Plasticity of N:P ratios in laboratory and field populations of *Trichodesmium* spp.

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ABSTRACT: We followed changes in N:P ratios in batch cultures of the planktonic marine cyanobacterium *Trichodesmium* (IMS 101) grown in 2 different media and in field populations from 4 different oceanic regions. Cultures grown on low P media showed a rapid rise in N:P ratio upon depletion of phosphate. Ratios exceeding 125 were reached in 1 experiment before attaining stationary phase. A transect across the North Atlantic Ocean along 32° N showed a monotonic decrease in the N:P ratio of field collected colonies, dropping from about 60:1 on the western side of the basin to about 30:1 on the eastern side. A second cruise sampled colonies and surface slicks in waters along the north coast of Australia, where ratios of N:P were generally lower than in the North Atlantic, ranging from 11:1 to 47:1 with an average of 22:1. A comparison of rising and sinking colonies collected at 8 stations in the Gulf of Mexico shows a higher mean N:P ratio among sinking colonies than floating colonies. Overall, the average N:P in the Gulf of Mexico was about 68:1. N:P ratios of *Trichodesmium* around the Hawaiian Islands were very consistent between 2 consecutive years of sampling, with an average colony N:P for both years of about 38:1. Our research demonstrates high variability in the cellular N:P in *Trichodesmium* both in the laboratory and in the field. *Trichodesmium* N:P ratio may provide an index to the relative severity of P limitation in these diazotrophs. Geochemical and ecological modeling efforts which rely on using the N:P ratio of diazotrophs in deriving nitrogen fixation rates should account for the variability of these ratios *in situ*.

KEY WORDS: *Trichodesmium* · N:P ratio · Nitrogen fixation · Diazotroph · Cyanobacteria

INTRODUCTION

Trichodesmium spp. is a colonial marine cyanobacterium that is widely distributed throughout the tropics and subtropics in oligotrophic waters (Capone et al. 1997, Karl et al. 2002, Carpenter et al. 2004). It is a notable for its ability to fix atmospheric dinitrogen (Capone et al. 2005). While major advances have been made in understanding the biology and spatial distribution of *Trichodesmium*, many questions remain regarding its elemental composition and stoichiometry

of growth (Mulholland & Capone 2001). Letelier & Karl (1996) reported that the particulate organic carbon to particulate organic nitrogen (POC:PON) ratio of *Trichodesmium* is close to the Redfield value (6.3:1) but that the PON to particulate organic phosphorus (PON:POP, hereafter biomass N:P) ratios of around 42 (colonies) to 52 (free trichomes) are substantially higher than the Redfield value (i.e. 16:1). While these results are consistent with earlier studies in the Pacific by Mague (1977) and Karl et al. (1992), the relative plasticity of these cellular ratios with respect to geo-

graphical location and physiology are not known. Comparative analyses of laboratory populations of *Trichodesmium* and other diazotrophs under different growth conditions and of diverse field populations are necessary to understand the potential variance in this key cellular parameter. In addition, constraining diazotroph biomass N:P ratios is important for improving geochemical estimates of nitrogen fixation in the ocean.

Several recent geochemical studies of nitrate and phosphate fields in sub-euphotic zone waters of the tropical and subtropical North Atlantic have reported large excesses of regenerated nitrate (NO_3^-) relative to phosphate (PO_4^{3-}). A parameter, termed N^*, first developed by Michaels et al. (1996) and more fully by Gruber & Sarmiento (1997), was applied to data from the Geochemical Ocean Section Study (GEOSEC) cruises from 1972 to 1978. N^* represents the degree to which NO_3^- concentration deviates from Redfield proportions with PO_4^{3-} concentration and is generally some variant of:

$$N^* = [NO_3^-] - 16 \times [PO_4^{3-}] \qquad (1)$$

Positive gradients of N^* indicate possible exogenous sources of nitrogen (e.g. N_2 fixation) or removal of phosphorus while gradients of decreasing N^* indicate differential removal of nitrogen (e.g. by denitrification) relative to P.

In the Atlantic, there is a distinct N^* maximum in the thermocline of the tropical and subtropical North Atlantic, with higher values in the northern gyre than in the southern gyre. These anomalies have been interpreted to result from N_2 fixation (Michaels et al. 1996, Gruber & Sarmiento 1997). Gruber & Sarmiento (1997) estimated total N_2 fixation by integrating the excess N signal along isopycnal surfaces and assuming that the biomass N:P ratio of N_2 fixers was greater than Redfield (16:1). They found the rates derived by this approach to be substantially greater than earlier estimated (e.g. Carpenter 1983a). However, basin scale gradients are not well characterized and use of regional gradients may yield lower integrated rates than previously suggested (Hansell et al. 2004). While Gruber & Sarmiento (1997) and Hansell et al. (2004) differ in their assumptions about the size and magnitude of these gradients, they both assume an N:P ratio for N_2 fixers of 125:1. However, the derived estimate of N_2 fixation is sensitive to the N:P chosen, and as the N:P ratio of diazotrophs is decreased, the estimate of N_2 fixation rate increases.

Using the N:P ratio of field and laboratory populations of *Trichodesmium* as a diagnostic marker, it may be possible to assess the relative extent of P limitation in the ocean and also to contribute essential data to the wider questions of interpreting the N^* signal and of the role of oligotrophic oceans in global N_2 fixation. We therefore examined the response of the biomass N:P ratio of cultures of *Trichodesmium* grown under different initial phosphate concentrations and determined the N:P ratio of natural populations of *Trichodesmium* at 4 diverse oceanic locations.

MATERIALS AND METHODS

Culture growth curve experiments. We examined *Trichodesmium* IMS 101 in batch culture in order to determine the variability in their biomass N:P ratios. The cultures were maintained in the laboratory statically in an incubator with cool white fluorescent bulbs (irradiance = 90 µmol quanta m^{-2} s^{-1}) on a 12:12 h light:dark cycle at 27°C. All cultures were grown on YBC II media, a defined nitrogen free media of salinity 36 PSU (Chen et al. 1996), shaken once per day, and transferred on average once every 2 to 3 wk when densities increased or culture health declined. We here report the results from 2 growth curve experiments which were each conducted for a duration of approximately 1 mo.

The first growth curve experiment (Expt 1) ran from 7 May to 1 June 1999, and was carried out in duplicate under 2 different conditions. Media 1 was standard YBC II media (Chen et al. 1996), containing 50 µM PO_4^{3-} and here termed 'high phosphate'. Media 2 was the same except it contained only 5 µM PO_4^{3-} ('low phosphate'). Inocula for both treatments (low and high phosphate) were raised in standard YBC II with 50 µM phosphate. Samples were taken from each treatment flask every other day for 25 d. At each time point, 5 ml of culture was preserved in 2% glutaraldehyde for later trichome counts using light microscopy. We assumed 100 cells per trichome (Carpenter 1983a). Sub-samples were also collected for N_2 fixation determination and for filtration onto combusted 25 mm GF/F filters for PON, POC and POP determinations. The volume sampled was adjusted throughout the growth curve to accommodate increasing cell density. POP analysis was conducted by the Nutrient Analytical Services Laboratory (NASL) at the Chesapeake Biological Laboratory (CBL) using the persulfate digestion method of D'Elia et al. (1977) and total particulate C and N was measured on an elemental analyzer (Europa ANCA CN) interfaced to a mass spectrometer (Europa 20/20) using the integrated mass signal for each element. Ten ml samples were filtered onto 5 µm polycarbonate membrane filters and analyzed for chlorophyll content using a methanol extraction and subsequent measurement of chlorophyll by fluorescence, as described by MacKinney (1941).

A second growth curve (Expt 2) similar in design and culture conditions to Expt 1 was performed in triplicate at the University of Southern California from 11 February 2000 through 3 March 2000. In Expt 2, parent cultures for inocula had been raised under the 2 respective conditions of low and high phosphate. In addition to the standard suite of parameters, concentrations of PO_4^{3-} were also analyzed over the course of this experiment on a Lachat continuous flow injection colorimetric nutrient analyzer. POP and PON contents were measured as PO_4^{3-} and NO_3^- after potassium persulfate digestion similar to the method used in Expt 1 (Raimbault et al. 1999) and also analyzed on the Lachat. In all cases, bacteria were present in the culture under both media conditions at low ($<10^4$ ml^{-1}) initial densities but reached densities of 10^6 ml^{-1} by stationary phase. N:P ratios are expressed as molar ratios.

The acetylene reduction procedure was used to estimate N_2 fixation rate (Capone 1993). Ten ml volumes of culture in serum vials sealed with silicone septa were amended with 1 ml of purified acetylene Samples were incubated at 27°C. Sub-samples (100 µl) of the gas phase were removed and analyzed by flame ionization gas chromatography about every 30 min over a time course of several hours as described by Capone (1993). Assays were conducted at every time point along the growth curve and were initiated at approximately the same time in the late morning or early afternoon every day, with light

incubation at 27°C. N_2 fixation was not measured during dark periods.

Field collections. Field collections were made during cruises aboard the RV 'Maurice Ewing' (27 October through 29 November 1999) in tropical northern Australian waters (primarily the Arafura, Timor and Coral Seas); aboard the RV 'Seward Johnson' in the Sargasso Sea and Atlantic Ocean on a cruise track from Bermuda to Tenerife, Canary Islands (9 May through 24 May 2000); aboard the RV 'Longhorn' in the Gulf of Mexico (July 2000); and aboard the RV 'Kilo Moana' (September to October 2002) and the RV 'Roger Revelle' (July to August 2003) in the North Pacific Subtropical Gyre (NPSG) (Fig. 1).

During the November 1999 cruise north of Australia, fresh samples of *Trichodesmium* spp. were generally collected by gentle plankton tows from the upper 20 m of the water column. In cases where *Trichodesmium* was extremely abundant and could be seen in patches or 'slicks' on the surface of the water, samples were collected by dropping a plastic bucket directly over the side of the ship. Some 'slick' samples were also collected by deploying a Zodiac from the ship and bucket sampling directly off the side. Only colonies were collected by hand picking them with disposable plastic inoculating loops into filtered seawater (GF/F) stored in polycarbonate bottles for filtering.

Samples of colonies were filtered onto precombusted GF/F filters. Particulate filters were kept frozen during the cruise and then dried in a 60°C drying oven at the
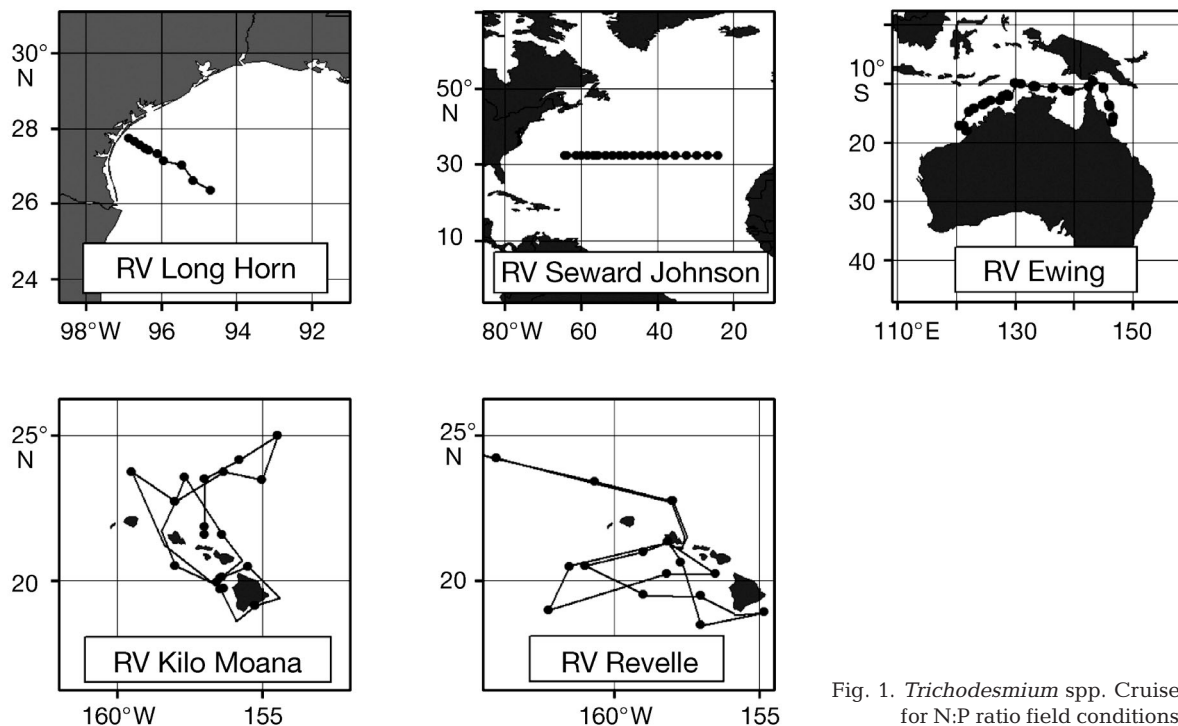

Fig. 1. *Trichodesmium* spp. Cruise tracks for N:P ratio field conditions

termination of the cruise for later persulfate digestion (Raimbault et al. 1999) and analysis on a Lachat continuous-flow auto analyzer system at the Marine Science Institute (University of Texas at Austin). Similar collections were undertaken in the Sargasso Sea and Atlantic Ocean. In the Gulf of Mexico, *Trichodesmium* brought aboard were separated on the basis of buoyancy status (floating or sinking) and these groups were analyzed for particulate matter separately (Villareal & Carpenter 2003). On the first NPSG cruise, colonies of *Trichodesmium* were picked out of the plankton tow using a plastic inoculating loop while filaments of the closely related *Katygnemene* spp. were allowed to aggregate on the surface of the tow material held in a bucket, then gently removed and placed in freshly GF/F filtered seawater. Both types of samples were poured into the barrel of a syringe and then gently filtered onto an in line GF/F filter. On the 2003 NPSG cruise, *Trichodesmium* colonies were picked directly onto filters with an inoculating loop after rinsing in filtered seawater. In all environments, both puff and tuft colony morphologies were collected.
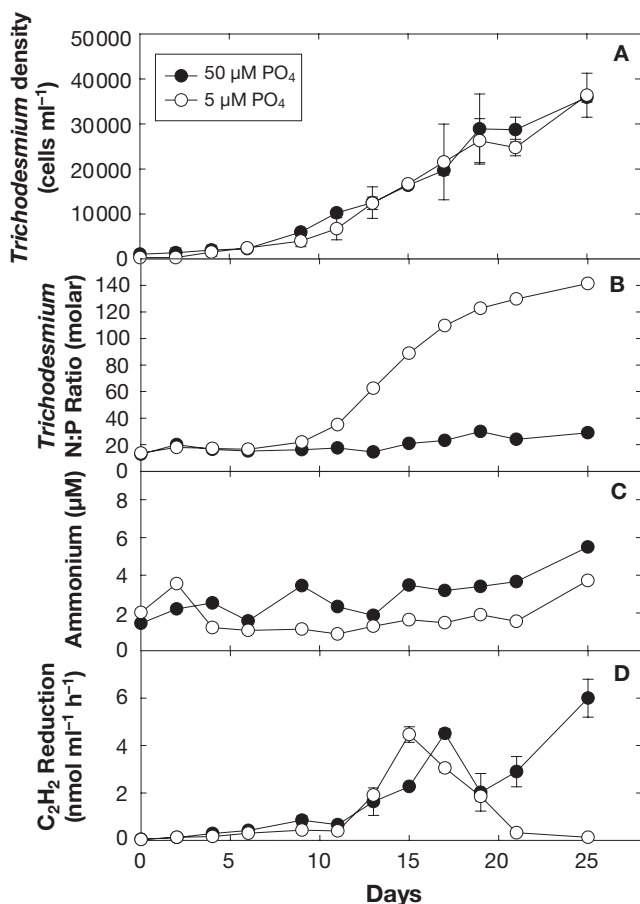
RESULTS

Culture growth experiments

During the 25 d Expt 1, cultures grown on each media followed very similar trends, with 6 d lags followed by rapid growth. Biomass doubling times were 3.5 d for the high phosphate culture and 3.4 d for the low phosphate culture, with density finally reaching >30 000 cells ml^{-1} by Day 25 (Fig. 2).

In Expt 2, the culture grown in high phosphate media reached only 23 000 cells ml^{-1} at Day 18, maintaining a 2.3 d doubling time throughout before leveling off; the culture grown in low phosphate media exhibited a doubling time of 2.5 d and peaked at 10 000 cell ml^{-1} at Day 14 followed by a sharp decline to 10 cells ml^{-1} by the end of the growth curve at Day 21 (Fig. 3). For both culture experiments, doubling
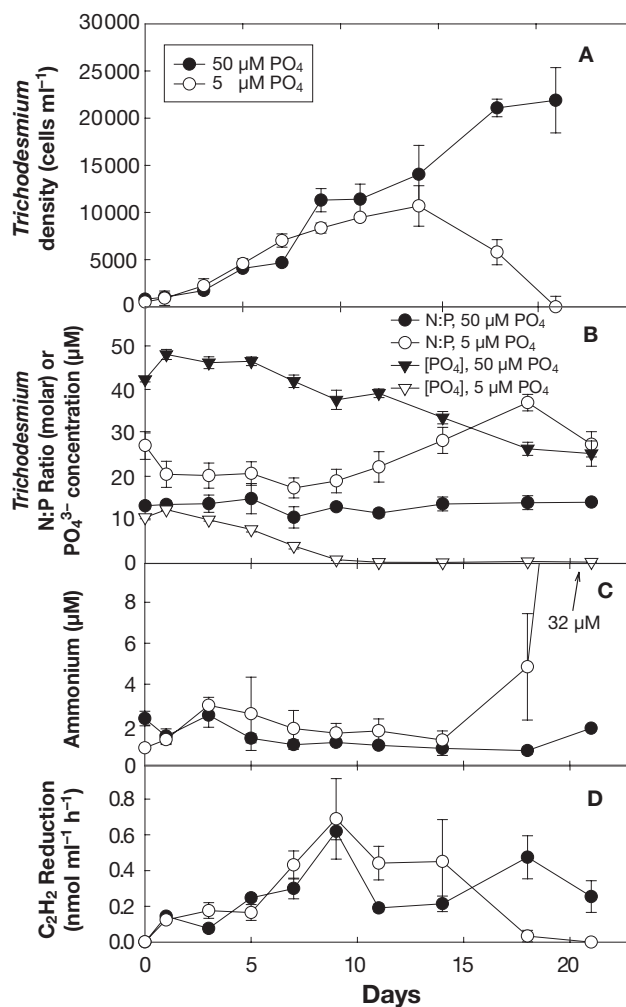

Fig. 2. *Trichodesmium* IMS 101 growth curve 1. (A) Cell counts, (B) N:P ratio, (C) ammonium concentrations and (D) C_2H_2 reduction rates. Error bars are ±SE

Fig. 3. *Trichodesmium* IMS 101 growth curve 2. (A) Cell counts, (B) N:P ratio and PO_4^{3-} concentrations, (C) ammonium concentrations and (D) C_2H_2 reduction rates. Error bars are ±SE

times of 2 to 4 d were similar to growth rates reported by Chen et al. (1996) and Mulholland & Capone (1999, 2001).

Chlorophyll *a* trends closely mirrored cell counts with chlorophyll levels in low phosphate media cultures declining during the last days of the growth curve (Mulholland & Capone 1999), while chlorophyll levels in standard YBC II media with high phosphate continued to increase or level off during the final days of the growth curve (data not shown). Ambient NH_4^+ concentrations in culture filtrates were measured in each of the 2 growth curves during Expts 1 and 2, and typically fluctuated between 1 and 4 µM over the duration of the growth curve (Figs. 2C & 3C). The ammonium levels for the 2 experiments increased during the last time point as the cultures reached stationary phase, with a sharp increase at the final point in the low phosphate treatment of Expt 2. N_2 fixation in the 2 cultures was comparable over most of the time course, except for the final 2 time points when rates in the P depleted cultures fell to low levels while being maintained in the high P media (Figs. 2D & 3D).

Ambient phosphate was measured in the filtrate only during Expt 2 and was initially approximately equivalent to the 50 µM high phosphate media conditions in the high P treatment and about 10 µM PO_4^{3-} in the low phosphate media (Fig. 3B). The somewhat higher concentration than expected (5 µM) in the low PO_4^{3-} treatment may have been due to a minor contamination artifact or analytical error. Phosphate concentrations decreased from 48 to 25 µM in the standard YBC II media-grown culture and from an initial concentration of 10 to 0 µM on Day 11 in the low phosphate media when the N:P ratio began to increase (Fig. 3B).

The biomass N:P ratios of cultures grown in high phosphate YBC II were near or below the Redfield ratio of 16:1 (Figs. 2B & 3B) and ranged from 7 to 18 in a preliminary experiment (data not shown), 8 to 11 during Expt 1 and 11 to 15 during Expt 2. However, cultures grown on 5 µM KH_2PO_4 media, while initially near Redfield N:P, generally increased their biomass N:P ratio as the growth curve progressed. The biomass N:P of low phosphate media-grown culture increased from 8 to 140 in Expt 1 and from 13 to 37 during Expt 2.

A 2-factor ANOVA and mixed model design was performed on each full data set of each growth curve and tested against the theoretical Redfield ratio of 106 C:16 N:1 P. Cultures grown with high and low phosphate availability in Expts 1 and 2 both had N:P ratios significantly different than Redfield ($p < 0.05$). The mixed model statistic showed that cultures grown on high phosphate during Expts 1 and 2 had biomass N:P ratios significantly lower than 16 and cultures grown on low phosphate in both experiments had ratios significantly higher than 16 ($p < 0.05$). In Expt 2, the off-

set in the biomass N:P ratio between high and low phosphate cultures initially and across the growth curve was significantly different ($p < 0.05$) (see above). Ambient phosphate in the filtrate of low phosphate cultures decreased in parallel with the sharp increase in biomass N:P, while the high phosphate cultures showed only a slight decrease in ambient phosphate as phosphorus was incorporated into biomass. Expt 2 also showed a sharp decrease in trichome density and chlorophyll *a* content in low phosphate cultures at the same time that PO_4^{3-} was depleted from the media.

A simple phosphorus mass balance of Expt 2, with both POP in *Trichodesmium* and ambient PO_4^{3-} in filtrate, showed that at least 77 % of the 50 µM initial PO_4^{3-} from YBC II media was accounted for by POP and at least 65 % of the 5 µM initial PO_4^{3-} from the low phosphate media was recovered as POP. The remainder was likely in the unmeasured dissolved organic phosphorus (DOP) pool, or alternatively a result of incomplete digestion of POP (or both).

Field collections

The field populations sampled represented a range of conditions and densities of *Trichodesmium*. During the Australian cruise of October to November 1999, *Trichodesmium* spp. was abundant and highly variable, and surface slicks were observed at many stations throughout the cruises, reaching densities over 10^5 colonies^{-1} (Mulholland et al. 2002). The Atlantic cruise occurred in May 2000, relatively early for high densities of *Trichodesmium* in this region (McCarthy & Carpenter 1979). Densities were less than 0.05 colony^{-1} at every station along the cruise track at 32° N latitude with the exception of Stn 43, where densities were 2 colonies^{-1}. Colony densities in the Gulf of Mexico were 2 to 5 colonies^{-1} at most stations with 1 station showing a surface slick of 51 colonies^{-1}. Densities of colonies on both the September 2002 and the August 2003 Pacific cruises ranged from less than 1 to up to 12 colonies^{-1} with an average of about 2 colonies^{-1}.

Ambient nutrient concentration varied greatly across the Australian transect. Surface NO_x (NO_2 plus NO_3) values ranged from 0 to 2.2 µM across stations while PO_4^{3-} values varied from 0 to 0.3 µM. At most stations, the ratio of $NO_x:PO_4^{3-}$ was well below 16:1, averaging about 3.9. Surface nutrient concentrations of NO_x and PO_4^{3-} were below the detection limit in the North Atlantic. In the Gulf of Mexico, PO_4^{3-} values were very low (~0.03 µM) to unmeasurable, while NO_x was detected at most stations in the 0.02 to 0.08 µM range. Surface values for NO_3^- were generally below the limit of detection for the 2 North Pacific cruises, while PO_4^{3-}
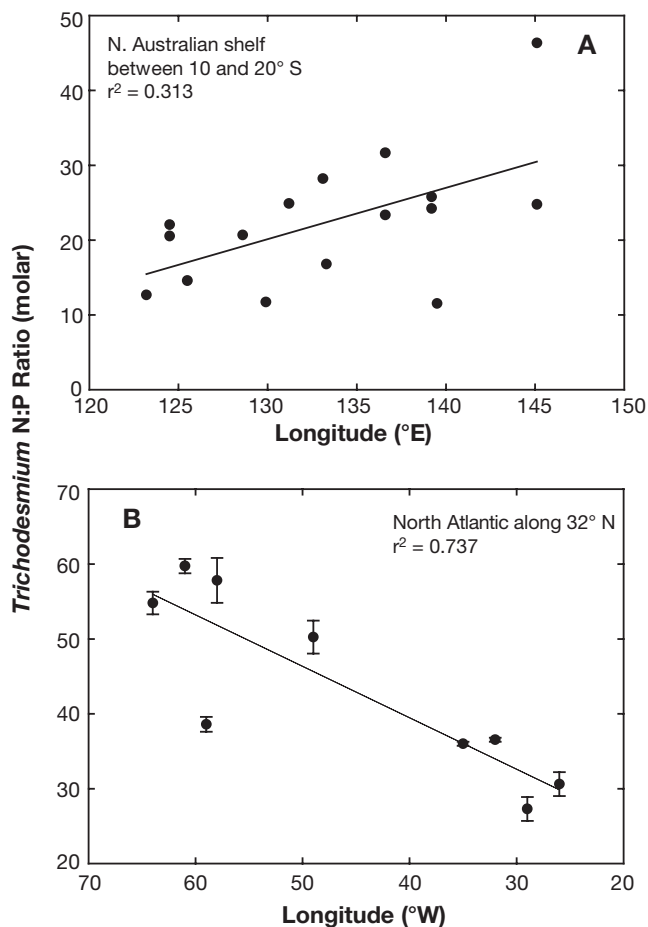
Fig. 4. *Trichodesmium* spp. N:P ratios of field populations. Samples were performed in duplicate for (A) the Australian transect and in triplicate for (B) the North Atlantic transect. Values in (B) are ±SE. Note differences in *y*-axis scale
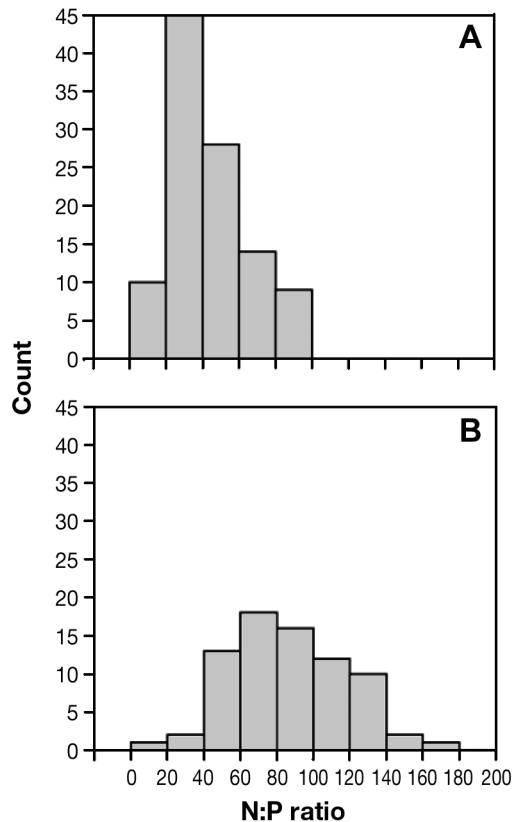

Fig. 5. *Trichodesmium* spp.. N:P ratios of (A) floating and (B) sinking colonies (Gulf of Mexico)
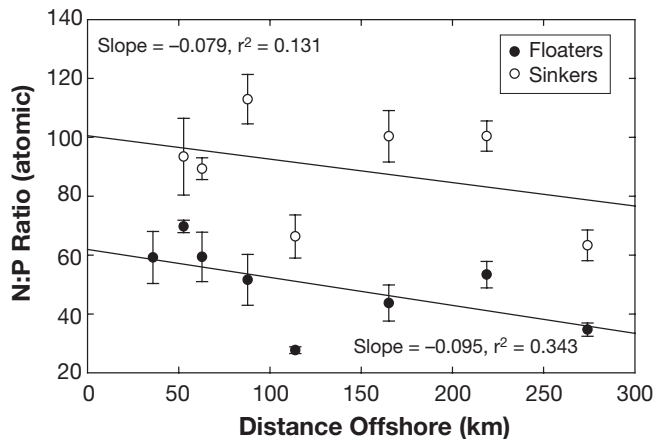

Fig. 6. *Trichodesmium* spp.. N:P ratios of floating and sinking colonies (Gulf of Mexico). Averages for each station ±SE, n = 3

concentrations during the September 2002 cruise averaged about 0.03 µM and ranged from 0.005 to 0.108 µM on the August 2003 cruise.

The N:P ratio along a 2-way transit of northern Australia (Fig. 4A) ranged from about 11 to 47, with an average of 22. There was a weak trend of decreasing N:P toward the west. There was no apparent relationship between the N:P ratio of *Trichodesmium* and surface PO_4^{3-} concentration ($r^2 = 0.005$). A decreasing trend in the PON:POP ratio in *Trichodesmium* spp. colonies was readily apparent across the west to east Atlantic transect along 32° N (Fig. 4B), with highest values of *Trichodesmium* N:P of 60 at the western extreme, and much lower values of about 30 at the eastern end of the transect.

In the Gulf of Mexico, the biomass N:P of floating colonies ranged from 27.7 to 69.7, with an average of approximately 50. A frequency histogram of colony N:P showed substantial variation within the buoyancy category. Mean biomass N:P ratio varied more widely in sinking colonies, but in both cases the mean ratio was 50% or less of the maximum ratio (Fig. 5). As previously shown (Villareal & Carpenter 2003), sinking colonies in the Gulf of Mexico had significantly higher (p < 0.0001) N:P ratios compared to floaters, ranging from 63 to 100 and averaging about 90. The overall

average of the samples collected (both floating and sinking colonies) was 68.4. The N:P ratio of colonies decreased moving offshore (Fig. 6).

The depth pattern of the N:P ratio in *Trichodesmium* colonies was distinct for floating and sinking colonies, and varied along the transect (Fig. 7). Except for Stn 1, floating colonies tended to have higher N:P ratios near the surface and were relatively uniform at depth. In most cases, the N:P ratio of sinking colonies far exceeded that of rising colonies collected from the same depth. There was, however, no clear pattern with depth for sinking colonies.

Biomass N:P ratios of samples of *Trichodesmium* collected in the Pacific in 2002 and 2003 averaged 36.2 and 40.1, and were not significantly different (p > 0.05) from each other. *Katygnemene* collected in 2002 had an average biomass N:P of 33.9 and was not significantly different (p > 0.05) from the *Trichodesmium* collected that same year. Neither *Trichodesmium* nor *Katygnemene* biomass N:P ratios showed correlation to PO_4^{3-} concentrations (Fig. 8).

DISCUSSION

Both laboratory cultures and field populations of *Trichodesmium* exhibited a broad range of N:P ratios, depending on their nutrient environment. Furthermore, our data suggest that the biomass N:P ratio of *Trichodesmium*, and other diazotrophs such as *Katygnemene*, respond to, and may be a useful diagnostic of, phosphorus limitation.

Laboratory cultures show a distinct response in biomass N:P ratios to limiting phosphorus and alter their

Fig. 8. N:P ratios of *Trichodesmium* spp. colonies in the North Pacific subtropical gyre in 2002, 2003, and *Katygnemene* in 2002 compared to PO_4^{3-} concentrations

biomass N:P substantially before starvation is manifested in cell biomass, chlorophyll, or N_2 fixation rates as occurs later in the growth curve. The lower biomass in low phosphate medium compared to high phosphate medium further confirms that cells were phosphate depleted in the low phosphate experiments. While cultures grown with low phosphate have N:P ratios that were initially similar to those cultures grown on higher phosphate media, the divergence is marked and large once phosphate levels are depleted. In contrast, the N:P ratio of each culture grown on standard YBC II media remained relatively constant and lower than the Redfield ratio, indicating phosphate sufficiency throughout the growth curves.

Phosphate concentrations in the filtrate were inversely related to the trend in the biomass N:P ratios, as would be expected in phosphate-depleted cultures (Fig. 9). In YBC II media cultures, the ambient dissolved phosphate levels remained relatively constant throughout the duration of the growth curve, dropping to no more than 40% of the initial concentration by the
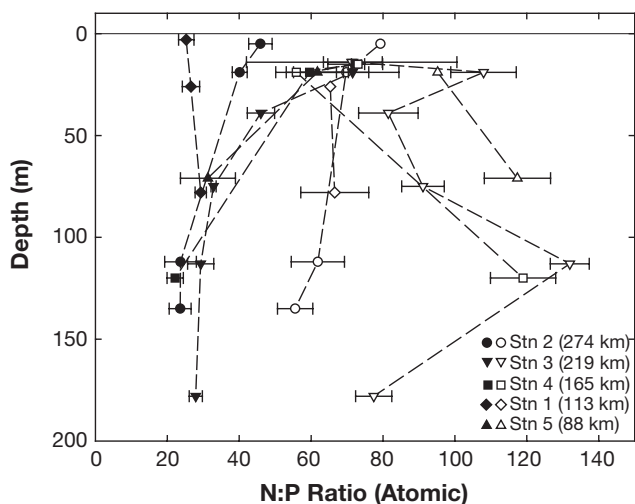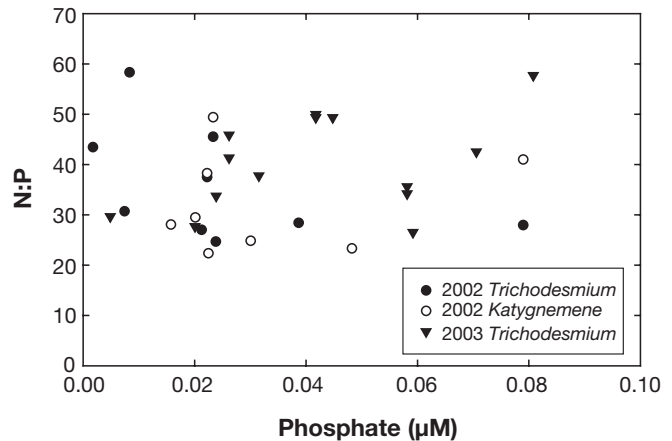

Fig. 7. *Trichodesmium* spp.. Depth profiles of N:P ratios for colonies (Gulf of Mexico). Distance offshore for each station given in parentheses. Open symbols = sinking, closed symbols = floating. Error bars are ±SE
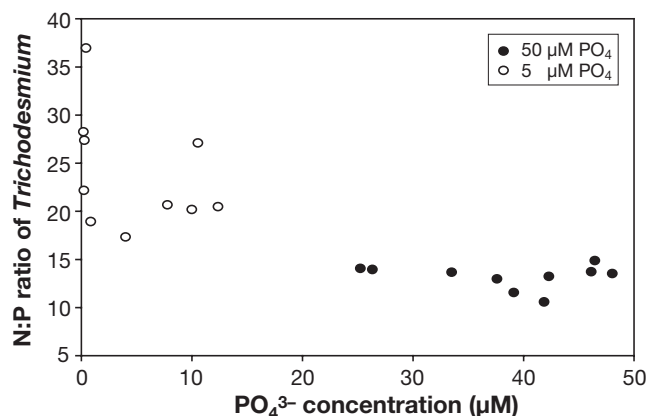

Fig. 9. Phosphate concentration vs. N:P ratio in culture Expt 2

final time point, indicating that phosphorus limitation was not reached. In contrast, the low-phosphate grown cultures had a much more significant decrease in ambient phosphate over time as the limited nutrient supply was depleted.

There were, however, differences in the 2 experiments. In Expt 1, the growth rate of *Trichodesmium* was apparently not limited by P over the time course, but large changes in biomass N:P ratio were seen. Thus, the biomass N:P ratio is very sensitive, and increases when *Trichodesmium* reaches P depletion. The growth rate was also the same in the 2 treatments in the beginning of Expt 2. However, towards the end of this experiment, biomass in the low phosphate cultures dropped off sharply. Here, biomass N:P levels did not increase as much as in Expt 1, yet biomass became limited in the end. Different pre-treatment (P replete for both in Expt 1 but different conditions in Expt 2 with each treatment being raised under the respective experimental regime of either high or low phosphate) accounts for the initial higher biomass N:P ratio seen in the low phosphate treatment compared to the high phosphate treatment in Expt 2. The differences in the growth curves may also be accounted for by the differences in pre-conditioning. In Expt 1, the sustained growth in the low phosphate treatment while biomass N:P increased may have resulted from the mobilization of P reserves (generally as polyphosphate granules in *Trichodesmium*) or perhaps as phosphates (Dyhrman et al. 2006). It is likely that initial inoculum in the low phosphate treatment in Expt 2 would have had far lower P reserves with the result that biomass increase ceased at an earlier point in the time course.

The culture experiments described here show that the biomass N:P ratio in *Trichodesmium* responds strongly to P availability and may have utility as a field indicator of P stress. We do not suggest that it is possible to relate the actual numbers from the cultures to the field, however. Batch culture experiments are similar to what might be seen in a bloom as cells undergo progressive nutrient exhaustion, and as the conditions are highly controlled, we know that the observed result is from the depletion of P in the media. When *Trichodesmium* is encountered in the field, its nutritional history is not known and growth could be balanced (which was not the case in the culture experiments). However, we know from the controlled culture experiments that the trend of higher biomass N:P ratios comes from P starvation. It is important to note that it does not necessarily indicate that P is the limiting nutrient to growth of *Trichodesmium* in the field as other P sources may be available (Sohm & Capone 2006).

Substantial variation was noted in *Trichodesmium* biomass N:P ratios both within and among the field sites (Table 1). Highest biomass N:P ratios in *Trichodesmium* biomass were found in the Gulf of Mexico samples. The sinking colonies had the highest biomass N:P overall, and the average biomass N:P of all samples combined was higher than at any other site. The N:P loading ratio in the Gulf from the Mississippi River is generally large and in excess of Redfield (Turner et al. 2003 and thus may explain the high biomass N:P seen there). Very high ratios were also found in the North Atlantic, with a trend towards higher N:P ratios on the western side of the basin. Phosphate in surface waters of the North Atlantic was below detection limits at all stations. The lowest average biomass N:P ratios of colonies were found on the North Australian transect, with intermediate values in the North Pacific samples. In the 2 Pacific samplings (RV 'Maurice Ewing' and RV 'Roger Revelle') we often encountered *T. erythraeum* rather *T. thiebautii* which is more common in the North Atlantic. *T. thiebautii* has a larger cellular diameter (typically about 7 μm) compared to *T. erythraeum* (typically about 3 μm) and tends to form larger colonies (100 to 200 vs. 50 to 150 trichomes per colony, respectively; Carpenter 1983b, Carpenter et al. 2004). Hence the lower per colony N and P content is not surprising.

Table 1. Summary of N:P ratios and PON and POP content of colonies (col) for the various cruises. na = not available; n/a = not applicable

Cruise	Year	Sample	No. of stations	Average N:P	SE	nmol N col	SE	nmol P col	SE	N:P from Avg
RV 'Maurice Ewing'	1999	Colonies	16	22.4	2.20	75.3	0.02	3.24	0.00	23.3
RV 'Longhorn'	2000	Sinkers	7	89.5	6.94	108.5	17.7	1.29	0.19	84.1
	2000	Floaters	8	49.9	4.90	121.2	21.6	2.59	0.30	46.8
RV 'Seward Johnson'	2000	Colonies	9	43.5	4.08	na	na	na	na	
RV 'Roger Revelle'	2003	Colonies	14	40.1	2.53	28.7	2.08	0.742	0.042	38.6
RV 'Kilo Moana'	2002	Colonies	10	36.2	3.35	83.1	21.8	2.06	0.33	40.3
		Free trichomes	10	33.9	3.27	n/a	n/a	n/a	n/a	
		Katygnemene	8	32.1	3.44	n/a	n/a	n/a	n/a	

The *Trichodesmium* IMS 101 culture is putatively *T. erythraeum* (Orcutt et al. 2002). For comparison with the field studies, and assuming 150 trichomes per colony, the P content of the high phosphate culture in the second experiment averaged 3.1 ± 0.22 µmol P per colony, and 27 ± 3.52 µmol N per colony while the low phosphate culture averaged 2.5 ± 0.16 µmol P per colony and 10.2 ± 3.21 µmol N per colony. The N:P ratios varied as described in Figs. 2 & 3 from near-Redfield to approximately 125 under severe P depletion.

Recent geochemical (Wu et al. 2000) and biological (Cotner et al. 1997, Sañudo-Wilhelmy et al. 2001, Dyhrman et al. 2002, Mulholland et al. 2002, Ammerman et al. 2003) evidence from the North Atlantic suggest that phosphorus may be in much shorter supply, relative to Fe, for surface diazotrophs. In addition, the phosphocline is generally deeper than the nitracline in the Sargasso Sea (Michaels et al. 1994, Wu et al. 2000). In the North Atlantic Ocean and Sargasso Sea, a highly oligotrophic basin, our research provides further evidence for severe phosphate depletion and suggests that phosphorus levels may be sufficiently low to exercise control on diazotrophs. The high *Trichodesmium* biomass N:P ratios and low phosphate concentrations in the Gulf of Mexico also suggest a potential for severe P limitation.

Across the northern Australian coast, throughout the Arafura, Timor, and Coral Seas, *Trichodesmium* biomass N:P ratios were generally lower, ranging from 11 to 47, with an average much closer to the Redfield ratio at 22. This suggests a higher availability of phosphorus, possibly a result of the relatively shallow sediments or proximal terrestrial influences, or both. Indeed, phosphate concentrations, while low, were usually measurable, and in the surface appeared to be generally in excess of nitrate (low soluble inorganic N:P). Compared with results from the culture experiments and the North Atlantic and Gulf of Mexico transects, it appears that *Trichodesmium* in the waters north of Australia are not highly P limited. Indeed, at most sites, the N:P of DIN to DIP was lower than the Redfield ratio (data not shown), suggesting P sufficiency there.

In the NPSG, N:P ratios of *Trichodesmium* biomass were also closer to Redfield than in the apparently P-limited Gulf of Mexico and western North Atlantic (generally <50 compared to >50). In addition, the ratios were consistent for 2 consecutive years. *Katygnemene*, a diazotroph very closely related to *Trichodesmium*, exhibited the same N:P ratio, as the *Trichodesmium* collected that same year, suggesting they do not respond differently to phosphate concentrations. The relatively lower N:P ratios found in the Pacific, and the failure of ratios to correlate with PO_4^{3-} concentrations, implies that *Trichodesmium* is not P-limited in the NPSG. However, colonies could be P-stressed at certain locations, as biomass N:P was >50 for some stations.

Comparing the values for N:P in the field with those found in culture experiments, it appears that *Trichodesmium* in all basins are dealing with some level of P deficiency, except for certain areas north of Australia. Ratios below Redfield are likely caused by luxury consumption and storage of phosphate. It remains to be seen at what N:P ratio these stores run out and colonies truly become P-limited. Kustka et al. (2003) suggest that an N:P ratio of about 40 is the critical ratio for P limitation, based on a comparison of N_2 fixation rates to N:P ratios. Our present findings roughly agree with their study.

While several studies have observed field populations of *Trichodesmium* with respect to molar biomass N:P ratio, the data are relatively limited in space and time. Mague et al. (1977) reported an N:P ratio of 20:1 for *Trichodesmium* colonies collected at one station north of the Hawaiian Islands. Letelier & Karl (1996) reported molar N:P ratios for free trichomes of 52 and 45 for May 1990 and October 1991, respectively, and of 42 ± 6.15 (SD) for colonies from Stn ALOHA. Letelier & Karl (1998) examined the N:P ratio of sinking and rising colonies collected from 5 and 100 m. Near surface colonies averaged an N:P ratio of about 43 regardless of buoyancy, while rising colonies from depth had a lower, although not significantly different, N:P ratio (~34) than sinking colonies (~45). These values fall in the mid-range relative to our Gulf of Mexico and Atlantic samples. Sañudo-Wilhelmy et al. (2001) found a relatively low N:P ratio (~18) in their subtropical North Atlantic transect between 10 and 16° N, with higher values (~50) further south between 0 and 6° N. Karl et al. (1992) reported very high biomass N:P ratios (125:1) in surface particulates after a *Trichodesmium* bloom event, providing further evidence of a very flexible cell quota for *Trichodesmium*. White et al. (pers. comm.) have also found wide variability in the N:P ratios of *Trichodesmium* continuous cultures as a function of P availability.

Recently, Sañudo-Wilhelmy et al. (2004) reported that a significant portion of cellular P is adsorbed to the cell surface of *Trichodesmium* colonies. The extent of PO_4^{3-} adsorption, which is variable, has implications for the use of bulk N:P as an indicator of P stress. High concentrations of adsorption of PO_4^{3-} relative to that bound organically would lower the bulk N:P ratio relative to the actual PON:POP. We did not correct for this extracellular P in our measurements, and thus the intracellular N:P ratio is likely greater than the numbers reported here. While extracellular phosphorus is not part of the so-called 'biological pool', a recent experiment suggests *Trichodesmium*

can internalize the extracellular pool at a rate of approximately 25 % d⁻¹, and thus it is important to cells (Fu et al. 2005).

Plasticity in N:P ratios is not restricted to diazotrophs: other marine cyanobacteria also exhibit considerable placticity in their cellular N:P ratios. *Synechococcus* and *Prochlorococcus* strains showed biomass N:P ratios of 21 to 33 under nutrient-replete conditions and 59 to 109 under P limitation (Bertilsson et al. 2003). A separate study found ranges of 15 to 24 for *Prochlorococcus* and 7 to 36 for *Synechococcus* (Heldal et al. 2003). Such plasticity is also commonly noted in eukaryotes. For example, Goldman et al. (1979) reported an N:P ratio range of 15:1 to 100:1 for *Monochrysis lutheri*. Karl et al. (2001) found considerable seasonal and interannual variability in N:P ratios of both soluble and particulate material at Stn ALOHA in the NPSG. This included a secular trend of increasing N:P ratio in the particulate pool over the 9 yr observation period.

Understanding the cellular stoichiometry of marine plankton, and in particular diazotrophs, has important implications in a number of arenas (Mahaffey et al. 2005). In a geochemical analysis of nutrient fields in the North Atlantic, Gruber & Sarmiento (1997) integrated the excess N accumulating along isopycnals, and derived an estimate for the amount of N_2 fixation needed to account for this by assuming an N:P ratio for diazotrophs well above Redfield to account for the ingrowth of the nitrate. They chose a value for *Trichodesmium* biomass of 125 N:1 P, gleaned from the study by Karl et al. (1992). Gruber & Sarmiento (1997) recognized the sensitivity of their N^* based estimate to the N:P ratio selected. Given our evidence, a lower value may be appropriate. Using a value of 45 (rather than 125) would increase their N_2 fixation estimate for the Atlantic by about 50 %. However, as noted by Hansell et al. (2004), large uncertainties exist in the N fixation estimates based on N^*. Hansell et al. (2004) estimates suggest N_2 fixation rates of approximately 10 to 25 % of that suggested from the Gruber & Sarmiento (1997) analysis.

As the modeling of marine systems advances and incorporates functions such as N_2 fixation, knowledge of the elemental stoichiometry of diazotrophs becomes critical (Hood et al. 2000). However, most coupled biogeochemical models assume Redfield C:N:P stoichiometry, although this approach is slowly changing. Correct representation of the stoichiometry of N_2 fixers is important if models are meant to be representations of the real world. Our research shows that *Trichodesmium* does not conform to Redfield stoichiometry with respect to its N:P ratios and that these ratios respond to, and are indicative of, P limitation in this abundant and cosmopolitan diazotroph.

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