% at the peak of nitrogen fixation (Mulholland and Capone, 2001; Breitbarth unpubl. laboratory data). In contrast, C:P and N:P ratios from field samples of *Trichodesmium* vary tremendously with a minimum N:P value of 14 (Kustka et al., 2003b) to a maximum of 125 for blooming *Trichodesmium* (Karl et al., 1991). The variability of the N:P ratio is not unexpected and has been reported before in other groups of phytoplankton, even in cultures growing under optimal conditions (Geider and La Roche, 2002). In contrast, data on C:P and N:P stoichiometry from *Trichodesmium* cultures are sparse (Berman-Frank et al., 2001a). In contrast to the field studies, an elemental N:P ratio of 16, in agreement with Redfield, was reported in *Trichodesmium* grown under optimal laboratory conditions (Berman-Frank et al., 2001a), leading the authors to conclude that natural *Trichodesmium* populations tend to be P limited in general. However, P cellular quotas may be high in *Trichodesmium* grown under nutrient-replete conditions as a result of luxury P uptake and may not represent the critical N:P ratio at which P may become limiting (Geider and La Roche, 2002).

The iron requirements and the optimal C:N:P:Fe molar ratio of *Trichodesmium* (http://www.nioz.nl/projects/ironages Appendix 7) has been the subject of debate (Raven, 1988; Berman-Frank et al., 2001a; Sanudo-Wilhelmy et al., 2001; Kustka et al., 2003b). It is generally agreed that the iron requirements should be higher in diazotrophs because of the high Fe content of the nitrogenase enzyme and because of the additional energetic requirements imposed by diazotrophy, which are partly met by an increase in photosynthetic proteins (Raven, 1988; Kustka et al., 2003a). Estimates of the difference in iron requirements between photosynthetic diazotrophs and other photoautotrophs range between 2.5 (Sanudo-Wilhelmy et al., 2001; Kustka et al., 2003a) and 100 fold higher (Raven, 1988). However, recent theoretical estimates (Kustka et al., 2003a) and laboratory studies (Berman-Frank et al., 2001a; Kustka et al., 2003b) have converged to iron use efficiencies (IUE) that are 5–10 fold higher than those of a generic phytoplankton grown on ammonium. In carbon-based growth rate measurements at various iron concentrations, the minimum Fe requirement for a moderately iron-limited photosynthetic diazotroph growing at 0.1 d^{-1} has been estimated to be 38–48 μmol:mol Fe:C in *Trichodesmium* cultures, whereas the ratio is reduced to 8 μmol:mol Fe:C under NH_4^+ replete conditions. Revised theoretical calculations of IUE in photosynthetic diazotrophs are in agreement with these experimentally measured values (Kustka et al., 2003a). However, these estimates are representative of the Fe:C ratio for iron-limited growth rate equivalent to 70% of the maximum observed growth rate in cultures. As the maximum growth rate approaches, the relationship with the Fe:C ratio becomes non-linear, reflecting the saturation of growth with Fe. At the maximum growth rate of 0.14 d^{-1}, the Fe:C ratios were around 180–250 μmol:mol (Berman-Frank et al., 2001a; Kustka et al., 2003b). It is not clear yet whether this large increase in the Fe:C is necessary to achieve the maximum growth rate in *Trichodesmium* or whether it represents luxury consumption and storage of iron. Compared to the reported Fe:C ratios in diatoms of 1–7 μmol Fe: mol C, this represents 5–100 fold higher Fe requirements in diazotrophs (Sunda and Huntsman, 1997; Berman-Frank et al., 2001a). Biochemical models predict that up to 50% of the cellular Fe could be associated with the nitrogenase enzyme, while 38% is contained in the photosynthetic apparatus under diazotrophic growth (Kustka et al., 2003b), In contrast, the photosynthetic burden accounts for 77% of the cellular Fe during ammonium-based growth in *Trichodesmium*.

4.6. Iron acquisition and metabolism

In laboratory experiments, both the cellular Fe content and the Fe uptake rate increased proportionally (log/log) to the total Fe concentration in the culture media (Kustka et al., 2003b). In many phytoplankton species, uptake rates increase linearly at low Fe concentrations but the slope of this relationship declines at higher Fe concentrations (Sunda and Huntsman, 1995). In *Trichodesmium*, Fe uptake is proportional to total iron concentration well into the region of oxyhydroxide precipitation (Kustka et al., 2003b), a behaviour that is also observed in some coastal species of diatoms (Sunda and Huntsman, 1995) and that allows for luxury Fe consumption. The luxury uptake of Fe-hydroxide (colloidal) in *Trichodesmium*

would be advantageous both in high iron input coastal regions (e.g.. coastal Australia) and regions of episodic iron input such as the oligotrophic North Atlantic. In support of this view, Wang and Dei (2003) report that, although Fe bound to low molecular compounds is preferred, *Trichodesmium* can obtain iron from colloid-bound iron. Nevertheless, very little is known about the mechanisms by which *Trichodesmium* acquire iron in nature. *Trichodesmium* can scavenge Fe from a variety of siderophores, but uptake rates are higher when Fe is bound to dihydroximate siderophores. In addition, inorganic Fe (III) supported high Fe uptake rates in *Trichodesmium* colonies (Achilles et al., 2003). It is not clear yet whether *Trichodesmium* itself can produce siderophores (Rueter, 1988), but siderophore 'piracy' from associated heterotrophic bacteria (Hutchins et al., 1999) may play an essential role in the Fe chemistry in *Trichodesmium* colonies. In other marine cyanobacteria, *idiA*, an iron-regulated gene is thought to be part of an iron transporter system (Webb et al., 2001). This gene is also present in *Trichodesmium*, although its function has not been clearly demonstrated. In addition, several intriguing observations have been reported by (Rueter, 1988).

Trichodesmium appears capable of assimilating iron directly from dust (Rueter, 1988) and *Trichodesmium* cell lysate can dissolve iron dust. However, the latter appears to have no relation with iron limitation because the ability of the cell lysate to solubilise dust is directly correlated with biomass rather than iron limitation itself. These two observations are interesting given the ecology of *Trichodesmium*. Being positively buoyant and sometimes found at the very surface, *Trichodesmium* would perhaps be capable of dissolving dust particles and improve the retention of iron in the mixed layer of tropical waters. *Trichodesmium* has a high rate of autolysis (Berman-Frank et al., 2004) and the released dissolved organic matter that can act as a strong iron ligand would greatly increase the recycling of iron in surface layers, after a *Trichodesmium* bloom, contributing to the iron retention in oligotrophic systems.

4.7. Energetic demand

The requirements of nitrogenase for ATP are higher than those of NADPH. The relative contribution of photosynthetic electron transport (PET), respiration and other processes such as the Mehler reaction has not been determined, but it appears that a very well-regulated balance between the three processes tied to a circadian rhythm is key to allowing co-occurrence of photosynthesis and nitrogen fixation within the same cell (Berman-Frank et al., 2001b). The Mehler reaction which can contribute to both the reduction of O_2 and to the production of ATP (Kana, 1993) is discussed in Section 5.1. In addition to providing energy and reductant, respiration is probably needed for the production of short carbon building blocks for the assimilation of the N_2 gas (Berman-Frank et al., 2001b).

Trichodesmium contains the energy storage product poly-beta-hydroxybutyric (PHB) acid (Siddiqui et al., 1992a). Minimum ($1.6+/-0.9$ mg g^{-1} dry weight) and maximum ($2.3+/-0.8$ mg g^{-1} dry weight) values of this compound were observed at night and in the early morning, respectively. Nitrogenase activity at its peak is believed to be limited by carbon and energy supply and it is therefore possible that breakdown of PHB provides some of the necessary energy to support maximum nitrogenase activity.

4.8. Dissolved nitrogen release

Dissolved organic nitrogen (DON) release is significant in *Trichodesmium* accounting for up to 50% of the newly fixed nitrogen (Glibert and Bronk, 1994). High NH_4 uptake rates also imply that the turnover of N is much higher than the net accumulation in N biomass, implicating large NH_4 release rates. While the NH_4 release can be recycled towards *Trichodesmium* biomass in pure cultures of this organism, the NH_4 released in the field will also be available to other phytoplankton species (Mulholland and Capone, 2001). Dissolved free amino acids such as glutamine and glutamate are released throughout the day but with a maximum rate coincident with the peak in nitrogenase activity. The glutamine to glutamate ratio peaks around 1200, at midday. The diel cycles of both glutamine and glutamate suggest that the primary pathway of nitrogen assimilation in *Trichodesmium* is via the glutamine synthetase-glutamate synthase pathway (Carpenter et al., 1992; Capone et al., 1994). The lysis of *Trichodesmium* cultures releases mainly HMW DON (Gry Berg and Dan Repeta, unpubl. data), and it becomes therefore important to understand the factors that lead to cell lysis in nature. Natural cell death (apoptosis) and viral lysis are two possible mechanisms

for the rapid demise of *Trichodesmium* blooms (Ohki, 1999; Berman-Frank et al., 2004).

4.9. Export

Gas vacuoles in *Trichodesmium* prevent the sedimentation of the blooms, explaining their persistence in the Red Sea. Walsby (1991) has hypothesised that *Trichodesmium* grows best below the surface but subsequently rises to the surface due to its abundant gas vacuoles. Dense aggregation of *Trichodesmium* at depth is rare but it has been reported as deep as 240 m in the North Pacific and in the Red Sea (Sellner, 1991). This sinking at depth has been attributed to buoyancy regulation. The fate of a *Trichodesmium* bloom is generally that of nutrient recycling through the microbial loop. Grazing by *Macrosetella* sp. might be the only direct export of particulate matter from *Trichodesmium* via excretion of fecal pellets (see Section 3.6.).

5. Regulation of nitrogen fixation

5.1. Protection of nitrogenase against oxygen deactivation

The nitrogenase enzyme is oxygen sensitive and needs to be protected from the products of photosynthesis. In heterocystous cyanobacteria, spatial segregation of photosynthesis and nitrogen fixation is achieved by the confinement of nitrogen fixation to the heterocysts, differentiated cells that do not evolve oxygen. In contrast, a temporal segregation of photosynthesis and nitrogen fixation is usually found in unicellular cyanobacteria, in which nitrogen fixation appears confined to a small window within the dark period (Colon-Lopez et al., 1997).

Trichodesmium fixes nitrogen during the light period, concurrently with photosynthesis, and in the absence of well-differentiated heterocysts. This apparent paradox has been discussed (Zehr et al., 1993; Gallon et al., 1996 and references therein) and recent work indicates that the problem of nitrogenase protection against oxygen damage is most likely achieved through an intermediate situation, combining both temporal and spatial (diazocytes) segregation of nitrogen fixation and photosynthesis. The expression of genes involved in nitrogen fixation (*nifH*), cellular differentiation (*hetR*) and a global nitrogen regulator (*ntcA*) suggest that cellular differentiation occurs in diazocytes, cells intermediate between heterocysts and undifferentiated cells. New diazocytes are formed based on a circadian rhythm, where the *hetR* and nifH expression is separated by 6–8 h (peak to onset), which is typical for heterocystous cyanobacteria as well. Other evidence for spatial segregation comes from microscopic studies demonstrating the confinement of the nitrogenase, carboxysomes and other enzymes to specific regions of the trichome rather than throughout (Bergman and Carpenter, 1991; Lin et al., 1998; Paerl, 1999; Carpenter and Price, 1976; Carpenter et al., 1990). In warm waters, the lack of heterocysts may in fact confer an ecological advantage for *Trichodesmium* relative to heterocystous cyanobacteria (Staal et al., 2003). At higher temperatures, the lower oxygen solubility coupled with the increased membrane permeability of the diazocytes is advantageous over the low oxygen permability of the glycolipid layer of heterocysts, which is temperature independent. Higher respiration rates with increasing temperatures also allow dark respiration to be an efficient oxygen protection mechanism in warm waters.

It has often been suggested that oxygen-deplete zones form in the centre of aggregates (Paerl and Bebout, 1988). However, single trichomes can also fix nitrogen (Ohki and Fujita, 1988), and in situ immunofluorescence showed that nitrogenase-containing cells are also present on the outer periphery of trichome, and thus are also exposed to oxygen. Furthermore, both PSI and PSII can be active in cells containing nitrogenase, supporting an important role for temporal segregation in addition to spatial separation.

The photosynthetic apparatus of *Trichodesmium* has a strong bias against PS II (PS I:PS II>1, Berman-Frank et al., 2001a), and this can in itself be a important adaptation in facilitating a solution to the problem of O_2 protection (Kana, 1993). At least part of the protection of nitrogenase against oxygen is apparently achieved by a balance between photosynthesis and respiration at the cellular level. Temporal segregation of photosynthesis and nitrogen fixation is observed as a depression of photosynthetic activity at midday, during the peak activity of the nitrogenase enzyme (Berman-Frank et al., 2001b). Both nitrogen fixation and photosynthesis are strongly tied to a circadian

rhythm (Chen et al., 1996, 1998). The observed decrease in net oxygen evolution during the period of highest nitrogenase activity implies very efficient oxygen-scavenging systems, probably resulting from the activities of several biochemical pathways (Sellner, 1997) such that nitrogenase is rarely exposed to elevated O_2 levels. There are three pathways that can reduce oxygen concentration in the cell: photorespiration, the Mehler reaction and the classical dark respiration pathway through cytochrome oxidase reaction (Siddiqui et al., 1991; Li et al., 1980). Respiration rates are high in *Trichodesmium* resulting in a high light compensation point (averaging 100–200 μmole photons m^{-2}, ranging between 30 and 325 μmole photons m^{-2}) (Carpenter and Roenneberg, 1995), and exhibit a diurnal cycle (Kana, 1993). The involvement of respiration is also supported by a correlation between the abundance of nitrogenase and the respiratory enzyme cytochrome oxidase (Bergman et al., 1993). The light-dependent Mehler reaction is well developed in *Trichodesmium* supporting the formation of ATP with increasing light and decrease in O_2. Rubisco's oxygenase activity is probably acting to decrease O_2 at midday (Siddiqui et al., 1992b; Li et al., 1980; Carpenter and Roenneberg, 1995). It is not clear whether or not the Mehler reaction produces high oxygen radicals in cyanobacteria (Helman et al., 2003), but *Trichodesmium* certainly possesses superoxide dismutase that would help in removing oxygen radicals produced by other O_2 protection mechanisms (Cunningham and Capone, 1992). It is likely that this Mehler activity can double the supply of ATP available for nitrogen fixation (Kana, 1991). This dependence on irradiation levels in turn is consistent with the Mehler reaction and the pattern of nitrogen fixation (Chen et al., 1996, 1998). High photorespiration rates can account for up to 46% of the gross photosynthesis rate (Kana, 1993). Overall, it is possible that the physiological processes required for the protection of nitrogenase against O_2 are responsible for the restricted temperature growth range and low growth rate of *Trichodesmium*.

5.2. Regulation of nitrogen fixation by dissolved inorganic nitrogen

Nitrogenase can also be under the control of other fixed dissolved inorganic nitrogen sources although there are few quantitative estimates of the concentrations of alternate dissolved nitrogen sources required to inhibit nitrogenase activity. Moreover, the qualitative information is also contradictory. Pulses of 10 μM NO_3 added to *Trichodesmium* cultures resulted in decreased nitrogen fixation rates by 35% (Mulholland et al., 2001) to 70% (Holl and Montoya, 2003), with a recovery of nitrogen fixation activity at NO_3 levels of 0.3–0.4 μM. However, long-term growth of *Trichodesmium* with nitrate as a sole source of nitrogen is possible in pure cultures and results in the shut down of nitrogen fixation (Berman-Frank et al., 2001a). This suggests that nitrate can be utilised after an induction period in pure culture, but in the field *Trichodesmium* is unlikely to successfully compete for nitrate with other faster growing species. Other fixed nitrogen sources, such as urea or NH_4, can also inhibit nitrogen fixation (Fu and Bell, 2003b). In contrast, *Trichodesmium* isolate NIBB1067 can simultaneously utilize combined N-sources and fix N_2 (Mulholland and Capone, 1999, 2000; Mulholland et al., 1999a, b) by restricting the release and recycling of NH_4 to a period of the circadian rhythm where nitrogen fixation is at its minimum. This is supported by the finding that the glutamine synthease transferase/biosynthetic ratio changes by 20% over the course of a diel cycle (Mulholland et al., 1999a).

In addition, physiological studies contradict biochemical and gene expression studies that characterise *nif* gene expression as a function of fixed nitrogen source. In IMS 101, a short-term incubation with nitrate led to only a partial conversion of the active form of nitrogenase into the inactive form (Chen et al., 1998). In particular, sources of fixed nitrogen such as NH_4, NO_3 and urea have been tested as potential repression factors. NH_4 was the most effective of the three nitrogen sources at repressing *nifH* transcription while significant repression by nitrate was not observed even at very high concentrations (20 mM) (Gallon et al., 1996; Dominic et al., 1998). Although *Trichodesmium* grown with NH_4 contained nitrogenase (Ohki et al., 1991), this nitrogenase was larger than the active form and probably consisted entirely of a modified, inactive form (Zehr et al., 1993). Repression by NH_4 has also been observed at the nitrogenase activity level (Gallon et al., 1996). Gallon et al. (1996) report that the characteristic diel cycle of nitrogen fixation with a peak at midday during the light cycle,

followed by decreased activity, could correspond to nitrogen sufficiency that occurs after a few hours of active nitrogen fixation. This is supported by fluctuations in intracellular glutamine and glutamate levels (Carpenter et al., 1992). The expression pattern of the *glnA* gene encoding for glutamine synthetase also indicates coordination of nitrogen fixation with other assimilation enzymes (Kramer et al., 1996). *GlnA* shows a bimodal expression pattern with peaks in early morning and late evening, bracketing the maximal nitrogen fixation period during midday. More work is needed to provide quantitative measures of the inhibition of nitrogen fixation by alternate sources of fixed nitrogen.

5.3. Regulation of nitrogen fixation by light

Trichodesmium can grow at high photon flux in sub-tropical/tropical oligotrophic surface waters and can adjust its photosynthetic characteristics accordingly to its position in the water column and to seasonal changes in the light regime (Carpenter and Roenneberg, 1995). While photoinhibition of photosynthesis is rarely detected, even at 2500 μE m^{-2} s^{-1}, decreases in nitrogenase activity in *T. erythraeum* has been observed at the same irradiance (Carpenter et al., 1993). The relationship between light intensity and nitrogen fixation is poorly described and has been derived from the photosynthesis-photon flux relationships (Fennel et al., 2002; Hood et al., 2001). Model-based results suggest that in *Trichodesmium*, nitrogen fixation versus photon flux density is characterised by an initial slope (α) of 0.023 (μmol N (mg Chl-a h)$^{-1}$ (μmole photons m^{-2} s^{-1})$^{-1}$) and a mean photon flux for light inhibition of nitrogen fixation at 1195 μmole photons m^{-2} s^{-1} (Hood et al., 2002). However, laboratory studies on nitrogen fixation have not been conducted at photon flux exceeding 180 μmole photons m^{-2} s^{-1} and therefore the experimental evidence for photoinhibition of nitrogen fixation is lacking (Ohki and Fujita, 1988; Fu and Bell, 2003b).

6. Conceptual model of the genetic control of the growth and distribution of *Trichodesmium*

The genome of *Trichodesmium* IMS101 has been sequenced and is available at the Joint Genome Institute (http://genome.jgi_psf.org/draft_microbes/trier/trier.home.html). Some of the information summarised below was obtained from searching the genome information.

6.1. Temperature

Trichodesmium can grow at temperatures ranging between 20 and 34°C with optimal growth occurring at 27°C. The strong temperature dependence of *Trichodesmium* growth is probably a result of intrinsic physiological limitation due to the co-occurrence of nitrogen fixation and photosynthesis. Some recently published works indicate that the protection of the oxygen sensitive nitrogenase enzyme from the oxygen evolved during photosynthesis is achieved via the consumption of oxygen by various metabolic pathways, including dark respiration. While light absorption itself is not temperature dependent, the processes of carbon fixation and respiration are and the correct balance between energy acquisition and oxygen consumption is probably limited to a narrow range in temperature.

6.2. Circadian rhythm

Trichodesmium possess the cyanobacterial genes (*kaiABC*) that control circadian rhythms. The processes of respiration, photosynthesis and nitrogen fixation all show some diurnal variations with the peak in nitrogen fixation at midday corresponding to a dip in photosynthesis, which combined with the steady increase in respiration, maintain the correct oxygen concentration that allows nitrogen fixation to proceed over a narrow window.

6.3. Iron uptake

Trichodesmium possess at least two types of uptake system for iron. The ones that are readily identified from the annotated genome are a Fe (II) system paralogous to the FeoAB (ferrous uptake system). Trichodesmium also contains a Fe (III) uptake system that is an ABC-transporter system composed of a permease, an ATPase and a periplasmic Fe binding protein. The genes coding for permease and the ATPase are contiguous and may be regulated in concert. A third type of iron uptake system appears

to be related to hydroxamate siderophore utilisation (periplasmic binding proteins). The ferric uptake regulator (fur) protein is present in the genome and may be a global regulator of iron metabolism in *Trichodesmium* as it is for other cyanobacteria. It is not clear which of these systems is induced under iron-limitation. In other species of cyanobacteria, more than one system are induced under iron limitation.

6.4. Phosphorus uptake

Phosphorus uptake is done by the *Pst* operon, also a high affinity ABC-transporter system. In addition, *Trichodesmium* contains many genes for the transport of phosphonate, also arranged in an operon. It is not clear whether this system is involved in organic phosphorus uptake. Also present is a gene encoding for alkaline phosphatase, and the activity of this enzyme in *Trichodesmium* has been confirmed experimentally. Because *Trichodesmium* is found in areas where dissolved P concentrations are extremely low, one would expect that alternate P acquisition pathways would have evolved in this species.

6.5. Nitrogen uptake

Trichodesmium possesses genes for ammonium permease, urease (operon), nitrate permease, nitrite reductase and nitrate reductase (operon), and several amino acid transporters. It can therefore probably grow on any of these nitrogen sources if they are available in its growth environment. One major consequence is that nitrogen fixation is repressed by dissolved fixed nitrogen as supported by numerous published reports cited above. *Trichodesmium* possesses the genes *ntcA* and *ntcB*, a two-component system known to regulate nitrogen assimilation in cyanobacteria.

7. Perspectives for future work

The compilation of the data available on *Trichodesmium* has shown very clearly that the bulk of the physiological measurements have been collected from field samples before this species was axenic, or even isolated in a unialgal culture. There is therefore a need to confirm some of the available data with more controlled laboratory experiments. A look at parameterisation for nitrogen fixation in some recent biogeochemical models quickly identifies the areas where more work is needed. Most models or estimates of nitrogen fixation impose strong temperature dependence on this process, with a critical temperature ranging between 22 and 25 °C (Berman-Frank et al., 2001a; Fennel et al., 2002). The choice of this temperature optimum is at present anecdotal, and reflects the field distribution of *Trichodesmium*. The relationship between growth and temperature needs to be investigated in the laboratory under controlled conditions. Elemental composition data that include P and iron requirements are also few and need to be obtained for optimally growing laboratory cultures, in order to resolve the discrepancy between theoretical and measured cellular Fe quotas (Raven, 1988; Rueter et al., 1991; Berman-Frank et al., 2001a; Sanudo-Wilhelmy et al., 2001). Given the apparent tight regulation between photosynthesis, respiration, the Mehler reaction and nitrogen fixation, there is a need to better understand the interaction between temperature, light and the circadian rhythm in the balance of energy utilisation. Additionally, more quantitative data are needed on the inhibition of nitrogen fixation rates by dissolved organic and inorganic nitrogen sources.

Although we are beginning to acquire a good understanding of the processes controlling *Trichodesmium* growth in the ocean, the discovery of other potentially important unicellular marine diazotrophs (Mitsui et al., 1986; Zehr et al., 2001; Montoya et al., 2004) is opening a new window on the importance of nitrogen fixation in the ocean. This may lead to a paradigm shift regarding the marine nitrogen cycle, but awaits the isolation and cultivation of these new diazotrophs as well as improvements in the methods to detect them quantitatively in nature.

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Appendix I: Cell density and biomass in *Trichodesmium sp.* in the field

location/date	Biomass [1)]	cells l^{-1}	depth	comments	Reference
NP, ALOHA	4.6 x 10^4 tr m^{-3}	4.6 x 10^3	0-45 m	[2)]	Karl et al. 1995
	2 - 140 co m^{-3}	60 - 4.2 x 10^3	"	[2)]	Letelier + Karl 1996
	1.1-8.4 x 10^4 tr m^{-3}	1.1 - 8.4 x 10^3	"	[2)]	"
NP, ALOHA		4.2 x 10^3	upper 45 m	seasonal and annual variation	Letelier + Karl 1996
NP, ALOHA		8.4 x 10^3	upper 45 m		"
NA	1 - 5 x 10^3 co m^{-3}	3-15 x 10^4		density mean [2)]	Carpenter 1983
NA	*10 - 13 µg chl-a l^{-1}*				Subramaniam et al. 1999
NA, 10.3N ~20W off NW Africa	6.5 x 10^3 tr m^{-3}	6.5 x 10^2		"	Vidal et al. 1999
"	2.21 x 10^6 co l^{-1}	6.63 x 10^9	bloom	"	"
NA *north* 30N	10.5 - 4737 co m^{-3}	2.5-16.4 x 10^4		range [2)]	*compiled in* Carpenter + Romans 1991
NA *south* 30N	835 - 5500 co m^{-3}	0.25-1.6 x 10^5		range	
NA *south* 30N	*76.3 (50 - 250) µg chl-a l^{-1}*			range	Carpenter + Romans 1991
	2.5 - 12.5 µg chl-a m^{-2}			range integrated over50m	
	3500 co m^{-3}	1.05 x 10^5		density maximum [2) 3)]	
	1415 co m^{-3}	4.25 x 10^4		density minimum [2) 3)]	
SAR		6.50 x 10^4	25 m	[3)]	Carpenter + Price 1977
SAR		1.70 x 10^3	15 m	[3)]	"
western SAR		1.18 x 10^3	25 m	density maximum [3)]	Carpenter + McCarthy 1975
western SAR		8.30 x 10^2	1 m	"	"
CAR		3.00 x 10^5		[3)]	Carpenter + Price 1977
CAR		7.58 x 10^4	15 m	[3)]	"

location/date	Biomass [1)]	cells l^{-1}	depth	comments	Reference
AS	8.74 x 10^6 tr dm^{-3}	8.74 x 10^8	average		Somasundar et al. 1990
IO off Kenia	2 - 268 co l^{-1}	8.04 x 10^6	20-2000 m		Kromkamp et al. 1997
GBR - 92/93	1 x 10^3 tr l^{-1}	1.00 x 10^5		+ 10^3 since 1928/29	Bell 1999
GBR	4 x 10^4 tr l^{-1}	4.00 x 10^6		max. in a bloom	Bell 1991
	5.7 x 10^6	5.7 x 10^8		density maximum in a bloom	Suvapepun 1991
	1.76 - 3.14 x 10^5 tr l^{-1}	1.76-3.14 x 10^7		average in a bloom	"
	1.3 - 9720 x 10^3 tr l^{-1}	1.3 x 10^2 - 9.72 x 10^8		range in blooms	*compiled in* Carpenter and Capone 1991
		0.6-2 x 10^6			Furnas 1991
	4 x 10^4 tr l^{-1}	4 x 10^6			Revelante + Gilmartin 1982
min cells l^{-1}	⇒	*60*			
max cells l^{-1}	⇒	*6.63 x 10^9*			
range µg chl-a l^{-1}	*50 - 250*				
range µg chl-a m^{-2}	*2.5 - 12.5*				
integrated cell density		**cells m^{-2}**			
CAR		2.11 x 10^9			Carpenter + Price 1977
SAR		3.63 x 10^8			"
western SAR		6.40 x 10^7		50m integrated	Carpenter + McCarthy 1975
subsurface maximum		**depth** m			
single trichomes		2 - 5	NP, ALOHA, correlated with stratification, not correlated with SST		Letelier + Karl 1996
total		20 - 50 to >50			

Appendix I abbreviations and footnotes

NP = North Pacific
NA = North Atlantic
SAR = Sargasso Sea
CAR = Caribbean
AS = Arabian Sea
IO = Indian Ocean
GBR = Great Barrier Reef

[1)] tr = trichomes, co = colonies
[2)] conversion to cells l^{-1} see Table 2
[3)] converted from cells volume^{-1} as originally published

Appendix II a: Cells, trichome and colony sizes and conversion factors

parameter	data	units	comments	Reference
cell volume	1690	μm^3	radial colonies	Carpenter 1983
	540	"	spherical colonies	
cell size	7 – 15	µm	diameter, *T.erythraeum*	Bortstadt et al. 1991
	12 – 21	"	diameter, *T.hildenbrantii*	
	5 – 12	"	diameter, *T. thiebautii*	
trichome width	8 - 10	µm	*T.thiebautii*	Prufert-Bebout et al. 1993
trichomes colony^{-1}	7-8	trichomes	*T erythraeum,* average	Brycson + Fay 1981
	2 – 65		*T.erythraeum*, range	
	21 / 47	"	*T.thiebautii*, av./max longitud. colonies	Bell et al. 1999
	157 / 360	"	radial colonies	
	100 - 250	"		Carpenter + Romans 1991
	<10 - 372	"	seasonal + annual variability at Station ALOHA, NP	Letelier + Karl 1996
	132 - 241	"		
	several hundred	"		Carpenter + Price 1976
cells trichome^{-1}	100	cells		Carpenter + Price 1976
	6 - 250 (x ~ 100)	"	seasonal + annual variability at Station ALOHA, NP	Letelier + Karl 1996
	59	"	GBR, *T. thiebautii*	Bell et al. 1999
	3 - 340	"	GBR, range, *T.thiebautii*	
	50	"		Furnas 1991
cells colony^{-1}	29800 (s.d = 7800)	"	western SAR	Carpenter + McCarthy 1975
diameter of colony	0.5 - 3	mm	tropical NA	Carpenter + Romans 1991
dimensions of colony	3-5 x 1	"		"
	1 - 1000	"		Carpenter + Capone 1991

Appendix II b:
Elemental content of *Trichodesmium spp.* measured under various conditions

Carbon ng	Nitrogen ng	Phosphorus ng	location comment	reference
	content per colony			
$9.7x10^3$	$2,4x10^3$	120	North Pacific [1]	Mague et al. 1977
$1x10^4$	2×10^3	-		Carpenter 1983 [2]
$1.1x10^4$	$2.3x10^3$		Bahamas and Caribbean [3]	Carpenter 1993
$1.1x10^4$	2×10^3	-	North Atlantic	McCarthy and Carpenter 1979 [4]
	mean (range)			
1.02×10^4 (1.3×10^3)	2.13×10^3 (0.4×10^3)	120		
	content per filament			
50.0 – 51.6	9.2 – 9.8	0.40 – 0.48	North Pacific [5]	Letelier and Karl 1996
57.7	9.6	0.51	North Pacific [6]	"
	mean (range)			
53.1 (5.7)	9.5 (0.6)	1.39 (0.1)		
	content per cell			
-	0.05	-	Caribbean/Sargasso	Carpenter and Price 1977
-	0.99	-	Caribbean [7]	Carpenter et al. 1987
	mean (range)			
-	0.52 (0.94)	-		
1.1×10^{-2} ng N ng $cell^{-1}$	2 ng N ng $cell^{-1}$	-	West Pacific [7]	Marumo 1975 [8]

Appendix II abbreviations

NP = North Pacific
GBR = Great Barrier Reef
SAR = Sargasso Sea
NA = North Atlantic

Appendix II footnotes

1) ATP = 13 (µg colony^{-1}), ATP carbon = (3.3 µg colony^{-1})
ATP carbon : N = 1.4 : 1 (weight:weight)
C : ATP = 746 :1 (weight:weight)
Chl-*a* = 34 µg colony^{-1}
2) in Carpenter and Roennegerg 1995
3) *T. erythraeum* and *T.thiebautii* means
4) ATP = 17 + 21 (µg colony^{-1}), C:ATP = 650 + 540, spherical + fusiform respectively
5) trichome morphology
6) colony morphology
7) assuming 100cells trichome^{-1}
8) in Carpenter et al. 1987

Appendix III a: Carbon turnover times of *Trichodesmium sp*. – field data

turnover time d	**variable/location/ comments**	**reference**
0.93	CAR/BAH SST 24.5-26.5°C Jan-Feb 1992	Carpenter and Roenneberg 1995
7.8	CAR SST 28.3-29.3°C Sept. 1992	"
6.13	BAH SST 28.5-29.7°C Sept.1993	"
11.6	central CAR	Carpenter and Price 1977
18	eastern CAR	Li et al. 1980
mean (range) 8.9 0.93 - 18		

Appendix III b: growth rates of *Trichodesmium sp*. – field data

growth rate d^{-1}	**technique**	**variable/location/ comments**	**reference**
0.046	based on total part. carbon	NP	Mague et al. 1977
0.023	based on part. C doubling times	NP	"
0.13 (0.066 - 0.14)	based on N assimilation	NP	"
0.14	based on ATP	NP	"
0.55 - 0.88	based on part. N doubling times	various oceanic regions	Carpenter et al. 1987
0.009 - 0.025	N-fix and $^{15}NH_4$ uptake	SAR	Carpenter and McCarthy 1975
0.005	based on part. N doubling times	NA	McCarthy and Carpenter 1979
0.055	based on part. N doubling times	CAR	Li et al. 1980
0.06	3:1 C_2H_4:N fixed	western NP, July	Saino + Hattori 1978
0.16	"	Kuroshio Current, March	"
0.66		off Tanzania, Mar.	Bryceson and Fay 1981
0.02	6.3 : 1	CAR	Carpenter and Price 1977
0.01		SAR	"
0.03		central NA, May-June	McCarthy and Carpenter 1979
0.006 - 0.02		Florida Current, Jan - Oct	Taylor et al. 1973
0.77		average, CAR	Carpenter et al. 1987
0.56	assuming 100 cells trichome^{-1}	BAH	"
mean (range) 0.19 (0.005 - 0.88)			

CAR = Caribbean
BAH = Bahamas
SAR = Sargasso Sea
NP = North Pacific
NA = North Atlantic

Appendix III c: specific growth rates of *Trichodesmium sp.* – lab data

μ d^{-1}	**technique**	**species**	**reference**
0.16	chl-*a* based	*T. thiebautii*	Prufert-Bebout et al. 1993
0.12	carbon specific	IMS 101	Berman-Frank et al. 2001
0.12	cell number and chl-*a*	"	Mulholland and Capone 2001
0.14	carbon specific	"	Kustka et al. 2003
mean (range) 0.14 (0.12 - 0.16)			

Appendix IV: Photosynthetic characteristics of *Trichodesmium spp.*

I_k	P_{max}	R	I_c	α	*C assimilation*	location	reference
E	mgO_2 mg chl-$a^{-1}h^{-1}$	mgO_2 mg chl-$a^{-1}h^{-1}$	E			comment	
687	41.9	14.9	~150	-		CAR/ BAH [1]	Carpenter et al. 1993
324	36.9	24.9	~150	-		[2]	"
-	66 - 91	12 - 27	78 - 160	0.23 - 0.27		CAR/ BAH [1]	Roenneberg and Carpenter 1993
295	44.7	17.6	134	0.137	10.8 µg C colony^{-1}d^{-1}	CAR/ BAH [3]	Carpenter and Roenneberg 1995
142	8.29	4.93	96	0.055	1.29	CAR [4]	"
285	6.45	2.41	130	0.019	1.63	BAH [5]	"
~ 200	(at 600 µE)	(30) [6]	280	0.012	0.06 0.08 µg C colony^{-1}d^{-1}	BAH [1]	Kana 1991
-	-	(13 – 46) [6]	-	-		[1]	Kana 1993
~ 220	6.3 12.5	1.0 4.1	59 169	0.010 0.029		Belize [7]	Villareal 1995
~300	-	-	-	-	3.9 – 4.3 % of total production	NP	Letelier and Karl 1996
					0.1 – 22.3 mgC m^{-2} d^{-1}	CAR/ SAR	Carpenter and Price 1977
					0.38 µgC µgchl-$a^{-1}h^{-1}$	NP	Mague et al. 1977
					1.17 µgC µgchl-$a^{-1}h^{-1}$	CAR	"
					0.01 - 0.62 µg C l^{-1} h^{-1}		Goering et al. 1966
					55 to 275 mgC m^{-2} d^{-1}	NA	Carpenter and Romans 1991
					3.78 mgC mgChl-a^{-1}		Lewis et al. 1988
-	18.52	-	-	-		lab [8]	Ohki et al. 1991
-	17.70	-	-	-		lab [9] [10]	Ohki and Fujita 1988
-	10.63	-	-	-		lab [9] [11]	"
-	11.89	-	-	-		lab [9] [12]	"
-	7.90	-	-	-		lab [9] [13]	"
~ 90 - 330	(17 – 80)	-	-	0.18 - 0.23		lab [14]	own laboratory data
				mean (range)			
296 (142-687)	26 (6.3-91)	16 (1-46)	143 (59-280)	0.09 (0.01-0.23)			

Appendix IV abbreviations and footnotes:

CAR = Caribbean
BAH = Bahamas
NP = North Pacific
SAR = Sargasso Sea
NA = North Atlantic

[1] *T. thiebautii*
[2] *T. erhythraeum*
[3] SST 24.5-26.5C Jan-Feb 1992
[4] SST 28.3-29.3 September 1992
[5] SST 28.5-29.7 September 1993
[6] % gross photosynthesis
[7] off-shore and in-shore, Atlantic Barrier Reef, P_{max} and R units: nmolO_2 µgchl-*a*$^{-1}$
[8] NIBB 1067, N_2 fixation = 15.4 mol molchl*a*$^{-1}$ h^{-1}
[9] NIBB 1067
[10] single trichome and small bundles, exponential growth
[11] single trichomes, linear growth
[12] small bundles, linear growth
[13] spherical colonies
[14] IMS-101, [Fe] = 4 – 800 nM and growth period, P_{max} here = maximum electron transport rate

Appendix V a: Nitrogen fixation rates of *Trichodesium spp.* - field data

All data are based on acetylene reduction assays unless otherwise noted. Conversions between mol and weight units are done without further notice. Conversions between C_2H_4 and N_2 reduction are based on a 4:1 ratio unless a different factor is given in the original publication. See table 2 for conversion factors regarding biomass estimates. Biomass conversions can yield inaccurate nitrogen fixation rates as biomass ratios such as chl-*a* colony^{-1} are subject to uncertainties.

location	**N-fixed cell^{-1}**	**N-fixed trichome^{-1}**	**N-fixed colony^{-1}**	**N-fixed biomass^{-1}**	**N-fixation rate volume^{-1} time^{-1}**	**N-fixation rate area^{-1} time^{-1}**	*standardized* **N-fixation rate** mol N mol chl-a^{-1} h^{-1}	**temp.** °C	**note**	**Reference**
	0.08 pg N cell^{-1} h^{-1}					26 mmol m^{-2} y^{-1}	3.9	25	1)	Bell et al. 1999
NP ALOHA						31 – 51 mmol m^{-2} y^{-1}		n.a.	2)	Karl et al. 1997
						80 mmol m^{-2} y^{-1}		n.a.	3)	Gruber and Sarmiento 1997
						1.47 Tg y^{-1}		n.a.	4)	Somasundar et al. 1990
global / IO				2 µmol N mmol cellular N^{-1} h^{-1}		4.79 / 3.12 Tg y^{-1}	2.6	n.a.	5) 6)	Carpenter et al. 1983
		87 – 252 pgN trichome^{-1} h^{-1}				27 375 mmol m^{-2} y^{-1}	43 - 124		7)	Carpenter and Capone 1991
global						5.4 Tg y^{-1}				

location	N-fixed cell^{-1}	N-fixed trichome^{-1}	N-fixed colony^{-1}	N-fixed biomass^{-1}	N-fixation rate volume^{-1} time^{-1}	N-fixation rate area^{-1} time^{-1}	*standardized* N-fixation rate mol N mol chl-a^{-1} h^{-1}	temp. °C	note	Reference
global						15.3 Tg y^{-1}		n.a.	8)	Lipschulz and Ownes 1996
"						39 (13 – 105) mmol m^{-2} y^{-1}		n.a.	9)	Capone and Carpenter 1999
						65 Tg y^{-1}				
AS						0.34 Tg y^{-1}			10)	Subramaniam et al. 1999
"						14.6 – 47 mmol m^{-2} y^{-1}				Capone et al. 1998
		~0.25 - 8.0 nmol C_2H_4 1000 trich^{-1} h^{-1}					0.4 - 14	28.2 – 33.2	11)	Bryceson and Fay 1981
CAR			156 + 305 ngN col.$^{-1}$ h^{-1}				765 + 1497	n.a.	12)	Capone et al. 1994
CAR			273 + 457 ~				1340 + 2242	n.a.	13)	"
NA subtrop.			0.6 nmol col.$^{-1}$ h^{-1}				41	n.a.	14)	Mulholland and Capone 1999
				0.25 nmol N µg chl-*a*$^{-1}$h^{-1}			0.06	n.a.	15)	Paerl and Bebout 1988
				2.11 ~			0.46	~	16)	"
			2.11 ngN col.$^{-1}$ h^{-1}	0.26 ~			0.06	n.a.	17)	Carpenter and Price 1976

location	N-fixed cell^{-1}	N-fixed trichome^{-1}	N-fixed colony^{-1}	N-fixed biomass^{-1}	N-fixation rate volume^{-1} time^{-1}	N-fixation rate area^{-1} time^{-1}	*standardized* N-fixation rate mol N mol chl-a^{-1} h^{-1}	temp. °C	note	Reference
CAR		60 pgN trichome^{-1} h^{-1}					29	n.a.	18)	Carpenter et al. 1987
“		2.46 pmol C_2H_4 trich.$^{-1}$ h^{-1}					4.2	~	19)	"
“		2.53 ~					4.4	~	20)	"
BAH		4.04 ~					6.9	~	21)	"
“		4.87 ~					8.4	~	22)	"
SAR		4.97 ~					8.5	~	23)	"
CAR		5.75 ~					9.9	~	24)	"
“		6.45 ~					11.1	~	25)	"
	0.069 pg N cell^{-1} h^{-1}				0.0071 nmol N l^{-1} h^{-1}		3.4	26-27	26)	Carpenter and McCarthy 1975
	0.2 ~				0.046 ~		9.8	~	27)	"
						0.6 mmol m^{-2} y^{-1}		~	28)	"
SAR	0.033 ~					1.4 ~	1.62	n.a.	29)	Carpenter and Price 1977
“	0.077 ~					32.9 ~	3.78	~	30)	"
“	0.09 ~		2.11 ngN col.$^{-1}$ h^{-1}			147.5 ~	4.42 / 10.35	~	31)	"
“			0.26 ~		0.499 ~		1.28	~	32)	"

location	N-fixed cell^{-1}	N-fixed trichome^{-1}	N-fixed colony^{-1}	N-fixed biomass^{-1}	N-fixation rate volume^{-1} time^{-1}	N-fixation rate area^{-1} time^{-1}	*standardized* N-fixation rate mol N mol chl-a^{-1} h^{-1}	temp. °C	note	Reference
						304 - 1520 mmol m^{-2} y^{-1}			33)	Carpenter and Romans 1991
TA						913 ~			34)	"
CAR +BAH			159 – 266 ~				780 – 1305	n.a.	35)	Gilbert and Bronk. 1994
"			182 +/- 33 ~				893 +/- 162	~	36)	"
"			156 +/- 27 ~				765 +/- 132	~	37)	"
"			159 +/- 7 ~				780 +/- 34	~	38)	"
					0 – 0.016 ~			25 or SST	39)	Goering et al. 1966
IO			0.13 - 3.0 nm C_2H_4 col.$^{-1}$ h^{-1}			5729 mmol m^{-2} y^{-1}	2.2 - 52		40)	Kromkamp *et al.* 1997
"			0.24 ~			0.037 – 31.7 ~	4.1		41)	"
			620 pmol C_2H_4 h^{-1} max. rate	2.09 nmol N µg chl-*a*$^{-1}$h^{-1}			1.9	n.a.	42)	Mague et al. 1977
				1.08 ~			0.96	~	43)	"
				18.9 +/- 1.1 pmol C_2H_4 µg atom N^{-1} h^{-1}			6.1 x 10^{-3} +/- 3.6 x 10^{-4}	28	44)	Saino and Hattori 1978
				504 +/- 7.1 ~			0.16 +/- 2.3 x 10^{-3}	~	45)	"

location	N-fixed cell^{-1}	N-fixed trichome^{-1}	N-fixed colony^{-1}	N-fixed biomass^{-1}	N-fixation rate volume^{-1} time^{-1}	N-fixation rate area^{-1} time^{-1}	*standardized* N-fixation rate mol N mol chl-a^{-1} h^{-1}	temp. °C	note	Reference
				1.41 - 5.57 nmol C_2H_4 µg atom N^{-1} h^{-1}			0.46 - 1.8	n.a.	46)	Saino and Hattori 1982
				7.05 - 9.92 ~ 15.7 - 22.5			2.3 - 3.2	~	47)	"
							5.1 - 7.3	~	48)	"
				6.53 - 29.0 ~ 6.25 – 518.89 µm N mol C^{-1} h^{-1}			2.1 – 9.4	~	49)	"
							1.29 x 10^{-3} 1.1 x 10^{-1}	n.a.		Sanudo-Wilhemy et al. 2001
min							*1.29 x 10^{-3}*			
max							*2242*			

abbreviations

NP = North Pacific
IO = Indian Ocean
AS = Arabian Sea
NA = North Atlantic
CAR = Caribbean
BAH = Bahamas
SAR = Sargasso Sea
TA = Tropical Atlantic

Appendix V b **Nitrogen fixation rates of Trichodesium sp.**
lab data, all acetylene reduction unless otherwise noted

N-fixation rate area^{-1} or biomass^{-1} time^{-1}	unit	*standardized* **N-fixation rate biomass^{-1} time^{-1}** mol N mol chl-*a*$^{-1}$ h^{-1}	**temp.** °C **(light)** µE	**note**	**Reference**
max. 50 - 300	nmol ethylene µg chl-*a*$^{-1}$ h^{-1}	11 - 40	26 (90)	1) 4)	Chen et al. 1996
0.0086 - 1.046 % h^{-1}			28 (55-65)	1) 5)	Mulholland and Capone 1999
0.5 % h^{-1}			26 (30-40)	2) 6)	Mulholland et al. 1999
14 – 16 % d^{-1}			~	2) 7)	"
12.5	fmol N cell^{-1} h^{-1}	8.6	~	2) 8)	"
2.75	"	1.9	~	2) 9)	"
~0.3 - 0.6	"	0.2 - 0.4	~	2) 10)	"
14.5	µmol N l^{-1} d^{-1}		~	2) 11)	"
6 to 18	µmol N l^{-1} d^{-1}		~	2) 12)	"
3.5	mol C_2H_4 mol chl-*a*$^{-1}$ h^{-1}	0.9	25 (7W m^{-2})	2) 13)	Ohki and Fujita 1988
4.7	"	1.2	~	2) 14)	"
22	"	5.5	~	2) 15)	"
26	"	6.5	~	2) 16)	"
35	"	8.8	~	2) 17)	"
46.2	"	11.6	25	2) 18)	Ohki et al. 1991
1.32 - 3.11	"	0.3 – 0.7	25	3) 19)	Paerl 1994
5 - 13	"	1.1 - 2.9			Berman-Frank et al. 2001
0.02 - 0.18	pg cell^{-1} h^{-1}	1.1 - 7.8			Fu and Bell 2003
min		0.2			
max		40			

Appendix V a: Footnotes:

[1)] max. was 0.40 for L-colonies, values similar to T. erythraeum (Bell 1993 cited in this paper)
[2)] 51 equals the equivalent to 50% PN export at ALOHA
[3)] equals ~90% of input via atmosph.
[4)] Arabian Sea, estimate based on abundance data by Devassy (1983)
[5)] first value global annual rate, second value Indian Ocean annual rate (as cited in Somasundar et al. 1990)
[6)] cellular value meas. in NP gyre
[7)] in blooms
[8)] ocean wide calculation based on reviewed data
[9)] extrapolation to depth integrated N_2 fixation in latitudinal bands of the ocean, season corrected, 10 fold higher that 1982 estimate
[10)] 5.88×10^{11} m^2 bloom, Arabian Sea
[11)] related to windspeed + time of day+bundledness
[12)] light + dark, two years / Caribbean/Atlantic
[13)] light only, two years / Caribbean/Atlantic
[14)] diel variation
[15)] small colonies (<30 trichomes/colony)
[16)] large colonies (>75 trichomes/colony) - shading effect
[17)] equivalent to high sea state, comp.w/ Roenneb+Carp. 1993
[18)] based on av. from Car. (6.45 ethyl) w/ 3:1 mol. conversion factor
[19)] hand collect. by divers, Caribbean, ultra clean methods
[20)] net collected, Caribbean, ultra clean methods
[21)] Bahamas, ultra clean methods, in situ
[22)] Bahamas – standard methods
[19-22)] *no significant difference between the procedures*
[23)] Sargasso Sea, no diff. To Bahamas, ultra clean methods in situ
[24)] average, Caribbean found sign. diff. between days, standard methods
[25)] average, Caribbean, ultra clean methods
[26)] 3:1 used, vol. rate converted from ng at 1m depth, maximum av.
[27)] as 26), max. measured
[28)] 3:1 used, rate per area was integrated over 50m depth, 9 % of surface value at 3% light (45m), 10 h d^{-1}
[29)] Sargasso
[30)] Caribbean progr. decrease below 15m, 5% at 75m of rate at 15m
[31)] per cell: 15m depth Caribbean, max. measured, surface Sargasso Sea per area: Caribbean, colonies shaken (not stirred) - disrupted
[32)] maximum at 10 – 15 depth 125x higher in Caribbean than in Sargasso, colonies intact $p<0.05$
[33)] estimate based on turnover and colony density data
[34)] tropical NA
[35)] Caribbean + Bahamas - Jan/Feb92, ^{15}N method in correlation to (LMW DON release) estim.
[36)] Caribbean + Bahamas - Jan/Feb92, *^{15}N method*
[37)] Caribbean + Bahamas - Jan/Feb92, *^{15}N method* incorp. into low molecular weight comp. only
[38)] note that LMW and acet. rates are virt. equivalent
[39)] up to max. 19.0% uptake, vol. converted from liter
[40)] bloom , density=221000col./l), Indian Ocean 2.3'S - 4.25'S off Kenia, ratio 8:1
[41)] density=2-268col./l, ratio 8:1
[42)] acetylene reduction compared to ^{15}N method, results in ratio: 1.93 : 1 but authors recommend ratio 3 : 1, converted µg algal N to µg chl-*a* – 25:1
[43)] % h (0.40 = 0.6 nmol N/colony/h) diel var.
[44)] measured 20:45 – 22:25 h
[45)] measured 13:40 – 14:45 h
[46)] trichomes anaerobic
[47)] colonies anaerobic
[48)] colonies aerobic
[49)] trichomes aerobic

Appendix V b Footnotes:

[1] IMS – 101
[2] NIBB1067
[3] *Trichodesmium sp.*
[4] differences in media, circadian rhythm
[5] circadian rhythm, 14:10h light:dark
[6] N-fix - N-turnover (urea enriched (30µM))
[7] N-fix turnover rate at exp. growth, bell shaped curve (Chen et al. 1996) or flat (lower estimate)
[8] maximum measured value
[9] N-fix NO_3 enriched (150µM)
[10] N-fix urea enriched (30µM), calc. from data in paper
[11] mean fixation rate in non-enriched media based on PN increase
[12] at a Biomass of 55 µg chl-a^{-1} l^{-1} (mean) and 30-40 µE, depending on calc.
[13] large bundle shaped colonies
[14] spherical colonies
[15] single trichomes, linear growth
[16] small bundles, linear growth
[17] single trichome and small bundles, exponential growth
[18] constant: 0.18 d^{-1}, exp. phase at 12 h light:dark, 15W m^{-2}
[19] at 100 µE differences depending on aggregation and age/growth phase of culture

Appendix VI a: **Elemental composition of *Trichodesmium spp.* measured under various conditions** (all field data)

C:N	N:P - molar ratio -	C:N:P	location comment	reference
5.98*	-	-	BAH and CAR [1)]	Carpenter et al. 1993
7.0	-	-	NA [2)]	Lewis et al. 1988
6.8 - 7.0**	-	-		Saino and Hattori 1978
6.4 - 6.5**	-	-	NP [3)]	
6.0*	-		BAH and CAR [4)]	Carpenter et al. 1993
4.71*	44.3*	209:44:1*	NP	Mague et al. 1977
5.4*	-	-		Carpenter 1983
6.53*	-	-	WP [5)]	Marumo 1975 [5)]
6.4 (4.6 - 7.5)	29 (14 - 52)	185:29:1 (C:P 79 - 310)	WP	Kustka et al. 2003
6.06 - 6.30*	-	-	NA	McCarthy and Carpenter 1979
7.32	42.76	313:43:1***	NP [6)]	Letelier and Karl. 1998
6.32	43.75	277:44:1***	NP [7)]	
6.61	44.8	296:45:1***	NP [8)]	"
5.88	34.19	201:34:1***	NP [9)]	"
6.14 - 6.34	45 - 52	303:49:1***	NP [10)]	Letelier and Karl 1996
6.4	42	269:42:1***	NP [11)]	"
7.13	125	891:125:1	NP [12)]	Karl et al. 1991
8.9	21	188:21:1	RS	Nagvi et al. 1986
	mean (range)			
6.5 (4.6 - 7.5)	47.5 (14 - 125)	313:48:1 (79:17:1- 891:125:1)		

Appendix VI b: **Elemental composition of *Trichodesmium spp.* measured under various conditions** (laboratory data)

C:N	N:P - molar ratio -	C:N:P	treatment	note	reference
12	4.8	58:4.8:1	IMS101 Fe-limited	[13]	Berman-Frank et al. 2001
12	13	156:13:1	IMS101 Fe-replete		"
	23.6 - 140		1 µM DOP		Mulholland et al. 2002
	23.8 - 150		3 µM DOP		
	5.7 - 35.2		50 µM DOP	[14]	

Appendix VI abbreviations and footnotes:

BAH = Bahamas
CAR = Caribbean
NA = North Atlantic
NP = North Pacific
WP = West Pacific
RS = Red Sea

* coverted from weight to molar ration with C/12:N/14,
** converted in a similar way based on the assumption that the data were given on weight basis, not indicated by the authors
*** calculated based on C:N and N:P values given in the paper

[1] 15m, *T.thiebautii*
[2] in a bloom
[3] first value at day second value at night ($p < 0.05$), *T.thiebautii*
[4] based on *T. erythraeum* and *T.thiebautii* means
[5] assuming 100cells/trichome, *in:* Carpenter et al. 1987
[6] neg. buoyant colonies at 5m
[7] pos. buoyant colonies at 5m
[8] neg. buoyant colonies at 100m
[9] pos. buoyant colonies at 100m
[10] trichome morphology, calc. based on means(C:N=6.24; N:P=48.5), PC:Chl = 187 - 199 (w:w)
[11] colony morphology, PC:Chl = 163weight:weight)
[12] in a bloom
[13] see Appendix VII e for Fe-ratios
[14] cultures grown on DOP (glycerol-P) as sole source for Phosphorus, variations in elemental rations are due to growth phase

Appendix VII: cellular Fe stoichiometry and iron uptake rates of *Trichodesmium*

Iron requirements for diazotrophic growth – compiled field and laboratory data.

field/lab	**Fe:C** µmol:mol	variable/ comments	**Reference**
lab	13 - 168	[1]	Berman-Frank et al. 2001
"	7.1 - 214	[2]	
"	38 - 48	requirement for diazotrophic vs. NH_4^+ replete growth	Kustka et al. 2002
"	8		"
field	20 - 500	different oceanic regions	"
"	18 - 222	Australia (north coast)	Kustka et al. 2003
"	333		Rueter et al. 1991
"	20		Sanudo-Wilhemy et al. 2001
	7.1 - 500	***over all range***	

the theoretical minimum value of the Fe:C ratio for diazotrophic growth of *Trichodesmium* equals 13.5 (Kustka et al. 2002)

[1] total iron treatments: 2.13 to 2030 nM with EDTA added to the media at a constant concentration (20 µM), resulting in log [Fe'] (denoting the sum of the total inorganic Fe species) from -11.78 to -7.8
(see table below for the effect on elemental stoichiometry)

[2] total iron treatments: 0.04 to 4 nM with EDTA added to the media at a constant ratio to the total iron concentration, resulting only in a minor change of log [Fe'] from -7.95 to -7.8

total Fe:C quotas, the intracellular quotas are 60 – 70 % lower

Effects of iron concentrations on *Trichodesmium* elemental composition and physiology.
modified after Berman-Frank at al. (2001)

total [Fe]	**log [Fe']**	**chl a :C**	**Fe:C**	**C:N**	**C:P**	**N:P**	**C:N:P:Fe**
nM		µg : µmol	µmol:mol	mol:mol	mol:mol	mol:mol	molar
2.1	-10.78	0.018	13	12	58	4.8	58 : 4.8 : 1 : $7.5x10^{-4}$
16.9	-9.88	0.17	30	8.9	55	6.2	55 : 6.2 : 1 : $1.7x10^{-3}$
51.1	-9.40	0.19	33	9	60	6.7	61 : 6.7 : 1 : $2.0x10^{-3}$
1768	-7.86	0.25	48	16	66	4.8	66 : 4.8 : 1 : $3.2x10^{-3}$
2030	-7.80	0.29	168	12	156	13	156 : 13 : 1 : $2.6x10^{-3}$
field			806	6.13	154	25	154 : 25 : 1 : $1.3x10^{-2}$

k_s for μ_c	**μ_c at k_s**
16.13 nM Fe	0.062 d^{-1}

Note: k_s for μ_c and μ_c at k_s were derived from this publication

Iron and nitrogen measurements on *Trichodesmium* collected in the Caribbean.
modified after Rueter et al. (1991)

	Fe colony^{-1}	**protein N colony^{-1}**	**Chl-*a* colony^{-1}**	**N_2 – fixation**	**N:Fe**	**chl-*a* : Fe**	**nmol N fix mol Fe^{-1} h^{-1}**
	nmol	nmol	nmol	nmol N fixed colony^{-1} h^{-1}	mol:mol	mol:mol	
range	0.135 - 0.470	51 - 204	0.044 – 0.098	0.0054 – 0.6616	166 - 707	0.128 – 0.709	0.03 – 3.01
mean	0.231	97	0.080	0.136	465	0.387	0.65
examples of particular interest from the data set	**0.470** highest value	78	0.060	0.0914	**166** lowest value	**0.128** lowest value	0.19
	0.220	**57** near low limit	**0.090** near max. limit	**0.6616** highest value	259	0.410	**3.01** max value
	0.140 near low limit	99	0.069	**0.0162** low value	**707** highest value	0.439	0.12

Iron uptake rates and specific growth rates measurements of *Trichodesmium* collected in the Caribbean.
modified after Rueter et al. (1991)

	Fe colony^{-1}	**^{55}Fe uptake**	**Fe specific growth rate**	**Fe based doubling time**
	pmol	pmol colony^{-1} min^{-1}	min^{-1}	d^{-1}
range	22 - 68	0.06 - 0.26	0.0014 - 0.0040	0.12 – 0.34
mean	55	0.05	0.0029	0.20

Appendix VIII: Nutrient uptake rates of *Trichodesmium sp.*

PO_4 uptake	**NO_3** uptake	**NO_2** uptake	**NH_4** uptake	**urea** uptake	**Glu** uptake	**note**	**reference**
~0.8 - ~7.9 nmol $cell^{-1}$ d^{-1}	80.6 nmol $cell^{-1}$ d^{-1}					1)	Ramamurthy and Krishnamurthy 1967
~0.2 - ~1.2 nmol $cell^{-1}$ d^{-1}	24.2 – 104.8 nmol $cell^{-1}$ d^{-1}					2)	"
	~ 0.58 fmol N $cell^{-1}$ h^{-1}	~ 12.5 fmol N $cell^{-1}$ h^{-1}		~ 1.66 fmol N $cell^{-1}$ h^{-1}		3)	Carpenter + McCarthy 1975
	< 0.2 fmol N $cell^{-1}$ h^{-1} 5)		20 fmol N $cell^{-1}$ h^{-1}		0.4 – 0.6 fmol N $cell^{-1}$ h^{-1}	4)	Mulholland et al. 1999
	25 fmol N $cell^{-1}$ h^{-1}					6)	"
				3 - 6 fmol N $cell^{-1}$ h^{-1}		7)	"
	0.0003 - 0.0043 % h^{-1}		0.04 - 1.84 % h^{-1}	0.0007 - 0.11 % h^{-1}	0.015 - 0.37 % h^{-1}	8)	Mulholland and Capone 1999
			18 µmol N l^{-1} d^{-1}				Mulholland and Capone 2001

1) changing N:P ratio 20:1 - 20:15 (increasing P), *T. erythraeum*, lab, 24h incubation
2) changing N:P ratio 1:1 - 25:1 (increasing N), *T. erythraeum*, lab, 24h incubation
3) *T. erythraeum*, lab

4) NIBB1067, lab

NH_4 K_s = 0.26 µM	NH_4 V_{max} = 13 /h x $10e^{-3}$
NO_3 K_s = 39 µM	NO_3 V_{max} = ND
urea K_s = 6.75 µM	urea V_{max} = 19 /h x $10e^{-3}$
Glu K_s = 1.34 µM	Glu V_{max} = 15 /h x $10e^{-3}$

5) calculated from data given in paper
6) NO_3 uptake max - NO_3 enriched (150µM), lab, NIBB1067
7) urea enriched (30µM), lab, NIBB1067

8) field

NH_4 K_s = 3.5 µM	NH_4 V_{max} = 22 /h x $10e^{-3}$
NO_3 K_s = 0.4 µM	NO_3 V_{max} = 0.2/h x $10e^{-3}$
urea K_s = 31 µM	urea V_{max} = ND
Glu K_s = 3.1 µM	Glu V_{max} = 4.5 /h x$10e^{-3}$

The Bunsen gas solubility coefficient of ethylene as a function of temperature and salinity and its importance for nitrogen fixation assays

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The Bunsen gas solubility coefficient of ethylene as a function of temperature and salinity and its importance for nitrogen fixation assays

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Abstract

The acetylene reduction assay is a common method for assessing nitrogen fixation in a variety of marine and freshwater systems. The method measures ethylene, the product of the conversion of the gas acetylene to its reduced form by nitrogenase. Knowledge of the solubility of ethylene in aqueous solution is crucial to the calculation of nitrogen fixation rates and depends on the temperature and salinity of the assay conditions. Despite the increasing interest in marine nitrogen fixation, no gas solubility (Bunsen) coefficients for ethylene in seawater are published to date. Here, we provide a set of equations and present semiempirically derived Bunsen coefficients for ethylene in water (ranging from 0.069 to 0.226) for a range of temperatures and salinities that are relevant for aquatic nitrogen fixation. We apply these data to nitrogen fixation scenarios at different temperatures and salinities and stress the importance of using accurate Bunsen coefficients in nitrogen fixation assays.

The global nitrogen cycle is balanced by two opposing biochemical pathways: denitrification, which releases N_2 to the atmosphere, and biological nitrogen fixation, which fixes N_2 gas. In the ocean, a change in the rate of either of these pathways will result in a change in the global oceanic nitrogen inventory. Estimates of total global and marine nitrogen fixation increased from 90 to 250-500 and 14 to 80-200 $\times 10^6$ tons of nitrogen year^{-1}, respectively, over the past 30 y (Capone and Carpenter 1999). Karl et al. (2002), in a recent review on marine nitrogen fixation processes and their importance for the biogeochemistry of the oceans, state that these estimates of marine nitrogen fixation are subject to large uncertainties. It is therefore of wide scientific interest to verify and improve generally applied methods to measure nitrogen fixation.

Most commonly, nitrogen fixation is measured using the acetylene reduction assay (ARA). The acetylene reduction assay can be applied to various types of marine and freshwater environments such as water column studies, sediment incubations, seagrass meadows, and so on. This assay measures the production of ethylene (C_2H_4) in a sample after incubation with acetylene (C_2H_2), which is an alternative substrate for the N_2 reducing enzyme nitrogenase. Ethylene is easily detected with high sensitivity using gas chromatography (Capone 1993; Capone and Montoya 2001; Montoya et al. 1996; Stewart et al. 1968). The solubility of C_2H_4, required for the calculation of N_2 fixation, is dependent on the temperature and salinity at which the assay is carried out.

The amount of C_2H_4 dissolved in an aqueous phase can be calculated based on the amount of C_2H_4 detected in the overlying gas phase by applying the Bunsen gas solubility coefficient. The Bunsen coefficient (α) represents the solubility of a real gas, where α is the unit volume of gas (v_0), reduced to T_o = 273.15 K and p_o = 1 atm (101.325 kPa), which is absorbed by unit volume (V) of solvent at the temperature of the measurement when the partial pressure of the gas is equal to one standard atmosphere (p_o)

$$\alpha = \frac{v_0}{V}. \quad (1)$$

Therefore, the amount of gas present in the aqueous phase equals

$$n_{aq} = \frac{p_{gT} \times \alpha \times V_{aq}}{R \times T_0} \quad (2)$$

where n_{aq} denotes the total amount of gas (mol) dissolved in the aqueous phase, p_{gT} is the partial pressure of the gas in the headspace (atm) at the temperature of measurement, α is the Bunsen coefficient at the temperature and salinity of the

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measurement, V_{aq} is the volume of the aqueous phase (liter), and R is the gas constant (0.08206 atm L mol^{-1} K^{-1}).

The application of the Bunsen coefficient has been common practice throughout nitrogen fixation studies applying ARA (Flett 1976; Capone 1993; Capone and Montoya 2001). As with any analysis of dissolved gases in liquids, the accuracy of the measurement largely depends on the correct gas solubility coefficients. Flett et al. (1976) discussed the temperature effect on the solubility of ethylene in freshwater and stated that α = 0.122 at 20°C and 1 atm. To the best of our knowledge no published values of ethylene Bunsen coefficients as functions of temperature and salinity are available. Therefore, deriving and supplying appropriate Bunsen coefficients for nitrogen fixation studies in aquatic systems is necessary.

In the present paper we derive Bunsen coefficients appropriate for a variety of marine systems ranging in temperature from 0°C to 35°C and salinities from 0 to 40. Our calculations can be readily applied to determine Bunsen coefficients for any temperature and salinity. The example calculations demonstrate that significant errors can be generated in ARA dependent nitrogen fixation rates when the effects of salinity and temperature on ethylene solubility are not taken into account.

Materials and procedures

All temperatures in this section are referred to as absolute temperature in Kelvin. Experimental data for the solubility of ethylene in water and aqueous electrolyte solutions are published by Clever et al. (1970), Grollman (1929), McAuliffe (1966), Morrison and Billett (1952), Narasimhan et al. (1981), Onda et al. (1970*a* , 1970*b*), Orcutt and Seevers (1937), Taft et al. (1955), Truchard et al. (1961), Wu et al. (1985), Yano et al. (1968), and Yano et al. (1974). The aforementioned publications are compiled and evaluated in the IUPAC Solubility Data Series by Hayduk et al. (1994). Recently, R. Battino (pers. comm. unref.) published gas solubility measurements for ethylene in water in Clever and Battino (2003). Additionally, technical solubility coefficients (λ), Ostwald coefficients (β), and Bunsen coefficients (α) of ethylene in water as a function of temperature are summarized in D'Ans et al. (1967), Dean (1999), and Wilhelm et al. (1977).

The technical solubility coefficient is defined as the volume of gas in cm^3 at 273.15 K and 1 at (technical atmosphere, 1 at = 0.9678 atm) partial pressure that is absorbed by 1 g of solvent:

$$\lambda = \frac{\alpha}{D} \times 0.9678 \qquad (3)$$

where D is the density of the solvent (in g cm^{-3}) at the temperature of the measurement. Additionally, Ostwald coefficients (β) for ethylene in water can be converted into Bunsen coefficients as

$$\beta = \alpha \times \frac{(T/\mathrm{K})}{273.15}. \qquad (4)$$

The Ostwald coefficient is defined as the solubility of a real gas in unit volume gas per unit volume of pure solvent at the temperature of measurement, where the partial pressure in the gas phase is one standard atmosphere. Commonly, gas solubility is also expressed as mole fraction (x), which can be converted to α as follows:

$$\alpha = \frac{x \times R \times T_o}{p_o} \times \frac{D}{M} \qquad (5)$$

where D equals the density of the solvent at the temperature of measurement and M represents the molecular weight of the solvent. Since Flett's (1976) original description of the ARA method, α has become the standard parameter describing the C_2H_4 solubility, and we have retained this convention. The available gas solubility coefficients from the literature, converted to α, are summarized in Fig. 1. We used these data to calculate Bunsen coefficients at relevant temperatures and salinities for marine nitrogen fixation studies. Further, we applied these Bunsen coefficients to a theoretical scenario of measured nitrogenase activity via ARA at different temperatures and salinities.

Semiempirical calculation of ethylene solubility coefficient (α) in seawater—We adapted the equation for the temperature dependence of gas solubility at a constant salinity from Weiss (1970) and obtained a fit of α versus temperature (Fig. 1) based on the measurements by Clever et al. (1970), Grollman (1929), McAuliffe (1966), Morrison and Billett (1952), Onda et al. (1970*a*), Orcutt and Seevers (1937), R. Battino (pers. comm. unref.), Taft et al. (1955), Truchard et al. (1961), Wu et al. (1985), and Yano et al. (1968). Ethylene gas solubility coefficients published by D'Ans et al. (1967), Dean (1999), and Wilhelm et al. (1977) agree well with the data used but are either derived from Morrison and Billett (1952) or have undefined primary sources and therefore were not used in the calculations.

$$\ln \alpha_0 = a_1 + \frac{a_2}{(T/\mathrm{K})} + a_3 \ln(T/\mathrm{K}) \qquad (6)$$

$$a_1 = -189.757$$

$$a_2 = 10092.7$$

$$a_3 = 26.9729$$

In addition to the temperature, the salt content influences the solubility of gases in liquids. The logarithm of the gas solubility in freshwater (α_0) over the gas solubility at a specific salt concentration (α_I) as a function of the ionic strength of the solution yields a linear dependency, where the slope (k) is the empirical salt coefficient and can be used to correct α relative to different ionic strengths I (Setschenov 1889):

$$\log_{10}\left(\alpha_0 / \alpha_I\right) = k \times I. \qquad (7)$$

The ionic strength of a solution is defined as $I = 0.5 \sum_i \left(c_i/c^o\right) z_i^2$, where c_i is the molar concentration and z_i is the charge of the ith ion. The salt effect coefficient itself is temperature dependent.

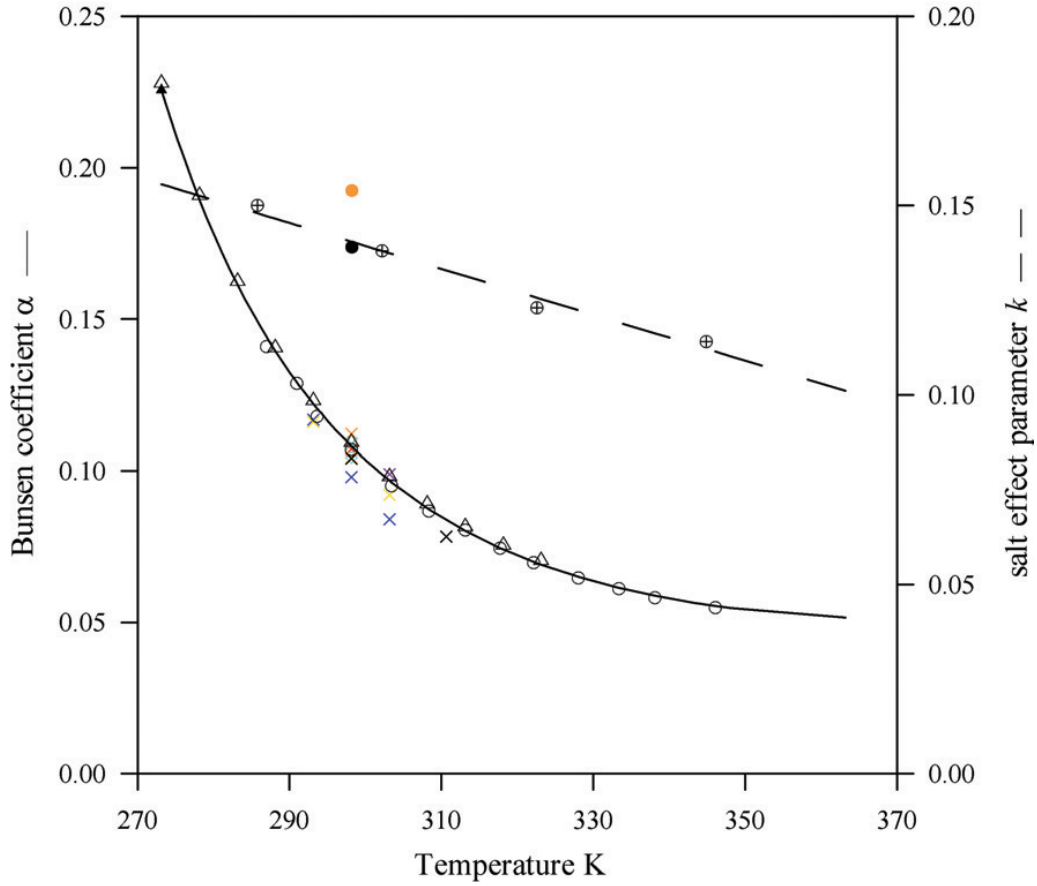

Fig. 1. Temperature dependence of the Bunsen coefficient α (for freshwater) and of the salt effect coefficient *k* (for NaCl). The temperature dependence is largely described by Morrison and Billett (1952) – ○ [black] and R. Battino (pers. comm. unref.) – △ [black]. Further measurements accounted for were published by Clever et al. (1970) – x [purple], Grollman (1929) – x [black], McAuliffe (1966) – ◇ [cyan] Narasimhan et al. (1981) – x [blue], Orcutt and Seevers (1937) – x [cyan], Taft et al. (1955) – x [yellow], Truchard et al. (1961) – ▲ [black], Wu et al. (1985) – x [red] and Yano et al. (1968) – x [orange]. The solid line represents the fit of the experimental data based on equation 6. Data for the salt effect coefficient (*k*) are based on measurements of Morrison and Billett (1952) – ⊕ [black], Onda et al. (1970a) – ● [black] and Yano et al. (1974) – ● [orange] and the dashed line represents a linear fit of *k* based on equation 8.

We derived a temperature function for *k* based on data for NaCl given by Morrison and Billett (1952) and Onda et al. (1970*a*). A linear function was found to be sufficient, since the application of a quadratic function had an insignificant effect on the calculated Bunsen coefficients as a function of temperature and salinity (Fig. 1):

$$k = k_1 + k_2 \times \left(T / \mathrm{K}\right). \quad (8)$$

$$k_1 = 0.3212$$

$$k_2 = -6.063 \times 10^{-4}$$

The ionic strength of seawater at the desired salinity can be derived from the molar concentration of Cl^- ions (0.54588 mol L^{-1}) in seawater at a salinity (S) of 35:

$$I = 1.278 \times [\mathrm{Cl}^-] / \mathrm{mol\ L}^{-1}$$

or

$$I = 0.01993 \times \mathrm{S}. \quad (9)$$

Thus one can derive Bunsen coefficients as a function of temperature and salinity (Table 1) based on

$$\alpha = \exp\left[\left(a_1 + \frac{a_2}{(T / \mathrm{K})} + a_3 \ln(T / \mathrm{K})\right) - 2.303 \times k \times I\right]. \quad (10)$$

Assessment

We derived semiempirical Bunsen coefficients for a range of seawater salinities and temperatures based on laboratory data obtained for freshwater of different temperatures and for NaCl solutions of different concentrations and temperatures. Of the

Table 1. Bunsen coefficients (α) for ethylene in water of different temperatures and salinities (S) at 1 atm

Temperature (°C)	S = 0	S = 5	S = 10	S = 15	S = 20	S = 25	S = 30	S = 32	S = 35	S = 40
0	0.226	0.218	0.210	0.203	0.196	0.189	0.182	0.180	0.176	0.170
2	0.210	0.203	0.196	0.189	0.182	0.176	0.170	0.167	0.164	0.158
4	0.196	0.189	0.183	0.176	0.170	0.164	0.159	0.156	0.153	0.148
5	0.189	0.183	0.177	0.171	0.165	0.159	0.154	0.151	0.148	0.143
6	0.183	0.177	0.171	0.165	0.159	0.154	0.149	0.147	0.144	0.139
8	0.172	0.166	0.160	0.155	0.150	0.144	0.140	0.138	0.135	0.130
10	0.161	0.156	0.151	0.146	0.141	0.136	0.131	0.130	0.127	0.123
12	0.152	0.147	0.142	0.137	0.133	0.128	0.124	0.122	0.120	0.116
14	0.143	0.139	0.134	0.130	0.125	0.121	0.117	0.116	0.113	0.109
15	0.139	0.135	0.130	0.126	0.122	0.118	0.114	0.112	0.110	0.107
16	0.136	0.131	0.127	0.123	0.119	0.115	0.111	0.109	0.107	0.104
18	0.128	0.124	0.120	0.116	0.113	0.109	0.105	0.104	0.102	0.099
20	0.122	0.118	0.114	0.111	0.107	0.103	0.100	0.099	0.097	0.094
22	0.116	0.112	0.109	0.105	0.102	0.099	0.095	0.094	0.092	0.089
24	0.111	0.107	0.104	0.100	0.097	0.094	0.091	0.090	0.088	0.085
25	0.108	0.105	0.101	0.098	0.095	0.092	0.089	0.088	0.086	0.084
26	0.106	0.102	0.099	0.096	0.093	0.090	0.087	0.086	0.084	0.082
28	0.101	0.098	0.095	0.092	0.089	0.086	0.083	0.082	0.081	0.078
30	0.097	0.094	0.091	0.088	0.085	0.083	0.080	0.079	0.078	0.075
32	0.093	0.090	0.087	0.085	0.082	0.080	0.077	0.076	0.075	0.072
34	0.089	0.087	0.084	0.081	0.079	0.077	0.074	0.073	0.072	0.070
35	0.088	0.085	0.082	0.080	0.078	0.075	0.073	0.072	0.071	0.069

major electrolytes in seawater (NaCl, $MgCl_2$, Na_2SO_4, $CaCl_2$, KCl, $NaHCO_3$, KBr, and CsBr), Hayduk et al. (1994) summarize salt effect coefficients for NaCl, Na_2SO_4, KCl, $NaHCO_3$, and KBr at 298.15 K. However, the salt effect coefficient as a function of temperature is only reported for NaCl (data from Morrison and Billett 1952). In our analysis we exclusively used NaCl-based data to derive the temperature-and salinity-dependent salt effect coefficient. The composition of seawater was taken into account by using the ionic strength of seawater as given by Eq. 8. Thus, we assumed that all single charged electrolytes such as NaCl ($\sum z_i^2 = 2$) have a similar salt effect coefficient, whereas double charged electrolytes such as Na_2SO_4 ($\sum z_i^2 = 6$) have a three times higher *k* value. Actually, salt effect coefficients (in parentheses) of single charged electrolytes NaCl (0.139) (Onda et al. 1970*a*), KCl (0.136), and KBr (0.118) (Yano et al. 1974) are indeed in relatively close agreement with each other, whereas Na_2SO_4 (0.394) is 2.8 times higher and thus lies within the expectation (Onda et al. 1970*a*). However for electrolytes such as $NaHCO_3$ (0.195), a simple ionic strength–based method seems less accurate (Onda et al. 1970*b*). Nevertheless, the mole fraction (x_i) of NaCl in seawater relative to the total salt content is very high (0.837). Despite the lack of data for $MgCl_2$ (x_i = 0.104) and the minor importance of KBr (x_i = 0.0015) and $NaHCO_3$ (x_i = 0.005), we are confident that the use of the salt effect coefficient of NaCl, in combination with the application of the ionic strength of seawater, is sufficient to account for the salting-out effect of seawater (Millero 1996; Schwarzenbach et al. 1993). To the best of our knowledge, there are no other data for the salting-out effect of NaCl than those given by Morrison and Billett (1952), Onda et al. (1970a), Yano et al. (1974) (0.154), and the measurements published in D'Ans et al. (1967) (0.140) available. Based on the recommendation by Hayduk et al. (1994), preference was given to the measurements by Morrison and Billett (1952) and Onda et al. (1970*a*). The semiempirical approach presented here yields the most representative Bunsen coefficients for ethylene in seawater to date. Direct measurements of ethylene solubility in natural seawater are needed to verify or further improve these calculations.

The practical details of the ARA are well described (Capone 1993; Capone and Montoya 2001). In general the acetylene reduced to ethylene during ARA is detected using flame ionization detection–gas chromatography. In the following, an ARA case study serves as an illustrative example for the crucial role of the Bunsen coefficient of ethylene in nitrogen fixation studies.

In ARA, the total amount of ethylene produced in the assay vessel is determined by measuring the partial pressure of ethylene in the headspace. The amount of ethylene present in the gas phase is then given by the ideal gas law:

$$n_g = \frac{p_{gT} \times V_g}{R \times T} \tag{11}$$

where n_g is the amount of ethylene in mol, p_{gT} equals its partial pressure (atm) at the temperature of measurement T (K), V_g is

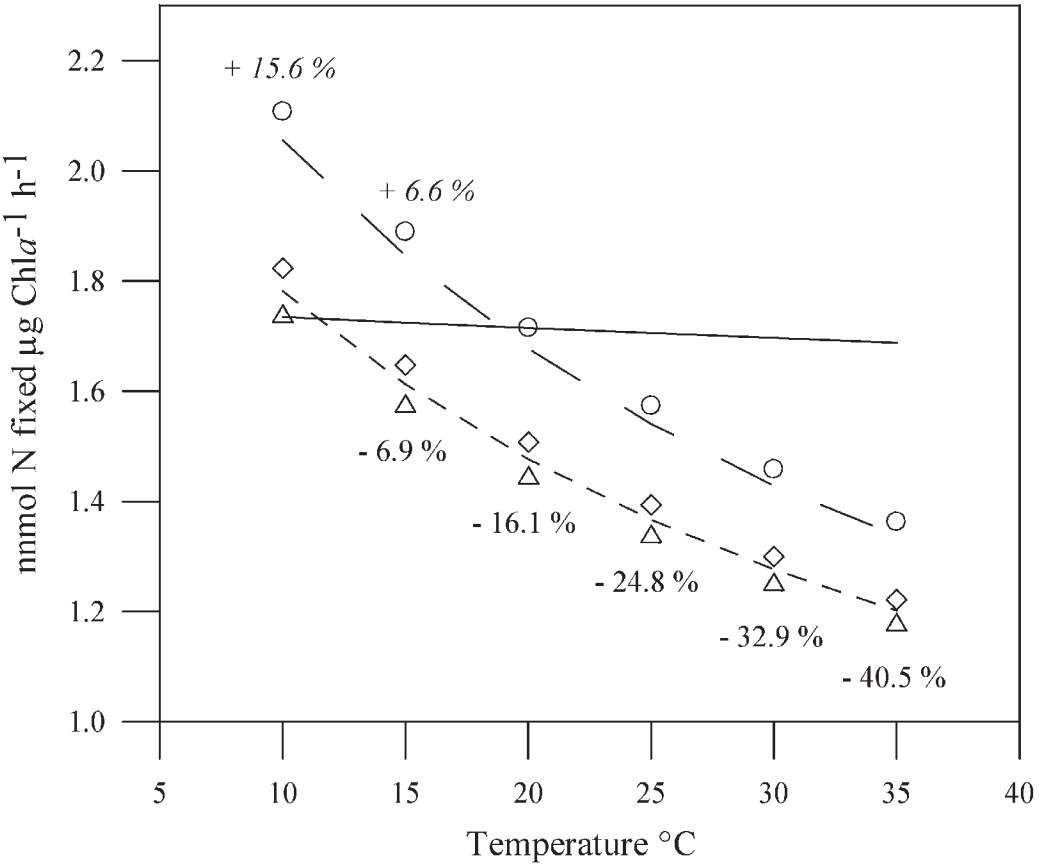

Fig. 2. Theoretical nitrogen fixation rates at temperatures and salinities relevant for marine nitrogen fixation using semiempirically derived Bunsen coefficients. ○ represents values at S = 0, ◇ at S = 30, and △ at S = 40. The solid line represents theoretical nitrogen fixation rates using a Bunsen coefficient of 0.122 independent of T and S in comparison with fixation rates based on T and S dependent Bunsen coefficients as derived in this work (– – –). Percentages illustrate difference between nitrogen fixation rates at a salinity of 35 (– – –) and 5 (— — —, values in *italics*) versus α = 0.122.

the headspace volume in liters, *R* equals the gas constant = 0.08206 atm L mol^{-1} K^{-1}. Because the amount of C_2H_4 present in the aqueous phase can be calculated based on Eq. 2, the total amount of C_2H_4 produced in the assay equals the sum of Eq. 2 and Eq. 11.

These calculations were applied to a theoretical scenario of 85 ppm ethylene production detected in a gas sample from a 20.2-mL headspace vial generally used in our laboratory (19 mL culture and 1.2 mL headspace) over a range of temperatures and salinities. We assumed a biomass of 50 µg chlorophyll *a* (Chl *a*) L^{-1} and an incubation time of 2 h. These values were based on common conditions and observations for ARA-dependent nitrogen fixation studies using cultures of the cyanobacteria *Trichodesmium* (strain IMS 101) in our laboratory. We calculated theoretical nitrogen fixation rates using a ratio of C_2H_2 reduced : N_2 reduced of 4:1 (Montoya et al. 1996) for temperatures between 10°C and 35°C and salinities ranging from 0 to 40 ppm (Fig. 2) using the Bunsen coefficients from Table 1. Additionally, we compared these results to calculated nitrogen fixation rates based on identical settings, but using a Bunsen coefficient of 0.122 independent of temperature and salinity. This exercise illustrates how differences in α result in significant differences in ARA determined nitrogen fixation rates.

With the exception of Flett (1976), Bunsen coefficients are generally not reported in studies of marine nitrogen fixation. Identical to Dean (1999) and in agreement with our calculations, Flett (1976) reports α = 0.122 as a value for freshwater at 20°C but gives no information on α as a function of salinity and temperature. Our calculations show that Bunsen coefficients range from 0.069 to 0.226 for temperatures (0°C to 35°C) and salinities (0 to 40) relevant for aquatic nitrogen fixation studies (Table 1). As a function of temperature and salinity, nitrogen fixation rates range between 1.18 (salinity = 40, temperature = 35°C) and 2.11 nmol N fixed (µg Chl *a*)$^{-1}$ h^{-1} (S = 0, T = 10°C). In contrast, nitrogen fixation rates only vary from 1.69 to 1.73 nmol N fixed (µg chl-*a*)$^{-1}$ h^{-1} over a temperature range from

10°C to 35°C when a Bunsen coefficient of 0.122 is used (Fig. 2). Employing $\alpha = 0.122$ in assays with a temperature higher than 19°C thus yields overestimation of nitrogen fixation rates regardless of the salinity and an underestimation in freshwater assays at temperatures lower than 19°C. If the ARA is conducted in oceanic environments ($S \approx 35$), underestimation and overestimation of nitrogen fixation will occur if $\alpha = 0.122$ is employed below or above 11.5°C, respectively (Fig. 2). Because gas solubility is negatively correlated with temperature and salinity, the strongest deviations from results that apply $\alpha = 0.122$ arise at high temperatures and salinities (>40% for $T = 35$, $S \geq 35$). These deviations are large compared to the precision of the gas phase analysis. Gas chromatography using flame ionization detection responds linearly to the concentration of C_2H_4, and a detection limit as low as 2.5 ppb can be achieved. Thus errors in a calibration curve generated by imprecise calibration standards are obvious and can be excluded. Note that the ratio of the volume in the aqueous phase to the volume of the headspace has an effect on the sensitivity of the method, and optimum volume ratios may need to be determined for each assay setup individually (Montoya et al. 1996).

Discussion

Whereas high salinities and temperatures are of particular interest for studies of nitrogen fixation in vast regions of oligotrophic tropical and subtropical oceans, lower temperatures and brackish waters are encountered in temperate fjords and semi-enclosed oceans such as the Baltic Sea, which experience seasonal episodes of nitrogen fixation. Here, a salinity range of 5 to 15 and a water temperature of 10°C to 20°C are common in regions possessing cyanobacterial summer blooms. For example, blooms of *Nodularia* spp., *Anabaena* spp., and *Aphanizomenon* spp. regularly occur during late summer in the Bothnian Sea and the Gulf of Finland where salinity averages 5 to 6 and water temperature 10°C to 14°C. Similar blooms have also been reported from the Western Baltic where the water temperature ranges from 15°C to 20°C in the summer with predominant salinities around 15 (Gallon et al. 2002; Lozán et al. 1996). At such a wide range of temperature and salinity, the Bunsen coefficients corrected for temperature and salinity are important for the accuracy of the N_2 fixation rate estimates. In some systems, α can deviate as much as 28% from 0.122 ($\alpha = 0.156$, $T = 10$°C, $S = 5$) and nitrogen fixation rates can be underestimated by 15.6% if measurements employ $\alpha = 0.122$. If studies are carried out in freshwater systems, deviations can even be larger (Table 1, Fig. 2).

In the open ocean salinity shows much less considerable change ($S \approx 35$) and temperatures regularly do not exceed 30°C (Levitus and Boyer 1994). The application of $\alpha = 0.122$ deviates up to 36% from appropriate values ($\alpha = 0.078$, $T = 30$°C, $S = 35$) and results in a bias toward too high N_2 fixation rate measurements by ~33% (Table 1, Fig. 2). The salt effect on the solubility of ethylene in seawater compared to the temperature effect can sometimes be negligible in oceanic systems. In contrast, N_2 fixation rates can be overestimated by more than 40% in tropical lagoons and other semienclosed water bodies when temperatures are elevated up to 35°C and the salinity is increased above 35 (Fig. 2).

The application of the ARA as a true measure for nitrogen fixation relies on the fact that nitrogenase is the only enzyme that reduces acetylene to ethylene and is based on a theoretical ratio between the rate of acetylene reduction to cellular N_2 reduction of 4:1 (Postgate 1982; Montoya et al. 1996; Gallon et al. 2002). However, this ratio frequently deviates from its theoretical value. Gallon et al. (2002) demonstrates that even though $^{15}N_2$ nitrogen fixation measurements accurately measure incorporation of nitrogen into cellular material, measurements of acetylene reduction more truly reflect the gross rate of N_2 fixation. The $^{15}N_2$ assay can underestimate the actual amount of N_2 fixed, because the population under investigation can release a significant portion of its newly fixed nitrogen (Bronk 2002) stressing the importance of the ARA for field measurements of nitrogen fixation. In our view, it also supports the need for increased accuracy of this method as provided here.

Comments and recommendations

Based on our calculations of Bunsen coefficients we agree with the validity of the recommendation by Flett et al. (1976), Capone (1993), and Capone and Montoya (2001) stating that appropriate gas solubility coefficients for ethylene must be applied for calculating nitrogen fixation rates and advise future authors to report the gas solubility coefficients used in their assays. Using available experimental data we provide interpolation formulas and also a convenient table (Table 1) for α values at temperature/salinity combinations of interest in aquatic research. Further, we stress the need for measurements of gas solubility coefficients of ethylene in seawater under temperature and salinity conditions that are relevant to marine nitrogen fixation studies. Estimates of the oceanic nitrogen budget as well as biogeochemical models, largely rely on ARA results to calculate N_2 fixation rates (Capone and Carpenter 1999; Fennel et al. 2001; Hood et al. 2001). The tropical/subtropical cyanobacterium *Trichodesmium* spp. or *Richelia* spp. have been assumed to be the major representatives of oceanic nitrogen fixers. Zehr et al. (2001) recently discovered unicellular diazotrophs, but their distribution as well as their contribution to the oceanic nitrogen budget is unknown. Thus measurements of marine nitrogen fixation may be extended to regions of different temperature and salinity than assessed to date. The need for standardized methods of nitrogen fixation measurements is apparent, and our contribution is intended to be a step toward that goal.

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Global warming may decrease nitrogen fixation by *Trichodesmium*

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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 into the ocean. Incorporating *Trichodesmium* in ocean biogeochemical circulation models relies on field-based correlations between temperature and *Trichodesmium* abundance. Temperature affects *Trichodesmium* growth directly by controlling physiological rates or indirectly through its influence on mixed layer depth, light and nutrient regimes. Here we present an empirical relationship between temperature and diazotrophic growth. This relationship and global warming scenarios from state-of-the-art climate models predict a future decline in *Trichodesmium* abundance and nitrogen input that could significantly affect global nitrogen cycling.

Main text

Present total marine N_2-fixation is estimated at 110 Tg yr^{-1} (*1*). A major fraction of up to 80 Tg yr^{-1} is directly attributed to *Trichodesmium* (*2*), which is estimated to account for up to 47% of the primary production in the tropical North Atlantic Ocean (*3*) and contributes to export production via nitrogen fueling of the phytoplankton community (*4*). *Trichodesmium* is generally limited to oligotrophic waters and its observed temperature distribution range (20°C - 30°C) is used to constrain N_2-fixation in ocean biogeochemical circulation models. The upper temperature limit is set by current sea surface temperature (SST) maxima and not by observed physiological constrains of high temperature on *Trichodesmium* distribution. Parameterizations based solely on field correlations cannot differentiate between direct and indirect effects of temperature on *Trichodesmium* growth and thus are of limited predictive value.

Therefore, we derived growth and N_2-fixation temperature tolerance and optima ranges of *Trichodesmium* based on controlled laboratory experiments. *Trichodesmium* grows and fixes nitrogen at temperatures between 20-34°C. N_2-fixation (suppl.[1]) and maximum specific growth rates (suppl.[2]) of the axenic *Trichodesmium* IMS-101 strain peak at 27°C (0.13 mmol

N mol POC^{-1} h^{-1}, μ_{max} carbon specific = 0.25 day^{-1}) (Fig. 1A). The photosystem II acclimates up to a maximum quantum yield at 27°C and maintains a high efficiency up to the maximum temperature of 34°C (suppl.figure). We infer an optimum temperature range for diazotrophic growth between 24 and 30°C. Growth and N_2-fixation rates are significantly reduced at lower or higher temperatures. Analogous, positive correlations of *Trichodesmium* abundance and water temperature were observed in field studies, and Capone et *al.* (*2*) used the 20°C sea surface temperature (SST) isotherm as poleward boundary to describe the distribution of *Trichodesmium*.

In order to extrapolate our results to the field and to predict future changes in the distribution of *Trichodesmium*, we employed global warming scenarios of 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, Fig 1B, suppl.[3]). This warming results in A: a poleward shift of the 20°C isotherm, predicting an 11% areal increase of *Trichodesmium's* potential geographic distribution; B: maximum predicted SSTs of still less than 34°C, which will not limit the potential distribution of *Trichodesmium* in tropical waters; and C: a decrease in the area characterized by optimum growth and fixation conditions (24-30°C) by about 16% (Fig 1C).

Because of the much higher fixation and growth rates of *Trichodesmium* in the 24-30°C SST range, the effect of C is likely to outweigh that of A. We thus expect a net decrease of N_2-fixation by *Trichodesmium* by the end of this century. Note, that our estimate is based on SST only and does not account for possible changes in nutrient supply which, to date, are more difficult to predict than SST.

Other future predictions of marine nitrogen fixation diverge. In contrast to our findings Boyd and Doney (*5*), 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 regimes. Time series measurements near Hawaii (*4*) support this trend. Although, SSTs in this area of the North Pacific are estimated to increase by almost 3°C (Fig. 4b) they do not exceed the physiological optimum range. Nevertheless, large regions of the tropical and subtropical oceans are predicted to fall outside the optimum range. Particularly temperature shifts above 30°C in N_2-fixation hotspots will result in significant changes of the regional nitrogen budgets. In the North Atlantic, for example, SSTs will exceed 30°C in the Caribbean Sea as well as in equatorial waters off West Africa, all of which are currently hotspots of N_2 fixation in a model based on field observations, MLD and light (*6*). 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. Whether or not other (i.e. latitudinally shifted) hotspots develop largely depends on feasible physical and chemical conditions, which might not be met elsewhere. A community shift towards other (unicellular) diazotrophs is hypothetical, but the effect of warming SST on these is completely unknown.

In conclusion our results suggest reduced fixed nitrogen input by *Trichodesmium* due to global warming processes based on the fundamental limitation of temperature on growth. The areal change in feasible SST sets constrains on the potential distribution of *Trichodesmium*. We expect that, within these limits, a combination of other controlling factors such as MLD, light, nutrient regimes (including iron) will further restrict *Trichodesmium* distribution. 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.

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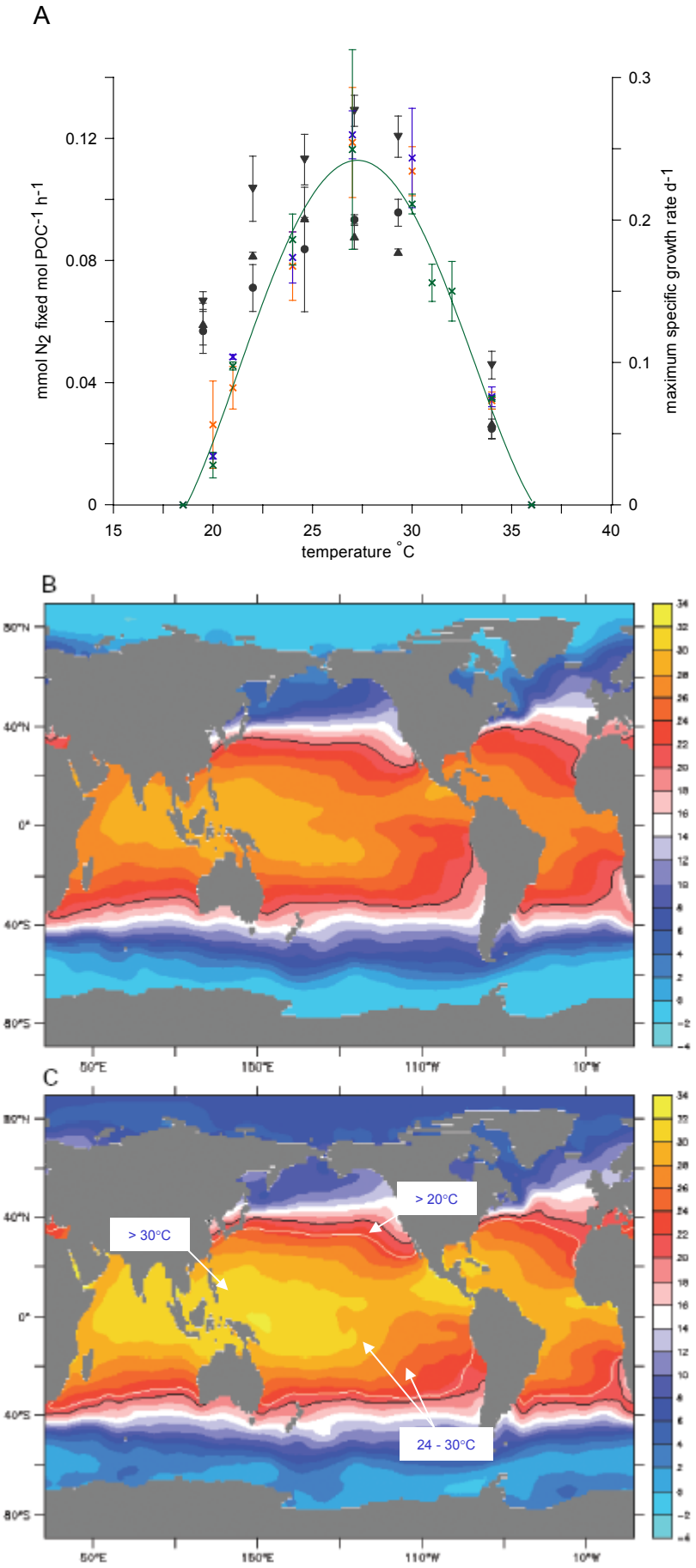

A
mmol N2 fixed mol POC-1 h-1
maximum specific growth rate d-1
temperature °C
0
0.04
0.08
0.12
0.1
0.2
0.3
15
20
25
30
35
40
B
C
> 20°C
> 30°C
24 - 30°C

Figure caption:

1A:

Maximum carbon (x, orange), nitrogen (x, blue) and chlorophyll-*a* (x, green) specific growth rates as a function of temperature. The green line denotes the function of chlorophyll-a specific growth based on the polynomial function:

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

where x denotes temperature in °C. Triangles and circles describe carbon specific nitrogen fixation as a function of temperature. Different symbols denote individual measurement series.

1 B+C:

The observed present-day annual mean sea surface temperature (B) (suppl.[3]) in comparison to the annual mean sea surface temperature incremented by the modeled increase over the period 1990 to 2090 (C) based on HadCM3. The black line (B+C) indicates the maximum latitudinal boundary of the 20°C isotherm and the white line (C) indicates its 1990 distribution superimposed on the 2090 model.

supplemental material:

Footnotes – Methods:

(suppl.[1])

Nitrogen fixation rates were measured using the Acetylene Reduction Assay (ARA) (D. G. Capone, *Handbook of Methods in Aquatic Microbial Ecology*, (1993).), while calculations were modified after Breitbarth et *al.* (E. Breitbarth, M. M. Mills, G. Friedrichs, J. LaRoche, *Limnology and Oceanography: Methods* **2,** 282-288, (2004)). Three replicates at each temperature were incubated simultaneously. A ratio of C_2H_2 reduced : N_2 reduced of 4:1 was used (J.P. Montoya, M. Voss, P. Kaehler, D.G. Capone, *Applied and Environmental Microbiology* **62**(3), 986-993, (1996)) and nitrogen fixation data were normalized to POC content of the incubated culture material.

(suppl.[2])

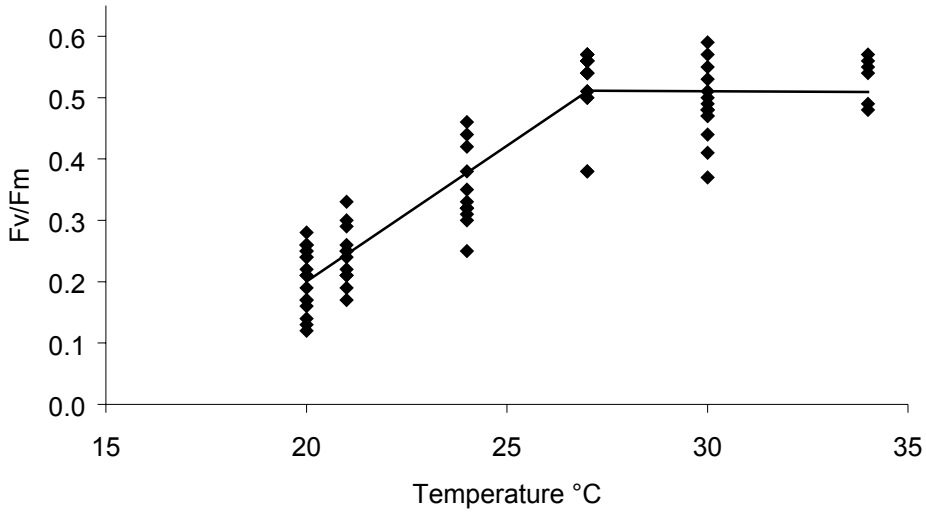
Maximum specific growth rates were determined by identifying the exponential growth phase in the batch cultures and applying a linear fit to the respective log-transformed POC, PON and chl-a values. The slope of the linear fit represents the growth rate.

(suppl.[3])

We applied sea surface temperature (SST) increase predictions based on two coupled atmosphere-ocean general circulation models (HadCM3 and GFDL) to current annual SST (S. Levitus, T. Boyer, in *World Ocean Atlas,* NOAA Atlas NESDIS 4, U.S. Department of Commerce, Washington, D.C., vol. 4. (1994)). The HadCM3 model is based on the assumption that future emissions of greenhouse gases will follow the IS92a 'business as usual' scenario

(http://www.met-office.gov.uk/research/hadleycentre/models/modeldata.html). This prognosis is generally verified by similar calculations using the GFDL R30 climate model which is also based on the IS92a climate scenario until 1990 and assumes a 1% CO_2 level increase per year thereafter. (http://www.gfdl.noaa.gov/~kd/ClimateDynamics/NOMADS/index.html).

supplemental figure



Photosynthetic quantum use efficiency of exponentially growing batch cultures acclimated to the respective temperatures measured as variable fluorescence versus maximum fluorescence (Fv/Fm) of the Photosystem II. The Fv/Fm increases up to 27°C and remains at high levels up to the maximum feasible growth temperature of 34°C.

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 the marine nitrogen cycle 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 diazotrophic growth from controlled laboratory experiments with implications for modeling approaches. We further supply a 3-step model to assess nitrogen fixation by *Trichodesmium* in batch cultures. Axenic *Trichodesmium* IMS-101 was grown at light intensities between 15 and 1350 µmol quanta m^{-2} s^{-1}. Growth rates increased up to 180 µmol quanta m^{-2} s^{-1} and did not vary significantly up to light intensities of 1100 µmol quanta m^{-2} s^{-1} (μ_{POC} ~ 0.26 d^{-1}), after which cultures were photoinhibited. Nitrogen fixation rates varied significantly as a function of growth phase. When normalized to Chl-*a*, N_2 fixation rates further are significantly affected by light intensity during mid-exponential growth (0.74 – 4.45 mol N fixed mol Chl-*a*$^{-1}$ h^{-1}), which was not the case if nitrogen fixation rates were normalized to POC (0.42 – 0.59, averaging 0.5 mmol N mol POC^{-1} h^{-1}). Thus, POC can be used to estimate the nitrogen input by *Trichodesmium* into the ocean. Nitrogen fixation rates level at a maximum of 350 nmol N fixed l^{-1} h^{-1}.

Introduction:

Trichodesmium is an unusual cyanobacterium in that it simultaneously fixes nitrogen and carbon in daylight conditions (Bergman et al. 1997; Gallon et al. 1996). This pattern has only been reported for two other cyanobacteria, *Lyngbya majuscula* and *Symploca* sp. (Fredriksson et al. 1998; Jones 1990). *Trichodesmium* is very abundant and has been 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 – 200 Tg yr^{-1} with *Trichodesmium* contributing 80 - 110 Tg N yr^{-1} (Capone and Carpenter 1999; Gruber and Sarmiento 1997; Karl et al. 2002).

Nitrogen fixation is an integral part in ocean biogeochemical circulation models (OBCM) and *Trichodesmium* is used as a model organism representing diazotrophic growth (Fennel et al. 2001; Hood et al. 2001; Hood et al. 2004; Hood et al. 2002). The parameterization of nitrogen fixation in OBCM's has been improved during recent years as *Trichodesmium sp.* received an increasing amount of scientific attention. While forcing variables affecting growth, nitrogen and carbon fixation of this cyanobacterium such as macro- and micro-

nutrient availability were determined to a large extent, irradiance as a factor has not been fully described (Berman-Frank et al. 2001; Kustka et al. 2003a; Kustka et al. 2003b; Mulholland and Capone 1999; Mulholland et al. 1999; Rueter et al. 1991; Sanudo-Wilhelmy et al. 2001). Fennel et al. (2001) and Hood et al. (2001) supplement the lack of precise information on irradiance versus nitrogen fixation patterns by adapting parameters from photosynthesis versus irradiance (P vs. I) functions. Carpenter et al. (1993) subjected field collected *Trichodesmium* from one sampling depth to light intensities up to 2500 µmol quanta m^{-2} s^{-1} photosynthetic active radiation (PAR), being the full surface irradiance, and described a P vs. I resembling nitrogen fixation pattern as a function of irradiance with indication of photoinhibition in *T. erythraeum*. Hood et al. (2002) based model equations for nitrogen fixation of *Trichodesmium sp.* on field observations and used a similar approach to assess nitrogen fixation versus irradiance parameters. In general it is problematic to distinguish between different forcing variables such as light, temperature or nutrient availability on the observed patterns in a natural environment. Thus parameter values from field data are subject to uncertainties and laboratory experiments are needed to verify observations and to isolate the effect of individual forcing variables.

The effect of short-term exposure to light intensities up to 180 µmol quanta m^{-2} s^{-1} on acetylene reduction of *Trichodesmium* has been studied under laboratory conditions (Fu and Bell 2003; Ohki and Fujita 1988). Nitrogen fixation rates increase with irradiance, whereas the applied maximum irradiances were relatively low for sub-tropical environments and a saturating irradiance was not clearly determined. In all experiments cultures were not grown at the respective light intensities. Plant cells adjust in various ways to different light regimes. Physiological responses to short-term shifts in the light regime, i.e. pigment acclimation, are discussed elsewhere (Chapter V and manuscript in prep). Here we focus on long term acclimations of *Trichodesmium* physiology grown under different light conditions.

For laboratory experiments using batch cultures, growth phase has to be considered as a physiological factor. Various authors (Berman-Frank et al. 2001; Chen et al. 1998; Mulholland and Capone 2001) have described nitrogen fixation and carbon fixation rates as a function of growth phase as well as of daytime. In general, nitrogen fixation rates are elevated during the exponential growth phase and show a maximum at midday.

Published laboratory experiments to date use light conditions with between 10 and 14 hours of full light intensity and dark period for the remaining time (L:D cycle). We conducted two factorial 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 effect of light intensity and the type of the diurnal light curve on diazotrophic growth of *Trichodesmium*. The experiments further aim to describe a light dependent stoichiometry of particulate organic carbon and nitrogen (POC, PON), Chlorophyll-*a*, and total protein content of cultures grown at different light intensities. As Chl-*a* content per cell in phytoplankton can be adjusted to acclimate to light intensity (Geider et al. 1997), normalization of nitrogen fixation rates to Chl-*a* versus POC as a biomass measure is compared. Finally, results compiled in this study are synthesized into a model to describe diazotrophic growth and nitrogen input into seawater 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 dissolved nitrogen added (Chen et al. 1996). Cultures were transferred during the exponential growth phase into 2l polycarbonate bottles, three replicates for each light intensity. An incubator (Rumed, Germany) was set up to imitate the natural solar cycle of 12h at 0N 90W Julian day 1. The cycle was modified by setting light intensities >97% as 100% resulting in a 2 hour peak intensity. Light intensities 15, 50, 180, 300, 600, 900, and 1100 µmol quanta m^{-2} s^{-1} photosynthetic active radiation (PAR) were created using neutral density screening and verified by measuring light penetration into an incubation bottle containing water using a submersible 4pi PAR sensor (LiCOR Inc, USA). Additionally cultures were grown at 50 and 900 µmol quanta m^{-2} s^{-1} PAR in the same incubator with the light cycle set up at 1h dusk and dawn each and a 10h period of full light intensity. Over the course of the growth period, samples for all parameters were generally taken at 11:00. 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.

Chlorophyll-*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 24h (modified after Welschmeyer (1994)). This simple extraction method was previously

compared to the application of mechanical disruption of the cells and proved to be as efficient for Chlorophyll-*a* analysis of *Trichodesmium* (E. Breitbarth, unpublished data).

Elemental analysis of particulate organic nitrogen PON and particulate organic carbon POC:
Culture material was filtered on pre-combusted GF/F filters, frozen at -20°C for intermediate storage and finally dried for 48h at 45°C. Filters were analyzed for PON and POC content on an elemental analyzer (Euro-EA, Hekatech, Germany) equipped with a chromium-oxid/cobalt-oxid oxidation reactor, a copper reduction reactor and a CHN column at an oven temperature of 45°C. Carrier gas flow (He) was set at 96ml min^{-1}. Measurements were corrected for blank values using measurements of similarly treated filters without culture material (Sharp 1975).

Nitrogen fixation measurements
Nitrogen fixation was measured using the Acetylene Reduction Assay (ARA) as described in Capone (1993) and Capone and Montoya (2001) while calculations were modified after Breitbarth et al. (2004). Gas samples were analyzed on a Shimadzu GC-19B equipped with a flame ionization detector and a 30m wide bore capillary column (0.53 mm, AluminaPlot®, Resteck, USA). Using 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 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 Chlorophyll-*a* content of the cultures. The ARA is prone to error and often yields a highly variable data outcome. The coefficient of variation (CV*, corrected for bias in small sample size of 3 replicates (Sokal and Rohlf 1995)) was used to estimate the variation between triplicate measurements. Data resulting in a CV* > 25 were excluded from the analysis after identifying the source of error, whereas all data were used in the statistical calculations. Common errors include false biomass determinations (disagreement in POC/PON and Chl-a values, aggregate formation by *Trichodesmium* and thus patchy distribution in the vial) and leaking crimp seals or blocked syringe needles (high biomass with exceptionally low ethylene production rates).

PAM fluorometry:
A PhytoPAM equipped with Optical Unit ED-101US/MP (Walz, Germany) was used to measure the ratio of variable to maximal fluorescence (Fv/Fm) of *Trichodesmium* in

response to different light intensities (Kolbowski and Schreiber 1995). Cellular fluorescence signals were recorded every second day over the complete growth period of the cultures. Samples were dark-adapted for 10 minutes 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/CO3 buffer (La Roche et al. 1993). Total protein was analyzed according to the bicinchoninic acid method using BCA protein assay reagents (Pierce, USA) and a 96-well plate reader. The absorbance signal was calibrated versus 0 – 1500 µg protein l^{-1} standards (Smith et al. 1985).

Statistic analysis:

All data were analyzed by applying 1-way ANOVA models and Fisher post-hoc tests using StatView (Version 5.0.1, SAS, USA). 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 at a natural cycle of different light intensities possessed typical microbial growth patterns with a clear exponential and stationary phase. The lag phase was reduced as cells were transferred during the exponential growth phase from the start culture.

Maximum PON and POC biomass was reached at 300 µmol quanta m^{-2} s^{-1} and maximum Chl-*a* biomass was reached at 180 µmol quanta m^{-2} s^{-1} at day 20 (Fig. 1, Tab. 1+2, Chl-*a* data not shown). Cultures grown under L:D cycle conditions reached higher biomasses at 50 and lower biomasses at 900 µmol quanta m^{-2} s^{-1} compared to cultures grown under natural light conditions during the same period of growth (Tab. 1). The L:D light cycle experiment was stopped at day 15 after PhytoPAM measurements indicated reduced cellular fluorescence and carbon as well as nitrogen specific growth declined, whereas the natural light cycle experiment was continued until day 20. Note that the highest POC biomasses are reached at 300 µmol quanta m^{-2} s^{-1} and the highest chlorophyll-a biomass is reached at 180 and 300 µmol quanta m^{-2} s^{-1}. Final biomasses of POC and chl-a diverge and the high

light treatments show a reduced chl-a biomass, which is due to photoacclimation and reflected in the chl-a : POC ratio (Tab. 3). Reduced biomasses at high light intensities are not significantly reflected in the maximum growth rates specific to each light intensity (Fig. 2), but are accounted for as an irradiance specific maximum biomass in the model.

The experiment imitating 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, $P < 0.05$) to higher light intensities and to each other. Carbon specific growth at light intensities ≥ 180 µmol quanta m^{-2} s^{-1} averaged 0.26 d^{-1} and did not differ significantly. Chlorophyll-a specific growth rates were similar to carbon specific growth (Fig. 2). Nitrogen specific growth rates were lower than carbon and chlorophyll specific growth rates at all treatments, whereas general trends were similar. Nitrogen specific growth rates ranged from 0.02 d^{-1} and 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 – 1100 µmol quanta m^{-2} s^{-1}.

Carbon and nitrogen specific growth rates at L:D light cycle conditions versus the imitated natural solar cycle were statistically identical at 50 µmol quanta m^{-2} s^{-1} but were significantly reduced at 900 µmol quanta m^{-2} s^{-1} at which they also differed from each other (0.19 d^{-1} carbon specific and 0.16 d^{-1} nitrogen specific growth rate, Fig. 2, nitrogen specific growth rates not shown).

The energy provided by the L:D light cycle treatments equals 1.48 times the energy supplied by the natural light treatment over the course of a day. Thus the 50 µmol quanta m^{-2} s^{-1} is equivalent to 74 and the 900 µmol quanta m^{-2} s^{-1} to 1330 µmol quanta m^{-2} s^{-1} of the 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) indicate photoinhibition of diazotrophic growth. In order to better compare the effect of these two light treatments, growth rate data are also plotted versus total PAR received per day in Figure 2.

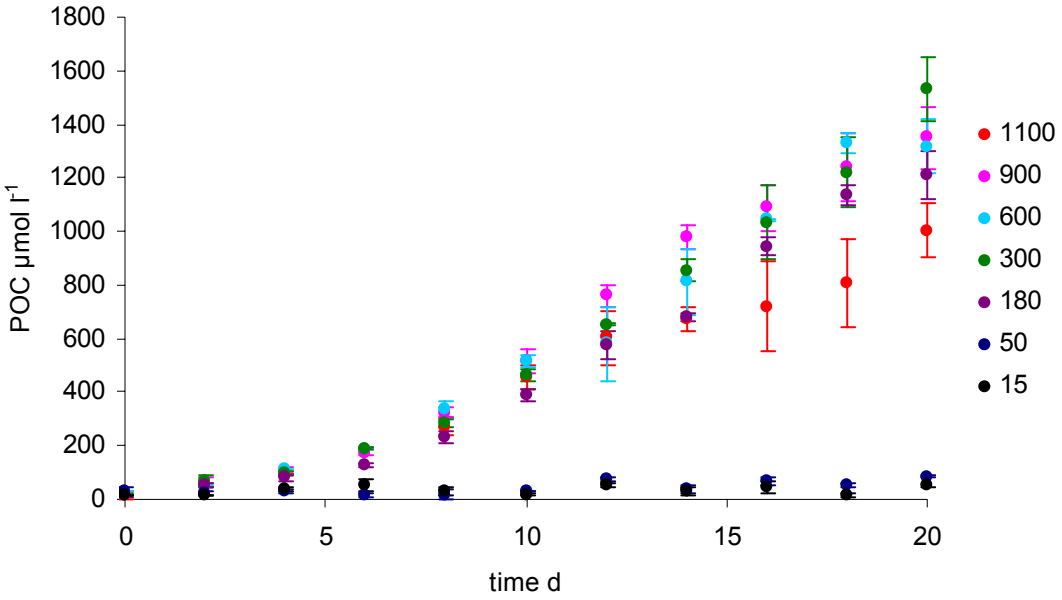


Figure 1: Carbon specific biomass as a function of light intensity and growth phase of the natural light cycle experiment. The light intensity is indicated on the right hand side in µmol quanta m^{-2} s^{-1} PAR.

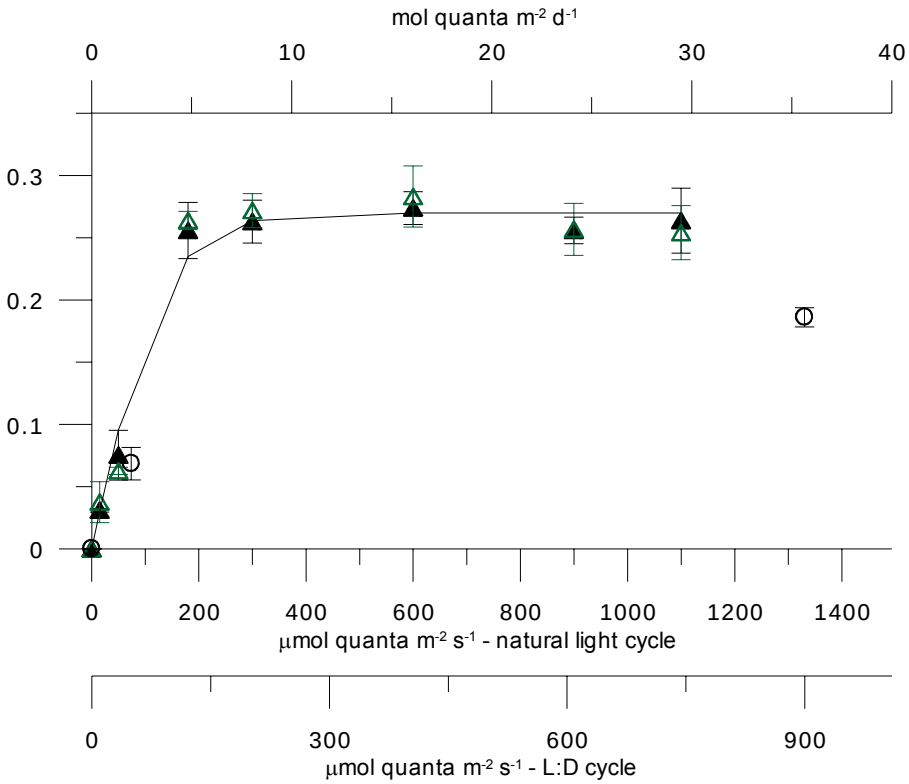


Figure 2: Carbon (black triangle) and chlorophyll-*a* (green triangle) specific growth rates of axenic *Trichodesmium* IMS-101as a function of light intensity. Carbon specific growth rates from the L:D cycle experiment are plotted as open circles. Light intensity from the L:D cycle experiment can be converted to the equivalent amount of light energy of the natural light cycle treatment (conversion factor = 1.48, both lower x-axis, see text). Growth rates are plotted versus total amount of photosynthetic active radiation per day (upper x-axis) for comparison. The solid line indicates carbon specific growth rates derived from the data based on equation 1. Error bars denote standard deviations.

Stoichiometry of PON, POC and Chl-*a*:

Like growth rates POC:PON ratios (mol:mol) were similar (overall ~ 5.4 – 5.6) at light intensities ≥ 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 – 1100 µmol quanta m^{-2} s^{-1} increased with growth phase from ~ 4.5 to 5.5 (days 2-10), values slightly decreased thereafter until day 16, recovered afterwards and averaged 5.7 – 5.9 (Tab. 4).
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 and a maximum of 0.058 was measured. For details see table 3 and figure 4.

Total protein content

Total protein contents of the cultures were analyzed on day 16. 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 as well as between all light intensities between 180 and 900 µmol quanta m^{-2} s^{-1}. The total protein content was significantly reduced at 15-50 and at 1100 µmol quanta m^{-2} s^{-1} compared to the rest of the treatments (post-hoc f-tests, $P < 0.05$). Night-time measurements taken 12h after the day-time measurements verified these trends differences to daytime measurements are compliant with growth rates (data not shown).

The POC:total protein ratio (weight:weight) resembles a similar pattern versus light intensity as the POC:PON ratio. Values range from 0.5 to 2.4 and are reduced at light treatment < 180 µmol quanta m^{-2} s^{-1} versus higher light intensities (Fig. 4). The low POC:PON ratios in the low light treatments indicate that here PON is mostly present as pure protein.

Cellular fluorescence and photosynthetic efficiency:

Over all Fv/Fm 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 had lower Fv/Fm

ratios than low light treatments it is only possible to clearly distinguish two groups of responses from day 14 on. Light intensities between 600 and 1100 µmol quanta m^{-2} s^{-1} possessed Fv/Fm ratios of 0.15 – 0.3 while cultures grown at lower intensities continued to yield higher Fv/Fm 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. 3).

Nitrogen fixation measurements versus light intensity and growth phase

We tested if 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 (rep. measures ANOVA, $P < 0.01$) (Fig. 5). Fixation rates were generally high in the beginning of the growth phase, 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} showed increased nitrogen fixation rates towards the end of the experiment (day 18+20). When normalized to POC, nitrogen fixation rates did not show a significant interaction with light intensity (ANOVA, $P = 0.25$, Fig. 5b). In contrast, Chlorophyll-*a* normalized nitrogen fixation rates are a function of light intensities (ANOVA, $P < 0.01$, Fig. 4a). This is particularly evident during mid exponential growth (days 8-12). 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}, while carbon specific nitrogen fixation rates averaged 0.5 mmol N_2 fixed (mol POC)$^{-1}$ h^{-1} (0.42 - 0.59). Frequently POC and Chl-*a* specific fixation rates at 180 µmol quanta m^{-2} s^{-1} where relatively low compared to rates at 50 and 300 µmol quanta m^{-2} s^{-1} (Fig. 5). For detailed results see tables 5+6.

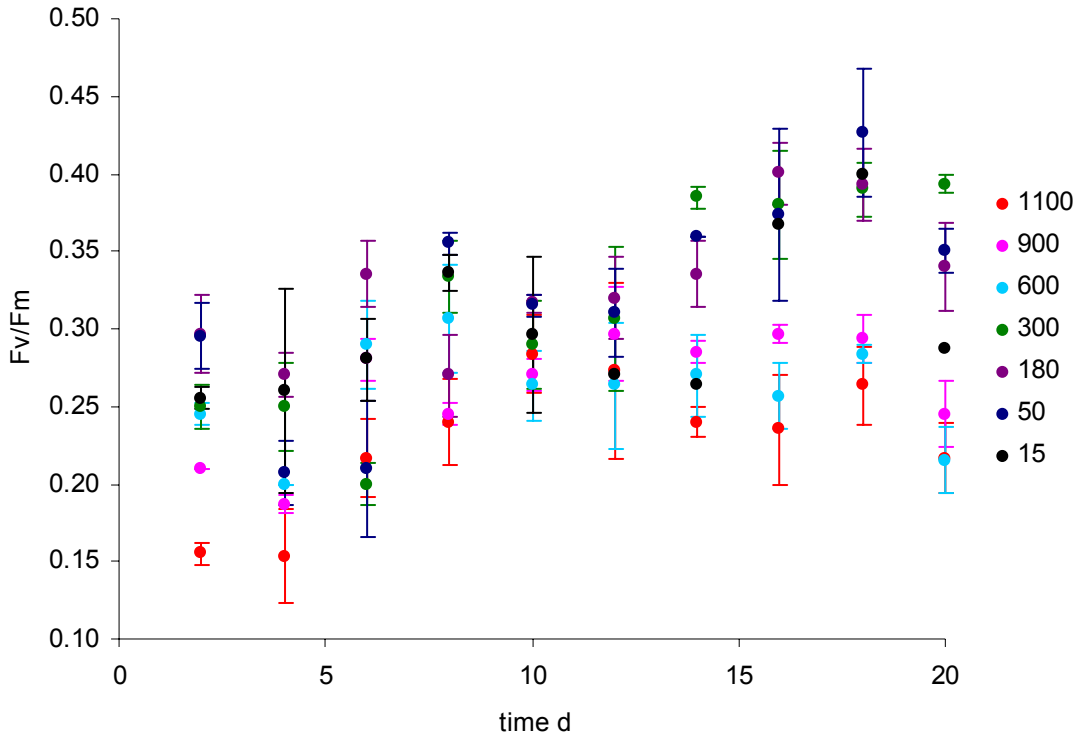


Figure 3: Photosynthetic quantum use efficiency (Fv/Fm) of *Trichodesmium* IMS-101 as a function of growth phase and light intensity. The light treatments are symbolized in the legend as µmol quanta m^{-2} s^{-1}. Error bars denote standard deviations.

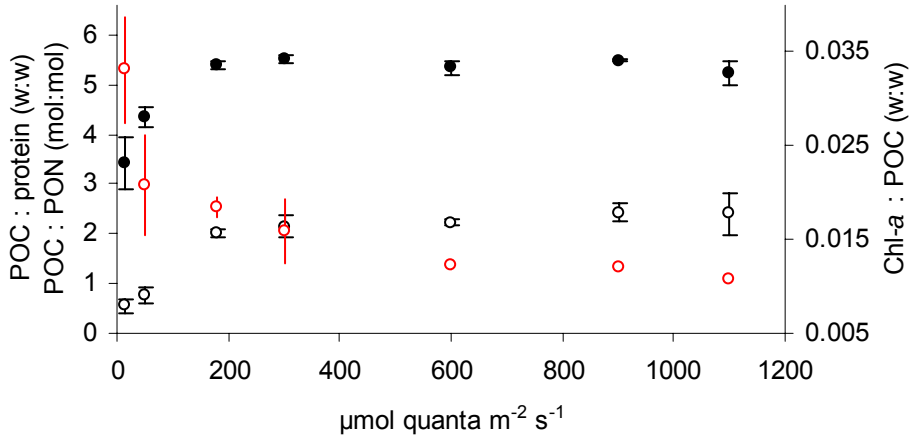
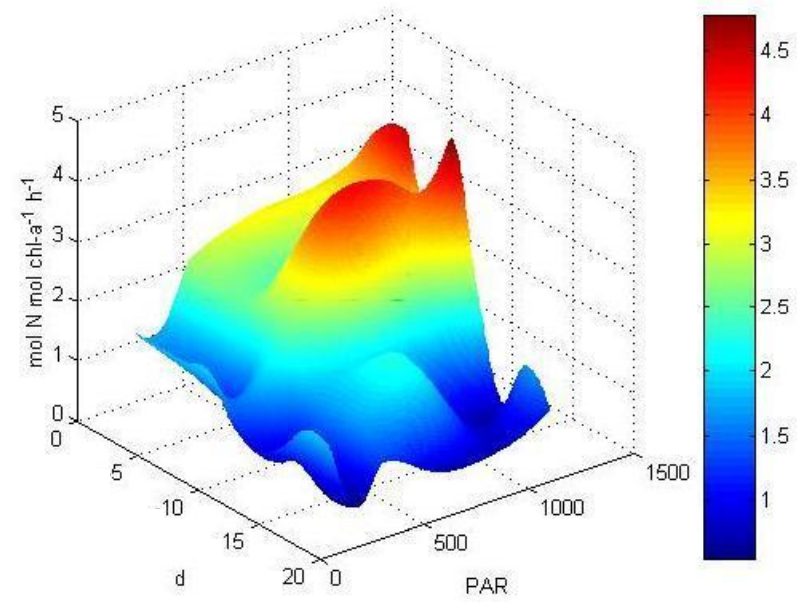


Figure 4: Overview of POC:total protein (open circles), POC:PON (solid circles) and Chl-a:POC (red circles) stoichiometry of *Trichodesmium* IMS-101 grown at different light intensities on day 16 of the natural light cycle experiment. Error bars denote standard deviations.

a



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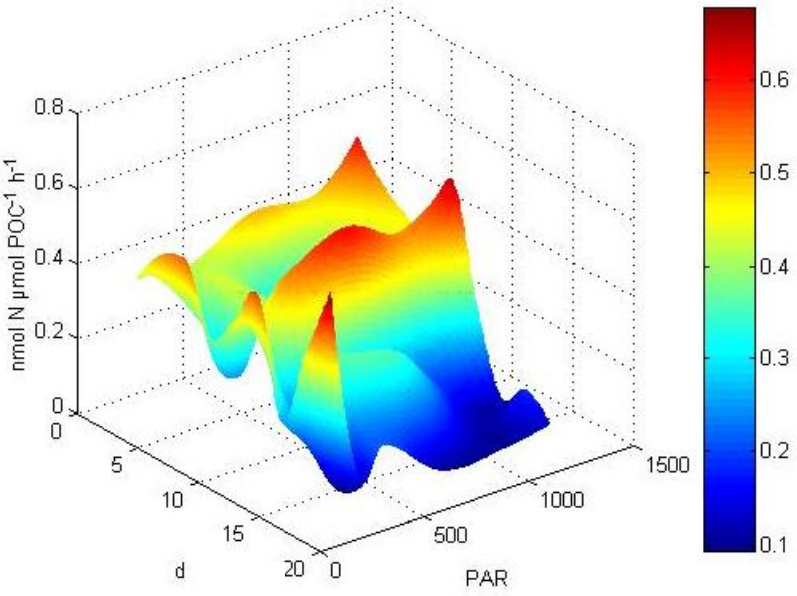


Figure 5: Chlorophyll-*a* specific (a) and carbon specific (b) nitrogen fixation rates as a function of growth phase (d = number of days, x-axis) and light intensity (µmol quanta m^{-2} s^{-1} PAR, z-axis). Shown are measurements from days 4-20. The datasets including standard deviations of the measurements are given in tables 5+6. Results from the experiment using a L:D cycle are not included but are presented in table 6 as well. The legends denote the nitrogen fixation rates in mol N fixed (mol chl-*a*)$^{-1}$ h^{-1} (a) and nmol N fixed (µmol POC)$^{-1}$ (b) plotted on the y-axis.

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

The data presented allow for a numeric approach to describe nitrogen input into seawater by *Trichodesmium* in batch culture incubations.

Model parameter, symbols, units and values used:

Description	Symbol	Value	Units
physical parameters			
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	1.8	µmol l^{-1} (µmol quanta m^{-2} s^{-1})$^{-1}$
POC biomass specific N_{fix} inhibition parameter	F_t		dimensionless

Specific growth rates under natural light conditions can be described based on equation 1, which is modified from Jassby and Platt (1975):

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

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})$^{-1}$) versus irradiance (I in µmol quanta m^{-2} s^{-1}) curve. Equation 1 is only applicable for light intensities 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 and a light inhibition term is not included.

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 the 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 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, the nitrogen fixation rate (nmol N l^{-1} h^{-1}) is directly correlated with the 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 and tuned to 1.8 µmol POC l^{-1} per unit light (µmol quanta m^{-2} s^{-1}).

At mid-exponential growth, observed maximum fixation rates average 350 nmol nitrogen fixed per liter at 300 µmol quanta m^{-2} s^{-1} (Fig. 5). Growth dynamics eventually down regulate nitrogen fixation rates due to NH_4^+ exuded from cells (Holl and Montoya 2003; Mulholland and Capone 1999; Mulholland et al. 1999; Mulholland et al. 2001). Reduced fixation rates at high irradiances in the later growth phase were observed (Fig. 5b, table 1) and thus equation 3 would only be valid 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 similar conditions as here. We applied the NH_4^+ data (not shown) to derive a simplified relationship between POC concentration and NH_4^+ in solution:

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

and derive 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:

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

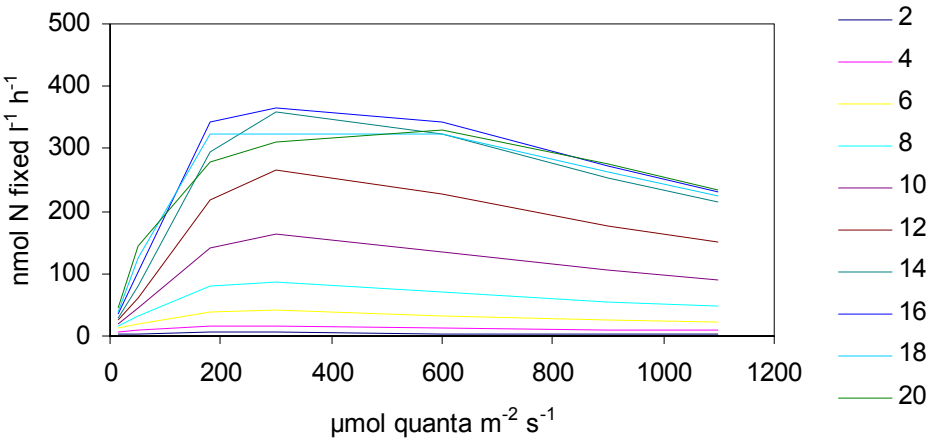
Where the parameters were tuned to the following values: a = 2, b = 8, c = 3.1.

Nitrogen fixation (N_{fix}, nmol l^{-1} h^{-1}) is described based on equation 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.

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

t_{max} denotes the maximum incubation time in days. The modeled nitrogen fixation rates are in agreement with the measured nitrogen fixation rates (Fig. 6).

a



b

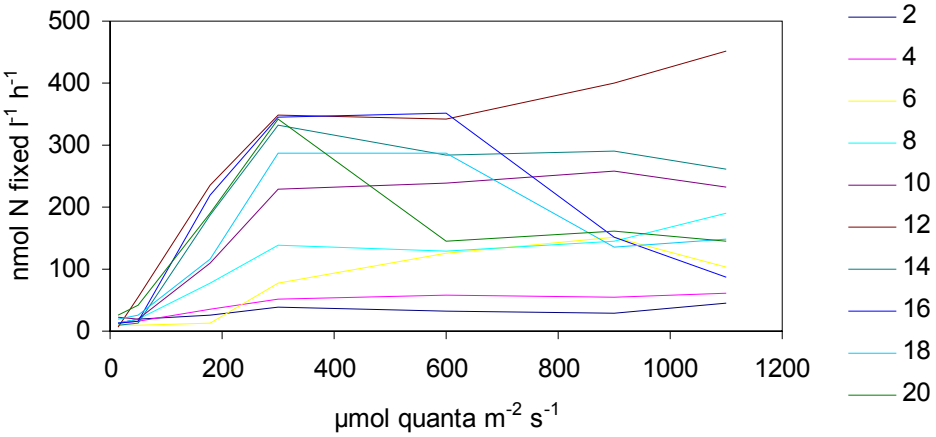


Figure 6: Results of modeled (a) and measured (b) nitrogen fixation rates as a function of light intensity and growth phase. The legend on the right denotes days of growth. In comparison to the measured rates the model fit yields lower nitrogen fixation rates during the early growth phase. The model applies photoinhibition regardless of the age of the culture, whereas this pattern may be affected by growth phase as indicated in (a). Fixation rates were stronger reduced at high light intensities during the late growth phase than during early exponential growth (a). Modeled and measured results agree reasonably well on maximum nitrogen fixation rates (~ 350 nmol N fixed l^{-1} h^{-1}) during mid and late exponential growth. See table 1 for exact values and standard deviations of nitrogen fixation and carbon biomass measurements.

Discussion:

Photosynthetic organisms occupy specific niches of light regimes. Light energy 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 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 cope with the paradox of photosynthetically producing oxygen while utilizing the nitrogenase enzyme, which is irreversibly blocked by oxygen. Thus *Trichodesmium* has to create an intracellular oxygen environment feasible for nitrogen fixation. Next to high respiration rates, the energetically costly photoreduction of oxygen in photosystem I (Mehler reaction) has been suggested to be the main oxygen protective mechanism in this non-heterocystous diazotroph (Carpenter and Roenneberg 1995; Kana 1991; Kana 1993). Overall, high energy requirements of phototrophs 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 irradiation in sub-tropical/tropical oligotrophic surface waters and data shown here are compliant to that (Capone et al. 1997; Carpenter and Roenneberg 1995).

We were able to elucidate the effect of different light intensities and light cycle characteristics on diazotrophic growth of *Trichodesmium* IMS-101. Growth rates increase up to light intensities of 180 µmol quanta m^{-2} s^{-1} and remain constant thereafter. Photoinhibition for diazotrophic growth was observed at high light intensities (> 1100 µmol quanta m^{-2} s^{-1}). POC normalized nitrogen fixation rates generally level at 300 µmol quanta m^{-2} s^{-1}. The effect that Chl-*a* normalized nitrogen fixation rates are a function of irradiance, while POC normalized fixation rates are not, is driven by the acclimation of Chl-*a* content per cell to specific light intensities (Fig. 5).

Light inhibition in *Trichodesmium* was demonstrated by the reduced growth rates of cultures subjected to 900 µmol quanta m^{-2} s^{-1} (L:D cycle conditions) versus cultures grown using a natural solar cycle. Carpenter and Roenneberg (1995) though conclude that *Trichodesmium* is adapted to high light regimes and can adjust its photosynthetic characteristics accordingly to its position in the water column and to seasonal changes in the light regime. While the authors detected no light inhibition of photosynthesis at 2500 µmol quanta m^{-2} s^{-1}, light inhibition of nitrogenase activity in *T. erythraeum* at the same irradiance was noticed in another study (Carpenter et al. 1993). The used culture organism, *Trichodesmium* IMS 101, is an isolate of *T. erythraeum* (Janson et al. 1999) and was grown at the light intensities at

which experiments were carried out at. Therefore cells were fully acclimated. The treatment of 900 µmol quanta m^{-2} s^{-1} for 10 h d^{-1} plus one hour of linear dusk and dawn phase equals a total quantum flux of 36 mol quanta 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 ~50% more light energy than cultures grown using a natural illumination cycle, which has to be considered in physiological experiments. We conclude that photoinhibition can affect *Trichodesmium* blooms at the sea surface. If in natural waters the surface irradiance is 2500 µmol quanta m^{-2} s^{-1}, the maximum light intensity chosen for this experiment resembles natural irradiance in 16 meters depth (based on a light attenuation coefficient of 0.05 m^{-1}). The span of irradiances used in our experiments approximately reflects light regimes in a natural water column from the upper meters to the 0.5 - 1% light level at ~ 100 meters depth. Typically *Trichodesmium* shows a biomass maximum 20 to 40 m depth and thus resides in a light environment of 300 to 900 µmol quanta m^{-2} s^{-1} (Capone et al. 1997). This preference matches the observed growth rate, biomass and nitrogen fixation maxima described here.

Higher energy supply, particularly between 180 and 300 µmol quanta m^{-2} s^{-1} light, is not utilized from the perspective of biomass accumulation. It is possible though, that increased gross photosynthesis or gross nitrogen fixation and thus metabolic activity within the cell result in higher turnover of carbon and nitrogen and thus exudation as DOC or DON (Glibert and Bronk 1994; Glibert and O'Neil 1999). This raises a concern regarding the conversion factor of ethylene reduced:dinitrogen fixed though. This factor can vary between 2 and 25 under different environmental conditions (Gallon et al. 2002), such as temperature (M.Mills and E. Breitbarth, unpublished data). It is likely that light intensity also influences the ratio of gross to net nitrogen fixation and thus the ethylene produced to nitrogen fixed conversion factor. 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 light irradiances which then is not available for biomass growth. The cells have to maintain a steady-state with regard to energy budget, physiological maintenance processes such as O_2 scavenging or photosystem repair and growth, which is met at 180 µmol quanta m^{-2} s^{-1}. Metabolic processes operate at optimum turnover rates, which is achieved between 180 and 300 µmol quanta m^{-2} s^{-1}. Higher rates would result in luxury nitrogen fixation, which is energetically inefficient. Measurements of cellular fluorescence (Fv/Fm) indicate reduced photosynthetic quantum use efficiency at light levels > 300 µmol quanta m^{-2} s^{-1} (table 1). Hence a larger proportion of light energy greater 300 µmol quanta m^{-2} s^{-1} received by the cell is not utilized, matching the growth and nitrogen fixation patterns described. Antagonistically, elemental stoichiometry suggest light limitation

below 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. 4, Table 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. Increased carbohydrate storage at high light intensities has also been suggested by (Letelier and Karl 1998) in order to regulate the buoyancy of *Trichodesmium* in the water column.

We derive an optimum light regime for diazotrophic growth between 180 and 1100 µmol quanta m^{-2} s^{-1}. The cut-off of the increase in growth rate at an 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) states a value of 280 µmol quanta m^{-2} s^{-1}. Growth rates are constant above this light intensity (μ_{POC} ~ 0.26 d^{-1}) and nitrogen fixation rates are 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 irradiances above 1100 µmol quanta $m^{-2}s^{-1}$, which in most cases is only reached at the sea surface. The application of Chlorophyll-*a* as a biomass measure and normalization parameter for physiologic rate measurements is unsuitable, as the cellular Chlorophyll-*a* content is adjusted to the light regime. The particulate carbon concentration is better suited for this application as it is the more conservative biomass parameter. Based on the presented findings we can state the following recommendations for the application in ocean biogeochemical circulation models. A: Diazotrophic growth of *Trichodesmium* can be described based on equation 1. Under nutrient and light replete conditions the 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}. B: Nitrogen fixation is a function of [POC] during exponential growth and can be simplified to 0.5 x POC (µmol l^{-1}) = N fixed (nmol l^{-1}). C: The maximum nitrogen fixation rate per unit volume is 350 nmol N fixed l^{-1} and expressed as a function of light intensity based on equation 6. D: 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 (Holl and Montoya 2003; Mulholland et al. 2001), but specific terms for a gradual NH_4^+ and NO_3^- down regulation of nitrogen fixation need to be developed. Comparing the modeling approach to the measured nitrogen fixation data (Fig. 6) reveals an apparent effect of growth phase on the light inhibition parameter, which requires further investigation. While down regulation by light inhibition was only measured at a later growth stage (higher NH_4^+ concentration), the model applies photoinhibition regardless of the age of the culture. Thus it may underestimate nitrogen

fixation at high light intensities in relatively young cultures. Nevertheless, the model represents nitrogen input, particularly maximum rates, reasonably well during mid and late exponential growth, which may be well applicable to physiological conditions in natural *Trichodesmium* blooms.

Surface abundance of *Trichodesmium* can be estimated using SeaWIFS imagery (Subramaniam et al. 2002). Hood et al. (2002) developed a model describing depth integrated nitrogen fixation using surface *Trichodesmium* Chl-*a* based on SeaWIFS data. Results presented here illustrate that Chl-*a* is not a suitable biomass indicator to normalize physiological rate measurements over different of light conditions as the Chl-*a*:POC ratio is acclimated to the respective light regime. Second, previous measurements of nitrogen fixation as a function of light intensity were conducted on non-light-acclimated cells that were only exposed to the specific light intensities for short periods of time. The here presented model is based on POC normalized nitrogen fixation measurements of light-acclimated *Trichodesmium* cultures and therefore may help to improve depth integrated nitrogen fixation models.

In conclusion, *Trichodesmium* is well adapted to high light regimes. 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, 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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Table 1: POC concentrations in μmol l^{-1} of *Trichodesmium* IMS-101 grown at different light intensities. Photosynthetic active radiation (PAR) is indicated in μmol quanta $m^{-2}s^{-1}$ as the mid-day peak light intensity during a natural solar cycle. The light intensities 75 and 1350 μmol quanta m^{-2} s^{-1} were converted from the L:D cycle experiment as described in the text. Bold numbers denote mean values and non bold numbers are standard deviations.

PAR / day	15	50	75 L:D 50	180	300	600	900	1100	1350 L:D 900
0	**14.7** 3.4	**30.4** 14.5	**47.9** 2.3	**13.0** 3.0	**15.7** 3.3	**20.2** 8.1	**23.5** 9.7	**16.1** 15.1	**47.1** 3.4
2	**14.4** 3.0	**21.2** 9.6	**46.3** 1.9	**52.2** 7.9	**66.4** 23.0	**51.4** 3.6	**63.2** 17.4	**51.0** 9.5	**77.5** 3.0
4	**40.0** 8.4	**27.5** 8.4	**55.9** 1.6	**78.7** 9.6	**98.3** 6.7	**110.0** 3.7	**106.8** 13.6	**95.4** 4.4	**130.3** 15.1
6	**51.9** 21.4	**13.7** 5.6	**89.0** 6.5	**127.4** 8.3	**188.2** 3.9	**189.5** 6.4	**175.2** 8.6	**189.4** 2.9	**200.6** 14.7
8	**30.5** 14.4	**18.0** 15.7	**80.3** 3.3	**229.7** 24.1	**286.0** 14.8	**333.5** 34.6	**323.4** 17.6	**271.4** 33.3	**255.9** 34.5
10	**18.0** 5.1	**29.2** 2.4		**391.5** 22.2	**464.4** 23.2	**515.1** 25.2	**517.4** 45.9	**457.3** 46.0	
11			**91.6** 4.1						**414.6** 16.5
12	**50.8** 7.9	**72.6** 7.6		**573.4** 52.9	**652.8** 5.3	**581.9** 137.5	**760.1** 40.2	**604.2** 100.7	
13			**103.8** 11.1						**552.4** 15.8
14	**31.7** 14.4	**37.6** 13.1		**681.4** 13.6	**854.7** 42.5	**810.5** 123.5	**977.7** 44.4	**671.4** 46.0	
15			**132.0** 9.7						**665.6** 63.4
16	**44.8** 20.5	**68.6** 15.2		**943.3** 34.0	**1032.9** 136.1	**1044.9** 3.2	**1088.3** 87.8	**717.9** 167.3	
18	**16.9** 7.9	**52.7** 6.7		**1135.3** 39.3	**1219.7** 129.9	**1328.6** 36.2	**1241.6** 127.9	**808.6** 163.2	
20	**55.7** 10.3	**85.5** 5.2		**1209.2** 88.6	**1534.7** 119.5	**1315.1** 100.9	**1349.3** 118.0	**1003.1** 100.2	

Table 2: PON concentrations in μmol l^{-1} of *Trichodesmium* IMS-101 grown at different light intensities. Photosynthetic active radiation (PAR) is indicated in μmol quanta $m^{-2}s^{-1}$ as the mid-day peak light intensity during a natural solar cycle. The light intensities 75 and 1350 μmol quanta $m^{-2}s^{-1}$ were converted from the L:D cycle experiment as described in the text. Bold numbers denote mean values and non bold numbers are standard deviations.

PAR / day	15	50	75 L:D 50	180	300	600	900	1100	1350 L:D 900
0	**7.1** 1.3	**10.7** 2.0	**12.3** 0.4	**8.1** 0.4	**7.0** 0.4	**8.3** 1.9	**6.6** 0.3	**7.2** 2.0	**10.8** 0.2
2	**6.8** 1.6	**6.4** 2.0	**14.6** 1.8	**10.4** 0.6	**13.9** 1.3	**12.5** 0.7	**13.7** 2.6	**13.5** 1.3	**16.6** 0.4
4	**8.3** 0.1	**13.7** 5.0	**17.0** 0.7	**16.2** 0.8	**18.4** 0.6	**22.9** 1.1	**22.8** 3.2	**20.0** 0.5	**27.1** 2.6
6	**6.5** 3.8	**4.1** 1.8	**19.8** 0.8	**22.2** 0.6	**33.0** 2.4	**36.1** 0.4	**35.9** 3.0	**37.1** 4.3	**37.1** 2.0
8	**10.8** 1.9	**7.9** 1.4	**14.5** 0.5	**44.1** 4.8	**51.0** 3.7	**58.3** 3.3	**59.0** 1.0	**48.9** 5.7	**40.7** 5.4
10	**6.6** 0.9	**8.9** 0.4		**65.2** 4.1	**76.7** 4.9	**88.8** 12.9	**87.0** 7.9	**76.5** 4.6	
11			**18.7** 0.6						**71.9** 4.3
12	**8.5** 1.6	**11.6** 1.2		**100.2** 9.8	**116.3** 5.0	**109.5** 20.4	**135.3** 5.1	**109.9** 12.5	
13			**21.3** 1.7						**97.8** 1.4
14	**8.2** 2.0	**12.6** 1.4		**124.0** 5.4	**149.6** 5.3	**150.2** 22.0	**171.8** 8.6	**125.0** 8.0	
15			**26.2** 2.1						**116.3** 7.0
16	**12.8** 4.2	**15.9** 3.8		**174.4** 3.9	**187.3** 22.2	**198.0** 4.8	**198.4** 15.7	**136.9** 25.6	
18	**9.4** 1.3	**12.9** 0.6		**205.7** 14.7	**213.3** 25.0	**232.7** 8.3	**218.5** 21.8	**146.4** 26.3	
20	**7.1** 1.9	**12.6** 1.9		**201.2** 14.1	**243.2** 22.8	**226.4** 10.8	**215.3** 14.8	**161.7** 12.5	

Table 3: Chlorophyll-*a* : POC stoichiometry (weight : weight) of *Trichodesmium* IMS-101 grown at different light intensities. Photosynthetic active radiation (PAR) is indicated in μmol quanta m^{-2} s^{-1} as the mid-day peak light intensity during a natural solar cycle. The L:D cycle experiment yielded no reliable chlorophyll-*a* measurements and thus the respective data were excluded. Bold numbers denote mean values and non bold numbers are standard deviations. The mean values for all data are calculated for days 4 – 18 of each light treatment and are given at the bottom of the table.

PAR / day	15	50	180	300	600	900	1100
0	**0.020** 0.001	**0.013** 0.005	**0.030** 0.006	**0.023** 0.004	**0.020** 0.009	**0.015** 0.004	**0.021** 0.006
2	**0.034** 0.005	**0.019** 0.002	**0.015** 0.002	**0.013** 0.003	**0.013** 0.002	**0.011** 0.003	**0.013** 0.003
4	**0.014** 0.002	**0.026** 0.008	**0.020** 0.004	**0.015** 0.002	**0.013** 0.001	**0.013** 0.001	**0.014** 0.001
6		**0.058** 0.020	**0.021** 0.001	**0.015** 0.000	**0.015** 0.001	**0.015** 0.000	**0.013** 0.001
8	**0.030** 0.008	**0.033** 0.010	**0.018** 0.001	**0.015** 0.001	**0.013** 0.001	**0.013** 0.001	**0.013** 0.001
10	**0.033** 0.002	**0.037** 0.004	**0.017** 0.001	**0.015** 0.002	**0.010** 0.003	**0.011** 0.004	**0.013** 0.001
11							
12	**0.015** 0.003	**0.016** 0.001	**0.018** 0.000	**0.018** 0.001	**0.012** 0.001	**0.013** 0.001	**0.011** 0.001
13							
14	**0.019** 0.004		**0.016** 0.006	**0.016** 0.001	**0.013** 0.000	**0.013** 0.000	**0.012** 0.000
15							
16	**0.033** 0.006	**0.017** 0.008	**0.018** 0.001	**0.016** 0.003	**0.012** 0.000	**0.012** 0.000	**0.011** 0.001
18	**0.038** 0.007	**0.021** 0.002	**0.014** 0.001	**0.011** 0.002	**0.009** 0.001	**0.011** 0.001	**0.009** 0.001
20	**0.018** 0.003	**0.022** 0.003	**0.019** 0.000	**0.014** 0.001	**0.012** 0.001	**0.010** 0.000	**0.010** 0.000
mean sd	**0.025** 0.011	**0.029** 0.016	**0.018** 0.013	**0.015** 0.002	**0.012** 0.002	**0.012** 0.002	**0.012** 0.002

Table 4: POC : PON stoichiometry (mol : mol) of *Trichodesmium* IMS-101 grown at different light intensities. Photosynthetic active radiation (PAR) is indicated in μmol quanta $m^{-2}s^{-1}$ as the mid-day peak light intensity during a natural solar cycle. The light intensities 75 and 1350 μmol quanta m^{-2} s^{-1} were converted from the L:D cycle experiment as described in the text. Bold numbers denote mean values and non bold numbers are standard deviations. The mean values for all data are calculated for days 4-18 (L:D cycle: 4-15) of each light treatment and are given at the bottom of the table.

PAR / day	15	50	75 L:D 50	180	300	600	900	1100	1350 L:D 900
0	**2.07** 0.11	**2.74** 0.77	**3.90** 0.14	**1.59** 0.34	**2.26** 0.55	**2.39** 0.45	**2.74** 0.29		**4.36** 0.27
2	**2.16** 0.49	**3.20** 0.92	**3.21** 0.48	**4.53** 0.34	**3.99** 0.11	**4.13** 0.51	**4.58** 0.41	**3.79** 0.72	**4.66** 0.18
4	**4.26** 0.38	**2.93** 0.03	**3.28** 0.19	**4.85** 0.35	**5.33** 0.20	**4.80** 0.22	**4.69** 0.10	**4.77** 0.24	**4.81** 0.10
6		**2.87** 0.89	**4.49** 0.23	**5.75** 0.44	**5.72** 0.40	**5.25** 0.12	**4.90** 0.17	**5.15** 0.64	**5.40** 0.12
8	**3.33** 0.24	**3.07** 0.64	**5.55** 0.05	**5.21** 0.06	**5.62** 0.24	**5.71** 0.36	**5.48** 0.22	**5.55** 0.13	**6.29** 0.16
10	**2.69** 0.52	**3.27** 0.12		**6.01** 0.04	**6.06** 0.39	**6.19** 0.26	**5.95** 0.10	**5.97** 0.38	
11			**4.92** 0.28						**5.77** 0.15
12	**5.98** 0.46	**6.23** 0.12		**5.73** 0.03	**5.62** 0.28	**5.28** 0.26	**5.62** 0.18	**5.48** 0.32	
13			**4.88** 0.21						**5.65** 0.12
14	**3.70** 0.98	**2.95** 0.81		**5.49** 0.13	**5.71** 0.10	**5.40** 0.15	**5.69** 0.06	**5.37** 0.10	
15			**5.05** 0.07						**5.71** 0.21
16	**3.42** 0.51	**4.34** 0.21		**5.41** 0.07	**5.51** 0.08	**5.34** 0.13	**5.48** 0.02	**5.22** 0.24	
18	**1.76** 0.57	**4.10** 0.57		**5.53** 0.21	**5.72** 0.10	**5.71** 0.11	**5.68** 0.02	**5.51** 0.12	
20	**7.92** 0.63	**6.84** 0.76		**6.01** 0.04	**6.32** 0.11	**5.80** 0.18	**6.26** 0.13	**6.20** 0.15	
mean sd	**3.57** 1.39	**3.83** 1.18	**4.69** 0.47	**5.50** 0.46	**5.66** 0.53	**5.43** 0.60	**5.44** 0.49	**5.30** 0.71	**5.61** 0.47

Table 5: Chlorophyll-*a* specific nitrogen fixation measurements of *Trichodesmium* IMS-101 (mol N fixed mol Chl-a^{-1} h^{-1}) grown at different light intensities. Photosynthetic active radiation (PAR) is indicated in μmol quanta m^{-2} s^{-1} as the mid-day peak light intensity during a natural solar cycle. The L:D cycle experiment yielded no reliable chlorophyll-*a* measurements and thus the respective data were excluded. Bold numbers denote mean values and non bold numbers are standard deviations.

PAR / day	15	50	180	300	600	900	1100
4	**1.85** 0.11	**1.72** 0.70	**1.69** 0.01	**2.60** 0.45	**3.00** 0.31	**3.09** 0.31	**3.31** 1.05
6	**1.35** 0.31	**1.02** 0.05	**0.33** 0.04	**2.04** 0.20	**3.38** 0.29	**4.43** 1.44	**3.02** 1.92
8	**1.37** 0.27	**1.38** 0.30	**1.39** 0.13	**2.35** 0.45	**2.31** 0.15	**2.85** 0.08	**4.17** 0.62
10	**1.62** 0.15	**1.78** 0.33	**1.44** 0.27	**2.42** 0.39	**3.27** 0.12	**2.94** 0.56	**2.58** 0.19
11							
12	**0.74** 0.08	**1.17** 0.16	**1.73** 0.09	**2.34** 0.27	**3.70** 0.21	**2.79** 0.20	**4.45** 0.14
13							
14	**1.01** 1.12	**0.83** 0.09	**0.87** 0.48	**1.75** 0.08	**1.97** 0.22	**1.75** 0.29	**2.31** 0.61
15							
16	**1.31** 0.50	**0.94** 0.09	**0.80** 0.13	**1.96** 0.00	**2.09** 0.46	**1.01** 0.05	**0.81** 0.12
18	**2.90** 0.43	**1.72** 0.27	**0.53** 0.05	**1.59** 0.30	**1.84** 0.62	**0.75** 0.33	**1.57** 0.23
20	**2.35** 0.10	**1.64** 0.10	**0.61** 0.06	**1.15** 0.14	**0.52** 0.02	**0.64** 0.02	**0.95** 0.16

Table 6: Carbon specific nitrogen fixation measurements of *Trichodesmium* IMS-101 (mmol N fixed mol C^{-1} h^{-1}) grown at different light intensities. Photosynthetic active radiation (PAR) is indicated in μmol quanta m^{-2} s^{-1} as the mid-day peak light intensity during a natural solar cycle. The light intensities 75 and 1350 μmol quanta $m^{-2}s^{-1}$ were converted from the L:D cycle experiment as described in the text. Bold numbers denote mean values and non bold numbers are standard deviations.

PAR / day	15	50	75 L:D 50	180	300	600	900	1100	1350 L:D 900
4	**0.75** 0.05	**0.39** 0.27	**0.10** 0.02	**0.40** 0.00	**0.39** 0.02	**0.47** 0.04	**0.46** 0.02	**0.57** 0.19	**0.31** 0.04
6	**0.19** 0.12	**0.14** 0.03	**0.11** 0.03	**0.10** 0.06	**0.53** 0.14	**0.58** 0.02	**0.76** 0.11	**0.49** 0.32	**0.20** 0.06
8	**0.36** 0.01	**0.59** 0.12	**0.08** 0.00	**0.30** 0.02	**0.34** 0.01	**0.25** 0.02	**0.40** 0.01	**0.53** 0.00	**0.48** 0.34
10	**0.19** 0.06	**0.25** 0.07	**0.13** 0.01	**0.28** 0.05	**0.38** 0.08	**0.39** 0.08	**0.45** 0.13	**0.46** 0.03	**0.39** 0.04
11			**0.33** 0.03						**0.42** 0.14
12	**0.55** 0.24	**0.42** 0.02		**0.42** 0.02	**0.49** 0.03	**0.56** 0.04	**0.48** 0.10	**0.59** 0.01	
13			**0.27** 0.06						**0.29** 0.10
14	**0.46** 0.00	**0.38** 0.02		**0.30** 0.04	**0.36** 0.01	**0.27** 0.04	**0.24** 0.04	**0.31** 0.06	
15			**0.23** 0.04						**0.15** 0.03
16	**0.17** 0.05	**0.27** 0.04		**0.21** 0.05	**0.26** 0.05	**0.37** 0.02	**0.17** 0.02	**0.12** 0.02	
18	**0.34** 0.02	**0.45** 0.05		**0.10** 0.02	**0.22** 0.01	**0.21** 0.00	**0.10** 0.05	**0.17** 0.02	
20	**0.46** 0.08	**0.61** 0.04		**0.16** 0.02	**0.21** 0.04	**0.09** 0.03	**0.10** 0.02	**0.12** 0.03	

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SYNTHESIS AND PERSPECTIVE FUTURE WORK

SYNTHESIS AND PERSPECTIVE FUTURE WORK

The review of published literature (chapter I) and laboratory results produced within this project (chapters III + IV) demonstrate that abundance and thus nitrogen and carbon fixation by *Trichodesmium* is co-limited by iron and phosphorus next to the controlling factors light and temperature. The effect of iron on *Trichodesmium* succession needs to be considered if iron fertilization experiments are proposed in sub-tropical oligotrophic waters. The results summarized here are further to be considered for policy making with respect to climate mitigation strategies.

Chapter II provides a set of equations and tables to be used in the determination of Bunsen coefficients for ethylene, a parameter that is required for assessing nitrogen fixation based on the acetylene reduction assay (ARA). This gas solubility coefficient is temperature and salinity dependent, but despite the increasing interest in marine nitrogen fixation, information thereon was lacking in the published literature. The potential impact of these newly calculated Bunsen coefficients was tested for nitrogen fixation scenarios at different temperatures and salinities. The results stress the importance of using accurate gas solubility coefficients in nitrogen fixation assays. The ethylene gas solubility coefficients and additional information provided improve a uniform application of the ARA to measure nitrogen fixation in a wide range of habitats and therefore are of potential interest for both limnologists and oceanographers working on nitrogen fixation.

Methodological considerations to assess nitrogen fixation pathways using the Acetylene Reduction Assay and ^{15}N stable isotope analysis

While the ARA measures the nitrogenase enzyme activity and thus a total substrate turnover regardless of product losses from the cell, the ^{15}N stable isotope analysis detects the incorporation of nitrogen into cellular material. Thus, a parallel application of ARA and ^{15}N stable isotope analysis allows to investigate the ratio of gross and net nitrogen fixation and gives insight in the ratio of particulate fixed nitrogen and cellular release of newly fixed nitrogen compounds. During the ^{15}N stable isotope analysis the dinitrogen in solution is enriched with $^{15}N_2$ gas. The $^{15}N_2$ incorporated into cells via nitrogen fixation can be detected using mass spectrometry. A comparison of both methods on the same organism under identical environmental conditions is generally used to yield a conversion factor of moles ethylene produced to moles $^{15}N_2$ incorporated into cells. This conversion factor is generally

used to calculate the amount of nitrogen fixed based on the amount of ethylene detected (see also chapter 1).

The application of this method comparison to different marine systems can result in a wide range of conversion factors (4 – 20 : 1) (Gallon et al. 2002). Even if subjected to blooms consisting of mainly one cyanobacterium (i.e. *Trichodesmium* sp.) this factor ranges from 1.9 – 7.2 with considerable variation (Montoya et al. 1996). The wide range of the conversion factor creates uncertainty about the precision of the nitrogen fixation measurements utilizing either of the two techniques (ARA or ^{15}N stable isotope analysis) and possesses the challenge to investigate the origin of deviation in natural systems from the theoretical value of 4 : 1. As the ARA measures the amount of acetylene (C_2H_2) reduced/ethylene (C_2H_4) produced, acetylene is added at saturating levels to the sample of interest, but still "competes" with dinitrogen gas for nitrogenase, which will reduce this substratum. The ARA is a direct measurement of nitrogenase activity but relies on the assumption that acetylene, if added at saturating levels, will completely block the nitrogenase enzyme from reducing dinitrogen gas. This assumption includes an error and therefore may cause false estimations of nitrogen fixation rates. The amount of C_2H_2 required to block nitrogenase needs to be individually determined for each assay set up.

Applying ^{15}N stable isotope analysis the amount of particulate ^{15}N detected theoretically represents an exact proportion of the amount of nitrogen fixed into the cells. In addition to the enrichment factor (relative amount $^{15}N_2$ added to the sample) which is a source of error, several sources of loss have to be considered. Nitrogen fixing cells may release some of the newly fixed nitrogen into their environment. This can occur either "actively" as release of NH_4^+ by nitrogen fixing cells of *Trichodesmium* into the surrounding seawater, which also serves as nutrition for adjacent non-N_2 fixing cells (Mullholland and Capone 2001), or via leaching of various forms of DON and amino acids (Flynn and Gallon 1990, Glibert and Bronk 1994, Glibert and O'Neil 1999). The proportion of nitrogen fixed and immediately released will therefore not be detected in the particulate matter via ^{15}N stable isotope analysis. Preliminary results (Breitbarth and Mills, unpublished) show an effect of temperature in the ratios of ethylene produced : ^{15}N reduced in cultures of *Trichodesmium*. *Trichodesmium* was simultaneously incubated for ARA and ^{15}N stable isotope analysis at a temperature range from 17 – 32°C and data indicate that at the optimum temperature for nitrogenase activity in *Trichodesmium* (27°C, Chapter III) the ratio of ethylene produced : $^{15}N_2$ incorporated was lowest (~7-8, Fig 1). Ratios increased with reduced temperatures to 37 and at elevated temperatures to a maximum of 49. While this study is preliminary and warrants the need for further investigation, the overall higher ratios than the theoretical value

of 4 indicate that a large proportion of the newly fixed nitrogen is released again as NH_4 and DON by the cells. Temperature has an affect thereon and thus influences the conversion factor.

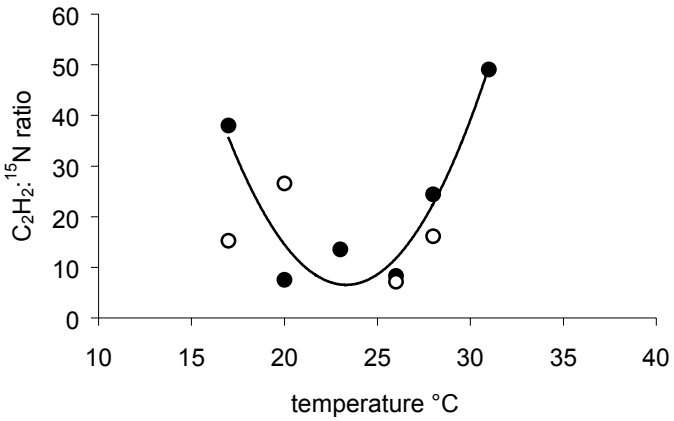


Figure 1: $^{15}N_2$ stable isotope incorporation versus C_2H_2 reduction. A direct comparison of nitrogen fixation measured using the Acetylene Reduction Assay and the ^{15}N stable isotope method shows strong deviations from theoretical acetylene reduced to nitrogen incorporated ratio of 3. Two independent experiments yield lowest ratios in relatively close agreement of 7.1 and 8.2 respectively at the incubation temperature of 27°C. The ^{15}N : acetylene ratio increases at temperatures lower and higher than 27°C up to a maximum of 49 measured at 31°C. The solid line indicates a proposed trend of the ratio with temperature.

This pattern might be indicative for the physiological state of nitrogen fixing cells (Gallon et al., 2002). ^{15}N stable isotope analysis in contrast to ARA more likely underestimates nitrogen fixation as it only detects the amount of $^{15}N_2$ incorporated into the cells. Thus, the observed ranges in the conversion ratios between the two methods skew estimations of gross nitrogen fixation based on ^{15}N stable isotope analysis. For further method improvement the following tasks require investigation in the future:

1. the proportion and the chemical form of dinitrogen fixed that is released from diazotrophs
2. environmental factors that influence this pattern (i.e. temperature, light, nutrients)
3. differences of patterns in conversion factors of gross and net nitrogen fixation among species / different marine systems
4. development of an algorithm specific to environmental factors (2) that allows to apply the correct conversion factor for the ARA, allowing the ARA to be applied as an efficient and economical method to estimate gross and net nitrogen fixation

As further improvement of the nitrogen fixation measurement techniques it is also particularly important to assess the role of newly discovered unicellular nitrogen fixers. These diazotrophs might be very abundant, but the understanding of their impact on contemporary nitrogen cycles is still vague (Zehr et al. 2001; Montoya et al. 2004). The potential future role of unicellular nitrogen fixers with respect to CO_2 increase and global warming has not been addressed at this stage and proposes interesting and important research approaches.

Chapter III predicts a future decline in the oceanic fixed nitrogen input by *Trichodesmium* that could significantly affect global nitrogen cycling. Temperature dependent growth rates clearly elucidate ecologically feasible maxima and minima. Trends in nitrogen fixation are based on the application of the ARA with a fixed conversion factor (3:1) of ethylene produced to nitrogen fixed. The aforementioned preliminary results (Fig 1) indicate that detailed analysis of gross and net nitrogen fixation as a function of temperature are required to fully understand nitrogen fixation of *Trichodesmium* as a function of water temperature.

Diazotrophic growth and climate change

Sea surface temperature rise is one of the most predictable consequences of global change. In the ocean, temperature can affect phytoplankton growth directly by controlling physiological rates or indirectly through its influence on mixed layer depth, light and nutrient regimes. The direct effects of the increase in temperature are seldom considered in future scenarios concerning marine primary productivity and nitrogen fixation. Chapter III demonstrates that the fundamental effect of temperature sets constraints on the potential future distribution, growth and gross nitrogen fixation rate of *Trichodesmium.* Only within the limits of temperature tolerance, other factors such as nutrient regime or light, further constrain *Trichodesmium*.

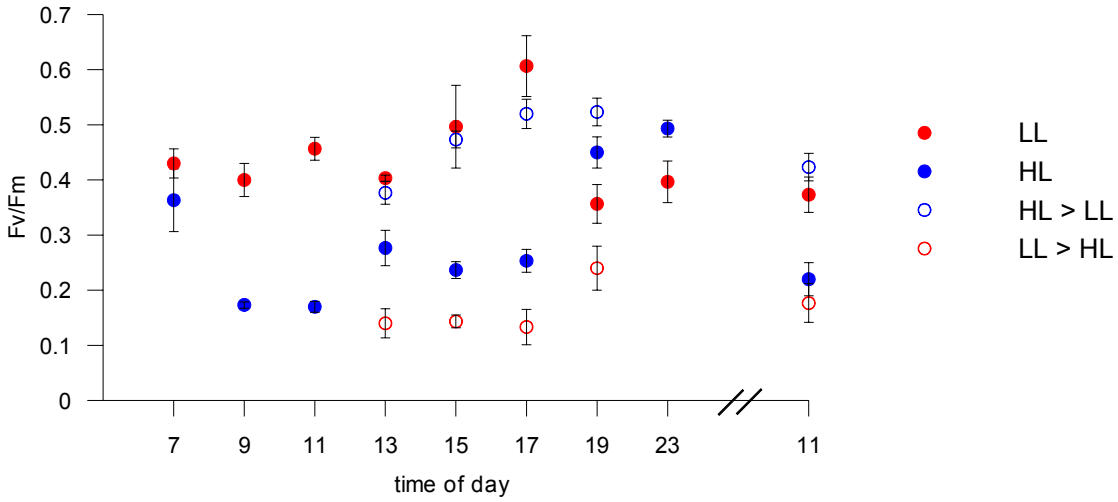
Thus, this approach is a contribution to an improved prediction on the effect of climate change on global biogeochemical cycles. A decline of marine N_2 fixation in the future will result in less new nitrogen available for phytoplankton primary production and thus consequently in reduced carbon export from the mixed layer. A decrease in *Trichodesmium* carbon and nitrogen fixation therefore directly and indirectly reduces the draw-down of atmospheric CO_2 into the oceans. Predicting future concentrations of atmospheric CO_2 and future CO_2 sequestration by the ocean is of great interest and chapter III demonstrates the importance of incorporating elementary biological principles in the biological components of

predictive CO_2 ocean uptake models, upon which indirect temperature effects can be studied.

Light and diazotrophic growth

Shallowing of mixed layer depth and increased stratification have been prognosed as an effect of atmosphere warming in the future (Houghton 2001). *Trichodesmium* inhabits the surface layer of preferably stratified waters in tropical environments and thus must be well adapted to high light regimes (Carpenter et al. 1993; Capone et al. 1997). 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. Chapter IV presents data on light dependent diazotrophic growth from controlled laboratory experiments with implications for modeling approaches. The comparison of a natural light cycle and a simple light:dark (L:D) set-up yielded that nitrogen fixation and growth of *Trichodesmium* depends on the total amount of energy (light quanta) supplied over the course of the day. As L:D cycles supply more energy than natural light cycles with similar peak light intensities, nitrogen fixation in L:D experiments can be over-estimated if this is not taken into account. Further, chapter IV includes a model describing nitrogen fixation by *Trichodesmium* as a function of light intensity and growth phase. Based on this model, *Trichodesmium* POC biomass can be used to estimate the nitrogen input by *Trichodesmium* into the ocean. Maximum nitrogen fixation rates by *Trichodesmium* are 350 nmol N fixed l^{-1} h^{-1}. Patterns of nitrogen fixation and photosynthetic quantum use efficiency as a function of short-term shifts in light regimes indicate rapid but not complete light acclimation (Fig. 2 a+b) (Chen et al. 1999). The model does not reflect physiological differences between vertical migrating *Trichodesmium* and cells that remain at a fixed depth and may be limited in that respect.

a



b

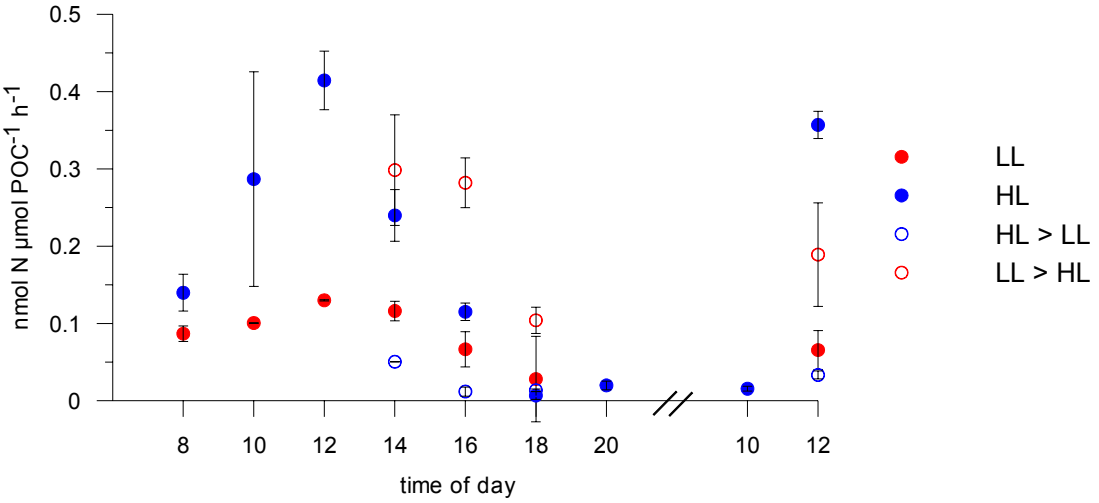


Figure 2: Changes of photosynthetic quantum yield efficiency (Fv/Fm) (a) and carbon specific nitrogen fixation (b) as a function of day time (over the course of two days) as well as the response of Fv/Fm to shifts from low (LL = 50 µmol quanta m^{-2} s^{-1}) to high light (HL = 900 µmol quanta m^{-2} s^{-1}) and vice versa. Cultures were shifted at 12:00. Fv/Fm values indicate rapid adjustment of the photosynthetic apparatus to changing light conditions. Nitrogen fixation rates of cultures shifted from the high light to low light regime approach fixation rates of cultures maintained at low light after 24h. In contrast cultures shifted from the low light to high light conditions still fix considerably less nitrogen after 24h compared to cultures grown under high light.

A conceptual model of nitrogen and carbon fixation in Trichodesmium:

Trichodesmium differs from other diazotrophs by fixing nitrogen and carbon during the light period, whereas nitrogenase is not confined in oxygen protecting heterocystous cells. This is a paradoxical paradigm since oxygen evolved during photosynthesis irreversibly blocks nitrogenase. A spatial and temporal decoupling of these two processes was previously suggested (Berman-Frank et al. 2001). Data presented here show that a temporal separation does not apply as carbon fixation is only reduced in favor of nitrogen fixation at sub-saturating light intensities (Fig 3). This may be due to energy limitation of the photoreduction of O_2 in photosystem I as a sufficient oxygen scavenging mechanism (Mehler Reaction). The data shown provide new evidence that *Trichodesmium* is well adapted to the ambient high light conditions of sub-tropical waters and that diazotrophic growth occurs with temporally only slightly shifted successive nitrogen and carbon fixation, which can not be interpreted as the main oxygen mitigation strategy.

Explanations for the enigma of daytime nitrogen and carbon fixation in this non-heterocystous cyanobacterium has challenged researchers working on marine nitrogen fixation ever since Dugdale et al. (1961) reported this paradigm. Berman-Frank et al. (2001) show a temporal segregation with nitrogen fixation peaking at midday and carbon fixation peaking in the morning and afternoon. They further provide indication for spatial segregation of nitrogenase containing cells within the trichome using antibody staining (see also Lin et al. (1998)), and consider these patterns as the major mechanisms allowing for daytime diazotrophy in *Trichodesmium*. Nevertheless, the authors also show the presence of the D1 protein (PS II) and nitrogenase within the same cells and conclude a crucial importance of the Mehler Reaction as an oxygen mitigation mechanism, as indicated by high peroxide concentrations at the walls of the nitrogen fixing cells.

Here nitrogen and carbon fixation as a function of light intensity were assessed using the same axenic *Trichodesmium* IMS-101 strain as Berman-Frank et al. (2001), but in contrast a natural light cycle (versus L:D light cycle) in order to better mimic natural light regimes was applied. The findings of carbon and nitrogen fixation during the light period (Fig 3 a+b) follow the expression of photosynthesis (*psbA*) and nitrogen fixation (*nifH*) genes in part (Chen et al. 1999). The *psbA* and *nifH* gene expression is phase shifted by approximately 6h and the photon use efficiency (PUE) of nitrogenase is elevated in the morning in analogy with *nifH* gene expression. Expression of *psbA* and *nifH* are completely down regulated at night (Chen et al. 1999) and carbon and nitrogen fixation are absent. Whilst the photoreduction of oxygen in PS I is required during daytime, it remains enigmatic that the

expression of *psaA* peaks at night and how PS I operates in darkness (Chen et al. 1999). Even though *psaA* expression is lowest at the peak of nitrogen fixation, it is not absent. One possible explanation is, that the oxygen production by nitrogen fixing cells indeed is less than that of carbon fixing cells and PSI activity implied by *psaA* expression together with respiration may be sufficient to allow for effective oxygen scavenging within these cells. Nevertheless, as the presence of D1 in nitrogen fixing cells clearly implies oxygenic photosynthesis, it seems plausible that the photosynthetic reactions here solely serve as an energy provider (i.e. for carbohydrate synthesis and nitrogen fixation) and not for net carbon fixation as detected by ^{14}C incorporation. Carpenter and Price (1976) already showed by autoradiography that ^{14}C incorporation is confined to the peripheral regions of trichomes. Even though nitrogen and carbon fixation in *Trichodesmium* occur simultaneously, the phase shifted expression of photosynthesis and nitrogen fixation genes may be part of a regulatory mechanism that involve de-novo synthesis of nitrogenase each morning and eventual degradation by oxygen from photosynthesis in the afternoon (Colon-Lopez et al. 1997; Colon-Lopez and Sherman 1998; Chen et al. 1999). Further supporting this, PSII independent carbon fixation has been demonstrated in diazotrophs and might also occur in *Trichodesmium* (Misra and Tuli 1993). A potential temporal separation of photosynthetic oxygen production and carbon fixation in *Trichodesmium* requires investigation.

Trichodesmium grown at light intensities 15 and 50 μmol quanta m^{-2} s^{-1} were characterized by low (< 5) POC:PON stoichiometry, while cells grown at higher light intensities (up to 1100 μmol quanta m^{-2} s^{-1}) possessed values greater 5.5 during exponential growth and approached near Redfield stoichiometry in the stationary phase (chapter IV). Similar changes in elemental stoichiometry have been attributed to high biomass specific nitrogen fixation during exponential growth (Mulholland et al. 2001), which indicate carbon fixation rates that are low relative to nitrogen fixation during that period. Similar to Berman-Frank et al. (2001), reduced carbon incorporation rates were also measured at midday in cultures receiving a peak light intensity of 50 μmol quanta m^{-2} s^{-1} (Fig 3b), but *Trichodesmium* does not show reduced carbon fixation rates if cultures received more light. The results demonstrate that cultures receiving higher light intensities have no depression of carbon fixation rates at the peak of nitrogen fixation rates. At high light intensities (> 900 μmol quanta m^{-2} s^{-1}) though, carbon fixation rates may be reduced at midday due to photoinhibition, which is a typical diel pattern of photosynthetic organisms. Further, carbon specific ^{14}C incorporation is a function of light intensity (Fig 3b) while carbon specific nitrogen fixation rates do not differ significantly at different light intensities (Fig 3a). Conclusively, a

specific nitrogen fixation rate must be achieved to maintain cellular metabolism and only if sufficient light energy is provided, net carbon uptake and cellular growth can increase (see growth rate in chapter IV). Photosynthetic energy yield at low light intensities appears to be invested into carbon energy storage (glycogen), which is consumed for nitrogen fixation, and at a lesser extent into particulate carbon fixation. Net-carbon uptake rate can not be interpreted as equivalent to photosynthetic activity. Thus, if ^{14}C incorporation is reduced, photosynthesis might well be running at high rates, providing the energy for carbohydrate synthesis and nitrogen fixation. Contrarily, Berman-Frank et al. (2001) show indication for a decline of photosynthetic quantum yield efficiency (Fv/Fm) and increased non-cycling electron turnover times during the mid light period and argue that this implies reduced PS II activity. Data presented here show a similar decrease of Fv/Fm (Fig 4) over the course of the light cycle. Additionally, information on the relative quantum yield (or photon use efficiency, PUE) of net-carbon fixation (^{14}C incorporation) and nitrogenase activity (acetylene reduction assay) is provided (Fig 5). While nitrogen fixation quantum yield efficiency is higher between mid-morning and the peak of light intensity, carbon fixation quantum yield efficiency levels over the course of the day or slightly decreases during maximum light intensities. As carbon specific nitrogen and carbon fixation increase from morning to mid-day, but their specific quantum yield does not, it is apparent that after reaching a maximum possible quantum use the fixation rate levels or even decreases with further increasing light intensities resulting in decreases PUE and Fv/Fm. I.e. if a more than sufficient amount of light energy is provided, harvesting systems can afford to run less efficient. Therefore the decrease in photosynthetic quantum yield efficiency can not be interpreted as indicative for reduced photosynthetic activity either. Berman-Frank et al. (2001) base the reduced Fv/Fm measured in field samples on decreased electron transport rates downward of PS II (shown as increasing turn over rates), but unfortunately do not provide information on the light regime. Markedly, the 180 μmol quanta m^{-2} s^{-1} treatment showed the highest PUE for nitrogen and carbon fixation. The specific growth rate does not increase further at light levels greater 180 μmol quanta m^{-2} s^{-1} (chapter IV), which infers that this light intensity represents or is close to the light compensation point for elementally balanced diazotrophic growth that yields close to Redfield cellular stoichiometry. The decrease on photosynthetic oxygen evolution, reported by Berman-Frank et al. (2001), likely reflects efficient oxygen scavenging by the Mehler Reaction and not decreased photosynthetic activity. Nevertheless, likewise to nitrogen fixation pathways (as described above), to completely understand carbon fixation pathways in *Trichodesmium*, measurements of gross and net carbon fixation, including DOC exudation, are required.

In conclusion, the proposed mechanism of temporal segregation of nitrogen and carbon fixation only applies to low light intensities and are due to energy limitation. Reduced carbon fixation at high light intensities might be due to photoinhibition. Net carbon fixation is not directly indicative for photosynthetic oxygen evolution and co-occurs with nitrogenase activity during the day if sufficient light energy is available to run both energetically demanding processes (Fig 2B). Therefore, the strategy how *Trichodesmium* manages nitrogen fixation during oxygen evolution appears to be on the cellular level to a large extent. Nitrogenase is confined to specific areas within the trichome which have been termed diazocytes (Lin et al. 1998; Berman-Frank et al. 2001). These diazocytes also show a cellular differentiation on the molecular level in that *hetR*, the gene for heterocyst formation, is expressed. A similar pattern is found in *Symploca*, another non-heterocystous Osciallatorian that is closely related to *Trichodesmium* and also fixes carbon and nitrogen simultaneously (Fredriksson et al. 1998; Janson et al. 1998). Oxygen is rapidly transported out of these cells by respiration and the Mehler Reaction (Kana 1991; Kana 1993; Berman-Frank et al. 2001). High respiratory activity might also provide additional energy (ATP) for nitrogen fixation as suggested for *Cyanothece* (Colon-Lopez et al. 1997) and photosynthetic oxygen production may be temporally separated from carbon fixation as found in *Plectonema* (Misra and Tuli 1993). Cellular membrane permeability increases with temperature and due to the necessity to reduce cellular O_2 levels it has been proposed that non-heterocystous cyanobacteria such as *Trichodesmium* are better adapted to warm waters than heterocystous cyanobacteria (Staal et al. 2003). The patterns of energy budgeting and cellular pO_2 control summarized here provide *Trichodesmium* with a unique adaptive advantage in high light, high temperature environments of tropical and subtropical oligotrophic oceans and partially explain their ecophysiological success as an important marine nitrogen fixer.

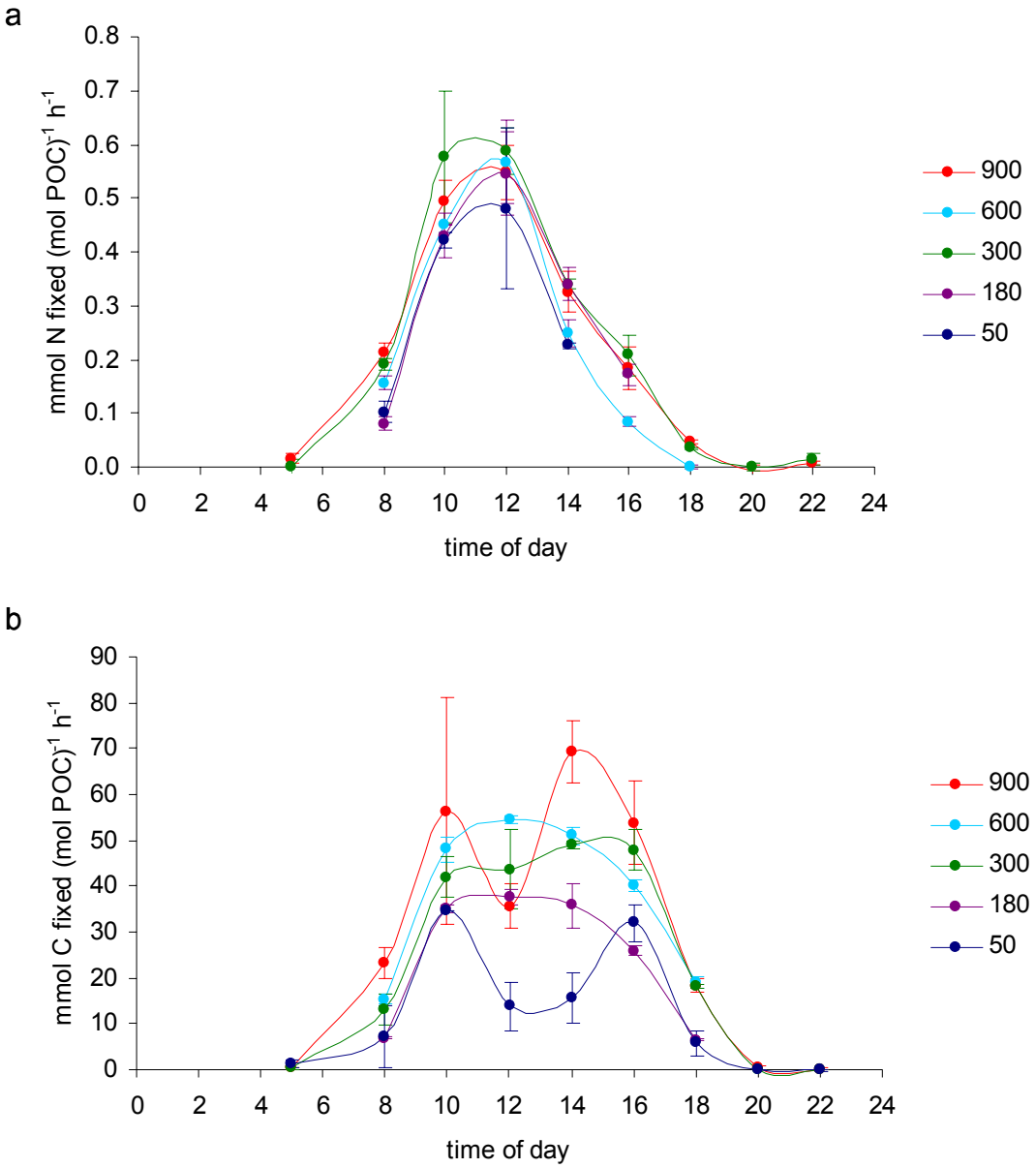


Figure 3: POC normalized nitrogen fixation (Acetylene Reduction Assay, a) and carbon fixation (^{14}C incorporation, b) as a function of daytime in *Trichodesmium* grown at different light intensities. Carbon and nitrogen fixation incubations took place simultaneously on exponentially growing cultures. Error bars denote standard deviations.

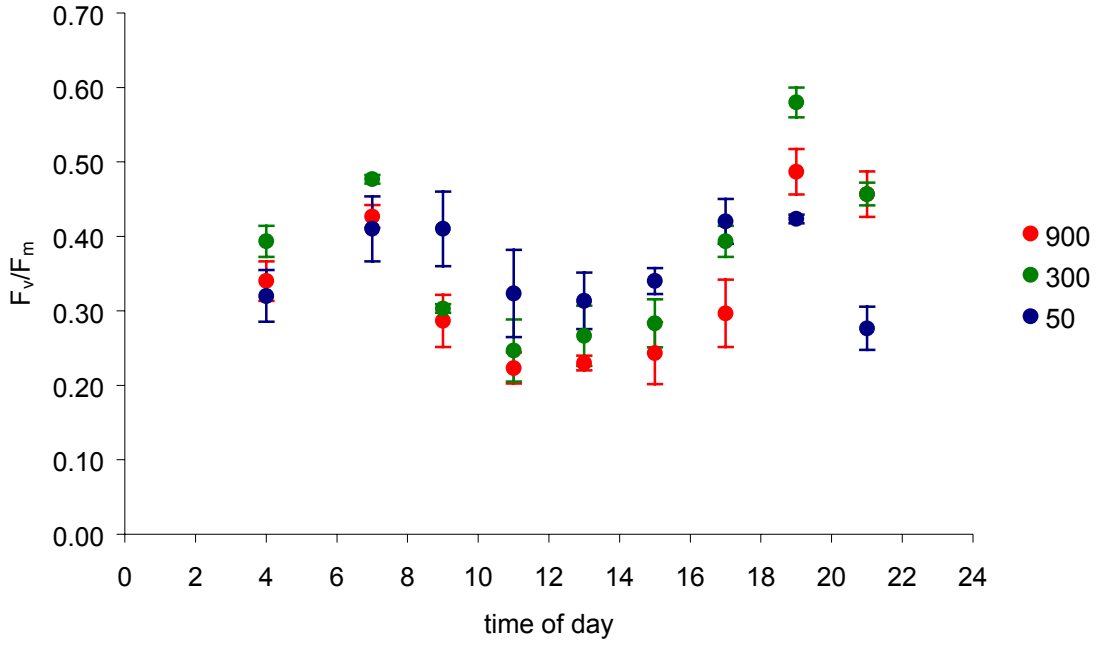


Figure 4: Photosynthetic quantum yield efficiency (F_v/F_m) of *Trichodesmium* as a function of daytime and light intensity. Measurements were performed at the same and from the same cultures as nitrogen and carbon fixation measurements shown in figure 3.

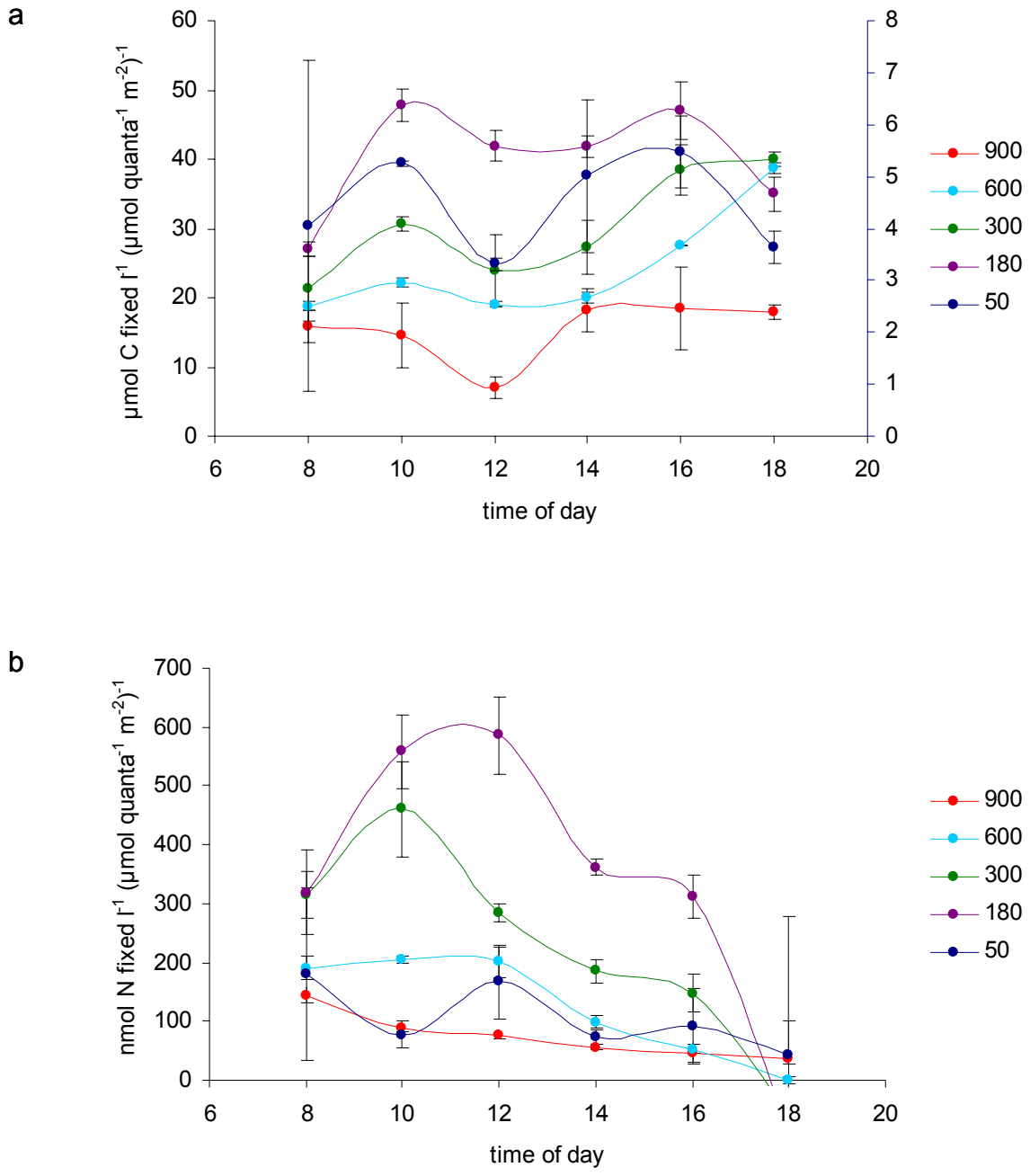


Figure 5: The relative quantum yield of net-carbon fixation (^{14}C incorporation, a) and nitrogenase activity (acetylene reduction assay, b) in *Trichodesmium* grown at different light intensities. Carbon and nitrogen fixation incubations took place simultaneously on exponentially growing cultures. The values on the 2nd y-axis apply to the results from the 50 µmol quanta m^{-2} s^{-1} treatment of the carbon fixation measurements (a).

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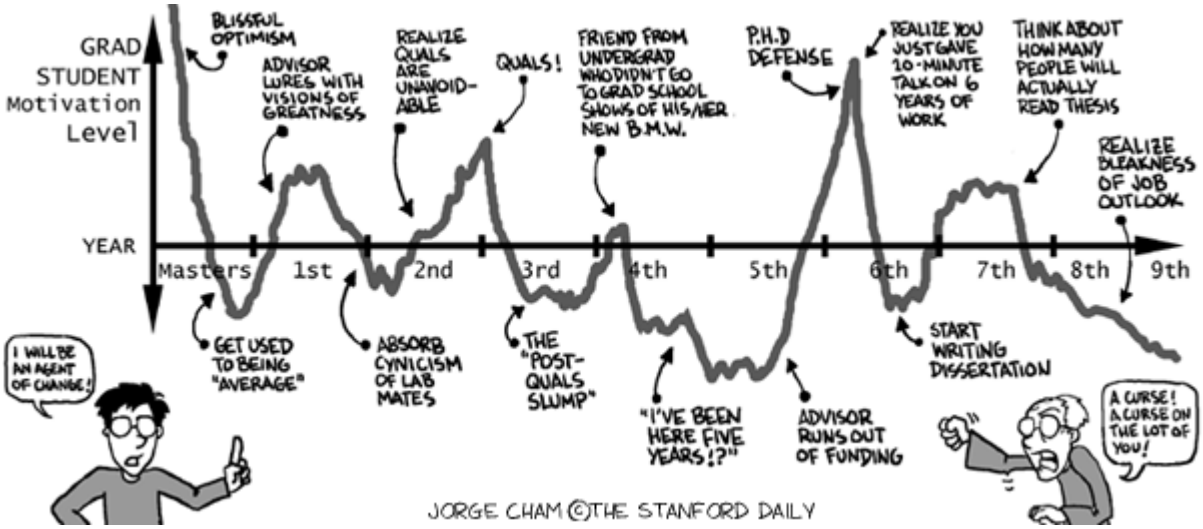

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