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Variable depth distribution of *Trichodesmium* clades in the North Pacific Ocean

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Summary

Populations of nitrogen-fixing cyanobacteria in the genus *Trichodesmium* are critical to ocean ecosystems, yet predicting patterns of *Trichodesmium* distribution and their role in ocean biogeochemistry is an ongoing challenge. This may, in part, be due to differences in the physiological ecology of *Trichodesmium* species, which are not typically considered independently in field studies. In this study, the abundance of the two dominant *Trichodesmium* clades (Clade I and Clade III) was investigated during a survey at Station ALOHA in the North Pacific Subtropical Gyre (NPSG) using a clade-specific qPCR approach. While Clade I dominated the *Trichodesmium* community, Clade III abundance was >50% in some NPSG samples, in contrast to the western North Atlantic where Clade III abundance was always <10%. Clade I populations were distributed down to depths >80 m, while Clade III populations were only observed in the mixed layer and found to be significantly correlated with depth and temperature. These data suggest active niche partitioning of *Trichodesmium* species from different clades, as has been observed in other cyanobacteria. Tracking the distribution and physiology of *Trichodesmium* spp. would contribute to better predictions of the physiological ecology of this biogeochemically important genus in the present and future ocean.

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Introduction

Cyanobacteria in the genus *Trichodesmium* are keystone members of the phytoplankton community in the tropical and subtropical oceans. As a diazotroph, *Trichodesmium* provides the oligotrophic waters it inhabits with a source of new nitrogen (N), playing an important role in the global cycling of carbon (C) and N (Letelier and Karl, 1996; Capone *et al.*, 1997; Goebel *et al.*, 2007; Benavides and Voss, 2015). Recently, the genus has also been shown to have high rates of phosphate reduction, producing reduced forms like phosphite and methylphosphonate in the western North Atlantic (Van Mooy *et al.*, 2015). Methylphosphonate hydrolysis could be a source of the potent greenhouse gas, methane, to the upper water column (Karl *et al.*, 2008; Beversdorf *et al.*, 2010; del Valle and Karl, 2014). In short, *Trichodesmium* is central to the cycling of C, N, and phosphorus (P) in these vast oligotrophic systems.

Due to its ecosystem-level significance, many years of study have focused on investigating the distribution and abundance of *Trichodesmium* populations. While the factors controlling *Trichodesmium* growth and nitrogen (N_2) fixation are increasingly understood (Bergman *et al.*, 2013), there are still major knowledge gaps in predicting patterns in marine N_2 fixation, in part because the distribution of *Trichodesmium* is not well characterized. The distribution and abundance of the genus is spatially and temporally heterogeneous, and is sometimes, but not always, correlated with gradients in temperature and salinity (Foster *et al.*, 2007; Rouco *et al.*, 2014), iron (Fe) and P concentrations (Tyrrell *et al.*, 2003; Moore *et al.*, 2009; Snow *et al.*, 2015) and with both anticyclonic and cyclonic eddies (Davis and McGillicuddy, 2006; González Taboada *et al.*, 2010; Guidi *et al.*, 2012; Olson *et al.*, 2015a,b). Furthermore, much of the information on *Trichodesmium* distribution and abundance has been derived from surveys in the North Atlantic Ocean, specially the Sargasso and Caribbean Seas, followed by some areas of the North Pacific and South Pacific Oceans (Luo *et al.*, 2012). The inherent patchiness of

Trichodesmium, coupled with restricted surveys of its abundance and distribution, may hinder accurate modeling of these populations. In addition, the present difficulties in revealing the factors controlling the distribution and physiological ecology of this genus may be linked to clade- or species-specific variability, which is widely known for other cyanobacteria (Flombaum *et al.*, 2013; Biller *et al.*, 2015), but largely unexplored in *Trichodesmium*.

The genus *Trichodesmium* comprises 6 different species that have been grouped in 4 different clades (Hynes *et al.*, 2012). Clade I and Clade III populations are considered the most abundant in most field populations, based on cellular morphology (Carpenter *et al.*, 1993; Hynes *et al.*, 2012), 16S rDNA diversity (Hmelo *et al.*, 2012), and recent quantitative studies in the western North Atlantic (Rouco *et al.*, 2014). Clade I includes *T. thiebautii*, *T. tenue*, *T. hildebrandtii*, and *T. spiralis*, and Clade III includes *T. erythraeum* and *T. contortum* (Hynes *et al.*, 2012). There are few cultured representatives of the various species, and the vast majority of experimental work has focused on *T. erythraeum* strain IMS101 and other representatives of Clade III (Bergman *et al.*, 2013). Both laboratory work with representatives of Clade III and Clade I, and field work with colonies of different morphologies, suggest that there may be considerable variation in physiology within the genus, with observed variability in response to CO_2 (Hutchins *et al.*, 2013; Gradoville *et al.*, 2014), the cycling of reduced P (Dyhrman *et al.*, 2009; Van Mooy *et al.*, 2015), N_2 fixation (Webb *et al.*, 2007; Hynes *et al.*, 2009), and temperature (Chappell and Webb, 2010). Yet, most field studies have focused on the *Trichodesmium* genus as a whole and quantified its abundance by performing microscopic counts of filament and colonies (Janson *et al.*, 1995), using molecular tools targeting the *nifH* gene (Church *et al.*, 2005; 2008; Foster *et al.*, 2007), or using a Video Plankton Recorder (VPR) to observe and enumerate colonies (Davis and McGillicuddy, 2006; Olson *et al.*, 2015a,b). A new method was recently developed to quantitatively survey *Trichodesmium* Clade I and Clade III populations (Rouco *et al.*, 2014), to investigate the differential factors driving the ecology of these two *Trichodesmium* populations with finer resolution than previous approaches have provided.

In this study, the Eularian dynamics and vertical abundances of *Trichodesmium* Clade I and III populations were investigated in whole water samples collected during two cruises (KM1217 and KM1219) at Station ALOHA in the North Pacific Subtropical Gyre (NPSG). Using a clade-specific qPCR approach developed and validated by Rouco *et al.* (2014), the survey took advantage of the extensive field campaign conducted at Station ALOHA during the summer of 2012 (Wilson *et al.*, 2015) with near-daily sampling of upper water-column physical and chemical characteristics. This data set provides a unique opportunity to explore the temporal and vertical dynamics of *Trichodesmium* clades in the NPSG, as well as to discern potential factors driving niche partitioning within the *Trichodesmium* genus.

Results and discussion

Trichodesmium *spp. abundance*

A qPCR-based method for quantifying Clade I and Clade III (Rouco *et al.*, 2014) was applied to a sample set of depth profiles (5–100 m) collected at Station ALOHA (22°45′ N, 158 00′ W) during August and September 2012 (see Supporting Information; Table S1). Samples were collected at the same time of day (local time 4 am) by draining 12 L of water onto 10 μm filters for analysis after Rouco *et al.* (2014). Average abundance of *Trichodesmium* spp. (Clade I + Clade III) in the upper 100 m was 995 cells L^{-1}, and ranged from 4 to 10 720 cells L^{-1}, showing high day-to-day variability (Fig. 1 and Supporting Information Fig. S1). Satellite and hydrographic data (Fitzsimmons *et al.*, 2015; Wilson *et al.*, 2015) showed no clear advection of water masses into the study area over the majority of the sampling period that might explain this variability. Sea Surface Height Anomaly (SSHA) was stable during the study period with no eddies moving through the region of Station ALOHA (Fitzsimmons *et al.*, 2015). The advection of a low salinity water mass was observed on Sept. 6 (Wilson *et al.*, 2015), coinciding with the final time-point of the time-series, but this time point did not drive any of the patterns in *Trichodesmium* spp. abundance discussed below. In addition, wind (7 m sec^{-1}) and sea state (moderate), known to influence *Trichodesmium* spp. distribution (Olson *et al.*, 2015b), were mostly uniform in the area and therefore were unlikely to influence the daily changes observed here. Notably, daily variability was also observed for other phytoplankton groups during this period (Wilson *et al.*, 2015), indicating that high sampling frequency is required to resolve phytoplankton population dynamics in detail, especially when studying *Trichodesmium* which is known to fluctuate dramatically both in space and time (Church *et al.*, 2009).

Trichodesmium is present both as free filaments and as single colonies in the field, whose sizes can span from ~1000 to 30 000 cells $colony^{-1}$ (Letelier and Karl, 1996). The qPCR method used here for cell enumeration (Rouco *et al.*, 2014) has previously been shown to significantly correlate with total *Trichodesmium* spp. abundance assayed with microscopy, and is robust both at a low cell number and across several orders of magnitude. Yet, as discussed by Rouco *et al.* (2014), the presence of a random colony in a CTD bottle could

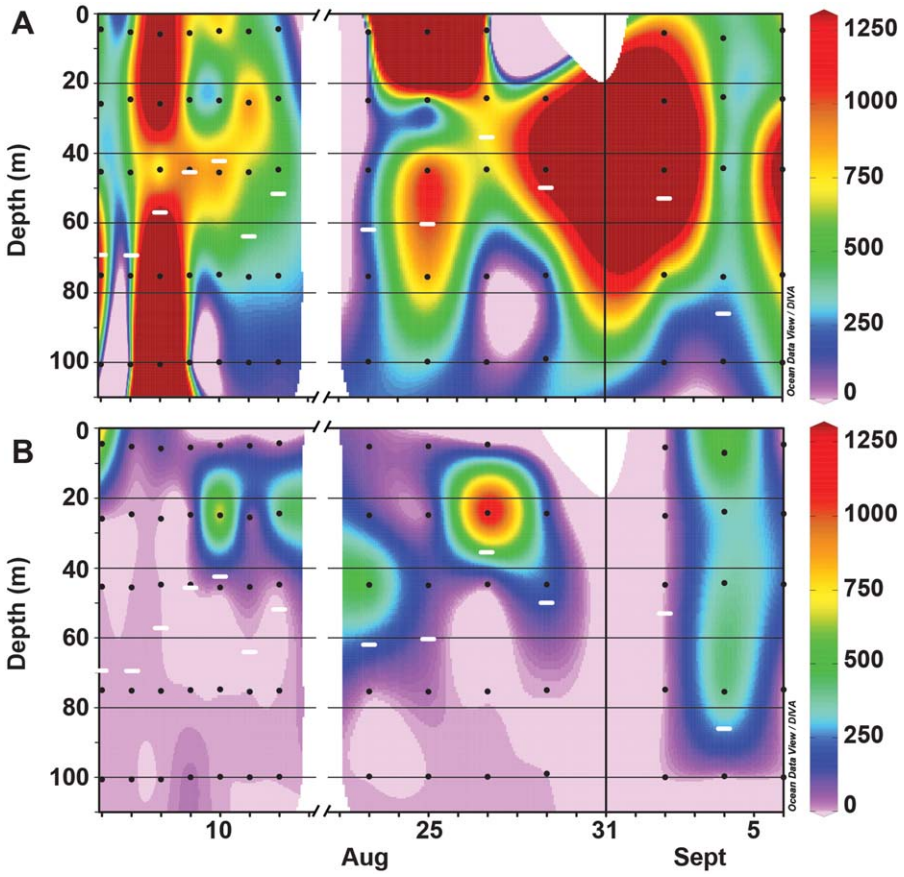

Fig. 1. Time-series of Clade I (A) and Clade III (B) abundance (cells L^{-1}) in the upper 100 m at Station ALOHA in the North Pacific Subtropical Gyre during August–September 2012. Samples were collected at the same time of the day from 12 L polyvinyl chloride bottles mounted on a rosette sampling device between August 4–14 (KM 1217) and August 23–September 6 (KM 1219). Absolute abundance values were calculated using a clade-specific qPCR approach targeting the *rnpB* gene with methods as described in Rouco *et al.* (2014). Black circles represent sample depth and white lines indicate mixed-layer depth (MLD). MLD was calculated based on a seawater potential density anomaly of 0.125 from the surface. Data were contoured using Ocean Data View 4.5.6 with the DIVA grid method (Schlitzer, R., http://odv.awi.de/). Note that for a better visual comparison between clades the scale in A and B are the same. Thus, all Clade I values above 1250 cells L^{-1} are grouped and represented with a dark red color.

occasionally drive the cell count for a sample higher by an unknown degree. Based on the low average cell number in our study (~900 cells L^{-1}) the majority of the *Trichodesmium* should be in the filament form (Letelier and Karl, 1996), and this was qualitatively confirmed by the rare presence of colonies observed in the net tows performed daily during the cruise, which integrated over many thousands of liters. Thus, while colony presence could potentially affect the cell concentration of individual samples, this likely did not affect the overall relationship observed in our study, which involved ~100 samples.

The qPCR-based abundances observed during the summer of 2012 fall within the range of microscopy-based abundances observed during a three-year study between 1989 and 1992, where average *Trichodesmium* abundance in the upper 45 m ranged from ~1000 to 9000 cells L^{-1} (Letelier and Karl, 1996). However, average total abundance (995 cells L^{-1}) was closer to the winter (October to March) than the summer (April to September) average values observed in Letelier and Karl (1996). Phytoplankton biomass and productivity during August 2012 were low in the vicinity of Station ALOHA and across the geographical area from 22 to 26°N and 152 to 160°W (Wilson *et al.*, 2015) compared with the historical time-series dataset (1989–2012) recorded by the Hawaii Ocean Time-series (HOT) program, and the factors driving this anomalously low productivity might have also affected *Trichodesmium* total abundance.

Overall, the average *Trichodesmium* abundance in the upper 100 m at Station ALOHA during the study period was ~8 times lower than the abundance observed in the western North Atlantic using the same method (Rouco *et al.*, 2014). Increased abundance of *Trichodesmium* in the western North Atlantic relative to other systems has

been observed using other molecular tools (Foster *et al*., 2007; Church *et al*., 2008), cell counts (Sohm *et al*., 2011), and image analysis from a VPR (Davis and McGillicuddy, 2006; Olson *et al*., 2015a). Although it is widely accepted that *Trichodesmium* has increased abundance in the western North Atlantic relative to other regions (Luo *et al*., 2012), an important consideration is that these Atlantic datasets typically include high station-to-station variability of up to three orders of magnitude (Foster *et al*., 2007; Rouco *et al*., 2014). This variability in *Trichodesmium* abundance may, in part, be driven by freshwater input from the Amazon River plume for studies that transect this feature (Foster *et al*., 2007; Subramaniam *et al*., 2008; Rouco *et al*., 2014; Hilton *et al*., 2015). Rouco *et al*. (2014) found stations outside of the plume region had *Trichodesmium* abundances similar to those observed in this study.

Trichodesmium *clade distribution*

The clade-specific assay employed here identified considerable variability in the abundance and distribution of both Clade I and Clade III populations at Station ALOHA (Fig. 1 and Supporting Information Fig. S1). On average, Clade I was the most abundant clade with an average value throughout the water column of 893, ranging from 4 to 10 644 cells L^{-1}, compared with the Clade III average abundance of 102, ranging from 0 to 1242 cells L^{-1} (Fig. 1). As a result, Clade I abundance over the study period significantly correlated with total *Trichodesmium* spp. abundance (Table 1, Table S2). However, Clade III populations increased in abundance on certain days at some depths, and even dominated (>50%) the *Trichodesmium* populations on others (e.g. August 6 at 5 m, August 10 at 25 m, and August 23 at 75 m; Fig. 1). This is in contrast to studies from the western North Atlantic where Clade III was always less than 10% of the *Trichodesmium* community (Rouco *et al*., 2014). Furthermore, Clade III abundances were not significantly correlated with Clade I abundances at Station ALOHA, as they were in the western North Atlantic (Rouco *et al*., 2014). The data presented here suggests that Clade III is not a uniformly small component of the community in all ocean basins, and current culture isolates like the genome strain IMS101 *T. erythraeum* (Clade III) may be better models for some systems, like the NPSG.

Trichodesmium *clade partitioning*

While Clade I populations were distributed throughout the upper 100 m of the water column with occasional peaks in abundance below 75 m, Clade III populations were only observed above the mixed layer depth (MLD), which ranged from depths of 35.5 to 86.0 m (Fig. 1). The contrasting depth distribution (Figs. 1 and 2) and lack of correlation between clades (Table 1, Table S2) is suggestive of spatial partitioning of *Trichodesmium* populations. Clade III abundance correlated negatively with depth, suggesting that factors related to depth (e.g., light, temperature, and nutrients) may drive niche partitioning within the genus. Depth segregation, mainly driven by light, has been observed in other groups of cyanobacteria, such as *Prochlorococcus* (Moore *et al*., 1998; West and Scanlan, 1999; Johnson *et al*., 2006), but is not known in *Trichodesmium*. Previous studies revealed inter-clade differences in the excitation spectrum of pigments in the *Trichodesmium* light-harvesting phycobilisome, with varied phycourobilin (PUB) to phycoerythrobilin (PEB) ratios in both field and culture representatives (Clade I: 1.31 ± 0.17 and Clade III: 0.83 ± 0.04; Neveux *et al*., 2006; Hynes *et al*., 2012). Both Neveux *et al*. (2006) and Hynes *et al*. (2012) suggested that this pigment differentiation could be key for the diversification of the *Trichodesmium* genus with depth, consistent with the observations herein. Unfortunately, a direct correlation between clade abundance and Photosynthetic Active Radiation (PAR) could not be explored in this study, since sampling was performed at night. Yet, Clade III was always observed above the midday 10% surface PAR depth ($PAR = 4.27 \pm 0.36$ mol quanta m^{-2} d^{-1}), which ranged from 49 m to 57 m throughout the sampling period, suggesting that light could in fact be a factor driving the observed distribution.

Clade III abundance also significantly correlated with temperature during the study period (Table 1, Table S2), with Clade III populations becoming a dominant (>50%) portion of the community when the temperature was above 25°C (Fig. 3). This observation could result from different temperature optima for growth between the two clades. There are few culture experiments that examine temperature optima for growth of different clade representatives in the same laboratory, where incubation conditions, including light, are internally consistent and most directly comparable. However, Chappell and Webb (2010) observed that a representative Clade I strain had a temperature optimum 2°C lower than two representative Clade III strains. Although extrapolation of these data to the field must be interpreted with caution, the temperature optima are consistent with an increase in competitive fitness for Clade III under higher temperatures, and could potentially explain the observed increase in Clade III abundance above 25°C.

Near-surface temperatures during July–August 2012 (25.3 ± 0.2) were 0.7–0.9°C lower than the respective monthly averages from the 1989 to 2011 climatology at Station ALOHA (Wilson *et al*., 2015). This suggests that Clade III populations could represent >50% of the *Trichodesmium* population under average summer temperatures and that the *Trichodesmium* community might be

Table 1. Pearson correlation coefficients (Pearson coef.) between total *Trichodesmium* abundance (Clade I + Clade III cells L^{-1}), Clade I or Clade III abundance (cells L^{-1}) and temperature (Temp), salinity, and depth. Significant correlations, corrected for multiple testing (Benjamini and Hochberg, 1995), are indicated in bold. Temperature and salinity profiles were obtained using a CTD package (SBE 911Plus, SeaBird).

	Clade I (cells L^{-1})	Clade III (cells L^{-1})	Temp (°C)	Salinity	Depth (m)
Total					
Pearson coef.	0.990	0.089	0.129	−0.007	−0.184
p	**0.000**	0.638	0.434	0.953	0.281
Clade I					
Pearson coef.		−0.052	0.080	0.012	−0.140
p		0.768	0.641	0.953	0.434
Clade III					
Pearson coef.			0.350	−0.136	−0.311
p			**0.009**	0.434	**0.023**
Temperature					
Pearson coef.				−0.393	−0.847
p				**0.004**	**<0.001**
Salinity					
Pearson coef.					0.420
p					**<0.001**

dominated by different clades in the winter versus summer period. This observation in the NPSG contrasts with previous work in the western North Atlantic where both clades strongly correlated with temperature, and Clade III abundances were routinely low at temperatures >25°C (Rouco *et al*., 2014). It is possible that different *Trichodesmium* species, or strains, in each ocean basin have different temperature optima, and this could be resolved with qPCR primers designed to distinguish *Trichodesmium* at the species level. Recent studies have revealed unique features in the *Trichodesmium* genome (Pfreundt *et al*., 2014; Hilton *et al*., 2015; Walworth *et al*., 2015) which suggest that species within this genus might have the potential for rapid evolution and adaptation in response to changing biogeochemical regimes, and this might explain differential patterns in clade abundance relative to temperature between these ocean basins. The relationship between clade abundance and temperature also may influence the potential selection and distribution of *Trichodesmium* clades with future ocean warming. Recent modeling work suggests that even subtle differences in growth responses to future ocean conditions may shift community structure (Dutkiewicz *et al*., 2015). Seasonal observations of *Trichodesmium* clade-specific abundance in this region would help identify the consistency of the relationship with temperature.

The significant correlation of Clade III abundance with temperature could have been driven by other factors that co-varied with depth and temperature, such as light, salinity, iron, nitrate or phosphorus, especially considering the strong correlation between depth and temperature (Table 1, Table S2 and Fig. 2). A relationship between nutrients, iron, and clade abundance could not be directly resolved in this study given the lower frequency of these biogeochemical measurements compared with the qPCR sampling. However, average vertical distribution patterns of phosphate (Fig. 2) and iron (Fitzsimmons *et al*., 2015) throughout July-August showed a decrease in both phosphate and iron with depth, and thus, their availability could have contributed to the observed depth segregation. In fact, phosphorus has already been suggested as a possible driver of niche partitioning in the North Atlantic (Rouco *et al*., 2014), and differential phosphorus (Dyhrman *et al*., 2009) and iron (Chappell and Webb, 2010) metabolism has been hypothesized within the *Trichodesmium* genus (Dyhrman *et al*., 2009). Previous studies in the NPSG (Karl *et al*., 1992; Letelier and Karl, 1996; White *et al*., 2006) have suggested that *Trichodesmium* colonies might regulate their buoyancy to mine phosphorus at depth (Villareal and Carpenter, 1990; Letelier and Karl, 1998; White *et al*., 2006). The differential depth distribution of *Trichodesmium* clades observed here could thus be associated with buoyancy capacities unique to species of the two clades. These differences may in turn be related either to a unique cell physiology, such as a differential production of carbohydrates that counteract positive buoyancy (Villareal and Carpenter, 1990), or to a differential aggregation of these species in colonies of different morphologies or sizes, since colony size appears to be an important factor controlling vertical migration below the MLD (White *et al*., 2006). The qPCR clade-specific quantification methods are destructive and the separation of clade representatives for activity assays or biochemical quantification of colony size, carbohydrate pools, or other measurements was not possible to further study this question. Finally, it is important to consider that

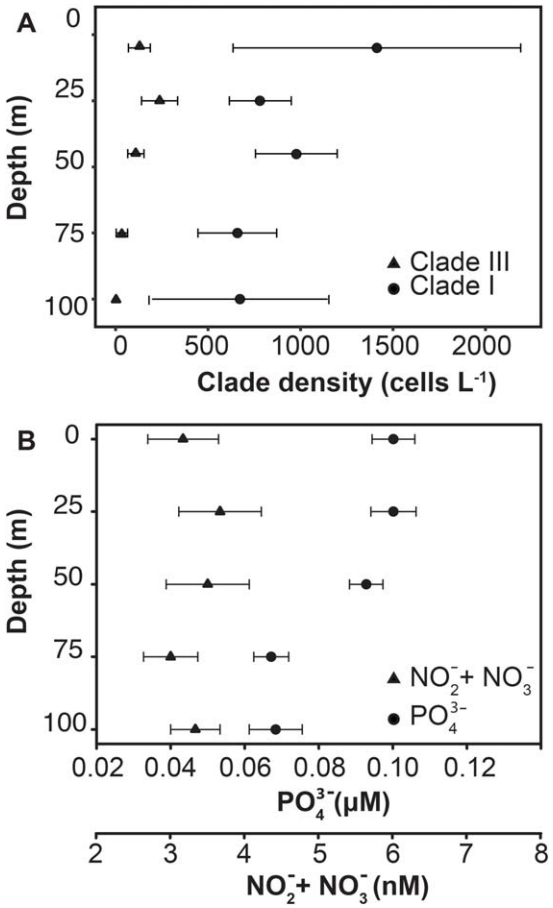

Fig. 2. Averaged vertical profiles of clade abundance and nutrient concentrations. Error bars correspond to standard error of the mean ($n = 14$ for clade abundance, n = 8 for PO_4^{3-}, and n = 6 for $NO_2^- + NO_3^-$). Nutrient analyses (PO_4^{3-} and $NO_2^- + NO_3^-$) were performed using protocols employed by the Hawaii Ocean Time-series (HOT) program (http://hahana.soest.hawaii.edu/hot/protocols/protocols.html).

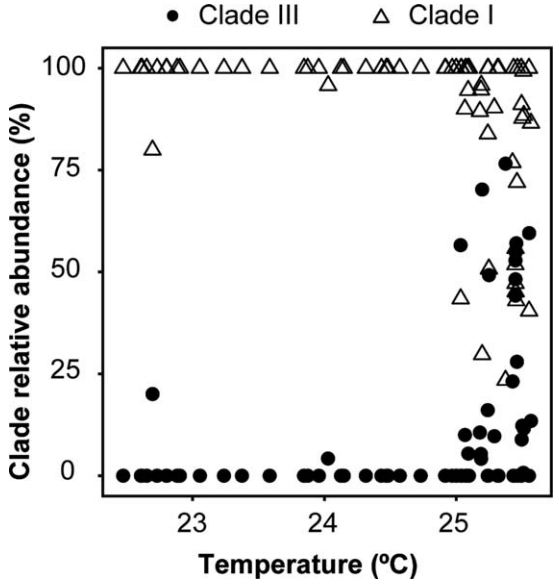

Fig. 3. *Trichodesmium* Clade I and Clade III relative abundance (clade %) as a function of temperature. The highest percentages of Clade III were observed at temperatures above 25°C.

Trichodesmium is tightly associated with an abundant heterotrophic epibiont community (Paerl *et al.*, 1989; Sheridan *et al.*, 2002), and these communities can play a role in both phosphorus and iron metabolism (Roe *et al.*, 2012; Van Mooy *et al.*, 2012). With the complexity of the *Trichodesmium* holobiont it is difficult to entirely discern if these depth patterns are associated with resource distribution related to the physiology of *Trichodesmium* spp. or if the patterns are also influenced by variability in the epibiont heterotrophic community.

To summarize, *Trichodesmium* spp. from Clades I and III show distinct depth distributions in the NPSG, and both are highly variable even within the context of this high-resolution dataset. The correlation between depth, temperature and Clade III abundance is suggestive of the important role that temperature, and other factors that co-vary with it, such as light or nutrients, may play as niche-defining features for species falling in this clade. If *Trichodesmium* clades have distinct physiological ecology and segregate their niche space, as is suggested herein, this would contribute to the known difficulties in predicting *Trichodesmium* distribution in the field (Davis and McGillicuddy, 2006; Luo *et al.*, 2012; Olson *et al.*, 2015a,b) and emphasizes that in some instances it might not be appropriate to include *Trichodesmium* populations as a single entity in biogeochemical models. Climate-related increases in global sea surface temperature and stratification are predicted in the future ocean (Moss *et al.*, 2010). Such changes could intensify the uncertainty associated with the prediction of the abundance and distribution of these populations. Higher temperatures could favor Clade III in the NPSG, leading to a differential adaptation of *Trichodesmium* clades in the NPSG, similar to what is predicted for *Prochlorococcus* ecotypes (Flombaum *et al.*, 2013; Biller *et al.*, 2015). A differential response of *Trichodesmium* strains to climate change, such as increasing CO_2, has been observed in laboratory cultures (Hutchins *et al.*, 2013) and field studies (Gradoville *et al.*, 2014), which may also favor certain clades in the future ocean. More work is required to track and assay the physiological ecology of *Trichodesmium* beyond the genus level to more fully parameterize the activities and distributions of this important diazotroph. Moving forward, this information stands to improve predictions of abundance and

distribution patterns of this biogeochemically important species in the present and future ocean.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Fig. S1. Time series of Clade I abundance (cells L^{-1}) in the upper 100 m at Station ALOHA in the North Pacific Subtropical Gyre during August-September 2012. Black circles represent sample depth and white lines indicate mixed layer depth. The color scale includes the whole range of abundance values for Clade I.

Table S1. Primer sequences, product sizes and efficiencies for the qPCR reactions targeting the *rnpB* gene. Primer sequences were obtained from Chappell and Webb (2010).

Table S2. Pearson correlation coefficients (Pearson coef.) between total *Trichodesmium* abundance (Clade I + Clade III cells L^{-1}), Clade I or Clade III abundance (cells L^{-1}) and temperature (Temp), salinity, and depth after removal of outliers with high cell density.