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Expression of the nitrogen stress response gene *ntcA* reveals nitrogen-sufficient *Synechococcus* populations in the oligotrophic northern Red Sea

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Abstract

Determining the nitrogen (N) status of phytoplankton is important for understanding primary production and N cycling in marine ecosystems. We assayed transcript levels of the N regulatory gene *ntcA* to assess the physiological N status of *Synechococcus* populations exposed to different N regimes in the meso- to oligotrophic Gulf of Aqaba, Red Sea. *Synechococcus* populations were N sufficient even in low-N environments when the ratio of dissolved nitrogen to phosphorus indicated that overall phytoplankton biomass was constrained by N. Ammonium supported *Synechococcus* N requirements under most conditions, but during a massive spring bloom in April 2000 alternative N sources were utilized. Evidence from *ntcA* clone libraries indicates changes in the genotypic makeup of *Synechococcus* populations under different N regimes, suggesting that the *Synechococcus* genotypes present in N-poor waters were those adapted for life in these environments. Thus, the success of *Synechococcus* in the open oceans is likely to be at least partially due to the selection of genotypes suited to life under prevailing N conditions rather than to prolonged manifestation of the N stress response, mediated by ntcA, in less well-adapted genotypes.

Low photosynthetic biomass, prevalent in vast oligotrophic expanses of the world's oceans, is often attributed to nitrogen (N) limitation of overall primary production and phytoplankton standing-stock (Fanning 1992; Tyrell and Law 1997). N limitation has been inferred from nutrient addition bioassays and from the ratio of dissolved nitrogen to phosphorus (DIN : SRP) in seawater (Redfield 1958; Graziano et al. 1996; Tyrell and Law 1997). A DIN : SRP ratio of 16 : 1 is indicative of nonlimiting conditions, whereas deviations from this ratio indicate nutrient limitation of phytoplankton community yields, with DIN : SRP < 16 indicating N limitation and DIN : SRP > 16 indicating P limitation. Yet these bulk assessments of nutrient limitation do not indicate whether individual phytoplankton species that populate low-nutrient environments are growth-rate limited by the supply of nutrients. Indeed, Goldman et al. (1979) argued that it is possible to have nutrient-limited phytoplankton biomass in oligotrophic environments with phytoplankton growth rates close to maximal. This may be due to high nutrient turnover rates and/or selection for phytoplankton species suited to life in low-nutrient regimes. Significant differences in cellular N and phophorus (P) requirements and nutrient source utili-

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zation and scavenging capabilities exist among diverse phytoplankton taxa as well as among closely related marine cyanobacteria (Dortch 1990; Moore et al. 2002; Bertilsson et al. 2003). Additionally, the N : P ratios indicative of N or P stress vary considerably for different phytoplankton species (reviewed in Geider and La Roche [2002]). Furthermore, Dyhrman et al. (2002) have shown that two cyanobacterial genera responded differently to the same ambient nutrient environment with one genus (*Plectonema*) being phosphate stressed while the other (*Trichodesmium*) was not. Therefore, in order to understand the role nutrient availability plays in regulating population dynamics and community structure, it is important to assess the nutrient status of the organisms of interest from a cellular property that is unique to the nutrient stress in question and that enables assessment in a taxon-specific manner. Molecular approaches are particularly suited for such a purpose (Scanlan and West 2002).

Small, unicellular, non–nitrogen-fixing cyanobacteria of the genera *Synechococcus* and *Prochlorococcus* are abundant components of oligotrophic marine ecosystems. These genera are closely related, yet each is a distinct and genetically diverse group of cyanobacteria (Toledo and Palenik 1997; Moore et al. 1998; Rocap et al. 2002; Fuller et al. 2003). *Prochlorococcus* has low cellular N requirements (Bertilsson et al. 2003; Heldal et al. 2003) and is extremely abundant in oligotrophic N-poor waters (Partensky et al. 1999*b*). *Synechococcus* populations, on the other hand, have higher cellular N requirements than *Prochlorococcus* (Bertilsson et al. 2003; Heldal et al. 2003) and are most abundant in waters freshly enriched with N (Waterbury et al. 1986; Glover et al. 1988; Lindell and Post 1995). While they remain an important component of the phytoplankton, *Synechococcus* numbers decline significantly in low-N environments (Waterbury et al. 1986; Lindell and Post 1995; Partensky et al. 1999*a*; DuRand et al. 2001). Therefore, *Synechococcus* populations may be experiencing N stress in oligotrophic waters. In this study, we assess how *Synechococcus* populations respond to changing N conditions and how they survive in low-N conditions in the northern Red Sea. To this end, we developed a taxon-specific molecular assay for assessing N stress in *Synechococcus* from *ntcA* gene expression (Lindell and Post 2001).

The transcriptional regulator encoded by *ntcA* mediates the N stress responses in cyanobacteria. Ammonium is the preferred and energetically cheapest source of N in these organisms (Flores and Herrero 1994). In the absence of ammonium, the cells induce pathways that enable growth on alternative inorganic and organic N sources such as nitrate, nitrite, and urea (Flores and Herrero 1994). In this first stage of N stress, NtcA up-regulates the transcription of its own gene as well as those required for the transport of these alternative N sources into the cell and their intracellular conversion to ammonium—the form of N assimilated into cellular organic compounds (Luque et al. 1994; Valladares et al. 2002). When no appropriate N source is available to support growth, the second stage of N stress is induced in which a series of physiological changes occur to attempt to maintain cell integrity until the supply of N is renewed. NtcA mediates the transcription of genes required for the survival of cyanobacteria exposed to N starvation (Luque et al. 2001; Muro-Pastor et al. 2001). Thus the physiological response to N stress requires immediate enhanced transcription of the *ntcA* gene, whether the N stress is due to the absence of the preferred ammonium or absence of any N source that supports growth (Luque et al. 1994). This enhanced transcription is specific for N stress and is induced once ammonium concentrations drop below 1 μmol L^{-1} in laboratory cultures of *Synechococcus* sp. strain WH7803, even in the presence of a suite of inorganic and organic N sources (Lindell and Post 2001). However, the threshold concentration for induction of *ntcA* expression may differ for the various *Synechococcus* genotypes found in nature and may depend on the flux of ammonium rather than on a set concentration.

The degree of *ntcA* transcript accumulation depends on the physiological N status of the cell. Cyanobacteria growing on ammonium display low basal *ntcA* transcript levels (Luque et al. 1994; Lindell and Post 2001; Bird and Wyman 2003). During N deprivation, *ntcA* transcript levels are at their maximum, remaining high for as long as the cells are starved for N (Lindell and Post 2001; Bird and Wyman 2003). In comparison, *ntcA* transcript levels are intermediate when alternative N sources support growth (Lindell and Post 2001; Bird and Wyman 2003). The response time for changes in *ntcA* transcript accumulation is rapid, occurring within 1 h of a change in the N status of the cell (Lindell and Post 2001). The *ntcA* assay assesses the N status of *Synechococcus* field populations by exploiting these intrinsic differences in *ntcA* accumulation with N availability. In this assay, transcript levels from untreated *Synechococcus* field populations are compared to chemically induced maximal and basal levels in these same populations (Lindell and Post 2001). In this way, the physiological N status of *Synechococcus* populations is determined, enabling us to differentiate between three distinct N states: (1) growth on ammonium—indicated by no induction of the N stress response and low basal *ntcA* transcript levels; (2) growth on N sources other than ammonium—indicated by induction of the N stress response with *ntcA* transcript levels intermediate between basal and maximal levels; and (3) N starvation—indicated by induction of the N stress response with maximal *ntcA* transcript levels.

At an enriched coastal site at the northern tip of the Gulf of Aqaba (ammonium and nitrate concentrations each reached 600 nmol L^{-1}), the *ntcA* assay showed that the N stress response was not invoked among *Synechococcus* populations (Lindell and Post 2001). While the power of the *ntcA* assay has recently been recognized, it has yet to be used to assess the N status of cyanobacterial populations in low-N environments (Scanlan and West 2002; Zehr and Ward 2002). Here we apply the *ntcA* assay to assess the N status of *Synechocococcus* populations at a coral reef site and an open-water station of the Gulf of Aqaba, Red Sea. The latter site undergoes predictable seasonal changes in phytoplankton biomass and nutrient concentrations (Genin et al. 1995; Lindell and Post 1995; Labiosa et al. 2003), with nitrate concentrations ranging from nanomolar to micromolar and *Synechococcus* abundances fluctuating from 10^3 to $>10^5$ cells per milliliter of seawater. Ammonium concentrations are low year-round (<100 nmol L^{-1}; Fuller et al. 2005). Using the *ntcA* assay, we found that despite low ambient

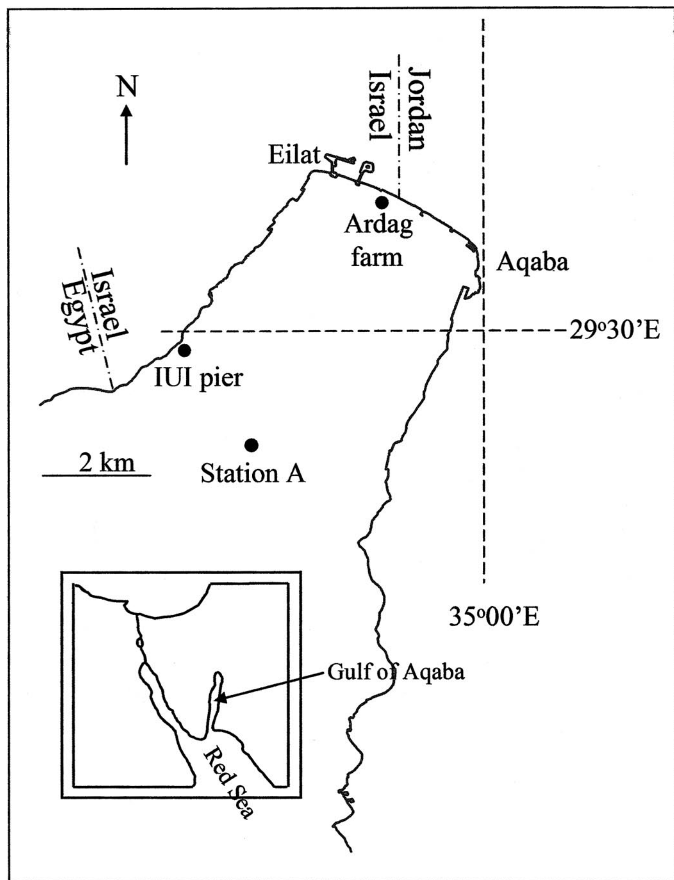

Fig. 1. Map of the northern tip of the Gulf of Aqaba showing the location of sampling Sta. A, the Interuniversity Institute (IUI) pier in the Coral Beach Nature Reserve, and the Ardag fish farm near the North Beach. The inset shows the position of the Gulf of Aqaba relative to the Red Sea.

ammonium concentrations, the N stress response was not induced in *Synechococcus* populations for most of the annual cycle and that these populations were N sufficient even when overall phytoplankton biomass was constrained by N.

Materials and methods

Sampling—Water samples were taken from a depth of 5 m (unless otherwise stated) from Sta. A (29°28′N, 34°55′E) in the northern tip of the Gulf of Aqaba, Red Sea (Fig. 1) from September 1998 to December 2000 in a single 12-liter Niskin bottle per sampling date. Water was prefiltered over a 20-μm-pore-size mesh to remove large plankton except for nutrient determinations.

Ammonium concentrations were determined onboard immediately after sampling using the fluorescent orthophthaldialdehyde method (Holmes et al. 1999) and a DyNA Quant TM 200 fluorometer (Hoefer). Nitrite, nitrate, and phosphorus (after a 20-fold concentration by the MAGIC method [Karl and Tien 1992]) were measured colorometrically on a QuickChem 8000 flow injection autoanalyzer (Lachat Instruments) with detection limits of 20 nmol L^{-1} for nitrate and nitrite and 10 nmol L^{-1} for phosphorus. DIN : SRP ratios were calculated from concentrations of dissolved nitrate + nitrite and phosphorus. It was not possible to ascertain the DIN : SRP ratio for August and September 2000 because of values below limits of detection for one or both nutrients in surface layers.

Chlorophyll *a* (Chl *a*) was extracted from 100-ml samples collected on GF/F filters in 90% acetone for 24 h at 4°C and measured on a TD700 fluorometer (Turner Designs). Abundances of phycoerythrin-fluorescing *Synechococcus* cells were determined on a FACScan flow cytometer after fixation in 0.4% paraformaldehyde (pH 8) and frozen in liquid nitrogen.

Samples for DNA extraction (5 liters) were filtered onto 0.45-μm-pore-size Supor-450 membranes (Gelman Sciences). Membranes were immersed in storage buffer (20 mmol L^{-1} EDTA, 400 mmol L^{-1} NaCl, 0.75 mol L^{-1} sucrose, 50 mmol L^{-1} Tris [pH 9] as per Gordon and Giovannoni [1996]) and stored at −80°C until nucleic acid extraction.

N stress response assay—The N stress response was determined by assessing mRNA transcript levels of *ntcA* in field populations of *Synechococcus.* Water samples (10 liters) were collected during the late morning (between 10:00 h and 12:00 h) because nitrogen utilization often requires light, and *ntcA* expression in *Synechococcus* sp. strain WH7803 varies over a diel cycle with greatest expression in the morning hours (Lindell 2000). Samples were divided into three equal volumes of 2.5–3.0 liters and incubated with (1) 100 μmol L^{-1} ammonium (NH_4^+), (2) 100 μmol L^{-1} L-methionine-D,L-sulfoximine (MSX), or (3) not amended, for 90 min at 25°C while illuminated with 200 μmol of photons m^{-1} s^{-1} (similar to light levels at 5-m depth). The addition of ammonium leads to basal transcript levels indicative of growth on this preferred N source, whereas the addition of MSX induces maximal transcript levels indicative of N starvation (Lindell and Post 2001). MSX starves a wide range of cyanobacteria of N by preventing NH_4^+ assimilation into organic compounds (Flores and Herrero 1994). Transcript levels in the unamended subsample provide the actual *ntcA* expression levels in *Synechococcus* field populations and are compared to the maximal (MSX-amended) and basal (NH_4^+-amended) levels in these same populations (Lindell and Post 2001). The addition of 100 μmol L^{-1} NH_4^+ and MSX ensures that the concentration of these compounds does not change significantly in these reference subsamples during the course of the incubations.

Differences in *ntcA* transcript levels between NH_4^+- and MSX-treated subsamples verify that field populations respond to these additions. The short incubation period required for this assay enables assessment of the N status in real time within a <2-h time resolution, and it minimizes detrimental effects associated with day- to week-long bottle incubations required in nutrient addition bioassays. Furthermore, while the *ntcA* assay involves addition of ammonium and MSX to subsamples that serve as references, experimental subsamples remain untreated, thereby circumventing physiological changes induced through N additions.

Following the 90-min incubation, each subsample was filtered onto a single Supor-450 membrane under a vacuum of 25 inches of Hg while illuminated with 200 μmol of photons

Table 1. Primer sequences for *ntcA* amplification. Note that the G15–16F primer mix is a 1 : 1.3 ratio of primers G15F and G16F. N = G,A,T,C; V = G,A,C; B = G,T,C; H = A,T,C; D = G,A,T; K = G,T; S = G,C; W = A,T; M = A,C; Y = C,T; R = A,G. Note that primer S50R has two differences to the MIT9313 *Prochlorococcus* clade.

Primer	Specificity	Nucleotide sequence
1F	General cyanobacterial	5′-ATH TTY TTY CCN GGN GAY CCN GC-3′
4R	General cyanobacterial	5′-AT NGC YTC NGC DAT NGC YTG RT-3′
1AF	General cyanobacterial	5′-ATH TTY TTY CCB GGG GAY CCD GC-3′
4AR	General cyanobacterial	5′-AT GGC YTC GGC KAT GGC YTG RT-3′
G15F	General cyanobacterial	5′-GAR TCN GGB GAA GAG ATC ACY GT-3′
G16F	General cyanobacterial	5′-GAR TCW GGW GAA GAR ATW ACW GT-3′
S50R	*Synechococcus* specific	5′-G CAG RTC RAT SGT GAT SCC SHG-3′

m^{-1} s^{-1} during the ~20-min filtration. Filters were immersed in storage buffer (20 mmol L^{-1} EDTA, 400 mmol L^{-1} NaCl, 0.75 mol L^{-1} sucrose, 50 mmol L^{-1} Tris [pH 9]), snap frozen in liquid nitrogen, and stored at −80°C until RNA was extracted from half of the filter (see below). Reverse transcription (RT) to produce complementary DNA (cDNA) followed by nested polymerase chain reactions (PCRs) were carried out to determine the relative amounts of *ntcA* transcript in the three treatments for each sample (*see below*).

Nucleic acid extraction—In order to assess the prevalence of *ntcA* in a wide range of organisms, we extracted genomic DNA from an assortment of cultured organisms. Genomic DNA from cyanobacteria or bacteria was extracted according to Scanlan et al. (1990) with the dialysis step omitted. Genomic DNA from eukaryotic algae was extracted according to Saunders (1993), except that the algae were not ground prior to extraction. Genomic DNA from field samples was extracted using the following protocol. Cells were treated with lysozyme (1 mg ml^{-1}) at 37°C for 30 min followed by an additional 30-min incubation at 37°C in the presence of proteinase K (0.1 mg ml^{-1}) and SDS (1%). Proteinase K was inactivated with a 10-min incubation at 55°C. DNA was extracted with phenol : chloroform : isoamyl alcohol (25 : 24 : 1/v : v : v) followed by a chloroform : isoamyl alcohol (24 : 1) extraction. Nucleic acids were then precipitated with 0.4 volume of 7.5 mol L^{-1} ammonium acetate and 1 volume of isopropanol and resuspended in nuclease-free water.

Total RNA was extracted from field samples using a hot-phenol method described previously (Lindell and Post 2001). Briefly, the samples were treated with lysozyme (1 mg ml^{-1}) at 37°C for 15 min, and the cells were lysed by heating in a microwave to near boiling in the presence of 1% SDS after bringing the pH of the buffer down to 7.5 with HCl. The samples were extracted after a 5-min incubation with phenol preheated to 65°C and the subsequent addition of chloroform–isoamyl alcohol (24 : 1/v : v). The filter dissolves in the organic phase during this treatment. Subsequent extractions of the aqueous phase were carried out with phenol–chloroform–isoamyl alcohol (25 : 24 : 1/v : v : v) and chloroform–isoamyl alcohol (24 : 1/v : v). Nucleic acids were precipitated with 0.4 volume of 7.5 mol L^{-1} ammonium acetate and 1 volume of isopropanol and resuspended in buffer TE2 (10 mmol L^{-1} Tris, 0.1 mmol L^{-1} EDTA [pH 8]). DNA was degraded using DNA-*free* (Ambion). The absence of DNA from RNA samples was verified prior to analysis by a negative result with nested PCR for the maximal number of cycles used in the reverse transcription–polymerase chain reaction (RT-PCR) protocol. RNA was quantified densitometrically from agarose gels with one-dimensional (1D) image analysis software (Kodak Digital Science).

PCR, cloning, and sequencing—*ntcA* was amplified from cyanobacterial isolates with general cyanobacterial primers 1F and 4R (*see Table 1 for primer sequences*), as described in Lindell et al. (1998), and yielded fragments 449 bp in length. Reactions were run in 50-μl volumes with 2 mmol L^{-1} $MgCl_2$, 0.2 mmol L^{-1} of each deoxynucleoside triphosphate, 1 μmol L^{-1} each primer, 1.25 U of *Taq* DNA polymerase (Promega), and 0.2–10 ng genomic DNA. *ntcA* was amplified from field samples using primers 1F and 4R or less degenerate general primers 1AF and 4AR (Table 1) at primer concentrations of 2.4 μmol L^{-1} and 0.8 μmol L^{-1}, respectively. PCR reactions were run on an MJ Research Thermocycler for 30–40 cycles of denaturation for 1 min at 94°C, annealing for 1 min at 55°C, and elongation for 1.5–2 min at 68–70°C following an initial 4-min denaturation step at 94°C.

The general primer G15–16F and the *Synechococcus*-specific (*Syn*-specific) primer S50R (at concentrations of 0.8 μmol L^{-1} each) were used to amplify a 344-bp fragment specifically from *Synechococcus* field populations, either directly from genomic DNA or in nested PCR reactions in which the template DNAs were *ntcA* fragments amplified with the general *ntcA* primers (Lindell and Post 2001). Reaction mixes and cycling conditions were the same as those described above, except that the $MgCl_2$ concentration was 1.5 mmol L^{-1}. The *Syn*-specific primer was designed by aligning *ntcA* from a wide range of cyanobacteria and choosing a region of near identity among *Synechococcus* isolates that was different from that of other cyanobacterial genera.

Amplified *ntcA* fragments were gel purified using the GeneClean II kit (Bio 101) or the Qiaex II Gel Extraction kit (Qiagen), ligated into the pGEM-T vector (Promega), and transformed by heat shock into the DH5α strain of *Escherichia coli* that was rendered chemically competent for transformation. Sequencing of *ntcA* clones was carried out with ABI 377 Prism DNA sequencers by the Genome Services Analysis Unit of the Life Sciences Institute, Hebrew Uni-

versity of Jerusalem. Sequences from cyanobacterial isolates were sequenced in both directions and verified with clones derived from independent PCR reactions.

PCR clones from field samples were chosen for sequencing to reflect the *ntcA* diversity present. In order to sequence as many different *ntcA* types as possible, the inserts/clones were prescreened by digestion with either of the restriction enzymes *Mse*I or *Pst*I. A number of clones from each restriction fragment pattern (when present) were chosen for sequencing from each sampling date. Therefore, no attempt was made to quantify the different *ntcA* field types but rather to document their presence. Only field clones whose deduced amino acid sequences were verified from independent samples and PCR reactions were used, thus excluding two potentially rare field clone types from our tree analyses. While clone libraries provide a qualitative indication of the prevalent groups, a more quantitative method (such as single cell assays or quantitative PCR) is needed to assess relative abundances of the individual *Synechococcus* genotypes under different environmental conditions.

The partial *ntcA* sequences from *Synechococcus* and *Prochlorococcus* cultures and from Red Sea field clones have been deposited in the GenBank database under the accession numbers AY885076–AY885120.

RT-PCR—Reverse transcription followed by nested PCR was carried out as previously described (Lindell and Post 2001). Briefly, 50 ng of total RNA was denatured at 70°C and reverse transcribed in 20-μl reactions with 2 pmol primer 4AR using 65 U Superscript II reverse transcriptase (Gibco-BRL) at 42°C in the presence of 40 U RNasin (Promega). Two microliters of the resultant cDNA was amplified in 50-μl reactions with primers 1AF and 4AR for 30 cycles under the reaction and cycling conditions described above. One microliter of the resultant PCR reaction was used in nested PCR reactions with primers G15–16F and S50R for 11–25 cycles in 20-μl reactions. The number of cycles carried out was empirically determined to ensure that amplification was still in the exponential phase for both PCR reactions. The amount of *ntcA* cDNA amplified from each subsample was quantified densitometrically from agarose gels with 1D image analysis software. Expression levels for each of the treatments are presented relative to total *ntcA* transcription (no addition, $+NH_4^+$, and +MSX) rather than by scaling to expression relative to one treatment.

Phylogenetic analysis—Nucleotide sequences were aligned using Clustal X and verified visually. Phylogenetic analysis was carried out on 404 bp (corresponding to the region between the general *ntcA* primers) with PAUP V4.0b10 software (Sinauer Associates, Inc.). The distance tree was inferred using HKY85 distances with minimum evolution as the objective function. Heuristic searches were performed with 100 random addition sequence replicates and the tree bisection-and-reconnection branch-swapping algorithm. Starting trees were obtained by stepwise addition of sequences. Distance, maximum parsimony, and maximum likelihood bootstrap analyses of 100 resamplings were carried out. *ntcA* fragments amplified from cyanobacterial isolates and field samples were grouped in subgeneric clades based on identity levels greater than 85%. Clade designations follow those from Rocap et al. (2002) and Fuller et al. (2003). Field clades with no currently known corresponding *Synechococcus* isolate in culture were designated as nonclassified (NC).

Results

Environmental conditions—Seasonal dynamics of total phytoplankton and *Synechococcus* populations followed those of N and P concentrations at Sta. A in the Gulf of Aqaba, Red Sea, during the 2-yr sampling period of our study (Fig. 2). In summer–autumn stratified waters (Jun–Sep), dissolved inorganic N (DIN = $NO_3^- + NO_2^-$), soluble reactive phosphorus (SRP), and overall phytoplankton biomass, determined from Chl *a* concentrations, were low (Fig. 2; Table 2). During mixing (Nov–Mar), DIN and SRP were injected from deeper waters into the photic zone, with a subsequent increase in phytoplankton biomass. At the onset of stratification, nutrient-rich waters became trapped in the surface layers and a phytoplankton bloom ensued. This spring bloom led to rapidly reduced DIN and SRP concentrations in the photic zone and a subsequent decline in phytoplankton biomass. By summer, DIN, SRP, and phytoplankton biomass were again low. *Synechococcus* abundances were greatest during the spring bloom, peaking immediately after the increase in overall Chl *a* concentrations, and declining dramatically subsequent to nutrient draw-down in both years (Fig. 2). The differences between 1999 and 2000 were due to the deeper winter–spring mixing event in 2000, which extended to below 600 m and injected more nutrients into the photic zone (Fig. 2). Ammonium concentrations ranged from 11 nmol L^{-1} to 61 nmol L^{-1} throughout the year, and while seasonal changes may have occurred, the lack of multiannual measurements prevents us from drawing clear conclusions (Fig. 2).

Concentrations of DIN and SRP (at 5-m depth) were low during the stratified period of both 1999 and 2000. Surface layer DIN : SRP ratios were well below the Redfield ratio of 16—ranging from 0.3 to 5, suggesting that phytoplankton biomass was strongly limited by N (Figs. 2, 3). On 13 August 2000, an episodic increase in DIN to 0.16 μmol L^{-1} led to DIN : SRP ratios likely to be greater than 16, as SRP was below limits of detection. Conversely, during winter–spring mixing of 1999, sufficient DIN was injected into the surface layers to transfer the system to one limited by P as indicated by DIN : SRP ratios ranging from 30 to 70 (Figs. 2, 3). Phytoplankton biomass during deep winter–spring mixing in 2000 was unlikely to have been limited by the availability of either N or P as suggested by measurable levels of both nutrients and DIN : SRP ratios close to 16. In this year, mixing extended to depths as great as 600 m (Fig. 2c), transporting phytoplankton below the photic zone for long periods of darkness; thus, phytoplankton biomass was likely to have been limited by light (Labiosa et al. 2003). Seasonal changes in DIN : SRP ratios, from low ratios indicative of N limitation during the stratification period to high ratios indicative of P limitation during deep mixing, are not unique to the Gulf of Aqaba; similar trends have been reported for the Sargasso Sea (Cavender-Bares et al. 2001).

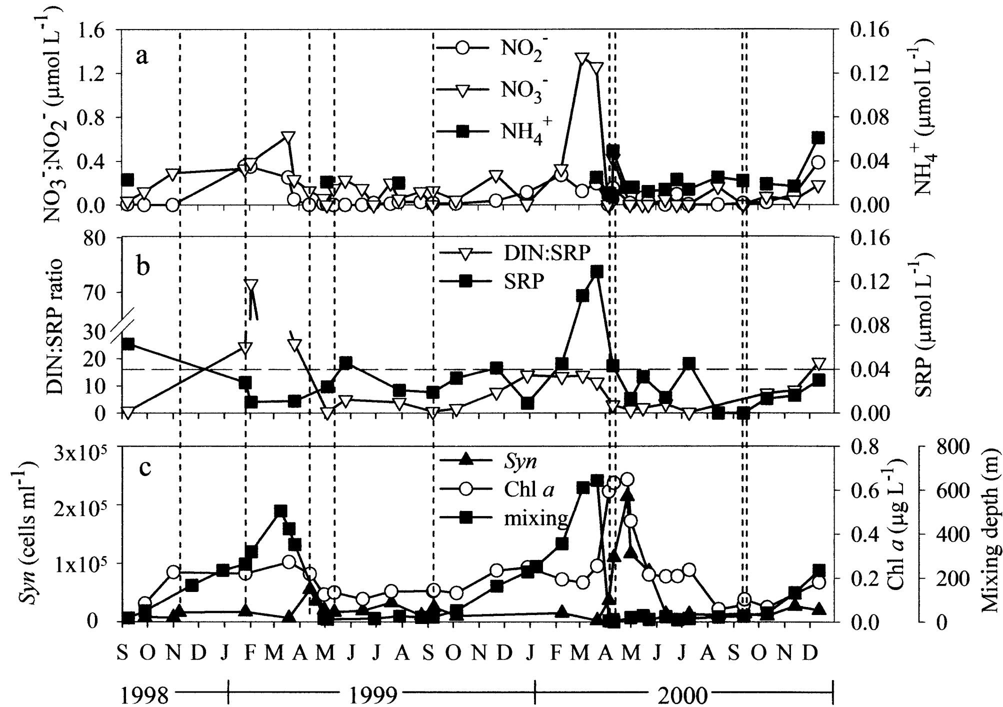

Fig. 2. Seasonal variation in inorganic nutrient concentrations and phytoplankton abundance. (a) Ammonium, nitrate, nitrite; (b) soluble reactive phosphorus (SRP), DIN : SRP ratios; (c) Chl *a*, *Synechococcus,* surface mixed-layer depth. Note that the scales for ammonium and SRP are 10-fold lower than for nitrate and nitrite. Water samples were collected from 5-m depth at Sta. A (29°28′N, 34°55′E) in the northern tip of the Gulf of Aqaba, Red Sea, from September 1998 until December 2000. The dashed vertical lines denote sampling dates presented in Fig. 6. The dashed horizontal line in panel b corresponds to a DIN : SRP ratio of 16. On 13 August 2000, DIN : SRP ratios were likely to be greater than 16 as SRP was below limits of detection and DIN was 0.16 μmol L^{-1}.

Synechococcus population detection and diversity determined from the ntcA *gene*—The *ntcA* gene is found in a wide variety of cyanobacteria and is specific to this group. Using the general *ntcA* primers (1A and 4F), we successfully amplified the *ntcA* gene from all cyanobacteria tested, with the exception of the freshwater *Prochlorothrix hollandica* (Fig. 4a shows results from representative cyanobacterial isolates). However, *ntcA* was not amplified from a wide variety of eukaryotic algae or from autotrophic or heterotrophic bacterial isolates (Fig. 4a shows results from representative strains). Furthermore, *ntcA* was amplified specifically from cyanobacteria within mixed planktonic communities (Fig. 4b). Shallow waters in the photic zone (18 m) yielded a fragment of expected size, whereas a PCR fragment was barely visible from deeper waters (190 m) where cyanobacterial abundances are low. Cloning and sequencing of these PCR fragments revealed them to be most similar to *ntcA* from known marine *Synechococcus* and *Prochlorococcus* isolates. Therefore, *ntcA* can be used to specifically identify cyanobacteria from within mixed communities consisting of a multitude of organisms from a wide range of taxa.

Phylogenetic relationships inferred from *ntcA* sequences

Table 2. Seasonal ranges of nutrient concentrations in nmol L^{-1} (NH_4^+ = ammonium; DIN = NO_3^- + NO_2^-; SRP = soluble reactive phosphorus), DIN : SRP ratios, chlorophyll *a* concentrations in μg L^{-1} (Chl *a*), and *Synechococcus* abundances in cells ml^{-1} (*Syn*) at 5-m depth during the period of this study. Seasonal periods of mixing and stratification were determined from temperature profiles. Nutrient values of zero indicate concentrations below limits of detection (10 nmol L^{-1} for NH_4^+ and SRP, and 20 nmol L^{-1} for DIN).

Year	Season	NH_4^+	DIN	SRP	DIN : SRP	Chl *a*	*Syn*
1999	Nov 98–Feb 99: winter–spring mixing	ND	290–730	10–28	24–71	0.22–0.23	8,000–16,000
	Mar–Apr 99: spring onset of stratification	ND	130–880	11–24	0.4–25	0.22–0.27	6,500–55,000
	May–Sep 99: summer–autumn stable stratification	20	0–220	19–46	0.6–4.9	0.11–0.14	17,000–33,000
2000	Nov 99–Mar 00: winter–spring mixing	25	120–1,470	10–129	7.7–13.8	0.18–0.26	2,000–15,000
	Apr–May 00: spring onset of stratification	0–49	0–570	13–33	1.1–2.9	0.21–0.65	37,000–214,000
	Jun–Sep 00: summer–autumn stable stratification	12–25	0–164	0–45	0.3–3.3*	0.06–0.22	12,000–14,000

* DIN : SRP ratios were likely to have been considerably higher on 13 August 2000 when DIN increased to 164 nmol L^{-1}, but SRP was below limits of detection.

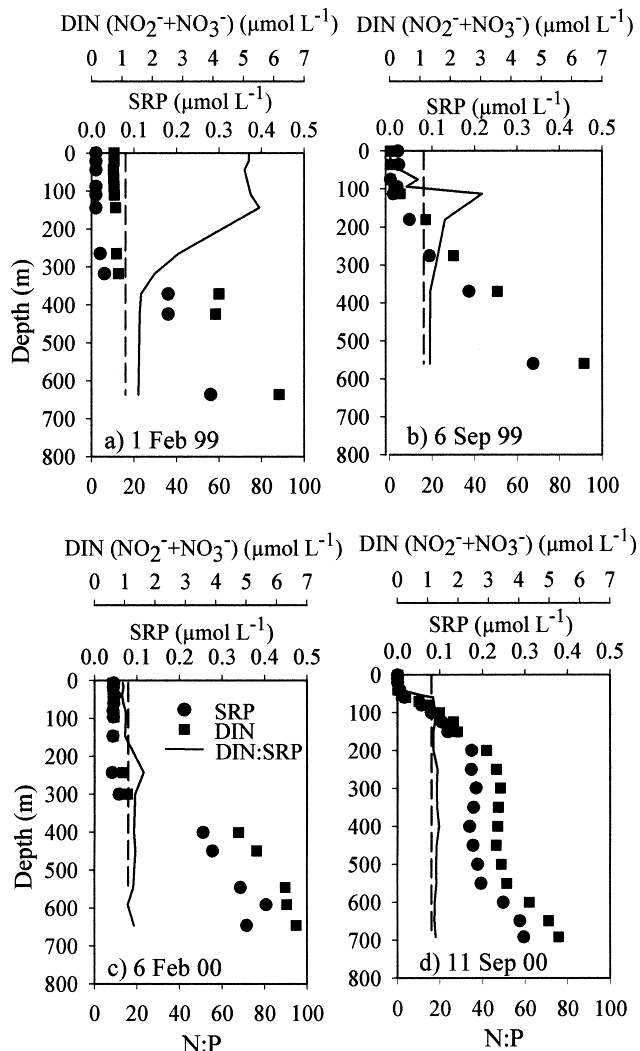

Fig. 3. Depth profiles of inorganic nutrients for representative dates during winter: February 1999 (a) and February 2000 (c); and stable stratification in autumn: September 1999 (b) and September 2000 (d). DIN, SRP, DIN : SRP. The dashed vertical line denotes a DIN : SRP ratio of 16.

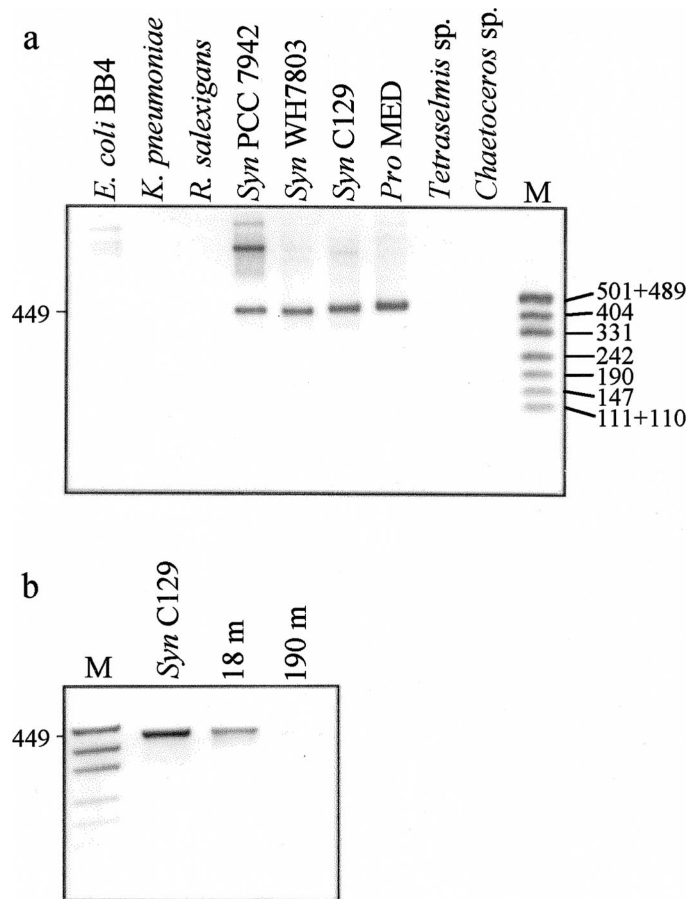

Fig. 4. Amplification of a 449-bp *ntcA* fragment from genomic DNA extracted from (a) bacterial or algal cultures and (b) Red Sea field samples. (a) Lanes shown are for the heterotrophic bacteria *Escherichia coli* strain BB4 and *Klebsiella pneumoniae*; the photosynthetic purple sulfur bacterium *Rhodospirillum salexigans*; the cyanobacteria *Synechococcus* sp. strains PCC7942, WH7803, and C129, and *Prochlorococcus* sp. strain MED; and eukaryotic algae prasinophyte *Tetraselmis* and the diatom *Chaetoceros.* Other cultures that tested negative for *ntcA* but are not shown include heterotrophic bacteria *Bacillus subtilis* strain LA1742, MED4 contaminant #1, #2, #3, #4, and the SS120 contaminant X; the photosynthetic purple sulfur bacterium *Ectorhodospira maresmortuis*; eukaryotic algae eustigmatophyte *Nannochloropsis* and prymnesiophyte *Isochrysis*; and the cyanobacterium *Prochlorothrix hollandica.* All other cyanobacteria tested were positive for *ntcA* and included *Synechococcus* sp. strains WH8018, WH8020, WH8102, WH8103, WH8109, CC9311, CC9305-3, C8015, RS9705, RS9708; *Prochlorococcus* sp. strains MIT9313, MIT9303, SS120, NATL2A, PCC9511, TATL1, NATL1, TATL2, MIT9312, MIT9201, RS810; *Trichodesmium* sp. strains RS9602, WH9601; and *Cyanothece* sp. strain BH68K. (b) Field samples were collected from Sta. A in the Gulf of Aqaba, Red Sea, from 18-m and 190-m depths on 23 September 1997. Sizes of the fragments in the pUC19/*Msp*I DNA marker (M) are shown to the right of panel (a).

from a variety of cyanobacterial isolates (Fig. 5a) are essentially congruent with those inferred from *rpoC1,* 16S rRNA, the 16S–23S intergenic spacer, and *psbA* sequences (Toledo and Palenik 1997; Rocap et al. 2002; Fuller et al. 2003; Zeidner et al. 2003). In all of these gene trees, *Synechococcus* and *Prochlorococcus* form a monophyletic group separate from other cyanobacteria. Within this monophyletic group, *Synechococcus* and *Prochlorococcus* fall into separate clusters that differ by more than 30% in their *ntcA* nucleotide sequences (with the exception of *Prochlorococcus* sp. strains MIT9313 and MIT9303, which cluster with *Synechococcus*). Within each cluster, the cultured isolates form a number of distinct subgeneric clades. Field sequences amplified from 20-m depth with the general *ntcA* primers fall within both *Synechococcus* and *Prochlorococcus* clusters (Fig. 5a). PCR with the *Syn*-specific primers (G15–16F and S50R) amplified *ntcA* fragments from environmental samples even when *Prochlorococcus* populations outnumbered *Synechococcus* 10-fold, and all sequenced clones clustered with *Synechococcus* strains. Furthermore, the full range of *Synechococcus* genotypes found with the general cyanobacterial primers was also obtained with the *Syn*-specific prim-

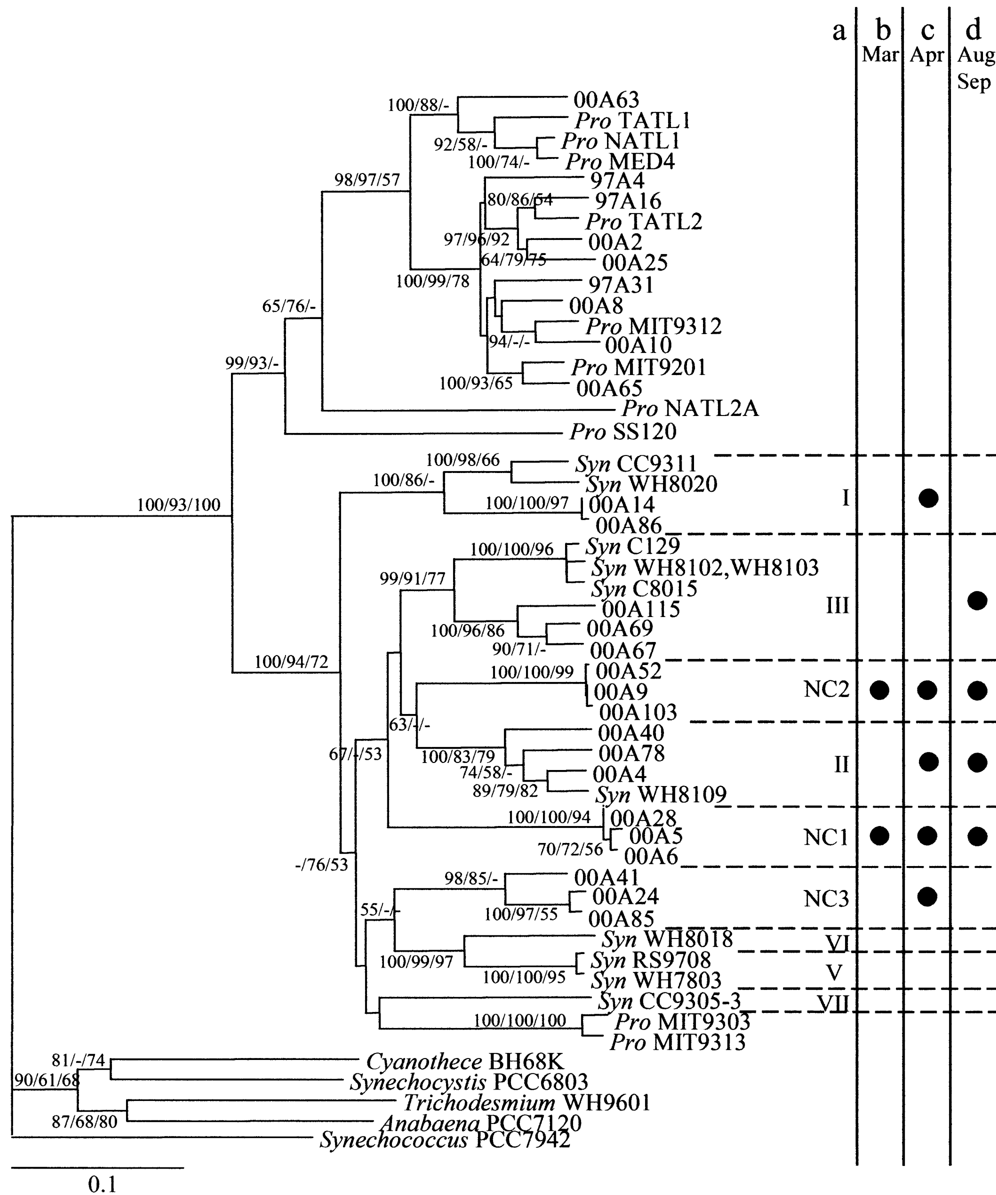

Fig. 5. *ntcA* sequences from cyanobacterial cultures and field samples in the Gulf of Aqaba, Red Sea. (a) *ntcA* gene tree showing the phylogenetic relationship of strains of marine *Synechococcus* (*Syn*) and marine *Prochlorococcus* (*Pro*) with other cyanobacteria and Red Sea field clones inferred from nucleotide distance tree. Field clones are designated by year, station ID, and clone number, respectively; e.g., 00A63 designates clone number 63 sampled from Sta. A in 2000. Bootstrap values from 100 replicates (>50%) are shown at branch nodes for distance, maximum parsimony, and maximum likelihood analyses. Dashed horizontal lines delineate subgeneric *Synechococcus* clusters with nucleotide identities of less than 85%. Roman numerals indicate clade designations used previously (Rocap et al. 2002; Fuller et al. 2003). Nonclassified (NC) clades are those field clones with no currently known corresponding *Synechococcus* isolate in culture. *Synechococcus*-like *ntcA* sequences obtained from 20-m depth at Sta. A during the following conditions: (b) vertical mixing, March 2000 ($n = 29$); (c) recent stratification, April 2000 ($n = 28$); (d) stable stratification, August–September 2000 ($n = 32$). Detection of sequences with greater than 85% identity to subgeneric clusters is represented by filled circles horizontally aligned with the respective clusters in panel (a). GenBank accession numbers of the *ntcA* sequences used to produce (a) are X60197 for *Synechococcus* sp. strain PCC7942; X71607 for *Synechocystis* sp. strain PCC6803; X71608 for *Anabaena* sp. strain PCC7120; U80855 for *Cyanothece* sp. strain BH68K; AF017020 for *Synechococcus* sp. strain WH7803; AF244902 for *Trichodesmium* sp. strain WH9601; BX572090 for *Prochlorococcus* sp. strain MED4; and AY885076–AY885120 for the *Synechococcus* and *Prochlorococcus* strains and Red Sea clones presented here.

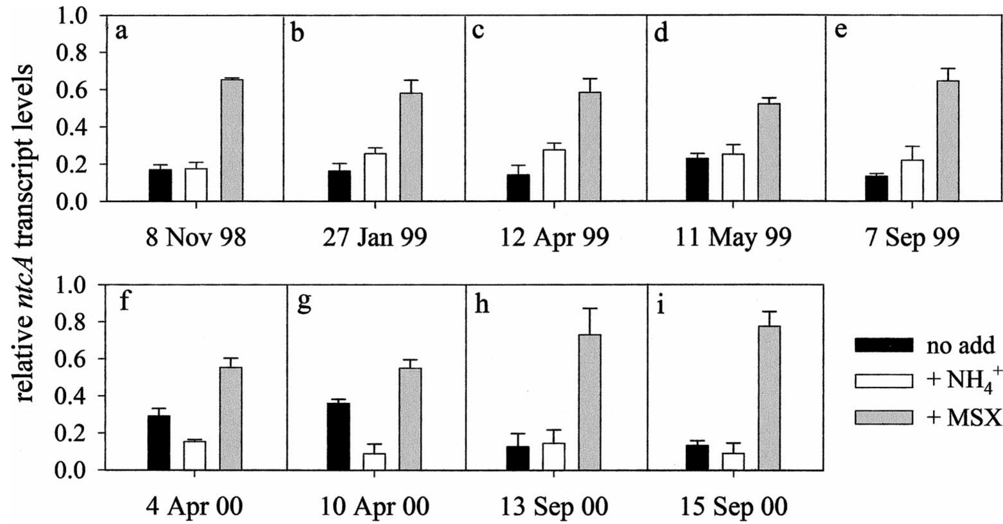

Fig. 6. Transcript levels of *ntcA* from *Synechococcus* field populations at different stages of the annual cycle sampled from 5-m depth at Sta. A in the Gulf of Aqaba, Red Sea. *ntcA* transcript levels of untreated subsamples (no add), subsamples treated with ammonium to induce basal levels ($+NH_4^+$) or chemically starved of N through the addition of MSX (+MSX) are shown as a fraction of total transcript levels (no add, $+NH_4^+$, and +MSX). *ntcA* transcript levels in the untreated subsamples were significantly below MSX-treated maximum levels on all sampling dates, determined from paired two-tailed *t* tests of means: 8 Nov 98 ($p < 0.01$, $n = 3$); 27 Jan 99 ($p < 0.05$, $n = 3$); 12 Apr 99 ($p < 0.01$, $n = 4$); 11 May 99 ($p < 0.01$, $n = 3$); 7 Sep 99 ($p < 0.01$, $n = 3$); 4 Apr 00 ($p < 0.05$, $n = 3$); 10 Apr 00 ($p < 0.001$, $n = 6$); 13 Sep 00 ($p < 0.05$, $n = 3$), and 15 Sep 00 ($p < 0.01$, $n = 3$). Transcript levels of the untreated subsamples were significantly greater than basal levels ($+NH_4^+$) on both sampling dates in April 2000: 4 Apr 00 ($p < 0.05$, $n = 3$); 10 Apr 00 ($p < 0.001$, $n = 6$). The untreated subsamples on other dates were not significantly greater than basal levels. n = number of nested PCR reactions per sampling date.

ers. Therefore, the *Syn*-specific primers amplify *ntcA* from the full range of *Synechococcus* genotypes among field populations in a taxon-specific manner.

Synechococcus abundances change with season in the Gulf of Aqaba (Lindell and Post 1995; Fig. 2). In order to assess whether the prevalence of various *Synechococcus* genotypes also change with season, we assessed *Synechococcus* diversity from *ntcA* clone libraries obtained from vertically mixed, N-replete waters in March 2000 (Fig. 5b), recently stratified waters with fluctuating N concentrations in April 2000 (Fig. 5c), and stably stratified waters with low N concentrations in August–September 2000 (Fig. 5d). Six distinct *Synechococcus ntcA* sequence types were detected in our clone libraries. Two of these genotypes were prevalent in libraries from all three seasons (clades NC1 and NC2). Another genotype (clade II) that was present during the spring and summer of 2000 in this study was previously found to be an abundant *Synechococcus* genotype in the Gulf of Aqaba throughout 1999 (Fuller et al. 2005). Three other genotypes were detected either in spring (clade I and NC3) or summer (clade III). Genotypes found in the 20-m clone libraries from a single season were also found in 60-m clone libraries from the same date (Penno et al. unpubl. data), suggesting that they may be prevalent under distinct environmental conditions. Furthermore, clones that clustered with the chromatically adapting *Synechococcus* strain CC9311 (clade I) were detected in recently stratified, but not stably stratified, waters in both the Gulf of Aqaba (Fig. 5) and the California Current of the Pacific Ocean (Palenik 2001). These results suggest that some *Synechococcus* genotypes were present year-round, whereas the prevalence of other *Synechococcus* genotypes varied with season and may be due to changes in water-column conditions.

Nitrogen status of Synechococcus *populations determined from the* ntcA *assay—Synechococcus* field populations utilized ammonium on all sampling dates during the 1998–1999 seasonal cycle as seen from basal expression of the *ntcA* gene (Fig. 6a–e). Therefore, even though ammonium was present at only nanomolar concentrations year-round, it was used preferentially over other N sources (such as nitrate, nitrite, or organic N) even in autumn and winter when nitrate concentrations were orders of magnitude higher than ammonium concentrations (Fig. 2). This suggests that a continuous flux of ammonium, regenerated through grazing and decomposition of organic matter, supported *Synechococcus* growth during these periods.

In contrast to findings from the 1998–1999 annual cycle, the N stress response was induced in *Synechococcus* populations in the spring of 2000. *ntcA* transcript levels were elevated above ammonium-utilizing basal levels but were below N-deprived maximal levels on the two sampling dates (6 d apart) in April 2000 at Sta. A (Fig. 6f,g). This indicates that the flux of regenerated ammonium was not sufficient to support the rapid net population growth of *Synechoccocus* during this period. (Note that total phytoplankton biomass

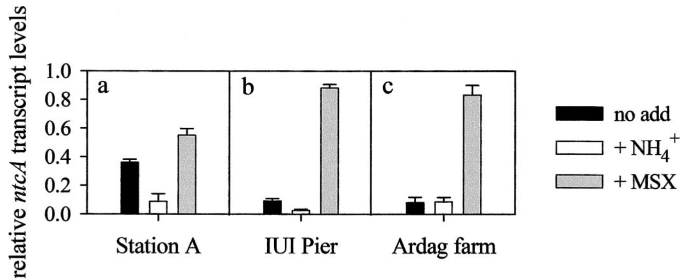

Fig. 7. Transcript levels of *ntcA* from *Synechococcus* field populations at different sites in the Gulf of Aqaba, Red Sea, during April 2000. (a) Sta. A (*see Fig. 6*) on 10 April 2000, (b) The IUI pier in the Coral Beach Nature Reserve on 12 April 2000, (c) The Ardag fish farm on 11 April 2000 (as per Lindell and Post [2001]). *ntcA* transcript levels in the untreated subsamples were significantly below MSX-treated maximum levels at all three sampling sites, determined from paired two-tailed *t* tests of means: Sta. A ($p < 0.001$, $n = 6$); the IUI pier ($p < 0.05$, $n = 3$); the Ardag fish farm ($p < 0.01$, $n = 3$). Transcript levels of the untreated subsamples were significantly greater than basal levels (+NH_4^+) for Sta. A ($p < 0.001$, $n = 6$) and the IUI pier ($p < 0.05$, $n = 3$), but not for the Ardag fish farm. n = number of nested PCR reactions per sampling site.

was close to the annual maximum during this sampling period, and that three *Synechococcus* genotypes not previously detected during winter mixing were present during the spring bloom.) These data further indicate that the subsequent induction of the N stress response enabled the utilization of other N sources and that these sources were sufficient to support the N requirements of the *Synechococcus* populations, as *ntcA* transcript levels were significantly below N-deprived maximal levels. Expression of the *ntcA* gene did not exceed ammonium-utilizing basal levels during stable stratification in September 2000 (Fig. 6h,i), indicating that these populations were again growing on ammonium.

In order to determine whether the N stress response was apparent in *Synechococcus* populations from more coastal regions (Fig. 1) in the Gulf of Aqaba during the 2000 spring bloom, we employed the *ntcA* assay at the Coral Beach Nature Reserve (sampled off the pier at the Interuniversity Institute for Marine Sciences), which had similar N concentrations as Sta. A during this period (data not shown) and was adjacent to a site anthropogenically enriched for N (the Ardag fish farm) with significantly higher ammonium concentrations (~600 nmol L^{-1}; see Lindell and Post [2001]) than Sta. A. *Synechococcus* populations from the coral reef displayed intermediate *ntcA* transcript levels (Fig. 7). Therefore, similar to the open-water populations at Sta. A during the spring bloom, the N stress response was induced in *Synechococcus* populations of adjacent coral reefs, showing that ammonium was not sufficient and that an alternative N source supported growth. In contrast, *Synechococcus* populations from the Ardag fish farm displayed basal *ntcA* expression (Lindell and Post 2001; Fig. 7), indicating that this enriched site contained sufficient ammonium to support *Synechococcus* growth.

Our results show that the N stress response was not induced in *Synechococcus* populations on most sampling dates at Sta. A, as well as at the Ardag fish farm during spring 2000, as indicated from basal *ntcA* transcript levels. The N stress response was induced during the spring bloom of 2000 at both Sta. A and a coral reef site, with *ntcA* expression elevated above basal levels. However, the lack of maximal *ntcA* expression on all sampling dates indicates that *Synechococcus* populations were not N starved in the Gulf of Aqaba even under oligotrophic conditions when DIN concentrations were near or at the detection limit (see Fig. 2). Thus *Synechococcus* populations were N sufficient with either ammonium, the preferred N source, or alternative N sources supporting growth.

Discussion

In this study we have, for the first time, employed *ntcA* expression as a direct measure of the N status of a major component of marine phytoplankton, the picocyanobacterium *Synechococcus.* Expression of this N stress–induced gene provides a specific assessment of N availability to *Synechococcus* populations. Using this assay, we have determined that *Synechococcus* populations were not N deprived in the stably stratified N-poor waters of the northern Red Sea, despite a progressive reduction in population size. Clearly the low DIN : SRP ratios, indicative of N limitation of overall phytoplankton standing-stock during that period, did not reflect the N status of the resident *Synechococcus* populations. Furthermore, our results indicate that *Synechococcus* is capable of differential utilization of N sources in situ. Ammonium was the N source being utilized for most of the year, despite low year-round concentrations. However, during the spring of 2000 ammonium availability was insufficient to support *Synechococcus* N requirements, and the utilization of additional N sources became necessary. Possible N sources utilized by *Synechococcus* include nitrate, nitrite, urea, cyanate, and amino acids (Palenik et al. 2003). Nitrate injected into the photic zone from deeper waters was the most abundant N species. It is thus enticing to speculate that *Synechococcus* populations were direct users of this N source in "new" primary production. However, the *ntcA* assay does not resolve which of these alternative N sources is being utilized.

Small flagellate and ciliate grazers are major recyclers of organic N to ammonium (Goldman and Dennett 1992; Selmer et al. 1993). A decoupling between phytoplankton growth and grazing pressure may have led to a disturbance in the balance between ammonium regeneration and ammonium uptake during the upswing of the massive *Synechococcus* bloom in spring of 2000. Such a decrease in ammonium supply relative to demand would have led to our observed response of the induction of *ntcA* expression by *Synechococcus* populations and the subsequent synthesis of transporters and enzymes required for the utilization of N sources other than ammonium. Fine-scale temporal sampling over the transition period of grazing and growth rates, ammonium regeneration rates, and N status of *Synechoccocus* populations from *ntcA* expression would enable us to assess these hypotheses.

Our findings argue against the paucity of N being the direct cause of the seasonal decline in *Synechococcus* abun-

dances—despite a positive correlation between N concentrations and *Synechococcus* numbers during stratification periods. Perhaps other nutrients covarying with N, such as P, were influencing *Synechococcus* population dynamics. In a recent study assessing P stress among cyanobacterial populations in the Gulf of Aqaba, Fuller et al. (2005) suggest that P stress might have been involved in the 1999 decline in *Synechococcus* populations based on the coincident onset of expression of the phosphate-binding protein, PstS. However, as Fuller et al. (2005) stated, most of the PstS expression measured was likely to have come from the 10-fold more abundant *Prochlorococcus* populations. The difference in cyanobacterial target populations for the *ntcA* and PstS molecular assays makes it difficult to directly compare the findings of these two studies: *ntcA* expression assessed N stress specifically in *Synechococcus* populations, whereas the PstS assay probed P stress in combined *Synechococcus* and *Prochlorococcus* populations. However, it is interesting to note that at the period of lowest N and P concentrations during September 1999, both *ntcA* transcript levels and PstS expression were low, indicating that the respective cyanobacteria assessed in each assay were not limited for N or P. Unfortunately, during periods of enhanced *ntcA* or PstS expression, the P stress or N stress assay, respectively, was not employed, preventing us from ascertaining whether the cyanobacteria present during these periods were differentially stressed for N or P or whether they were stressed for both nutrients at the same time.

Alternatively to nutrient control, mortality processes such as grazing and viral infection may regulate *Synechococcus* populations during periods of stable stratification. Indeed, in situ growth rates of cyanobacteria in oligotrophic waters are often close to the maximum known for these organisms (Vaulot et al. 1995; Brown et al. 1999; Crosbie and Furnas 2001) with grazing pressure keeping populations in check under stable environmental conditions (Reckermann and Veldhuis 1997; Brown et al. 1999; Calbet and Landry 2004). Furthermore, Muhling et al. (2004) have recently suggested that cyanophages may be regulating *Synechococcus* populations in the Gulf of Aqaba. However, decoupling between growth and mortality processes must occur during periods of net population growth for *Synechococcus* blooms to manifest themselves. We therefore propose that the pronounced changes in water column conditions—the end of deep mixing coupled with the entrapment of high N concentrations in newly stratified surface waters—enabled higher growth rates (potentially of certain *Synechococcus* genotypes; *see below*) and released *Synechococcus* populations from mortality control leading to the spring bloom. This is consistent with numerous reports of *Synechococcus* blooms subsequent to episodic or seasonal increases in N in surface waters (Glover et al. 1988; Lindell and Post 1995; Gin et al. 1999; DuRand et al. 2001). The decline in population size as stratification progresses may be due to the increased impact of grazer and virus populations subsequent to the increase in *Synechococcus* populations, leading to higher rates of mortality relative to growth in early summer. In this scenario, the reestablishment of a steady-state relationship between mortality processes and growth would keep *Synechococcus* populations at their summer low. Low summer *Synechococcus* populations may also be due to an as yet unknown biotic interaction with *Prochlorococcus.* Indeed, opposing oscillations in *Synechococcus* and *Prochlorococcus* abundances over yearly cycles in the Sargasso Sea and the Gulf of Aqaba have been known for some time (Olson et al. 1990; Lindell and Post 1995; DuRand et al. 2001).

The lack of the N stress response in *Synechococcus* populations from ammonium-poor waters initially surprised us in light of our findings that *ntcA* expression is induced in *Synechococcus* sp. strain WH7803 at ammonium concentrations <1 μmol L^{-1} (Lindell and Post 2001). On the one hand, this may reflect genotypic differences in the threshold for induction of *ntcA* expression between genotypes such as strain WH7803 (clade V) and the genotypes more prevalent in the Gulf of Aqaba (clades I, II, III, NC1, NC2, NC3) for which little to no physiological information exists. Alternatively, *ntcA* expression may be induced in response to the flux of ammonium rather than to a set concentration. Regardless, continuous expression of NtcA and the suite of N stress genes it up-regulates would come at a high metabolic cost for any of the *Synechococcus* genotypes. Indeed, our results suggest that this mode of long-term acclimation to low-N environments is unfavorable for maintaining sizeable *Synechococcus* populations. However, the N stress response in *Synechococcus* field populations is likely to play an important role. Transient expression of *ntcA* during the upswing of the spring bloom enabled *Synechococcus* populations to overcome a temporary insufficiency of ammonium and use alternative N sources.

Synechococcus genotypes present in N-poor waters are those adapted for life in low-N regimes or are those capable of acclimating to the changing N environments. Two genotypes (representatives of clades NC1 and NC2) were present in N-replete mixed waters, newly stratified bloom conditions with fluctuating N concentrations, as well as stably stratified low-N waters. These genotypes must therefore have sufficient phenotypic plasticity to acclimate to all these N conditions—even if this meant a transitory induction of the N stress response for the utilization of N sources other than ammonium during the spring bloom. However *Synechococcus* genotypes from four other clades were detected in our clone libraries under more limited sets of environmental conditions—often in only one season (Fig. 5). This suggests that these genotypes may be less successful under conditions prevalent during seasons other than those in which they were found. Indeed two genotypes found during the spring bloom were not detected during stably stratified N-poor waters and may be specialized for rapid growth after the onset of stratification traps cells and nutrients in the photic layer subsequent to deep-mixing events. We further speculate that the induction of the N stress response during the spring bloom may be in part due to differences in the physiology of the different *Synechococcus* genotypes present during this period.

Keeping in mind that we do not know the relative abundance of these different *Synechococcus* genotypes, we hypothesize that N availability and possibly that of covarying P may influence the genotypic makeup of the *Synechococcus* populations present, causing a shift in the presence of *Synechococcus* genotypes concomitant with changes in envi-

ronmental conditions. Clearly a comparative physiological assessment of the response of the different genotypes is required to assess their potential for acclimation to low ammonium concentrations. Furthermore, analysis of *ntcA* expression of specific genotypes, especially during the spring transition period, would provide an indication as to whether ammonium availability is indeed a driving force behind the seasonal differences in *Synechococcus* population structure.

In summary, the physiological N status of *Synechococcus* populations was reported to us in real time through expression of the N regulatory gene *ntcA*. We learned from this study that the success of *Synechococcus* in the open oceans is in part because of selection of genotypes suited to life under prevailing N conditions, rather than to prolonged manifestation of the N stress response in less well-adapted genotypes. Thus, *Synechococcus* populations, as well as other phytoplankton taxa (Goldman et al. 1979) presiding in oligotrophic seas worldwide, may not be growth-rate limited by N despite N's control of overall phytoplankton biomass. Rather the role of N availability in regulating *Synechococcus* population structure may be in temporary releases from grazing pressure and/or viral infection after transient influxes of N into the photic zone. Thus, potential environmental stressors such as low N, while impacting biomass and potentially genotypic composition, may not adversely affect the physiological N status of the *Synechococcus* genotypes residing in low-N environments.

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