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Gammaproteobacterial diazotrophs and *nifH* gene expression in surface waters of the South Pacific Ocean

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Abstract

In addition to the cyanobacterial N_2-fixers (diazotrophs), there is a high *nifH* gene diversity of non-cyanobacterial groups present in marine environments, yet quantitative information about these groups is scarce. N_2 fixation potential (*nifH* gene expression), diversity, and distributions of the uncultivated diazotroph phylotype γ-24774A11, a putative gammaproteobacterium, were investigated in the Western South Pacific Ocean. γ-24774A11 gene copies correlated positively with diazotrophic cyanobacteria, temperature, DOC, and ambient O_2 saturation, and negatively with depth, chlorophyll *a*, and nutrients, suggesting that carbon supply, access to light, or inhibitory effects of DIN may control γ-24774A11 abundances. Maximum *nifH* gene copy abundance was $2x10^4$ L^{-1}, two orders of magnitude less than for diazotrophic cyanobacteria, while the median γ-24774A11 abundance, $8*10^2$ L^{-1}, was greater than for the UCYN-A cyanobacteria, suggesting a more homogeneous distribution in surface waters. The abundance of *nifH* transcripts by γ-24774A11 was greater during the night than during the day, and the transcripts generally ranged from 0 to 7%, but were up to 26% of all *nifH* transcripts at each station. The ubiquitous presence and low variability of γ-24774A11 abundances across tropical and subtropical oceans, combined with the consistent *nifH* expression reported in this study, suggest that γ-24774A11 could be one of the most important heterotrophic (or photoheterotrophic) diazotrophs and may need to be considered in future N budget estimates and models.

Introduction

Biological nitrogen (N_2) fixation, the reduction of atmospheric N_2 gas to ammonium, is a process performed only by certain microorganisms (diazotrophs). This bioavailable N is an important source of N that supports primary production in oligotrophic marine ecosystems (Karl et al. 1997), where N availability often limits phytoplankton growth (Graziano et al. 1996, Mills et al. 2004). Rate measurements and geochemical estimates suggest N inputs via N_2 fixation are much smaller than the N loss processes through denitrification (including anaerobic ammonium oxidation) (Mahaffey et al. 2005). A better understanding of the identity, distributions, and activity of microorganisms contributing to N_2 fixation in the oceans is necessary in order to balance the oceanic N budget (Zehr and Kudela 2011).

The diversity and abundances of diazotrophs in the oceans have been studied using sequence diversity and abundances of the *nifH* gene, which encodes a key structural protein of the nitrogenase enzyme that catalyzes the N_2 fixation reaction (Zehr and Paerl 1998). The most significant N_2-fixing microorganisms in the ocean are thought to be the cyanobacteria *Trichodesmium* (Capone et al. 1997), symbiotic and free-living unicellular cyanobacteria (UCYN, including *Crocsphaera*) (Zehr et al. 2001, Montoya et al. 2004), and filamentous symbionts (of the order *Nostocales*) that associate with diatoms (Villareal 1994, Carpenter et al. 1999). Molecular surveys from various marine environments have also recovered a high diversity of *nifH* genes that cluster with non-cyanobacterial Bacteria (Zehr et al. 2003b) in surface waters and even below the epipelagic zone in the open ocean (Langlois et al. 2005, Hewson et al. 2007, Hamersley et al. 2011, Halm et al. 2012). The diazotrophic bacteria that have been obtained from open ocean epipelagic seawater cluster with a wide range of bacterial groups, including Alpha-, Beta-, Gamma-, and Deltaproteobacteria, and Firmicutes (Zehr et al.

63 1998, Zehr et al. 2003b). A majority of the diverse heterotrophic communities detected at the
64 DNA level in marine environments have not been detected in mRNA (Omoregie et al. 2004,
65 Man-Aharonovich et al. 2007, Short and Zehr 2007), thus the contribution of *nifH* gene-
66 containing heterotrophic bacteria to the N cycle in oceanic environments remains unclear. These
67 groups potentially contribute to the N_2 fixation activity that is often reported in the <10 μm small
68 size-fraction (Staal et al. 2007a, Bonnet et al. 2009, Benavides et al. 2011). A small number of
69 heterotrophic bacterial sequence types have been repeatedly found in open ocean clone libraries,
70 which includes a Gammaproteobacteria-affiliated group that we here call γ-24774A11 based on a
71 *nifH* sequence recovered from the South China Sea (Moisander et al. 2008). It is not known
72 whether it is free-living or forming symbioses such as the diazotrophic cyanobacterium UCYN-
73 A (*Candidatus* Atelocyanobacterium thalassa) (Thompson et al. 2012). Different
74 gammaproteobacterial *nifH* sequences have been recovered from tropical and subtropical (Zehr
75 et al. 1998) to polar (Diez et al. 2012) oceans. γ-24774A11 appears to have a broad geographic
76 distribution, including the Atlantic and Pacific Oceans, Mediterranean Sea, Red Sea, Arabian
77 Sea, and South China Sea, and its *nifH* transcripts have been detected in several studies (Falcon
78 et al. 2004, Bird et al. 2005, Church et al. 2005, Bombar et al. 2011, Turk et al. 2011). The
79 frequent observations and *nifH* transcription indicate that this group may play a more significant
80 role in oceanic N_2 fixation than the other *nifH* gene-containing heterotrophic bacteria. To date,
81 the γ-24774A11 phylotype remains uncultivated and no other genetic information besides the
82 *nifH* sequence is available from this bacterium. Its ecophysiology, genetic potential, and
83 contribution to oceanic N_2 fixation are unknown.

84 Diurnal cycles in oceanic cyanobacterial diazotroph N_2 fixation and *nifH* expression have
85 been described, but data on diel patterns in *nifH* expression in heterotrophic diazotrophs are

86 scarce. Diel patterns of N_2 fixation activity in the marine cyanobacteria *Trichodesmium*, UCYN-
87 A, and *C. watsonii* have been reported, and while probably partially regulated by circadian
88 rhythms (Chen et al. 1996), the cycles of nitrogenase activity are directly linked with access to
89 energy supporting the enzyme and strategies for protection against inhibition by O_2 (Postgate
90 1990, Rabouille et al. 2006). The diel cycle in the marine filamentous cyanobacterium
91 *Trichodesmium* is well characterized, with the majority of the N_2 fixation occurring during the
92 day (Staal et al. 2007b). The uncultivated UCYN-A, a heterotrophic cyanobacterium, has been
93 thought to express *nifH* primarily during the day (Church et al. 2005) while peak *nifH* expression
94 and N_2 fixation of *C. watsonii* occurs during the night (Tuit et al. 2004, Shi et al. 2010).
95 Although mRNA from heterotrophic bacterial groups has been detected in several studies, little
96 information is available on spatial and temporal variability in *nifH* gene expression of non-
97 cyanobacterial oceanic diazotrophs, particularly in natural conditions. Heterotrophs might be
98 expected to also have a diel pattern, since they may be dependent upon light generated
99 photosynthate from phytoplankton, or may be dependent on supplementary light driven energy,
100 perhaps through proteorhodopsins.

101 In this study, we detected the γ-24774A11 *nifH* gene sequence cluster in the low-nutrient,
102 low-chlorophyll waters of the Western South Pacific Ocean. We investigated its diversity
103 compared to other ocean regions, quantified abundances of the phylotype, and determined day
104 vs. night *nifH* expression with respect to depth in the epipelagic waters. Comparisons of the day-
105 night expression at nine stations across the region allowed us to determine the *nifH* expression
106 diel pattern of this diazotroph. The results were compared with those for the major
107 cyanobacterial groups, and the influence of environmental parameters was investigated. The
108 information on diel variability in gene expression and distribution patterns in comparison to

109 other diazotrophs further underscores the need to fully determine the importance of non-
110 cyanobacterial diazotrophs in oceanic N_2 fixation.
111
112 **Materials and Methods**
113 Samples were collected onboard *R/V Kilo Moana* in March-April, 2007 from Coral Sea,
114 Australia (155°E, 15°S), moving southeast (to 160°E, 30°S), then returning back to New
115 Caledonian and Fijian waters, reaching 170°W, 15°S. Samples were taken from 23 stations over
116 34 d (Figure 1).
117 The water column density structure, O_2, and *in situ* fluorescence were determined from
118 conductivity-temperature-depth profiles. Discrete samples were collected for extracted
119 chlorophyll *a,* (Welschmeyer 1994) nutrients (SRP, NO_3^-, NO_2^-) (Strickland and Parsons 1972,
120 Sakamoto et al. 1990, Karl and Tien 1992, Zhang 2000), dissolved organic carbon (DOC)
121 (Carlson et al. 2004), total dissolved nitrogen (TN) (Walsh 1989), and abundances of
122 picoplankton (Zehr et al. 2008), from the same depths as those for determination of abundances
123 and gene expression of diazotrophs. Methods are described elsewhere in detail (Moisander et al.
124 2010).
125 For DNA and environmental parameters, samples were collected from 8 depths between
126 0 and 175 m (Table S1). Approximately 4.5 L of surface water was sequentially filtered through
127 10-μm (polycarbonate, Osmonics) and 0.2-μm (Supor, Pall Gelman) filters. DNA was extracted
128 using a modified Qiagen Plant kit protocol (Moisander et al. 2008). Abundances of γ-24774A11
129 were determined by the quantitative Taqman® 5'-nuclease assay PCR (Table S2) (Short and
130 Zehr 2005, Moisander et al. 2010). Data for abundances of four groups of diazotrophs (UCYN-

A, *C. watsonii*, *Trichodesmium* spp., and filamentous cyanobacterial symbionts) are discussed elsewhere (Moisander et al. 2010) (Table S1).

Nested PCR with degenerate *nifH* primers (Zehr and Turner 2001) was used to create a *nifH* clone library, using previously reported amplification conditions (Moisander et al. 2008). Fourteen samples in which high γ-24774A11 abundances were recorded by qPCR were chosen for clone libraries. In order to further address potential microdiversity, γ-24774A11-specific primers were designed. Flanking regions in the 24774A11 partial *nifH* sequence generated by the degenerate primer approach were targeted (Oligoperfect, Invitrogen). The primers (Table S2) resulted in a product with an expected fragment length of 281 bp. The 24774A11-PCR cycling was initiated by 95°C for 2 min, followed by 30 s at 94°C, 30 s at 56°C, and 30 s at 72°C for 30 cycles, and finally 7 min at 72°C. Bands were excised, cloned into a pGEM-T (Promega) vector, and sequenced at the UC Berkeley sequencing facility. If bands were observed in negative controls they were sequenced. Sequences were trimmed using CLC Workbench (Aarhus, Denmark) and imported into a *nifH* database in ARB software (Ludwig et al. 2004). The sequences were aligned against a *nifH* database containing >23,000 sequences, originally aligned using a HMMER algorithm based on PFAM. Neighbor-joining trees were created for conceptually translated sequences using Kimura correction in ARB. The sequences from this study have GenBank accession numbers HQ229006-HQ229036 and KF619449-KF619537.

Expression of the *nifH* gene by γ-24774A11, UCYN-A, *Crocosphaera*, and *Trichodesmium* were investigated by qRT-PCR. RNA samples were collected around mid-day (filtration time 13:00-15:30) or midnight (filtration time 21:40-3:40) into acid-washed 4.5-L polycarbonate bottles using Niskin bottles from four depths in the euphotic layer, followed by filtration. Identical samples were collected at each time point and placed in a deck incubator for

12 h, then filtered during the opposite light phase. In a few cases holding in the incubator was not necessary, if sampling was conducted at the same station both during the day and night. RNA samples were filtered similarly to the DNA samples, and filters were placed in sterile tubes containing 350 µL RLT buffer (Qiagen RNeasy minikit) with 1% β-mercaptoethanol and ~50 µL of a mixture of 0.1-mm and 0.5-mm diameter glass beads (BioSpec Products). Tubes were frozen in liquid nitrogen and stored at -80°C.

RNA was extracted using a modified RNeasy protocol. Samples were homogenized over two 2-min intervals (Mini-Beadbeater-96, BioSpec Products), with 1-min cooling on ice between bead beatings. Tubes were centrifuged at 8,000 *g* for 2 min, filters were removed, and tubes centrifuged again. The supernatants were transferred to new 2-mL tubes and 250 µL of 100% ethanol was added. RNA purification was then carried out according to the manufacturer's protocol, including a 1-h on-column DNase digestion. Samples were eluted into 50 µL of RNase-free water and stored at -80°C. RNA was reverse-transcribed using SuperScript III First-Strand Synthesis System for RT-PCR (Life Technologies), with 8 µL of RNA extract. Gene-specific reverse primers nif2 and nif3 (Zehr and Turner 2001) were added at 5 pmol each. Negative control and no-qRT reactions were run for each sample using DEPC-treated water in place of the reverse transcriptase.

nifH gene expression was quantified using qRT-PCR assays targeting γ-24474A11 and four cyanobacterial diazotrophs (Table S2). Expression by UCYN-A, *Crocosphaera* and γ-24774A11 was measured from 0.2-µm filters. 10 µm filters from daytime were assayed for *Trichodesmium* and heterocyst-forming diatom symbiont *Richelia* (Het-1). qPCR was conducted on RT and no-RT products for each sample using Applied Biosystems (ABI) 7500 Real-Time PCR system using 2 min at 50°C followed by 45 cycles of 15 s at 95°C and 1 min at 60°C. The

25-µL reactions consisted of 12.5 µL ABI TaqMan Gene Expression Master Mix, 0.4 µM and 0.2 µM final concentrations of primers and probe, respectively, 2 µL template, and 8 µL water. Standards, consisting of eight 10-fold dilutions of linearized recombinant plasmids containing the relevant *nifH* targets (10^7-10^0 copies/reaction), were run on each plate. Standard curves were generated by linear regression of threshold cycle (Ct) vs. log number of gene copies per reaction. Linear regression R^2 values were ≥0.99 for all standard curves. Amplification efficiencies were calculated using the equation $E=10^{-1/m}$-1 where m is the slope of the standard curve. Efficiencies were >90% for all reactions. Standards, qRT and no-qRT samples were run in duplicate. Where amplification of no-qRT samples occurred, no-qRT gene copy values were subtracted from qRT gene copies to correct for DNA contamination. Six to eight no template controls were run on each plate. Inhibition tests were carried out for each primer/probe set by delivering 2 µL each of 10^5 standard and cDNA sample to the same well and assessing the percent efficiency of the reaction in relation to the amplification of the standard alone (%Efficiency=[1- ($Ct_{inhibition}$-$Ct_{standard}$)/$Ct_{standard}$]*100). Inhibition tests were conducted on a subset of 8 cruise samples (station 5). The reaction efficiencies of inhibition tests were 97.3% or greater, thus, all samples were considered uninhibited. The limits of detection and quantification have been empirically determined to be 1 and 8 copies per reaction, respectively (data not shown). Amplifications falling below the limit of quantification were designated as detected but not quantifiable, (DNQ).

Day and night time transcript abundances were compared by non-parametric paired tests (Wilcoxon Matched Pair Signed Ranks Test) for each *nifH* phylotype (IBM SPSS Statistics). Relationship of the abundances to environmental parameters was analyzed by linear regression (IBM SPSS Statistics). Data distribution was improved by log transformation. Abundances of

199 gene copies and transcripts in the water column (from 0 to 150 m and from 0 to 125 m,
200 respectively) were calculated by numerical integration.
201
202 **Results**
203 Phylogenetic analysis showed that amplified *nifH* gene sequences contained
204 cyanobacterial and non-cyanobacterial *nifH* genes. A *nifH* sequence that closely matched
205 UCYN-A was recovered from 9 samples (Figure 2). These sequences had a 100% identity to
206 *nifH* in the published UCYN-A (*Candidatus* Atelocyanobacterium thalassa) genome (Tripp et al.
207 2010). Sequences were recovered with a 99% amino acid identity with *C. watsonii* WH8501 and
208 *Trichodesmium erythraeum* IMS101, respectively. An additional *nifH* phylotype (clone
209 33523A05) was recovered that had 88% amino acid sequence identity to the UCYN-A *nifH*
210 sequence, and 96% amino acid sequence identity to a sequence from the North Pacific Ocean
211 (DQ821974). One additional sequence type fell within the cyanobacteria clade, and another
212 sequence was obtained that fell in Cluster 3 (as defined by (Zehr et al. 2003b).
213 Gene sequences were recovered using the nested *nifH* PCR approach that fell within the
214 non-cyanobacterial γ-24774A11 cluster (Figures 2, 3). Each of these sequences had one or more
215 amino acid substitutions in comparison with γ-24774A1-like sequences from prior studies. A
216 total of 88 sequences were obtained using the γ-24774A11–specific primers (Figure 3). The
217 majority of these sequences (83 of 88) were from one phylotype that is identical to the original γ-
218 24774A11 clone (Moisander et al. 2008). Five additional phylotypes were recovered that each
219 differed by one amino acid over the 93 residue γ-24774A11 sequence (Figure 3).
220 Sequences were recovered from the negative PCR bands in the nested reactions, which
221 were assumed to originate from PCR reagents (Zehr et al. 2003a). These sequences fell into their

own cluster within Alphaproteobacteria (Figure 2). We queried the *nifH* database with terms 'PCR contaminant' and 'negative' and included resulting sequences in our tree that were close matches with sequences we recovered from negative PCR bands. Previously reported negative control sequences, including *Derxia* sp. and *Burkholderia* sp. related sequences, were close to the sequences from the negative control reactions from this study.

γ-24774A11 distributions based on gene copies

Gene copy abundances of γ-24774A11 varied from non-detectable to $2*10^5$ gene copies L^{-1}, and decreased below approximately 100 m depth (Figure 4). The maximum abundance of γ-24774A11 was $2*10^4$ L^{-1} (at station 21) and thus two orders of magnitude lower than the maxima for UCYN-A, *C. watsonii*, or *Trichodesmium* (Table 1). The median abundance across all samples, $8x10^2$ copies L^{-1}, however, was higher for γ-24774A11 than for UCYN-A or *Trichodesmium* (Table 1).

The abundances of γ-24774A11 were compared to those of other diazotrophs and to several environmental factors (Moisander et al., 2010) (Table S1, S3). There was a significant positive correlation between γ-24774A11 abundance and temperature, DOC, and O_2 saturation ($p=0.000$, $n=156$-173, Table S3). O_2 saturation varied from 61 to 102% in the 0-150 m surface layers, decreasing towards the DCM (Figure S1), with no clear difference between day and night. The average euphotic zone depth was 100.6 m (±13.4) (Table S1), when using 1% PAR from PAR in 5 m as the bottom of the euphotic layer. There was a negative correlation between γ-24774A11 abundance and depth, density, Sigma-T, NO_3, NO_2, SRP, TN, chlorophyll *a*, and *in vivo* fluorescence (Table S3). The abundance of γ-24774A11 was positively correlated with abundances of *C. watsonii*, *Trichodesmium*, Het-1, and *Synechococcus* cell numbers (Table S3).

245

246 *nifH expression*

247 Unicellular diazotroph *nifH* gene expression was detected throughout the study area
248 during the day and night (Figure S2, Figure 5). Transcript abundances for γ-24774A11 were
249 significantly higher at night than during the day (p=0.012, n=36, Wilcoxon Matched-Pair Signed
250 Ranks Test; Figure 5, Figure S2, Table 2) and the same pattern was observed in *C. watsonii*
251 (p=0.000, n=36). In contrast, transcript abundances were not significantly different between day
252 and night for UCYN-A (p=NS, n=36). For all three unicellular diazotrophs, there was depth
253 dependence of transcript abundances, with greatest abundances at the top 75-100 m of the water
254 column (Figure S2). Daytime *nifH* transcript abundances were up to 10^7 L^{-1} for *Trichodesmium,*
255 and up to 10^3 *nifH* transcripts per L^{-1} for Het-1 (Table 2).

256 Integrated transcript abundances for the entire upper water column (0-125 m) (transcripts
257 m^{-2}) were calculated and the proportions of diazotroph phylotypes in the community compared
258 (Figures 5, 6). Integrated transcript abundances were the most constant for γ-24774A11, with
259 little variability among stations (Figure 5). Average integrated transcript abundances for UCYN-
260 A ($1*10^9$ transcripts m^{-2} during the day and $3*10^8$ m^{-2} at night) were 1-2 orders of magnitude
261 greater than for γ-24774A11 ($3*10^7$ during the day and $8*10^7$ at night), but had much greater
262 variability (range from no detectable transcripts to $8*10^9$ m^{-2} in UCYN-A, and from $6*10^4$ to
263 $3*10^8$ m^{-2} in γ-24774A11). The abundances were greatest at the southernmost station 10 for
264 UCYN-A, and at the mid-latitude station 6 for γ-24774A11. *C. watsonii* had the greatest
265 transcript abundances of the three unicellular diazotrophs, with a maximum of $9*10^{10}$ transcripts
266 m^{-2} at one of the northernmost stations (Stn 21) at night. The greatest transcript abundances for
267 *Trichodesmium* and Het-1 were also detected at station 21 (Figures 5, 6). For Het-1, the

transcript abundance range ($3*10^4$ to $1.3*10^8$ m^{-2}) and average ($4*10^7$) during the day were very close to the values for γ-24774A11. UCYN-A comprised the majority of transcripts at the southernmost stations (8, 10, and 12) during the day and at station 10 during the night (Figure 6). *Crocosphaera* dominated transcripts at eight of the nine stations during the night and at two northwestern stations during the day (stations 4 and 6). *Trichodesmium* comprised the majority of daytime transcripts at four stations near the end of the transect. Het-1 formed 10% of transcripts at station 4 and less than 5% at all other stations during the day. The contribution of γ-24774A11 to the total transcript pool was between 0 and 7.4% of all transcripts, with the exception of station 4 during the day (24% of transcripts), and station 24 at night (26% of transcripts).

Discussion

Understanding the ecophysiology of the contributors to marine N_2 fixation is key for characterizing the controls and limitations of the oceanic N cycle. In spite of the known biogeochemical importance of N_2 fixation, quantitative PCR studies suggest that diazotrophs in oceans are several orders of magnitude less abundant than dominant non-diazotrophic bacteria. Although diazotrophs could therefore be considered rare species, their unique functional niche is biogeochemically-important in the marine ecosystem. Here we describe the diversity, abundances, and N_2 fixation gene diel expression in a non-cyanobacterial, gammaproteobacterial diazotroph in comparison with the major oceanic cyanobacterial diazotrophs.

Diversity

290 The results suggest that the γ-24774A11 *nifH* phylotype generally has a wide distribution in
291 different oceans but that microdiversity is also important. The population in this study was
292 dominated by one *nifH* phylotype that has been detected in several oceans, including the Atlantic
293 (Langlois et al. 2005), South China Sea (Moisander et al. 2008, Bombar et al. 2011), North
294 Pacific (Church et al. 2005), and Red Sea (Foster et al. 2009). Additional closely-related
295 phylotypes were detected in this study, the North Pacific (Fong et al. 2008), Arabian Sea (Bird et
296 al. 2005), Mediterranean Sea (Man-Aharonovich et al. 2007), and Southern California Bight
297 (Hamersley et al. 2011).

298 The presence of *nifH* sequences as PCR reagent contaminants has been reported (Zehr et
299 al. 2003a, Bostrom et al. 2007), and frequently the same groups within Alphaproteobacteria have
300 been reported as contaminants. We conclude that all sequences from the negative controls in this
301 study that clustered closely with these Alphaproteobacteria, were indeed contaminating
302 sequences. The nested *nifH* PCR protocol is known to be sensitive to reagent contamination
303 (Zehr et al. 2003a), but the contaminants that appear in negative bands can be disregarded by
304 sequencing them and removing these sequences from further analysis.

305

306 *Abundance of γ-24774A11*

307 The results suggest that the maximum abundances of the γ-24774A11 phylotype
308 typically are lower than for the cyanobacterial diazotrophs (in this study they were two orders of
309 magnitude lower). However, median abundances were higher than for UCYN-A and
310 *Trichodesmium*, suggesting a more uniform distribution than of the cyanobacterial groups. The
311 ubiquitous presence could suggest a broad tolerance for certain environmental conditions. For
312 UCYN-A, the presence of conditions promoting the host organisms of this symbiotic

cyanobacterium may play a key role, although they thus far remain unknown. Abundances of UCYN-A appear to be patchy both horizontally and vertically, likely following stratification of its eukaryotic hosts. Such stratification or patchiness is not evident with γ-24774A11 in this study, suggesting it is not dependent on a specific host, but may be a free-living heterotroph or photoheterotroph. Alternatively, it may be benefiting from associations with a host that is ubiquitous, or may be associated with particulate organic matter (Le Moal and Biegala 2009). The greatest γ-24774A11 abundances were found in the <10-μm size fraction, suggesting that this diazotroph does not rely on a large host. However, the host may be small, or if the association with a host is loose, γ-24774A11 may dissociate during sample handling, as was found for UCYN-A (Thompson et al. 2012).

Influence of environmental conditions

N_2 fixation, catalyzed by the nitrogenase enzyme, is strictly environmentally controlled. Importantly, the enzyme is destroyed by O_2 (Fay 1992), and the process requires a large amount of energy. Many ecological strategies have evolved among diazotrophs to avoid O_2 inhibition. Cyanobacteria protect their nitrogenase from inhibition by O_2 using either spatial (specialized cells such as heterocysts in *Nostocales*) or temporal (day-night variability) separation, while heterotrophic bacteria may use colony formation to protect cells from O_2, allowing spatial separation of N_2 fixation from extracellular O_2 (Fay 1992). A well-known example of an obligate aerobe in soils that fixes N_2 aerobically by using respiratory protection is the Gammaproteobacterium *Azotobacter vinelandii*, currently classified in *Pseudomonadaceae* (Rediers et al. 2004). In this study, O_2 availability did not negatively control γ-24774A11 abundances or *nifH* expression, suggesting this bacterium may use respiratory protection or its

336 nitrogenase may be protected through low O_2 microzones in association with organic matter or a
337 host organism. The positive correlation between abundances of γ-24774A11 and O_2 could be due
338 to co-variation of O_2 with another controlling factor such as energy from bioavailable DOC or
339 light. Unlimited access to energy in cyanobacteria is thought to make them superior to
340 heterotrophic bacteria in terms of using N_2 fixation to support growth (Postgate 1990), and thus
341 far all of the known key open ocean oligotrophic diazotrophs that are active are cyanobacteria.
342 While some heterotrophic bacteria can fix N_2 in anaerobic or aerobic conditions, the organisms
343 need to consume large amounts of carbon to obtain energy. Carbon is known to be an important
344 growth-limiting factor for oceanic heterotrophic bacteria (Kirchman et al. 2000, Thingstad et al.
345 2008). We speculate that the most reasonable explanation for low N_2 fixation rates and low
346 growth rates of heterotrophic prokaryotes in the open ocean is a lack of sufficient energy in the
347 form of bioavailable carbon. In experiments conducted in parallel to this study, abundances of γ-
348 24774A11 increased over a 3-day incubation period relative to a control treatment, in response to
349 sugar additions (Moisander et al. 2011). This suggests that availability of energy may be a key
350 factor regulating the growth of γ-24774A11 and perhaps other heterotrophic diazotrophs. The
351 positive correlation with DOC in this study supports this hypothesis.
352 Although the availability of DOC may be a growth limiting factor, suggesting a positive
353 link with phytoplankton production, oligotrophy was thought to promote the abundance of the γ-
354 24774A11-related phylotype in the Arabian Sea (Bird et al. 2005). Similarly, in this study, γ-
355 24774A11 had a negative correlation with chlorophyll. The presence of ammonium inhibits the
356 enzyme synthesis in some diazotrophs (Postgate 1990), however N_2 fixation in some open ocean
357 diazotrophs appears to tolerate elevated ammonium (Dekaezemacker and Bonnet 2011, Masuda
358 et al. 2013). Nutrients increasing with depth with chlorophyll, especially DIN, may negatively

359 influence fitness of γ-24774A11. NO_3, NO_2, TN, and SRP all had a negative correlation with the
360 abundance of γ-24774A11.

361 Major oceanic pelagic bacterial groups and ecotypes, including Gammaproteobacteria,
362 have depth stratification that may vary depending on season, and light adaptation and nutrient
363 acquisition mechanisms (Johnson et al. 2006, Treusch et al. 2009). A study in the Southern
364 California Bight (Hamersley et al. 2011) suggested primarily a surface association for γ-
365 24774A11, while the phylotype was detected down to 300 m in the Arabian Sea (Bird et al.
366 2005). Surface-associated groups such as γ-24774A11 could supplement their energy from light
367 directly through rhodopsin or bacteriochlorophyll-based metabolisms. Alternatively, the surface
368 association could be an indication of an oligotrophic lifestyle, or an advantage gained from
369 phytoplankton photosynthesis products. Until isolates or genomic information of γ-24774A11
370 becomes available, its energy metabolism and nutritional requirements will remain unknown.

371 Abundances of γ-24774A11 correlated positively with other diazotrophs, particularly
372 with *C. watsonii*. The γ-24774A11 phylotype had a significant positive relationship with
373 temperature, shown also for *C. watsonii* in the area (Moisander et al. 2010). The overall
374 conditions that promote growth of *Trichodesmium* and *C. watsonii* also seem to promote growth
375 of γ-24774A11 (Table S3). The γ-24774A11distributions had a weaker relationship with UCYN-
376 A, and were more constant than distributions of UCYN-A and *Crocosphaera*.

377

378 *Transcript abundance*

379 Nitrogenase gene transcription is strongly regulated in response to the presence of O_2 and
380 combined N (Dixon and Kahn 2004, Martinez-Argudo et al. 2005). The consistent presence of
381 *nifH* transcripts from γ-24774A11 over large geographic distances suggests that this phylotype

382 contributes to N_2 fixation over wide spatial and temporal scales. Consistent *nifH* expression
383 during the day suggests it may occupy suboxic or anoxic microniches in live cells or dead
384 particulate matter, and possibly uses active respiratory protection or other physiological
385 mechanisms against O_2 inhibition as found in its relative, *A. vinelandii* (Oelze 2000).

386 Trancription by the γ-24774A11 phylotype has been reported previously during the day
387 and night (Falcon et al. 2004, Church et al. 2005). The *nifH* gene expression by γ-24774A11 had
388 a subtle diel pattern, with higher rates of expression at night, while such pattern was not obvious
389 in a previous study conducted over 2-days (Church et al. 2005). The results suggest γ-24774A11
390 N_2 fixation is controlled by different factors from UCYN-A, due to different ecological strategies
391 in these organisms. Greater transcription at night could mean that reduced localized O_2 tension at
392 night is beneficial for this organism. Although generally not thought to play a major role in
393 heterotrophic bacteria, circadian rhythms may be involved with non-photosynthetic bacteria such
394 as *Pseudomonas putida* (Soriano et al. 2010). Diel rhythms in gene expression found in open
395 ocean bacteria may thus reflect an inherent diel or circadian pattern even in heterotrophic
396 microorganisms, or be a secondary result from changing environmental conditions due to cyclic
397 pattern in availability of photosynthesis products (or O_2) in the euphotic layers. It has been
398 suggested that marine microbial gene expression may be synchronous across distant
399 phylogenetic and functional groups reflecting changing environmental conditions (Ottesen et al.
400 2013). It should be noted that because some samples were kept in the deck incubator up to 12 h
401 until the opposite light phase, it is possible that bottle effects influenced transcription.

402 Metatranscriptomic studies frequently use transcript abundances to estimate differential
403 expression of metabolic pathways in the environment. Transcript abundances in this study can be
404 used as a relative indicator of contributions of each of the diazotroph phylotypes to total *nifH*

transcription activity. Our data suggest that γ-24774A11 may contribute up to 26% of the *nifH* gene transcription at a given time. The transcript abundances suggest N_2 fixation rates in γ-24774A11 could be greater during the night than during the day, but the contribution to the total *nifH* gene transcript pool may be significant any time of the day. Although N_2 fixation rates associated with γ-24774A11 remain to be demonstrated, transcript data suggest that when integrated over long distances and over the diurnal cycle, the phylotype may play a role in marine N_2 fixation. Heterotrophic bacteria have been reported to be abundant and diverse in the marine environment. This study shows that at least one of these is widely distributed and has consistent *nifH* expression, and that its transcripts can be as or more abundant than in cyanobacteria. However, since it is difficult yet to measure N_2 fixation of individual groups, the significance of heterotrophic N_2 fixation remains an enigma.

Supplementary information is available at The ISME Journal website.

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598

599

600

601 Figure legends:

602 Figure 1. A, Temperature along the cruise transect in the surface. B, Abundance of γ-24774A11
603 in the surface as *nifH* gene copies log (value+1) L^{-1}.

604 Figure 2. Neighbor-joining *nifH* phylogenetic tree for the 359 bp *nifH* gene region with
605 representative sequences from the study. Station numbers and depths are indicated for sequences
606 from this study.

607 Figure 3. Neighbor-joining *nifH* phylogenetic tree for γ-24774A11 sequences from this study and
608 other select studies. Sequences from this study include those obtained with γ-24774A11 specific
609 primers. AO, Atlantic Ocean; MS, Mediterranean Sea; NPAC, North Pacific Ocean; Red Sea;
610 SCS, South China Sea; SPOT, Southern California Coastal waters.

611 Figure 4. Abundance of γ-24774A11 along the study transect as *nifH* gene copies (log value+1)
612 L^{-1}.

613 Figure 5. Integrated *nifH* transcript abundance in the 0-125 m water column (m^{-2}). γ-24774A11
614 (A), *C. watsonii* (B), UCYN-A (C), *Trichodesmium* and Het-1 (D).

615 Figure 6. Proportions of transcripts during the day (top) and at night (bottom) of the five
616 diazotroph phylotypes. The data are based on transcripts per m^{-2}.

617

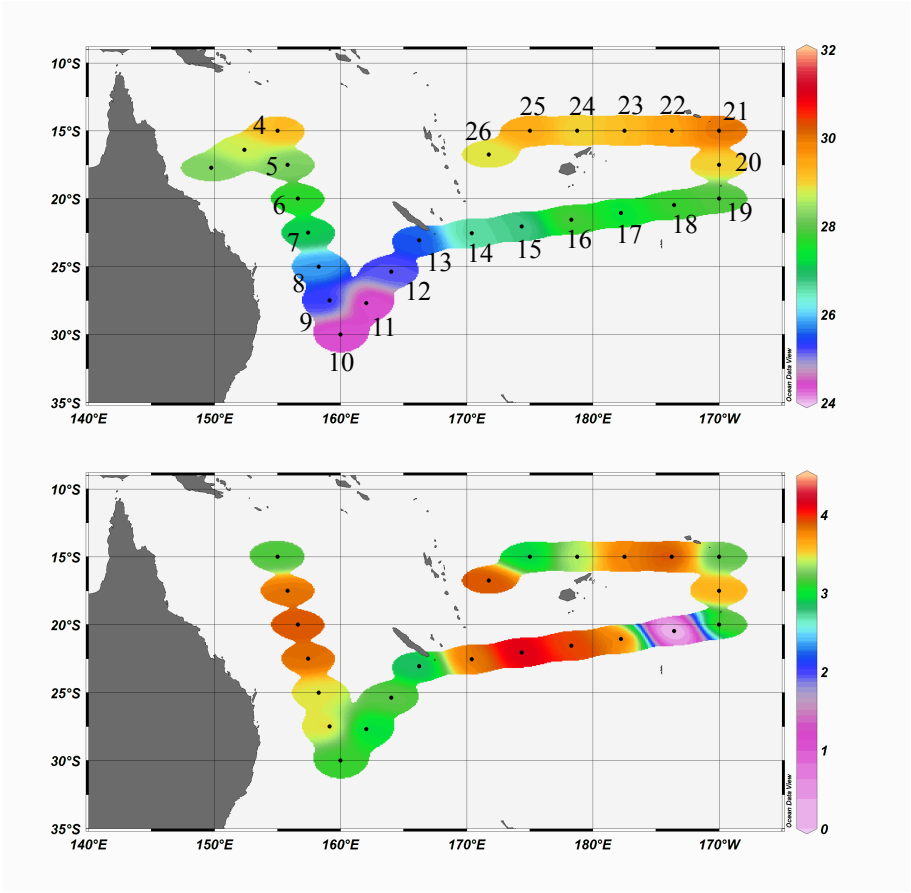
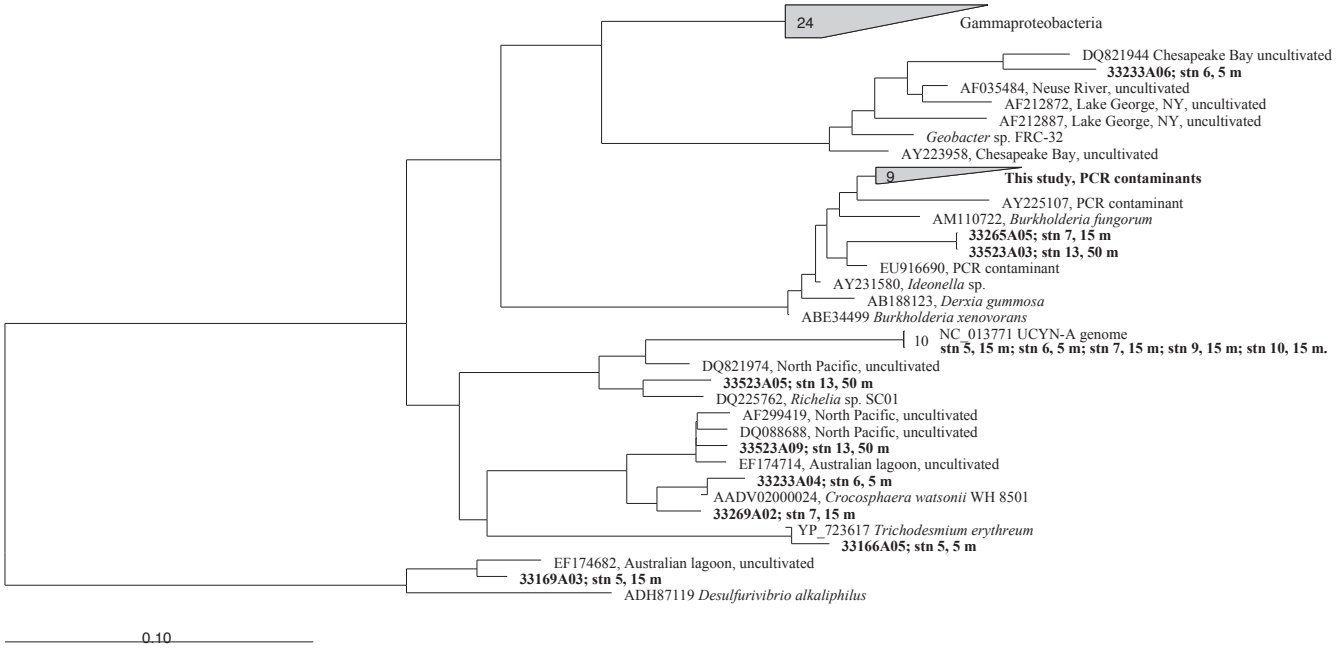
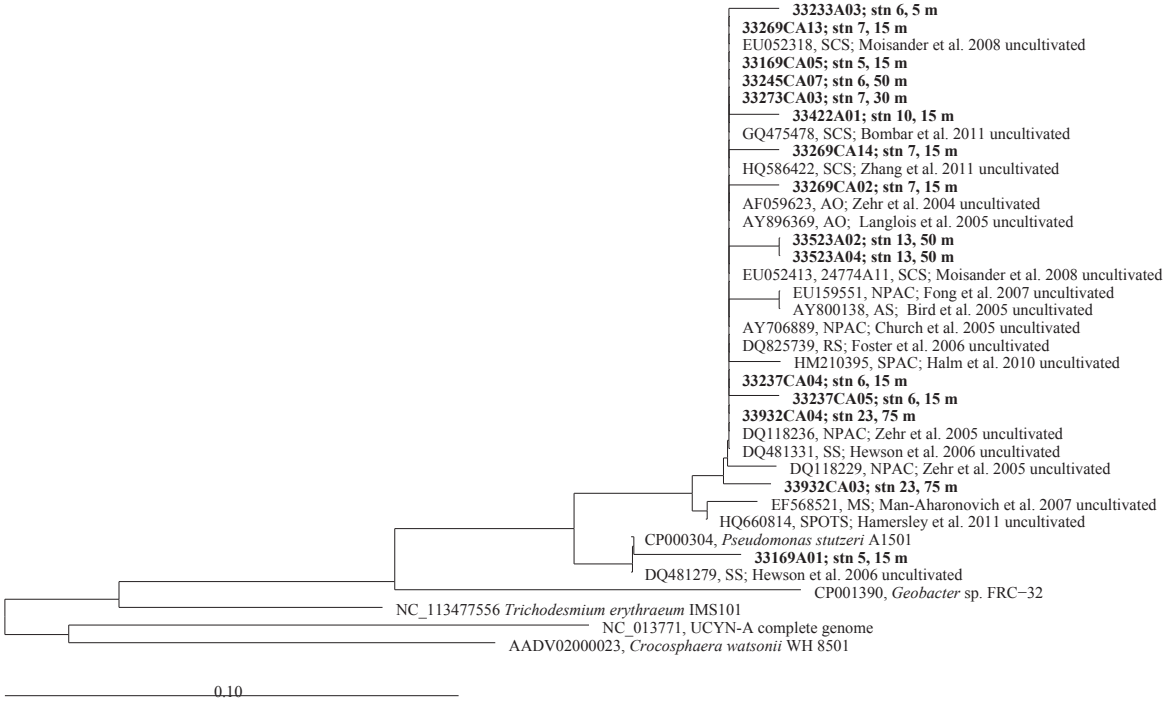


Figure 1. Moisander et al.


24
Gammaproteobacteria
DQ821944 Chesapeake Bay uncultivated
33233A06; stn 6, 5 m
AF035484, Neuse River, uncultivated
AF212872, Lake George, NY, uncultivated
AF212887, Lake George, NY, uncultivated
Geobacter sp. FRC-32
AY223958, Chesapeake Bay, uncultivated
9
This study, PCR contaminants
AY225107, PCR contaminant
AM110722, Burkholderia fungorum
33265A05; stn 7, 15 m
33523A03; stn 13, 50 m
EU916690, PCR contaminant
AY231580, Ideonella sp.
AB188123, Derxia gummosa
ABE34499 Burkholderia xenovorans
10
NC_013771 UCYN-A genome
stn 5, 15 m; stn 6, 5 m; stn 7, 15 m; stn 9, 15 m; stn 10, 15 m.
DQ821974, North Pacific, uncultivated
33523A05; stn 13, 50 m
DQ225762, Richelia sp. SC01
AF299419, North Pacific, uncultivated
DQ088688, North Pacific, uncultivated
33523A09; stn 13, 50 m
EF174714, Australian lagoon, uncultivated
33233A04; stn 6, 5 m
AADV02000024, Crocosphaera watsonii WH 8501
33269A02; stn 7, 15 m
YP_723617 Trichodesmium erythreum
33166A05; stn 5, 5 m
EF174682, Australian lagoon, uncultivated
33169A03; stn 5, 15 m
ADH87119 Desulfurivibrio alkaliphilus
0.10


33233A03; stn 6, 5 m
33269CA13; stn 7, 15 m
EU052318, SCS; Moisander et al. 2008 uncultivated
33169CA05; stn 5, 15 m
33245CA07; stn 6, 50 m
33273CA03; stn 7, 30 m
33422A01; stn 10, 15 m
GQ475478, SCS; Bombar et al. 2011 uncultivated
33269CA14; stn 7, 15 m
HQ586422, SCS; Zhang et al. 2011 uncultivated
33269CA02; stn 7, 15 m
AF059623, AO; Zehr et al. 2004 uncultivated
AY896369, AO; Langlois et al. 2005 uncultivated
33523A02; stn 13, 50 m
33523A04; stn 13, 50 m
EU052413, 24774A11, SCS; Moisander et al. 2008 uncultivated
EU159551, NPAC; Fong et al. 2007 uncultivated
AY800138, AS; Bird et al. 2005 uncultivated
AY706889, NPAC; Church et al. 2005 uncultivated
DQ825739, RS; Foster et al. 2006 uncultivated
HM210395, SPAC; Halm et al. 2010 uncultivated
33237CA04; stn 6, 15 m
33237CA05; stn 6, 15 m
33932CA04; stn 23, 75 m
DQ118236, NPAC; Zehr et al. 2005 uncultivated
DQ481331, SS; Hewson et al. 2006 uncultivated
DQ118229, NPAC; Zehr et al. 2005 uncultivated
33932CA03; stn 23, 75 m
EF568521, MS; Man-Aharonovich et al. 2007 uncultivated
HQ660814, SPOTS; Hamersley et al. 2011 uncultivated
CP000304, *Pseudomonas stutzeri* A1501
33169A01; stn 5, 15 m
DQ481279, SS; Hewson et al. 2006 uncultivated
CP001390, *Geobacter* sp. FRC−32
NC_113477556 *Trichodesmium erythraeum* IMS101
NC_013771, UCYN-A complete genome
AADV02000023, *Crocosphaera watsonii* WH 8501
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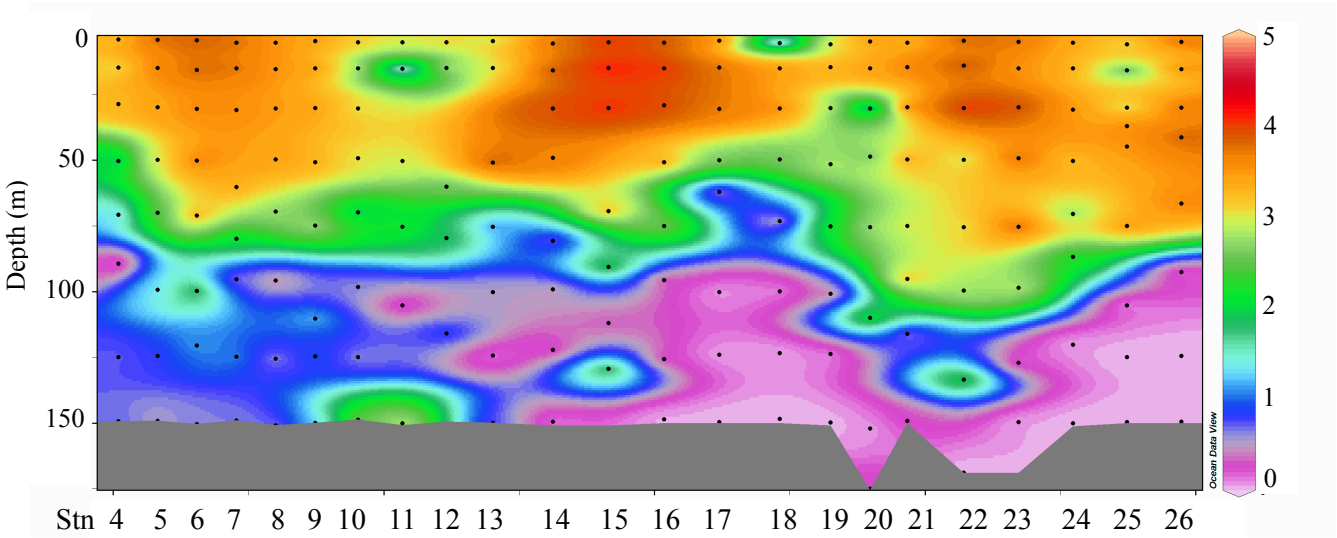
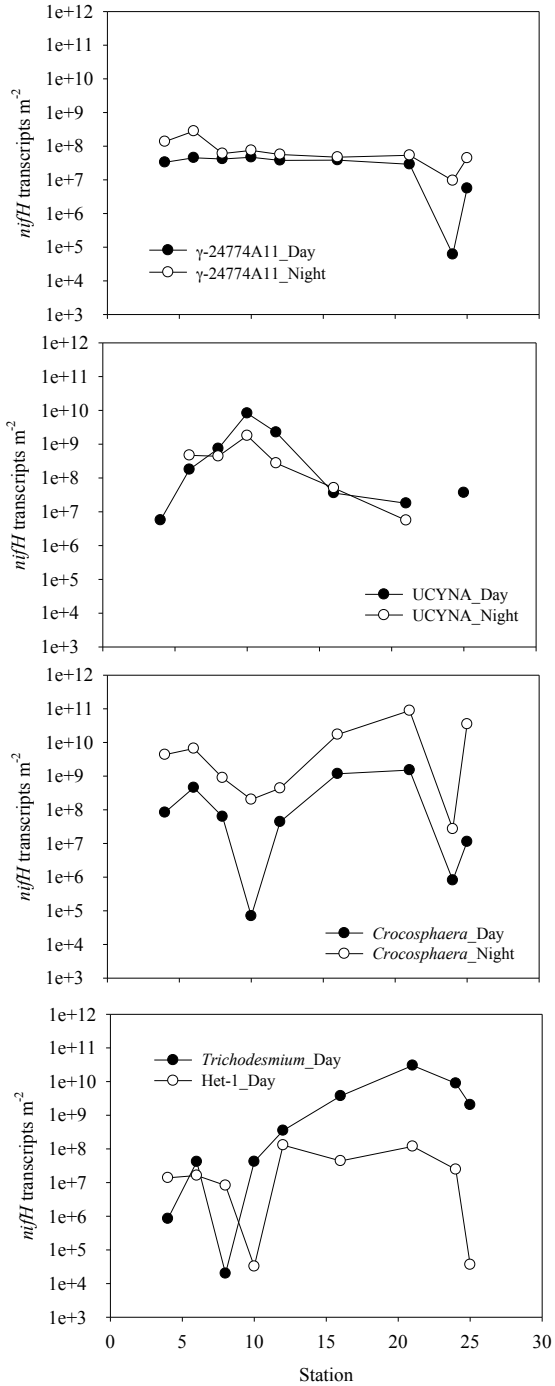


Figure 4. Moisander et al.



Moisander et al. *Figure 5*

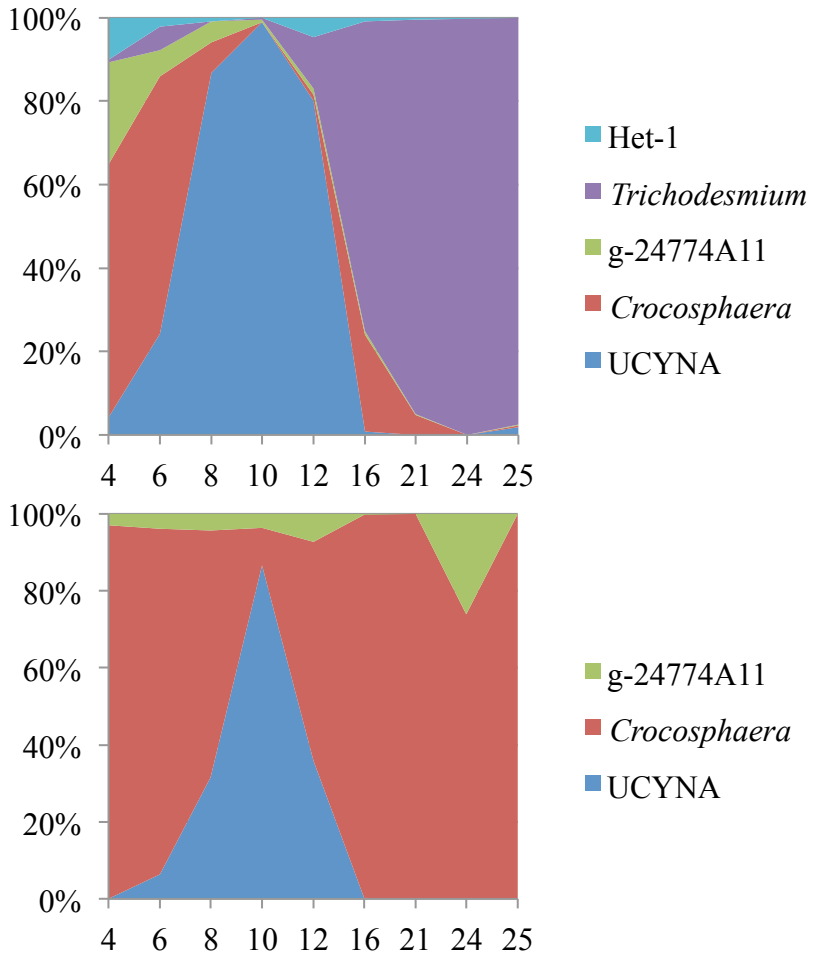


Figure 6. *Moisander et al.*

Table 1. Descriptive statistics of *nifH* gene copy abundances determined by qPCR (L^{-1}) for the different diazotroph groups. All sampling depths between 0 and 175 m were included from stations 4-26 (total of 22 stations).

	γ-24774A11	UCYN-A	*Crocosphaera*	*Trichodesmium*	Het-1
n	174	169	172	168	99
Mean	$2.5*10^3$	$9.7*10^4$	$1.2*10^5$	$1.4*10^5$	713
Std. deviation (±)	$3.8*10^3$	$2.6*10^5$	$6.3*10^5$	$5.7*10^5$	$2.2*10^3$
Median	787	50	$3.5*10^3$	328	21
Maximum	$2.0*10^4$	$1.9*10^6$	$7.9*10^6$	$6.3*10^6$	$1.7*10^4$

Table 2. Descriptive statistics of *nifH* gene transcript abundances (L^{-1}). Low median values reflect presence of a large number of datapoints with low values, as evidence of patchiness.

	UCYN-A		*Crocosphaera*		γ-24774A11	
	Day (n=36)	Night (n=36)	Day (n=36)	Night (n=36)	Day (n=36)	Night (n=36)
Mean	$9.5*10^3$	$2.3*10^3$	$3.4*10^3$	$1.6*10^5$	$2.5*10^2$	$6.9*10^2$
Std. Deviation	$3.2*10^4$	$5.8*10^3$	$1.1*10^4$	$4.8*10^5$	$4*10^2$	$1.1*10^3$
Median	1	0	1	2050	1	173
Maximum	$1.8*10^5$	$2.5*10^4$	$6*10^4$	$2.6*10^6$	$1.3*10^3$	$5.0*10^3$

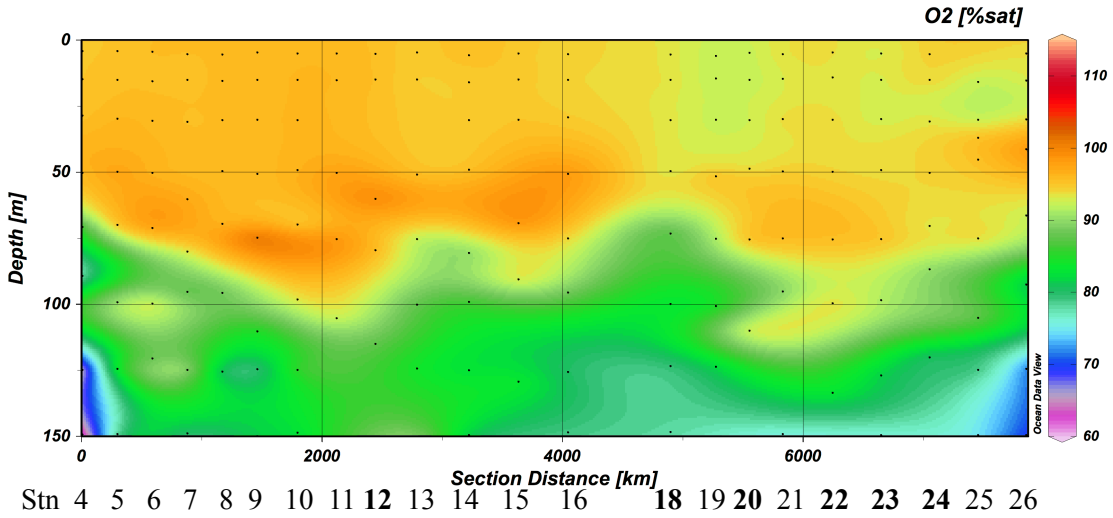


Figure S1. Oxygen saturation (%) in the study area. Stations where profiles were taken at night are shown in bold.

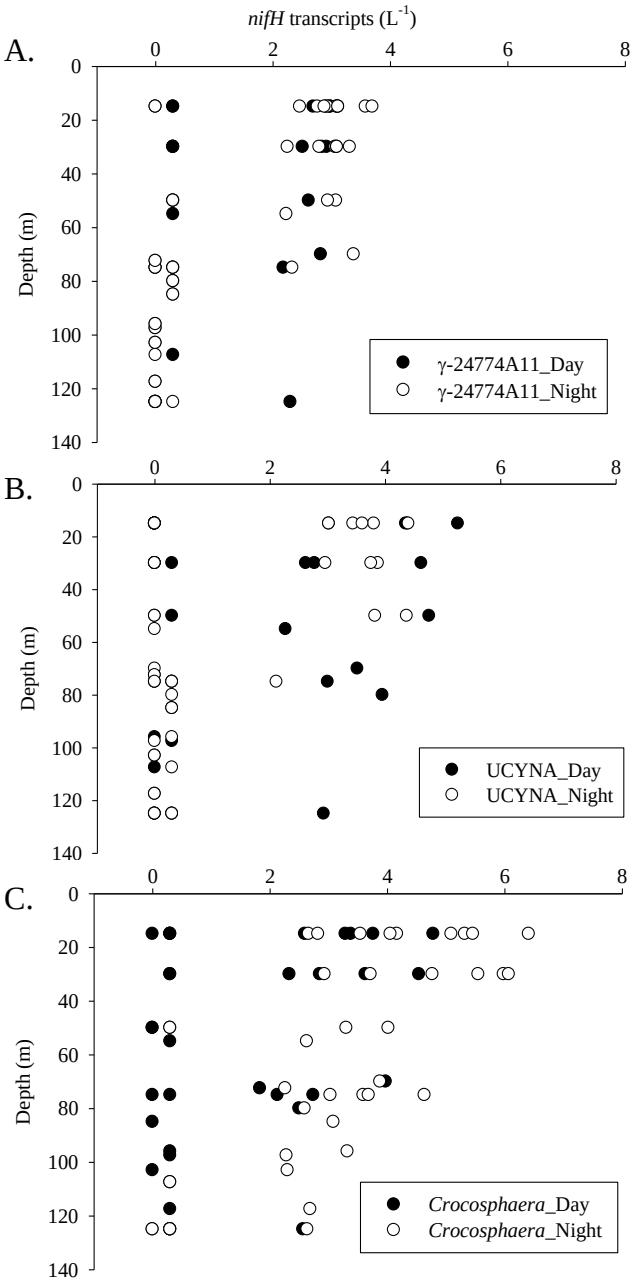


Figure S2. Abundance of *nifH* transcripts (L^{-1}) of γ-24774A11 (A), UCYN-A (B), and *Crocosphaera* (C) during the day and night.

Table S1. Sampling dates and locations, and parameters detected.

Station	yyyy-mm-dd	Local time on deck	Longitude [degrees_east]	Latitude [degrees_north]	Depth [m]	Temperature [C]	Salinity [psu]	Density [d]
4	3/18/07	11:50	155	-15	4.1	29.2	34.4	1021.60
4	3/18/07	11:50	155	-15	14.8	29.2	34.5	1021.69
4	3/18/07	11:50	155	-15	28.6	29.2	34.5	1021.76
4	3/18/07	11:50	155	-15	50.3	26.8	35.1	1023.05
4	3/18/07	11:50	155	-15	70.7	25.5	35.4	1023.78
4	3/18/07	11:50	155	-15	89.3	25.0	35.5	1024.14
4	3/18/07	11:50	155	-15	124.9	23.6	35.7	1024.87
4	3/18/07	11:50	155	-15	149.3	22.7	35.9	1025.34
5	3/19/07	10:45	155.8	-17.5	4.2	28.2	34.8	1022.16
5	3/19/07	10:45	155.8	-17.5	15.0	27.9	34.8	1022.33
5	3/19/07	10:45	155.8	-17.5	29.8	27.3	34.9	1022.69
5	3/19/07	10:45	155.8	-17.5	49.9	26.4	35.2	1023.24
5	3/19/07	10:45	155.8	-17.5	69.9	25.8	35.2	1023.56
5	3/19/07	10:45	155.8	-17.5	99.2	24.0	35.4	1024.36
5	3/19/07	10:45	155.8	-17.5	124.5	22.8	35.5	1024.91
5	3/19/07	10:45	155.8	-17.5	149.1	22.3	35.6	1025.22
6	3/20/07	13:15	156.61	-20	4.4	27.7	34.9	1022.47
6	3/20/07	13:15	156.61	-20	15.6	27.6	34.9	1022.55
6	3/20/07	13:15	156.61	-20	30.5	27.5	34.9	1022.66
6	3/20/07	13:15	156.61	-20	50.2	27.4	35.0	1022.76
6	3/20/07	13:15	156.61	-20	71.1	25.9	35.3	1023.62
6	3/20/07	13:15	156.61	-20	99.7	24.3	35.4	1024.25
6	3/20/07	13:15	156.61	-20	120.5	23.6	35.5	1024.68
6	3/20/07	13:15	156.61	-20	150.3	22.6	35.6	1025.16
7	3/21/07	13:06	157.43	-22.5	5.3	27.1	35.1	1022.82
7	3/21/07	13:06	157.43	-22.5	15.1	27.1	35.1	1022.86
7	3/21/07	13:06	157.43	-22.5	30.9	27.0	35.1	1022.96
7	3/21/07	13:06	157.43	-22.5	60.2	26.0	35.4	1023.61
7	3/21/07	13:06	157.43	-22.5	80.0	24.1	35.4	1024.26
7	3/21/07	13:06	157.43	-22.5	95.3	23.4	35.5	1024.58
7	3/21/07	13:06	157.43	-22.5	124.8	22.5	35.7	1025.14
7	3/21/07	13:06	157.43	-22.5	148.9	21.5	35.7	1025.52
8	3/22/07	13:20	158.27	-25	5.4	25.8	35.4	1023.41
8	3/22/07	13:20	158.27	-25	15.4	25.8	35.4	1023.46
8	3/22/07	13:20	158.27	-25	30.3	25.8	35.4	1023.52
8	3/22/07	13:20	158.27	-25	49.6	25.8	35.4	1023.61
8	3/22/07	13:20	158.27	-25	69.5	24.7	35.5	1024.08
8	3/22/07	13:20	158.27	-25	95.7	23.5	35.4	1024.55
8	3/22/07	13:20	158.27	-25	125.5	22.3	35.6	1025.11
8	3/22/07	13:20	158.27	-25	150.8	21.5	35.7	1025.53
9	3/23/07	12:45	159.12	-27.5	4.6	25.3	35.7	1023.78
9	3/23/07	12:45	159.12	-27.5	15.0	25.3	35.7	1023.82
9	3/23/07	12:45	159.12	-27.5	30.1	25.3	35.7	1023.90
9	3/23/07	12:45	159.12	-27.5	50.7	25.1	35.7	1024.07
9	3/23/07	12:45	159.12	-27.5	74.8	22.6	35.7	1024.94
9	3/23/07	12:45	159.12	-27.5	110.3	20.5	35.7	1025.65
9	3/23/07	12:45	159.12	-27.5	124.6	20.0	35.7	1025.84
9	3/23/07	12:45	159.12	-27.5	149.9	19.6	35.7	1026.07
10	3/24/07	12:15	160	-30	5.1	24.3	35.7	1024.13
10	3/24/07	12:15	160	-30	15.1	24.3	35.7	1024.18

Station	yyyy-mm-dd	Local time on deck	Longitude [degrees_ east]	Latitude [degrees_ north]	Depth [m]	Temperature [C]	Salinity [psu]	Density [d]
10	3/24/07	12:15	160	-30	30.3	24.1	35.9	1024.41
10	3/24/07	12:15	160	-30	49.2	24.0	35.9	1024.58
10	3/24/07	12:15	160	-30	69.8	22.1	35.7	1025.03
10	3/24/07	12:15	160	-30	98.2	20.8	35.8	1025.58
10	3/24/07	12:15	160	-30	124.9	20.1	35.8	1025.86
10	3/24/07	12:15	160	-30	148.6	19.5	35.7	1026.10
11	3/25/07	8:30	162.03	-27.69	5.1	24.3	35.9	1024.26
11	3/25/07	8:30	162.03	-27.69	15.2	24.3	35.9	1024.31
11	3/25/07	8:30	162.03	-27.69	50.2	23.8	35.9	1024.61
11	3/25/07	8:30	162.03	-27.69	75.3	22.0	35.8	1025.15
11	3/25/07	8:30	162.03	-27.69	105.3	20.0	35.8	1025.83
11	3/25/07	8:30	162.03	-27.69	150.1	18.7	35.7	1026.31
12	3/26/07	0:44	164.01	-25.38	5.2	25.2	35.7	1023.81
12	3/26/07	0:44	164.01	-25.38	14.9	25.2	35.7	1023.85
12	3/26/07	0:44	164.01	-25.38	60.1	23.4	35.7	1024.61
12	3/26/07	0:44	164.01	-25.38	79.5	22.1	35.7	1025.09
12	3/26/07	0:44	164.01	-25.38	115.0	20.7	35.8	1025.65
13	3/29/07	14:02	166.233	-23.05	4.7	25.5	35.5	1023.58
13	3/29/07	14:02	166.233	-23.05	14.9	25.5	35.5	1023.62
13	3/29/07	14:02	166.233	-23.05	50.9	24.0	35.5	1024.27
13	3/29/07	14:02	166.233	-23.05	75.3	22.7	35.6	1024.79
13	3/29/07	14:02	166.233	-23.05	100.2	22.1	35.7	1025.14
13	3/29/07	14:02	166.233	-23.05	124.3	21.1	35.7	1025.55
13	3/29/07	14:02	166.233	-23.05	150.0	20.7	35.8	1025.82
14	3/31/07	17:57	170.39	-22.55	149.5	21.0	35.7	1025.66
14	3/31/07	17:57	170.39	-22.55	125.0	21.7	35.6	1025.33
14	3/31/07	17:57	170.39	-22.55	99.1	22.2	35.6	1025.05
14	3/31/07	17:57	170.39	-22.55	80.6	22.7	35.6	1024.79
14	3/31/07	17:57	170.39	-22.55	49.1	24.5	35.4	1024.04
14	3/31/07	17:57	170.39	-22.55	30.3	25.8	35.4	1023.51
14	3/31/07	17:57	170.39	-22.55	15.9	25.9	35.4	1023.44
14	3/31/07	17:57	170.39	-22.55	5.7	26.4	35.4	1023.21
15	4/1/07	9:30	174.35	-22.05	129.3	19.5	35.7	1026.00
15	4/1/07	9:30	174.35	-22.05	90.5	20.9	35.7	1025.48
15	4/1/07	9:30	174.35	-22.05	69.3	22.8	35.7	1024.84
15	4/1/07	9:30	174.35	-22.05	30.2	25.3	35.8	1024.00
15	4/1/07	9:30	174.35	-22.05	14.9	26.3	35.7	1023.49
15	4/1/07	9:30	174.35	-22.05	5.1	26.7	35.5	1023.19
16	4/2/07	14:45	178.3	-21.55	148.5	20.6	35.7	1025.75
16	4/2/07	14:45	178.3	-21.55	125.6	21.4	35.6	1025.43
16	4/2/07	14:45	178.3	-21.55	95.6	22.2	35.6	1025.02
16	4/2/07	14:45	178.3	-21.55	75.0	22.8	35.5	1024.73
16	4/2/07	14:45	178.3	-21.55	50.7	24.8	35.5	1023.97
16	4/2/07	14:45	178.3	-21.55	29.2	27.4	35.2	1022.88
16	4/2/07	14:45	178.3	-21.55	15.1	27.7	35.2	1022.72
16	4/2/07	14:45	178.3	-21.55	5.3	27.9	35.2	1022.59
17	4/3/07	12:50	182.23	-21.05	149.6	21.1	35.6	1025.61
17	4/3/07	12:50	182.23	-21.05	123.3	21.5	35.6	1025.36
17	4/3/07	12:50	182.23	-21.05	100.1	22.2	35.6	1025.05
17	4/3/07	12:50	182.23	-21.05	62.1	23.6	35.5	1024.40
17	4/3/07	12:50	182.23	-21.05	50.0	24.8	35.4	1023.91

Station	yyyy-mm-dd	Local time on deck	Longitude [degrees_ east]	Latitude [degrees_ north]	Depth [m]	Temperature [C]	Salinity [psu]	Density [d]
17	4/3/07	12:50	182.23	-21.05	30.4	27.2	35.2	1022.91
17	4/3/07	12:50	182.23	-21.05	14.8	27.2	35.1	1022.77
17	4/3/07	12:50	182.23	-21.05	4.6	27.4	35.1	1022.68
18	4/5/07	23:38	186.45	-20.47	148.4	21.8	35.6	1025.37
18	4/5/07	23:38	186.45	-20.47	123.4	22.9	35.7	1025.00
18	4/5/07	23:38	186.45	-20.47	99.9	23.6	35.6	1024.65
18	4/5/07	23:38	186.45	-20.47	73.2	24.5	35.4	1024.15
18	4/5/07	23:38	186.45	-20.47	49.6	26.4	35.4	1023.42
18	4/5/07	23:38	186.45	-20.47	30.3	27.6	35.5	1023.00
18	4/5/07	23:38	186.45	-20.47	15.1	27.7	35.5	1022.92
18	4/5/07	23:38	186.45	-20.47	5.5	27.7	35.5	1022.88
19	4/6/07	13:40	190	-20	123.7	22.8	35.5	1024.96
19	4/6/07	13:40	190	-20	100.7	23.8	35.6	1024.58
19	4/6/07	13:40	190	-20	75.2	24.7	35.5	1024.11
19	4/6/07	13:40	190	-20	51.6	26.2	35.4	1023.54
19	4/6/07	13:40	190	-20	30.2	27.8	35.3	1022.78
19	4/6/07	13:40	190	-20	14.5	27.9	35.2	1022.66
19	4/6/07	13:40	190	-20	6.0	27.9	35.2	1022.61
20	4/7/07	21:40	190	-17.5	175.0	23.3	35.9	1025.33
20	4/7/07	21:40	190	-17.5	152.1	24.1	35.8	1024.91
20	4/7/07	21:40	190	-17.5	110.0	25.4	35.7	1024.22
20	4/7/07	21:40	190	-17.5	75.5	27.0	35.8	1023.63
20	4/7/07	21:40	190	-17.5	48.6	28.4	35.5	1022.86
20	4/7/07	21:40	190	-17.5	30.3	28.4	35.3	1022.60
20	4/7/07	21:40	190	-17.5	15.1	28.9	35.2	1022.33
20	4/7/07	21:40	190	-17.5	4.8	29.0	35.2	1022.22
21	4/8/07	13:49	190	-15	149.2	25.2	36.2	1024.88
21	4/8/07	13:49	190	-15	95.2	27.4	36.1	1023.86
21	4/8/07	13:49	190	-15	75.0	27.7	35.7	1023.37
21	4/8/07	13:49	190	-15	49.7	29.1	35.8	1022.87
21	4/8/07	13:49	190	-15	29.9	29.4	35.5	1022.45
21	4/8/07	13:49	190	-15	14.7	29.8	35.0	1021.91
21	4/8/07	13:49	190	-15	5.3	30.0	35.0	1021.76
22	4/9/07	22:50	186.25	-15	168.9	23.3	36.1	1025.39
22	4/9/07	22:50	186.25	-15	133.5	25.1	36.0	1024.61
22	4/9/07	22:50	186.25	-15	99.6	25.9	35.6	1023.93
22	4/9/07	22:50	186.25	-15	75.5	26.9	35.7	1023.56
22	4/9/07	22:50	186.25	-15	49.9	29.0	35.6	1022.70
22	4/9/07	22:50	186.25	-15	30.2	29.5	35.1	1022.13
22	4/9/07	22:50	186.25	-15	14.1	29.5	35.1	1022.06
22	4/9/07	22:50	186.25	-15	4.6	29.5	35.1	1022.02
23	4/11/07	23:45	182.5	-15	149.6	24.0	35.8	1024.92
23	4/11/07	23:45	182.5	-15	127.0	24.8	35.8	1024.54
23	4/11/07	23:45	182.5	-15	98.5	26.1	35.6	1023.88
23	4/11/07	23:45	182.5	-15	75.3	28.5	35.9	1023.21
23	4/11/07	23:45	182.5	-15	49.2	29.2	35.3	1022.41
23	4/11/07	23:45	182.5	-15	29.9	29.2	35.1	1022.20
23	4/11/07	23:45	182.5	-15	15.1	29.2	35.1	1022.13
23	4/11/07	23:45	182.5	-15	5.0	29.1	35.1	1022.09
24	4/12/07	1:42	178.75	-15	150.1	24.0	36.0	1025.09
24	4/12/07	1:42	178.75	-15	120.1	25.5	36.0	1024.49

Station	yyyy-mm-dd	Local time on deck	Longitude [degrees_ east]	Latitude [degrees_ north]	Depth [m]	Temperature [C]	Salinity [psu]	Density [d]
24	4/12/07	1:42	178.75	-15	86.8	26.8	35.7	1023.69
24	4/12/07	1:42	178.75	-15	70.4	27.5	35.7	1023.41
24	4/12/07	1:42	178.75	-15	50.3	28.6	35.6	1022.85
24	4/12/07	1:42	178.75	-15	30.8	28.8	35.0	1022.27
24	4/12/07	1:42	178.75	-15	15.0	28.8	35.0	1022.16
24	4/12/07	1:42	178.75	-15	5.3	28.9	35.0	1022.10
25	4/13/07	12:44	175	-15	149.6	23.9	35.8	1024.91
25	4/13/07	12:44	175	-15	124.9	24.9	35.7	1024.40
25	4/13/07	12:44	175	-15	105.2	26.0	35.6	1023.90
25	4/13/07	12:44	175	-15	75.0	27.5	35.6	1023.32
25	4/13/07	12:44	175	-15	45.2	29.2	35.0	1022.16
25	4/13/07	12:44	175	-15	37.0	29.2	35.0	1022.13
25	4/13/07	12:44	175	-15	30.1	29.5	34.3	1021.52
25	4/13/07	12:44	175	-15	15.8	29.4	34.2	1021.42
26	4/14/07	12:50	171.75	-16.75	149.5	22.6	35.8	1025.32
26	4/14/07	12:50	171.75	-16.75	124.5	24.0	35.9	1024.84
26	4/14/07	12:50	171.75	-16.75	92.6	24.6	35.5	1024.24
26	4/14/07	12:50	171.75	-16.75	66.4	26.1	35.3	1023.51
26	4/14/07	12:50	171.75	-16.75	41.3	27.9	35.2	1022.72
26	4/14/07	12:50	171.75	-16.75	30.0	28.8	34.8	1022.11
26	4/14/07	12:50	171.75	-16.75	15.3	28.8	34.8	1022.05
26	4/14/07	12:50	171.75	-16.75	5.1	28.8	34.8	1022.00

Table S1								
Station	SigmaT [kgm3]	Fluor [fu]	O2 [umol/kg]	O2 [%sat]	PAR [umol m-2 s-1]	UCYNA [copies L-1]	Crocosph. [copies L-1]	Tricho [copies L-1]
4	21.58	0.06	183.35	94.80	908	1	116873	26663
4	21.63	0.06	183.58	94.92	347	1	215639	49934
4	21.64	0.08	183.97	95.12	225	1	62392	30333
4	22.83	0.13	191.23	95.65	129	98	1804	981
4	23.48	0.37	175.29	85.91	48	15	272	141
4	23.75	0.75	159.02	77.39	12	38	236	1876
4	24.33	0.31	133.70	63.57	2	18	335	46
4	24.69	0.09	129.55	60.75	1	0	155	0
5	22.14	0.05	186.36	95.08	1600	7603	30304	42
5	22.27	0.07	188.11	95.52	611	46832	35917	61
5	22.57	0.11	191.09	96.19	174	194258	36098	118
5	23.02	0.12	197.06	97.89	106	48608	12591	864
5	23.26	0.25	194.43	95.65	32	154170	1199	0
5	23.93	0.60	189.48	90.59	14	62	244	0
5	24.37	0.41	183.49	85.95	3	1	133	0
5	24.57	0.23	170.85	79.36	1		385	0
6	22.45	0.05	188.84	95.61	1080	5.09E+04	166860	2102
6	22.48	0.06	188.62	95.33	521	43040	143411	172
6	22.53	0.07	189.88	95.86	333	43020	217046	723
6	22.55	0.09	189.86	95.79	73	53568	203081	2076
6	23.32	0.16	198.57	97.96	85	591311	59163	0
6	23.82	0.28	194.74	93.51	11	23	515	0
6	24.15	0.51	183.22	86.96	5	12	356	290
6	24.50	0.23	171.93	80.27	1	16	324	0
7	22.80	0.05	189.98	95.41	1400	59119	25766	18
7	22.80	0.05	189.76	95.29	212	66787	123064	2469
7	22.82	0.06	189.87	95.23	461	49716	40149	25
7	23.35	0.12	196.24	96.97	56	717604	29608	742
7	23.92	0.32	193.45	92.59	58	787	381	0
7	24.16	0.92	183.14	86.65	27	38	187	0
7	24.59	0.41	197.18	92.07	5	31	402	0
7	24.87	0.26	173.03	79.36	1	13	222	0
8	23.39	0.06	193.95	95.55	58	365403	12349	0
8	23.39	0.07	194.14	95.64	42	314115	7308	1483
8	23.39	0.08	194.31	95.73	14	401099	12894	2077
8	23.39	0.09	194.10	95.60	7	252201	4291	0
8	23.78	0.14	197.24	95.47	1	426	790	29
8	24.13	0.37	186.28	88.25	1	14	110	0
8	24.57	0.28	168.31	78.25	0	22	74	0
8	24.87	0.11	159.66	73.14	0	31	306	0
9	23.76	0.12	195.89	95.87	1460	495449	7829	0
9	23.76	0.05	195.60	95.72	566	473281	12125	5969
9	23.76	0.07	195.36	95.55	370	557817	9598	492
9	23.85	0.10	196.14	95.71	188	293036	8412	1140
9	24.62	0.19	219.03	102.34	54	367851	226	
9	25.17	0.44	186.61	84.09	12	92	282	0
9	25.30	0.32	176.08	78.55	6	126	161	0
9	25.41	0.21	184.15	81.62	2	80	101	9
10	24.11	0.07	199.11	95.84	159	878576	1547	124
10	24.11	0.08	199.52	96.04	84	1078165	904	1705

Station	SigmaT [kgm3]	Fluor [fu]	O2 [umol/kg]	O2 [%sat]	PAR [umol m-2 s-1]	UCYNA [copies L-1]	Crocosph. [copies L-1]	Tricho [copies L-1]
10	24.28	0.11	200.29	96.25	59	1640141	937	14241
10	24.36	0.12	200.81	96.25	55	1852388	1322	1586
10	24.72	0.52	202.36	93.76	26	767	115	0
10	25.15	0.46	206.16	93.44	3	67	148	0
10	25.31	0.35	194.08	86.81	1	251	379	0
10	25.45	0.19	186.01	82.35	0	155	25	0
11	24.24	0.07	199.06	95.89	11	909545	33228	135
11	24.24	0.08	199.19	95.95	1	593136	29684	0
11	24.39	0.12	204.41	97.74	0	651887	11420	0
11	24.82	0.18	213.29	98.81	0	208390	1096	0
11	25.37	0.52	208.32	92.95	0			
11	25.65	0.09	187.25	81.57	0	403	5563	135
12	23.79	0.10	195.60	95.49	0	361554	24317	88
12	23.79	0.09	195.87	95.62	0	414256	13515	29240
12	24.35	0.11	210.64	99.80	0	40551	3325	20
12	24.74	0.14	207.24	96.05	0	90	877	41
12	25.14	0.53	193.44	87.50	0	50	243	0
13	23.56	0.08	192.50	94.45	899	111644	3641	516
13	23.55	0.08	192.04	94.23	536	204141	2226	75125
13	24.05	0.23	198.76	95.10	118	4361	47752	1
13	24.46	0.61	195.02	91.32	13	229	2076	1
13	24.70	0.33	188.57	87.31	3	43	1469	
13	25.00	0.09	184.16	83.88	1	12	231	
13	25.17	0.08	197.15	89.20	1	42	1037	
14	25.01	0.11	178.63	81.13	0	85	234	6363
14	24.78	0.24	178.95	82.36	0	1	1	1
14	24.61	0.34	184.72	85.76	0	1	1107	435
14	24.44	0.56	190.98	89.41	0	1	1347	217
14	23.83	0.17	197.30	95.14	1	104845	10152	15041
14	23.38	0.16	191.75	94.49	1	340093	11485	45880
14	23.37	0.10	191.93	94.64	1	278614	33339	169384
14	23.18	0.13	192.11	95.59	1	131871	9891	477606.441
15	25.43	0.45	189.55	83.86	2	1	74	0
15	25.09	0.22	207.36	94.01	15	350	498	1
15	24.54	0.14	210.99	99.03	61	110309	1973	8855
15	23.87	0.09	193.93	95.09	169	260574	16245	68500
15	23.42	0.09	190.53	94.82	354	5680	30335	463093
15	23.17	0.08	189.05	94.60	1130	4313	115270	1095710
16	25.10	0.11	174.83	78.91	1	0	88	1
16	24.88	0.29	174.60	79.83	3	0	141	1
16	24.61	0.70	190.32	88.33	15	1	154	119
16	24.40	0.21	200.48	93.91	46	722	472	356
16	23.75	0.12	203.26	98.47	152	384345	12654	22235
16	22.75	0.09	187.13	94.48	343	862	153142	181864
16	22.66	0.07	185.23	93.96	640	1496	143060	413863
16	22.57	0.08	184.80	94.17	1111	83	40194	234490
17	24.96	0.04	163.70	0.09	2	0	1	1
17	24.82	0.06	169.24	0.14	2	0	1	1
17	24.61	0.28	171.47	0.09	3	0	2	1
17	24.13	0.69	182.88	0.20	109	0	640	151
17	23.69	0.14	193.77	0.11	199	57	3430	1942

Station	SigmaT [kgm3]	Fluor [fu]	O2 [umol/kg]	O2 [%sat]	PAR [umol m-2 s-1]	UCYNA [copies L-1]	Crocosph. [copies L-1]	Tricho [copies L-1]
17	22.78	0.09	186.05	0.18	452	0	4880	162165
17	22.70	0.06	185.62	0.12	563	0	5526	757309
17	22.66	0.05	185.35	0.13	1720	1	24885	285749
18	24.72	0.08	169.03	77.91	0	0	143	0
18	24.46	0.14	168.59	79.19	0	0	192	0
18	24.22	0.24	174.91	83.20	0	1	392	1
18	23.83	0.58	179.62	86.61	0			438
18	23.21	0.15	186.07	92.58	0	1175	5727	58647
18	22.86	0.10	183.68	93.28	0	348	118165	396683
18	22.86	0.09	183.91	93.56	0	293	184275	946468
18	22.85	0.09	183.52	93.37	0	1	60560	169387
19	24.42	0.21	174.36	81.64	3	0	518	1
19	24.14	0.45	175.51	83.74	12	1	459	1
19	23.78	0.23	188.10	90.96	52	1	1430	0
19	23.32	0.10	192.91	95.63	108	9001	45644	9882
19	22.65	0.06	181.30	92.21	179	0	72797	106
19	22.60	0.05	180.49	91.89	248	0	23633	185
19	22.58	0.05	180.69	92.02	390	0	116381	313
20	24.57	0.26	157.15	74.43	0	1	931	1
20	24.25	0.45	173.81	83.39	0	1	329	1
20	23.74	0.17	190.87	93.55	0	1	275	0
20	23.31	0.08	190.86	96.06	0	0	224	1
20	22.65	0.07	180.70	92.90	0	0	1	0
20	22.47	0.06	179.34	92.14	0	0	365	0
20	22.27	0.06	178.04	92.08	0	0	281256	25898
20	22.20	0.06	177.79	92.10	0	0	1489308	81591
21	24.23	0.25	154.38	75.67	2		9992	3774
21	23.45	0.24	175.85	89.30	27	0	6345	1
21	23.05	0.15	186.89	95.13	68	1066	52040	1285
21	22.65	0.12	181.80	94.67	178	8729	287172	162975
21	22.33	0.10	179.85	93.96	401	0	387860	426918
21	21.85	0.07	177.59	93.02	721	0	969934	1980530
21	21.74	0.06	178.15	93.61	324	1	288242	1545576
22	24.66	0.13	147.56	70.00	0	0	1411	343
22	24.04	0.35	170.41	83.26	0	0	2654	1953
22	23.50	0.20	189.83	93.78	0	0	4702	1519
22	23.24	0.12	189.53	95.24	0	1517	12312	28887
22	22.49	0.10	181.06	94.00	0	0	122762	23834
22	22.01	0.11	179.83	93.86	0	0	241625	673164
22	21.99	0.11	179.28	93.55	0	0	98253	711749
22	22.00	0.11	179.50	93.66	0	0	438273	718460
23	24.27	0.24	158.31	75.82	0	1	2877	118
23	23.99	0.42	167.35	81.32	0	83	1038	7838
23	23.46	0.17	183.78	91.07	0	11381	1745	1
23	22.88	0.11	184.44	95.25	0	5141	211492	2731
23	22.20	0.08	180.15	93.70	0	0	468619	7088
23	22.07	0.06	177.78	92.28	0	0	107633	10656
23	22.07	0.06	178.18	92.47	0	0	62135	4971
23	22.07	0.06	178.30	92.52	0	1	385293	11491
24	24.44	0.12	148.74	71.34	0	0	142	445
24	23.97	0.20	161.51	79.52	0	0	231	1051

Station	SigmaT [kgm3]	Fluor [fu]	O2 [umol/kg]	O2 [%sat]	PAR [umol m-2 s-1]	UCYNA [copies L-1]	Crocosph. [copies L-1]	Tricho [copies L-1]
24	23.32	0.43	179.04	89.79	0	119	816	5216
24	23.10	0.31	182.72	92.80	0	0	969	1357
24	22.64	0.21	182.59	94.15	0	0	7020	19145
24	22.14	0.18	181.56	93.72	0	0	133116	412041
24	22.10	0.17	183.34	94.62	0	0	148816	1938483
24	22.08	0.15	182.68	94.44	0	0	177373	6266845
25	24.26	0.14	156.43	74.77	1	0	789	52
25	23.86	0.29	163.69	79.59	3	0	890	0
25	23.45	0.56	177.35	87.80	10	0	534	19
25	23.00	0.19	185.62	94.20	46	25801	23705	14246
25	21.97	0.22	183.16	95.04	160	0	1696478	415875
25	21.97	0.23	183.95	95.45	287		7902926	
25	21.39	0.06	176.57	91.68	235	0	9741	49
25	21.36	0.05	176.89	91.64	349	0	580	138
26	24.67	0.10	148.47	69.49	1	0	348	0
26	24.30	0.24	149.37	71.57	4	0	708	13
26	23.83	0.41	167.94	81.10	16	0	121	0
26	23.22	0.18	186.59	92.23	51	56343	3097	4810
26	22.54	0.13	192.67	98.14	122	62455	128320	19539
26	21.98	0.12	181.73	93.60	195	1702	353939	326792
26	21.98	0.10	180.68	93.08	379	909	257745	1522587
26	21.98	0.09	181.61	93.55	995	842	172009	577285

Table S1								
Station	Het-1 [copies L-1]	γ-24774A11 [copies L-1]	NO3 [nM]	NO2 [nM]	SRP [nM]	DOC [uM]	TN [uM]	chla [ug L-1]
4	180	1481	2.0	1.0	114.1	72.9	4.8	0.08
4	651	960	5.0	3.0	120.4	73.2	5.1	0.09
4	270	1781	2.0	2.0	109.3	71.8	4.6	0.12
4	0	52	1.0	4.0	174.8	67.7	4.3	0.13
4	0	12	5.0	2.0	226.1	63.3	4.1	0.50
4	0	0	2312.0	242.0	299.4	64.6	6.2	0.42
4	0	4	7769.0	35.0	535.1	57.1	10.6	0.18
4	0	4	8206.0	16.0	698.4	53.9	11.5	0.05
5	0	6468	1.4	1.0	115.0	66.3	5.1	0.06
5	57	4264	1.6	3.0	95.5	69.7	4.4	0.07
5	0	2498	4.0	3.0	86.8	71.4	5.6	0.16
5	164	1104	10.9	2.0	119.5	66.7	4.4	0.18
5	0	280	63.1	8.0	121.6	62.3	4.9	0.38
5	0	20	734.5	104.0	161.0	60.1	5.4	0.28
5	0	5	2665.6	32.0	236.6	58.4	6.4	0.06
5	16	2	3919.5	13.0	445.0	51.6	8.8	0.19
6	213	7793	7.1	1.0	113.5	65.5	5.0	0.02
6	133	7005	2.5	0.0	123.4	67.9	4.3	0.02
6	258	3986	15.2	2.0	101.2	70.1	5.1	0.09
6	66	5943	2.5	1.0	103.9	66.0	4.5	0.09
6	325	3806	5.0	1.0	92.2	67.1	4.2	0.15
6	21	162	25.6	2.0	90.7	65.3	5.5	0.18
6	0	13	816.0	230.0	194.3	61.1	6.3	0.31
6	0	3	3017.5	61.0	321.3	57.5	8.0	0.35
7	106	6760	3.6	1.0	98.4	68.2	5.2	0.03
7	514	6357	1.5	1.0	107.1	56.4	4.2	0.03
7	129	3748	7.4	7.0	110.2	60.5	4.2	0.10
7	472	2552	1.7	1.0	120.3	65.7	4.8	0.12
7	0	94	2.0	2.0	111.1	63.5	4.7	0.26
7	0	2	1370.4	133.0	208.7	58.8	5.0	0.51
7	0	14	1827.5	290.0	212.6	58.4	6.7	0.14
7	0	3	5314.0	21.0	374.3	49.2	8.8	0.09
8	133	2905	2.3	2.0	93.4	62.1	4.7	0.07
8	91	2941	3.1	2.0	70.0	68.2	5.0	0.07
8	76	3025	3.8	2.0	83.2	66.8	5.1	0.09
8	10	3042	2.6	1.0	94.0	64.5	5.4	0.03
8	33	511	2.7	3.0	108.1	63.7	5.4	0.22
8	0	1	4.6	2.0	138.4	60.3	6.4	0.14
8	0	1	3391.9	43.0	357.6	55.8	8.2	0.19
8	0	2	4778.4	17.0	396.6	52.6	8.5	0.08
9	108	3167	7.5	5.0	55.3	69.2	4.7	0.10
9	107	4336	9.4	4.0	92.2	67.2	4.8	0.10
9	0	3023	2.9	1.0	80.8	68.1	5.3	0.10
9	350	2470	11.5	3.0	67.1	67.0	5.4	0.10
9		928	2.5	2.0	53.2	65.2	5.3	0.17
9	0	19	12.8	3.0	89.5	67.5	5.7	0.04
9	0	11	2308.7	31.0	277.1	54.6	7.5	0.05
9	0	10	3907.4	112.0	171.5	56.7	7.1	0.12
10	100	1329	5.4	1.0	55.0	68.0	4.3	0.08
10	0	1228	15.6	2.0		68.6	4.6	0.11

Station	Het-1 [copies L-1]	γ-24774A11 [copies L-1]	NO3 [nM]	NO2 [nM]	SRP [nM]	DOC [uM]	TN [uM]	chla [ug L-1]
10	11	888	5.1	2.0	43.0	61.3	4.2	0.10
10	4	637	5.9	0.0	39.1	71.3	4.4	0.10
10	9	43	30.9	7.0	111.1	64.6	4.1	0.26
10	0	3						
10	0	2	824.5	90.0	151.9	59.9	5.8	0.29
10	0	790	2028.5	21.0	290.4	53.6	7.4	0.12
11		839						0.09
11	760	0						0.04
11		876						0.13
11		205						0.18
11		0						
11		1193						
12		1543	20.0	1.0	65.0	67.9	5.0	0.10
12	0	1419	28.0	1.0	71.0	69.6	5.0	0.07
12		310	8.0	1.0	72.0	65.1	4.5	0.10
12		107	10.0	3.0	95.0	60.6	4.9	0.15
12		10						
13		678	6.0	0.0	79.0			0.13
13	777	783	10.0	3.0	77.0			0.13
13		10952	57.0	3.0	70.0			0.25
13		7	27.0	28.0	104.0			0.53
13		2	738.0	108.0				0.11
13		0	1862.0	30.0	226.0			0.07
13		4	618.0	195.0	85.0			0.01
14		0	1858.5	17.6	216.0	62.9	6.7	0.08
14		0	1902.1	48.0	148.0	58.6	6.7	0.19
14		1	292.3	106.6	118.0	66.6	5.6	0.45
14		1	5.6	10.4	127.0	57.3	4.4	0.46
14		4662.01	0.3	0.9	59.0	61.2	4.2	0.21
14	134	8126.68	0.9	0.9	63.0	70.7	4.8	0.18
14	146	9992.58	0.0	0.5	56.0	70.2	5.5	0.18
14		6400.93	4.8	0.7	49.0	70.8	4.3	0.33
15		304	1626.9	47.0	223.0	53.9	5.7	0.37
15		313	0.0	1.4	86.0	61.7	4.4	0.18
15		3504	0.8	2.4	40.0	66.5	5.1	0.11
15	975	12815	1.3	1.5	54.0	68.8	4.8	0.11
15	2823	16685	0.7	1.0	46.0	71.4	4.6	0.14
15		13872	5.5	5.6	40.0	66.4	4.7	0.17
16		0	1353.6	13.9	190.0	57.1	6.2	0.12
16		0	623.5	26.5	161.0	56.8	6.0	0.26
16		0	692.0	88.7	129.0	58.7	5.5	0.54
16		466	1.4	1.2	54.0	65.5	4.4	0.22
16		3369	0.5	2.3	43.0	71.4	4.8	0.13
16	491	9543	9.7	3.3	40.0	75.9	5.6	0.11
16	1941	12285	0.3	1.3	38.0	73.5	4.6	0.10
16		8615	2.0	1.5	32.0	74.9	4.7	0.10
17		0	1813.8	8.4	203.0	45.0	5.8	0.01
17		0	1914.3	14.5	187.0	53.5	7.2	0.05
17		0	1221.6	27.4	147.0	52.6	5.8	0.30
17		0	874.7	57.4	173.0	60.1	6.6	0.55
17		450	15.3	1.7	82.0	62.7	4.6	0.16

Station	Het-1 [copies L-1]	γ-24774A11 [copies L-1]	NO3 [nM]	NO2 [nM]	SRP [nM]	DOC [uM]	TN [uM]	chla [ug L-1]
17	307	5087	6.7	1.1	42.0	74.5	6.0	0.11
17	172	6217	8.8	1.5	42.0	75.5	9.9	0.14
17		6554	8.0	1.4	26.0	74.7	5.0	0.12
18		0	2399.0	27.1	249.0	53.1	6.3	0.05
18		0	1023.0	41.6	269.0	57.2	5.4	0.14
18		0	212.8	170.4	199.0	61.6	5.1	0.31
18		0						
18		376	6.6	2.0	75.0	69.4	4.9	0.28
18	514	5464	1.5	0.9	109.0	75.5	5.4	0.18
18	3123	4026	6.2	5.4	110.0	72.7	4.8	0.18
18		0	7.5	1.4	89.0	77.0	5.7	0.15
19		0	48.9	76.8	121.0	62.4	4.7	0.33
19		0	4.8	1.6	127.0	68.8	4.4	0.40
19		160	3.7	1.4	105.0	61.1	4.8	0.20
19		919	3.6	0.8	96.0	71.3	4.6	0.09
19	0	1411	4.5	1.4	129.0	75.5	4.7	0.04
19	953	1703	5.7	1.9	105.0	78.2	4.6	0.05
19		1771	2.7	3.1	131.0	75.6	4.8	0.04
20		1	1437.0	167.8	208.0	50.6	5.0	0.04
20		0	2.8	3.3	145.0	59.6	5.0	0.05
20		533	3.4	0.7	129.0	69.3	4.6	0.03
20		816	0.0	0.0	123.0	73.6	5.7	0.04
20		213	0.9	0.3	119.0	74.1	4.6	0.08
20	0	1	2.4	0.8	171.0	75.5	4.3	0.17
20	0	2685	0.5	0.5	146.0	76.7	5.1	0.34
20		4001	0.0	0.7	144.0	76.3	4.9	0.19
21		0	977.2	176.7	437.0	61.7	7.1	0.15
21		2822	0.4	1.1	159.0	72.6	5.0	0.09
21		1125	0.3	1.4	166.0	73.2	6.9	0.08
21		4228	3.9	0.7	111.0	73.6	7.1	0.12
21	64	19805	0.4	1.0	132.0	65.6	5.4	0.16
21	516	4400	0.3	1.1	135.0	76.4	4.7	0.12
21		1536	1.0	1.1	97.0	77.8	5.9	0.10
22		0	1976.8	16.3	304.0	45.7	8.7	0.14
22		235	3.1	15.8	160.0	60.1	7.6	0.21
22		638	2.3	1.9	79.0	73.5	7.3	0.15
22		907	0.0	1.4	129.0	61.6	4.5	0.11
22		607	0.0	1.4	133.0	62.8	5.8	0.11
22		14867	0.2	5.3	88.0	77.9	5.3	0.13
22	5705	10819	1.9	1.9	94.0	78.4	6.1	0.16
22		7988	0.1	1.3	111.0	76.2	7.4	0.16
23		0	1892.8	30.3	347.0	57.4	5.6	0.17
23		0	10.5	42.1	170.0	53.7	4.2	0.45
23		888	0.0	2.2	313.0	72.8	5.1	0.47
23		12667	0.0	3.1	154.0	69.2	5.2	0.10
23		8719	0.0	1.8	142.0	74.7	5.1	0.07
23	107	11302	0.0	2.1	95.0	73.8	5.9	0.05
23	1660	3324	0.8	3.3	153.0	77.7	6.4	0.06
23		6193	0.0	2.1	92.0	76.6	6.0	0.05
24	0	0	1154.7	24.7	301.0	60.2	6.2	0.50
24	0	0	126.6	77.4	194.0	63.2	5.6	0.19

Station	Het-1 [copies L-1]	γ-24774A11 [copies L-1]	NO3 [nM]	NO2 [nM]	SRP [nM]	DOC [uM]	TN [uM]	chla [ug L-1]
24	0	130	0.0	86.4	131.0	71.7	6.5	0.16
24	0	307	0.0	3.4	121.0	71.9	4.7	0.35
24	1709	1202	0.0	2.1	67.0	71.6	5.0	0.22
24	3957	3481	0.0	1.9	48.0	67.9	5.7	0.26
24	11289	2812	0.0	2.0	41.0	74.8	6.1	0.36
24	16512	2371	6.1	3.3	33.0	82.9	4.8	0.33
25		0	2629.3	24.9	300.0	52.4	6.8	0.04
25	0	0	870.7	38.8	217.0	52.1	4.2	0.16
25	0	0	0.0	5.0	113.0	50.5	4.0	0.46
25	20	4539	0.0	2.5	91.0	49.2	3.5	0.13
25	1665	2975			48.0	79.7	5.6	0.11
25		3750	0.0	4				
25	0	371	3.8	1.9	73.0	72.1	4.5	0.16
25	0	100	0.7	2.4	126.0	70.9	5.1	0.09
26	0	0	1967.1	14.8	329.0	58.8	6.8	0.09
26	0	0	1553.5	38.6	300.0	58.0	6.5	0.22
26	0	0	39.2	20.1	197.0	64.2	4.9	0.42
26	30	4246	0.0	3.4		68.8	5.9	0.34
26	0	8947	3.6	3.7	111.0	62.2	4.8	0.17
26	3434	8826	3.0	6.4	69.0	69.1	4.5	0.14
26	4327	6463	2.5	3.1	67.0	73.1	4.5	0.22
26	1876	7891	21.7	6.0	61.0	63.9	4.2	0.20

Table S1		
Station	Synechococcus [cells L-1]	Picoeukaryotes [cells L-1]
4	181797	7359
4	203836	18575
4	404526	36064
4	299593	11684
4	370953	52054
4	34210	345805
4	930	63476
4	1380	
5	97326	
5	349441	50615
5	823878	
5	1.09E+06	
5	494317	59860
5	22964	200718
5		105619
5	3624	76170
6	195632	24657
6	67793	14378
6	486783	52828
6	233960	26638
6	523840	9829
6	359709	
6	31049	256461
6	2783	85788
7	277292	23013
7	310996	33480
7		
7	270495	18313
7	528333	
7	58383	225406
7		
7	4531	
8		
8		
8		
8		
8		
8		
8		
8		
9	555128	4269
9	335526	9699
9		
9	223946	4962
9	265908	
9	355205	60611
9	31888	144029
9		
10	326088	
10	158841	

Station	Synechococcus [cells L-1]	Picoeukaryotes [cells L-1]
10		
10	660303	
10	1.28E+06	88680
10	111598	177022
10	4775	228964
10		
11	696933	65114
11	960290	77877
11	1154556	71352
11	1508966	76566
11		
11		
12	300042	48177
12	472750	49848
12	1114702	33301
12	1015131	26498
12		
13	3140088	206289
13	4451493	259544
13		
13	331536	150119
13	44247	229046
13		
13		
14		
14	30228	210417
14	439726	141047
14	2085751	192103
14	1400884	51253
14		
14	1203724	46061
14	1909752	88258
15	2249	143468
15	964741	63466
15	547477	75777
15		
15	581770	51127
15	502332	63179
16		
16	462243	115175
16	18762	112082
16	665542	18741
16	469670	48417
16		
16	363694	593308
16	371989	54015
17		
17	3726	9556
17	6939	34355
17	118862	18330
17	938694	

Station	Synechococcus [cells L-1]	Picoeukaryotes [cells L-1]
17		
17	1075356	
17	1136302	
18		
18		
18		
18		
18		
18		
18		
18		
19	81251	163725
19	349464	
19	2473251	
19	1643733	
19		
19	233447	
19	225340	
20		
20		
20		
20		
20		
20		
20		
20		
20		
21		
21	121922	
21	236385	
21	512270	
21		
21	459359	
21	419368	
22		
22	19182	22936
22	327248	8587
22	199158	9355
22	83151	
22		
22	396497	6291
22	377629	26417
23		
23	77887	54885
23	93658	58503
23	189669	27882
23	222107	5141
23		
23	209872	18135
23	247044	7477
24		
24	5636	36106

Station	Synechococcus [cells L-1]	Picoeukaryotes [cells L-1]
24	310853	44823
24	930110	59457
24		
24		
24	5810407	27460
24		
25		
25	5846	17391
25	679786	
25	767427	13856
25	848855	16575
25		
25	885932	20966
25		
26		
26	759	8923
26	30966	66756
26		
26	636504	
26	474052	
26	680301	
26	559489	

Table S2. Oligonucleotide primer and probe sequences used in this study.

Target	Assay	Forward primer (5'-3')	Probe (5'-3')	Reverse primer (5'-3')	Ref
UCYN-A	qPCR	AGCTATAACAACGTTTTATGCGTTGA	TCTGGTGGTCCTGAGCCTGGA	ACCACGACCAGCACATCCA	1
C. *watsonii*	qPCR	CGTAATGCTCGAAGGGTTTGA	CAAGTGTGTAGAATCTGGTGGTC CTGAGCC	CACGACCAGCACAACCAACT	2
Tricho	qPCR	GACGAAGTATTGAAGCCAGGTTTC	CATTAAGTGTGTTGAATCTGGTGG TCCTGAGC	CGGCCAGCGCAACCTA	1
Het-1	qPCR	CGGTTTCCGTGGTGTACGTT	TCCGGTGGTCCTGAGCCTGGTGT	AATACCACGACCCGCACAAC	3
γ-24774A11	qPCR	CGGTAGAGGATCTTGAGCTTGAA	AAGTGCTTAAGGTTGGCTTTGGCG ACA	CACCTGACTCCACGCACTTG	4
γ-24774A11	PCR	TCCACACGTCTTATTCTGCACT		AGAGCAAACAATGTAGATTTCCTG	5

[1] Church MJ, Jenkins BD, Karl DM, Zehr JP. 2005. Vertical distributions of nitrogen-fixing phylotypes at Stn ALOHA in the oligotrophic North Pacific Ocean. Aquat Microb Ecol 38:3-14

[2] Moisander PH, Beinart RA, Hewson I, White A, Johnson K, Carlson C, Montoya JM, Zehr JP. 2010. Distributions of unicellular cyanobacteria broaden the oceanic N_2 fixation domain. Science 327:1512-1514

[3] Church MJ, Short CM, Jenkins BD, Karl DM, Zehr JP. 2005. Temporal patterns of nitrogenase gene (*nifH*) expression in the oligotrophic open ocean. Appl Environ Microbiol 71:5362-5370

[4] Moisander PH, Beinart RA, Voss M, Zehr JP. 2008. Diversity and abundance of diazotrophs in the South China Sea during intermonsoon. The ISME Journal 2:954-967

[5] This study

Table S3. Results of linear regression analysis. Relationship of abundances of the γ-24774A11 phylotype (gene copies L^{-1}) to other measured parameters was investigated. NS, not significant.

Parameter	**R^2**	***p***	**n**
UCYN-A (gc L^{-1})	0.214	0.000	168
C. watsonii (gc L^{-1})	0.550	0.000	170
Het-1 (gc L^{-1})	0.464	0.000	98
Trichodesmium (gc L^{-1})	0.354	0.000	167
Synechococcus (cells L^{-1})	0.303	0.000	113
Picoeukaryotes (cells L^{-1})	-0.169	0.000	84
Latitude	- 0.002	NS	173
TN (μM)	-0.131	0.000	156
DOC (μM)	0.439	0.004	156
NO_3 (nM)	-0.571	0.000	163
NO_2 (nM)	-0.534	0.000	163
SRP (nM)	-0.462	0.000	160
Depth (m)	-0.442	0.000	173
Temperature (C°)	0.421	0.000	173
Salinity (psu)	-0.222	0.000	173
Density	-0.490	0.000	173
Sigma-T	-0.428	0.000	173
O_2 (%)	0.525	0.000	165
Chlorophyll a (μg L^{-1})	-0.096	0.000	167
Fluorescence	-0.321	0.000	173