CHAPTER 5

NUTRIENT LIMITATION OF MARINE CYANOBACTERIA

Molecular ecology of nitrogen limitation in an oligotrophic sea

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1 Introduction

Many seas in the (sub)tropical regions are characterized as warm, stratified water bodies, the surface layers of which are deplete in inorganic nutrients, low in chlorophyll *a* concentrations as well as in suspended particulate matter. The coastal areas of such seas are often decorated with diverse benthic communities, like seaweed meadows, coral reefs and sponge cover, many of them massive expanses of an astonishing beauty. These benthic communities with their dwelling fish populations form natural resources of great biodiversity and ecological value, which often harbor rare and endemic species. As these communities often thrive in overlying waters of high transparency, the single most important parameter influencing the viability and health of such communities is the quality of the pelagic waters that feed them. The main risks threatening (sub)tropical benthic communities are eutrophication and chemical pollution of the overlying waters due to the impact of ever increasing human activity. Whereas chemical pollution, like e.g. release of antifouling, minor oil spills, may have a localized effect in the direct vicinity of the point source, eutrophication effects extend into the marine food web as a whole. It thereby directly or indirectly affects phytoplankton productivity and community structure. Open waters of (sub)tropical seas carry phytoplankton communities that are adapted to thrive in nutrient-deplete waters. These photosynthetic communities are composed of a mixture of eukaryotic and prokaryotic species, most of which do not cause massive blooms, release significant amounts of toxin or are harmful in other ways. A single exception is formed by the infrequent occurrence of blooms of *Trichodesmium*, a filamentous, colony-forming, nitrogen-fixing cyanobacterium. Blooms of *Trichodesmium* appear at the sea surface and colony densities may be so high that they cause discoloration of surface waters (red tide). *Trichodesmium* blooms are potentially harmful due to their toxic potential and their limited trophic interactions with the marine food web. These blooms are short-lived and show a great extent of patchiness, but are assumed to have considerable effect on the marine

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food web in their vicinity. Little is known about factors that determine the set of conditions for the development of *Trichodesmium* blooms. This chapter describes aspects of the molecular ecology of marine phytoplankton communities. Specifically, some of the interactions between the diazotrophic *Trichodesmium* and non nitrogen-fixing picophytoplankton communities in the Gulf of Aqaba, northern Red Sea, are examined here.

2 The Gulf of Aqaba, northern Red Sea

The northern Red Sea is a warm water body, stratified during both summer and winter, that carries oligotrophic, nutrient-depleted surface waters (Veldhuis and Kraay, 1993; Li et al., 1998). The Gulf of Aqaba is an extension of the northern Red Sea, a deep basin (600-1800 m), separated from the northern Red Sea by a shallow sill (240 m) at the Straits of Tiran. High evaporation rates drive a thermohaline circulation with a continuous advection of nutrient poor surface waters from the Red Sea into the Gulf, counterbalanced by an efflux of more dense deep waters (Klinker et al., 1976; Wolf-Vecht et al., 1992). The Gulf of Aqaba is subject to a distinct yearly cycle of stable stratification in summer and deep convective mixing in winter (Wolf-Vecht et al., 1992; Genin et al., 1995). Surface waters in summer are characterized by a shallow, but stable thermocline with surface temperatures of approximately 26 °C declining to 20.7 °C in the deep layers.

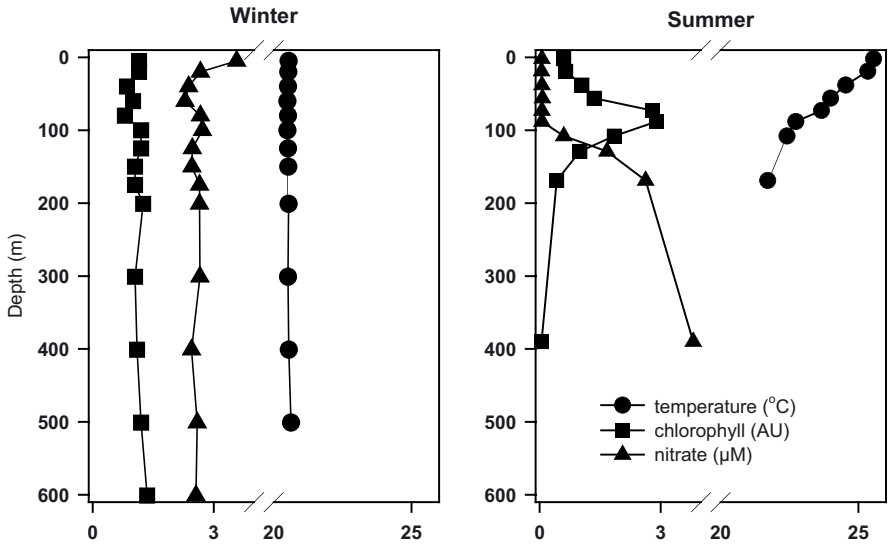

Figure 1. Typical depth profiles of temperature, nitrate and chlorophyll a *concentrations during winter mixing (left panel) and summer stratification (right panel) at sampling station A (29°28 N; 34°55 E) in the Gulf of Aqaba, northern Red Sea (Klinker et al., 1978; Genin et al., 1995; Lindell and Post, 1995).*

Lower air temperatures in fall cause a rapid erosion of the thermocline, leading to deep convective mixing during the winter months. Convective mixing may reach down to depths of 600 m or more in the northern part of the Gulf (Genin et al., 1995; Lindell and Post, 1995). This alternating pattern is reflected in a pronounced seasonal change in the trophic state of the Gulf, switching between mesotrophic to oligotrophic conditions as judged from inorganic nutrient and chlorophyll *a* concentrations in the surface layers (Fig. 1). Particularly interesting is the homogenous distribution of both nitrate (and other inorganic nutrients) and chlorophyll *a* during winter mixing. Phytoplankton and bacterial populations frequently show an even vertical distribution during this season, indicative of the extent and velocity of the deep mixing.

3 Phytoplankton communities

Phytoplankton chlorophyll *a* in surface layers of the Gulf of Aqaba is low in summer, with concentrations fluctuating between 0.02-0.04 µg per liter (Klinker et al., 1978; Genin et al., 1995; Yahel et al., 1998), considered characteristic for oligotrophic conditions. Chlorophyll *a* reaches maximal concentrations, in some years up to ~1.2 µg per liter, towards the end of the winter mixing period (Genin et al., 1995). Chlorophyll *a* concentrations in coastal surface waters fluctuate in a less dramatic fashion and averaged 0.45±0.12 µg per liter during winter 1997/1998 and declined to 0.22±0.06 µg per liter in the following summer.

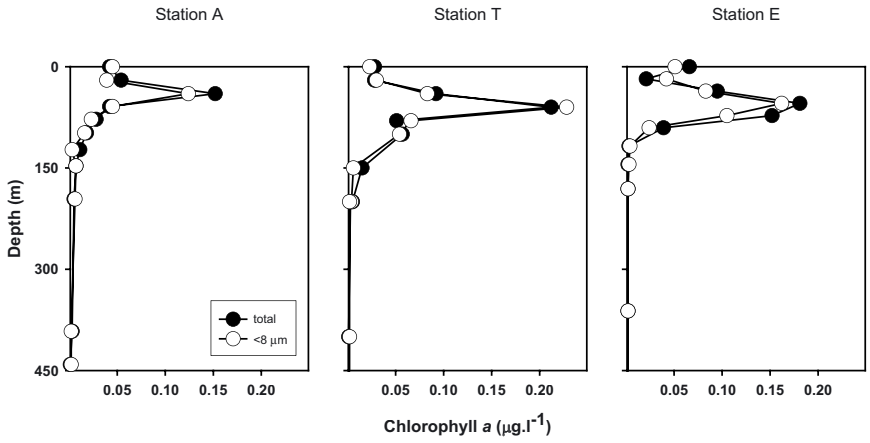

Figure 2. Depth distributions of chlorophyll a *concentrations at three distant locations in the Red Sea during June 1995. Station A is located in the northern tip of the Gulf of Aqaba, station T in the northern Red Sea and station E in the southern Red Sea. Note that the size fraction of < 8 µm (open circles) contains the bulk of chlorophyll* a *as determined on the non-filtered fraction (closed circles). 250 ml samples were filtered on 25 mm 0.2 µm pore size RC59 filters (Schleicher and Schuell) and extracted in 90% acetone.*

The phytoplankton community is in part made up of larger organisms (> 60 μm): various diatom and dinoflagellate species along with the filamentous, colony-forming, diazotrophic cyanobacterium *Trichodesmium* spp. Diatoms are more abundant during winter and early spring, but decline steadily during summer (Post et al., 2002). Dinoflagellates show only little variation in their cell numbers. *Trichodesmium* colonies are not observed during winter mixing, but they appear in surface waters during the stratification period and occasionally form blooms in both coastal and open waters (Post et al., 2002). However, more than 90% of phytoplankton chlorophyll *a* in coastal waters is contributed by phytoplankton cells of < 8 μm in diameter (Yahel et al., 1998); this percentage rises to > 95% in open waters (Fig. 2). Epifluorescence microscopy and flow cytometry later asserted that the bulk of this community was actually < 2 μm in cell size and thus belongs to the picophytoplankton size fraction. Picophytoplankton have a selective advantage over larger phytoplankton in (permanently) stratified, oligotrophic waters due to their high surface-to-volume ratios, which allows them to thrive at low nutrient concentrations (Chisholm, 1992), and their low sinking rates, which keep them suspended in the photic zone.

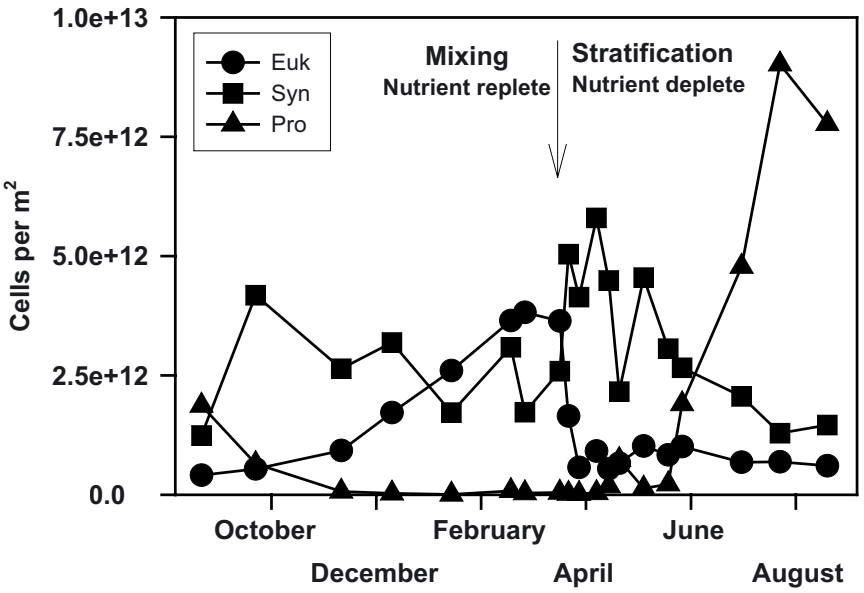

Figure 3. Seasonal change (1992-1993) in abundance of the major phytoplankton groups: eukaryotic algae (Euk), Synechococcus *(Syn) and* Prochlorococcus *(Pro) in the open water of the Gulf of Aqaba. The seasonal succession observed among these phytoplankton groups is typical for the Gulf of Aqaba after deep convective mixing (> 400 m) during winter. Abundance is presented as cell number integrated over the full depth of the water column. Arrow indicates the approximate time for cessation of mixing and onset of stratification.*

Thus the phytoplankton community in the Red Sea and Gulf of Aqaba is mostly composed of small eukaryotic algae (2-8 μm), and two groups of photosynthetic prokaryotes, the cyanobacteria *Synechococcus* (1-2 μm) and *Prochlorococcus* (0.6-1 μm). *Prochlorococcus* was found to be the dominant genus in the stably stratified Red Sea with high numbers (>100,000 cells per ml) during both summer and winter (Veldhuis and Kraay, 1993; Sommer et al., 2002). However, Figure 3 shows that in the Gulf of Aqaba the three phytoplankton groups are engaged in a distinct seasonal succession pattern as described by Lindell and Post (1995). Eukaryotic algae dominate phytoplankton in winter when the water column is deeply mixed and extensive amounts of nutrients are injected in the surface layers. At the onset of stratification eukaryotic algae decline strongly and a distinct *Synechococcus* bloom develops, fed by entrapped nutrients with nitrate as a prominent N-source. *Prochlorococcus* populations in the Gulf of Aqaba are most dynamic, they vary over 3 orders of magnitude, between > 250,000 cells per ml during summer stratification and virtual absence in winter, when waters of the Gulf are mixed down to 600 m depth (Lindell and Post, 1995). *Prochlorococcus* was rarely observed during the two months following the onset of stratification. After this period *Prochlorococcus* reappeared and rapidly increased their numbers to completely dominate the water column during the summer (Lindell and Post, 1995). The clear delineation between eukaryotic algal dominance under nutrient replete conditions and cyanobacterial dominance in stratified, nutrient deplete waters is especially striking. Thus the prokaryotic cyanobacterial groups among phytoplankton are most successful in nutrient-deplete surface waters in which unicellular *Synechococcus* and *Prochlorococcus* establish a stable abundance. In other (sub)tropical seas the two cyanobacterial groups also dominate in nutrient-depleted waters (Olson et al., 1990; Olson et al., 1990; Karl, 1999; Partensky et al., 1999). In addition, the nitrogen-fixing cyanobacterium *Trichodesmium* spp. is commonly found in such waters (Carpenter and Price, 1977; Carpenter and Romans, 1991; Karl et al., 1992; Capone et al., 1997; Karl, 1999) Lastly, diatom populations of oligotrophic waters often contain species which harbor the endosymbiotic, nitrogen-fixing cyanobacterium *Richellia* sp., like *Hemiaulus* spp. and *Rhizosolenia* spp. These diatom species were observed in the Gulf of Aqaba during summer stratification (Gordon et al., 1994). Overall, these findings suggest that cyanobacterial phytoplankton are better suited to cope with a limited supply of nutrients in marine environments. These observations raise questions with respect to how cyanobacteria cope with low nutrient supply and whether cyanobacterial populations are nutrient limited. Specifically, the study of nutrient effects on their biodiversity, adaptive potential and nutrient status will provide insights in phytoplankton population dynamics and conditions that lead up to blooms of the toxic cyanobacterium *Trichodesmium* spp. The semi-annual alternation in water column conditions of the Gulf of Aqaba with its changes in nutrient availability and the ensuing seasonal succession of phytoplankton are highly predictable and thus forms a unique model system for the study of phytoplankton - especially cyanobacterial - adaptation to nutrients in both short and sufficient supply.

4 Nutrient limitation

In general, marine phytoplankton productivity is thought to be limited by nutrients. The elements nitrogen (Kilham, 1988; Fanning, 1992; Tyrrell and Laws, 1997), phosphorus (Krom et al., 1991) and iron (Chavez et al., 1991; Kolber et al., 1994; Coale et al., 1996; McKay et al., 1997) are the macronutrients which are traditionally considered as potentially limiting phytoplankton productivity in oligotrophic seas. Low iron supply occurs in ocean provinces where desert dust deposition is low, specifically in High Nitrate Low Chlorophyll (HNLC) regions, e.g. provinces of the central Pacific (Chavez et al., 1991; Martin et al., 1994). Iron may also limit "new" primary production based on nitrogen fixation and specifically affect *Trichodesmium* productivity (Falkowski, 1997; Berman-Frank et al., 2001; Berman-Frank et al., 2001). In the Sargasso Sea such populations were found to experience iron stress (E. Webb, pers. comm.) and *Trichodesmium* in waters over the continental shelf off N-Australia had low iron quota (Kustka et al., 2003). The Gulf of Aqaba is considered to be iron-replete due to its proximity to land mass and high dust deposition (Post et al., 2002). Whether phytoplankton in the Gulf are controlled by nitrogen or phosphorus supply (or other factors) is still an open question. On the one hand, observations suggest prevailing N-limiting conditions. Inorganic nitrogen sources are at or below the detection limit in the photic layer (Lindell and Post, 1995), nitrogen contents of plankton biomass are low, short-lived blooms of the N_2-fixing cyanobacterium *Trichodesmium* develop (Post et al., 2002) and diatoms harboring diazotrophic endosymbionts appear in the stratified surface waters (Gordon et al., 1994). On the other hand, dominance of *Synechococcus* and *Prochlorococcus* with their low P contents (Bertilsson et al., 2003; Heldal et al., 2003), elevated alkaline phosphatase activities of picoplankton and of *Trichodesmium* colonies in surface waters of the Gulf (Li et al., 1998; Stihl et al., 2001) are indicative of low supply of inorganic phosphorus, possibly of phosphorus-limiting conditions. The establishment of N- versus P-limitation of phytoplankton is not a straightforward procedure as no available measurement provides an unequivocal answer. This becomes clear if one considers the following points:

1. More accurate and more sensitive methods for the chemical determination of dissolved nutrients are certainly important, but they do not directly address the question of nutrient limitation. Whereas e.g. ammonium concentrations in the sea can now be measured accurately (Holmes et al., 1999) at nanomolar levels (well below the Km values for known ammonium permeases), they do not provide direct information on the ammonium flux into cells and thus the nitrogen status of the phytoplankton community remains uncertain.
2. A wide variety of both P- and N-sources can be accessed by phytoplankton and not all of them are covered by standard determinations in water chemistry (e.g. phosphonate, urea, amino acids). The availability of other sources (dissolved organic N- and P-pools) is difficult to determine due to procedural problems and lack of information on the N-and P-species constituting these pools.
3. Bioassays based on nutrient additions (even low-level spikes of radioactively or stable-isotope labeled nutrients) to phytoplankton in enrichment experiments have

their own inherent problems. They may cause (co-)limitation by low trace metal availability, adverse effects due to sample enclosure (bottle effects, lack of turbulence, reduced gas exchange, exclusion of grazers, etc.).

There is thus a need for the development of an alternative method by which undisturbed natural samples can be interrogated regarding their nutrient status. Modern molecular techniques may provide the appropriate answer as they can directly assess this nutrient status. Firstly, one can identify the genes required for the assimilation of a given nutrient, e.g. the *narB* gene, which encodes nitrate reductase, is commonly found in marine *Synechococcus* isolates (Moore et al., 2002; Rocap et al., 2003). However, the gene is absent from all *Prochlorococcus* strains tested and apparently this genus is incapable of nitrate utilization altogether and thus distinct from *Synechococcus* (Moore et al., 2002; Rocap et al., 2003). Secondly, one can identify gene products (mRNA, protein) that are known to be induced and become strongly expressed under nutrient stress conditions. The principles of this approach have been reviewed in detail by Scanlan and Wilson (1999). At present, methods are available for detection of P-stress in marine cyanobacteria ranging from the expression of P-transport related periplasmic proteins in single cells (immunoassays using α-PstS antibodies) to the alkaline phosphatase activities of isolated phytoplankton aggregates and whole plankton community level (Scanlan et al., 1997; Li et al., 1998; Stihl et al., 2001; see Dignum et al., Chapter 4 in this volume). Below we summarize the stress responses of marine cyanobacteria to a low supply of combined nitrogen in the Gulf of Aqaba.

5 Cyanobacterial nitrogen stress responses

Marine cyanobacteria, like all phytoplankton groups, may either use "regenerated" N-sources like ammonium and urea or they may utilize "new" N-sources, e.g. by the assimilation of nitrate and fixation of molecular dinitrogen. Regenerated N-sources are those nitrogenous compounds that are recycled by heterotrophs (grazers, bacteria) within the photic layer. New N-sources are those N-compounds that originate from outside the photic layer (deep waters, atmosphere). Surprisingly little is known about cyanobacterial utilization of organic N-compounds, although their capability for urea and amino acid utilization has been recognized (Capone et al., 1994; Collier et al., 1999; Palinska et al., 2000; Lindell and Post, 2001; Valladares et al., 2002). Ammonium is the preferred source of inorganic nitrogen in cyanobacteria (Glibert and Ray, 1990; Flores and Herrero, 1994; Lindell et al., 1999; Lindell and Post, 2001). It may be imported from the environment into the cell by either passive diffusion of the uncharged ammonia or via active uptake of the ammonium ion. Ammonium is directly assimilated into organic matter via the concerted activities of glutamine synthetase (GS) and glutamate synthase (GOGAT) (Flores and Herrero, 1994). In the absence of sufficient ammonium, the cyanobacterial cell undergoes a series of adaptive processes in order to obtain the nitrogen required for growth and survival. The initial responses to ammonium deficiency include the induction of higher affinity ammonium uptake systems (Montesinos et al., 1998) and the

synthesis of proteins required for the utilization of other nitrogenous compounds such as nitrate, nitrite, urea and amino acids (Flores and Herrero, 1994; Herrero et al., 2001). Most marine *Synechococcus* have the potential for utilization of nitrate, nitrite and urea (Moore et al., 2002), and the genome of the oceanic *Synechococcus* WH8102 (Palenik et al., 2003) contains additional genes and open reading frames with similarity to amino acid and oligopeptide transporters known from other organisms. However, the *nifHDK* genes, which encode the main constituents of the nitrogenase enzyme complex, are lacking in the genomes of both *Synechococcus* and *Prochlorococcus*. Thus, these extremely abundant cyanobacteria do not engage in nitrogen fixation. The utilization of alternative nitrogen sources is energetically more expensive than that of ammonium as, in most cases, they require both active transport over the cell membrane as well as the conversion to ammonium before assimilation into organic compounds (Guerrero and Lara, 1987; Flores and Herrero, 1994). Hence it is not surprising that elevated levels of ammonium prevent the utilization of alternative nitrogen sources, e.g. nitrate and nitrite, by directly inhibiting their assimilation and by repressing the protein synthesis required for their assimilation at the level of gene transcription (Guerrero and Lara, 1987; Suzuki et al., 1993; Flores and Herrero, 1994; Luque et al., 1994). Once all external nitrogen sources have been exploited, the cell enters a stage of nitrogen deprivation. During adaptation of the cell to nitrogen stress, growth may continue transiently as many physiological changes take place including the specific degradation of phycobiliproteins which results in chlorosis in both marine and freshwater *Synechococcus* (Wyman et al., 1985; Grossman et al., 1994). This process drives the reuse of cellular nitrogen for the synthesis of proteins required for survival under nitrogen-deprived conditions (Grossman et al., 1994; Gorl et al., 1998). Growth is halted once both external and internal nitrogen supplies have been exhausted.

Synthesis of the nitrogen regulatory protein, NtcA, is an essential step in cyanobacterial adaptation to ammonium-deplete conditions (Vega-Palas et al., 1990). This transcriptional activator is subject to negative control by ammonium at the level of gene expression (Luque et al., 1994; Lindell et al., 1998). *ntcA* expression is down-regulated to basal levels in the presence of ammonium. In the absence of ammonium, NtcA enhances the expression of its own gene as well as of those required for the uptake and assimilation of nitrogen sources like nitrate and nitrite (Luque et al., 1994). NtcA may further be involved in the expression of genes required for urea utilization (Collier et al., 1999; Valladares et al., 2002). A mutant strain of marine *Synechococcus* WH7803, carrying an inactivated *ntcA* gene, is incapable of growth on nitrate and nitrite (Fig. 4) and does not degrade phycobiliproteins in a timely manner under nitrogen-deplete conditions (Moyal and Post, unpublished results). A lack of phycobiliprotein degradation was also observed in a *ntcA* mutant strain of freshwater *Synechococcus* PCC7942 (Sauer et al., 1999). The mode of action of NtcA in the chlorosis process of marine *Synechococcus* under N-depleted conditions has yet to be elucidated. *ntcA* expression in marine *Synechococcus* WH7803 occurs at three distinctly different levels of transcript accumulation: a low basal level in ammonium-grown cells, an intermediate level in nitrate-grown cells and maximal expression in nitrogen-deprived cells (Lindell et al.,

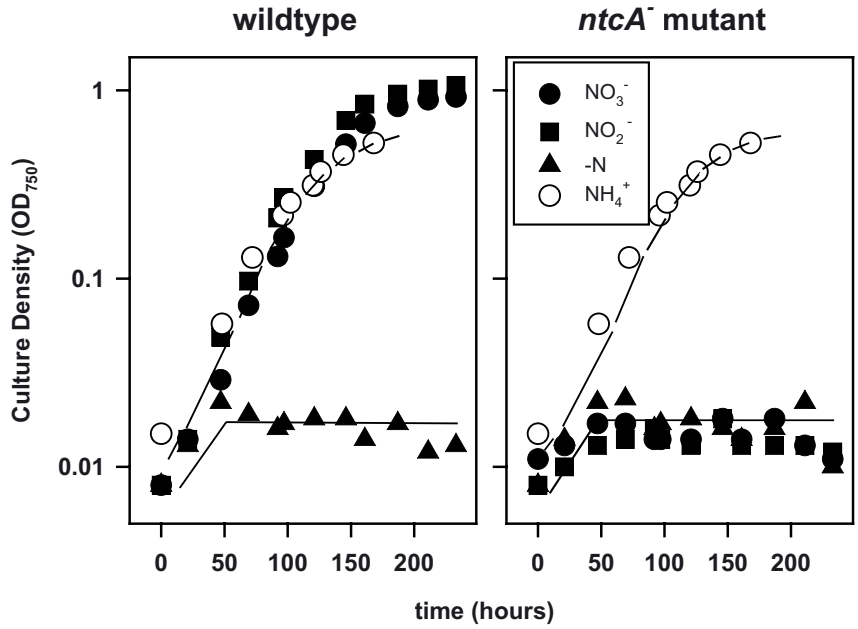

Figure 4. Autotrophic growth of marine Synechococcus *strain WH7803 wildtype and ntcA⁻ mutant cells in a mineral seawater medium with different inorganic N-sources (ammonium, nitrite and nitrate) or deplete of a combined N-source (-N). Data provided by A. Moyal and A.F. Post.*

1998; Lindell and Post, 2001). Such an expression pattern can form the basis for the development of molecular probes that assess the N-status of natural *Synechococcus* populations (see below).

Prochlorococcus, a genus closely related to *Synechococcus*, also carries the *ntcA* gene (Lindell et al., 2002). As it co-occurs with *Synechococcus* in overlapping niches in the surface ocean (Partensky et al., 1999), it experiences similar conditions with respect to N-nutrition. In contrast to *Synechococcus*, *Prochlorococcus* is much less versatile in its utilization of N-compounds. None of the many *Prochlorococcus* isolates studied is capable of nitrate utilization and only few isolates can utilize nitrite (Moore et al., 2002). Whole genome analyses indeed confirmed these physiological characteristics as *Prochlorococcus* spp. lack the genes needed for nitrate assimilation (Dufresne et al., 2003; Rocap et al., 2003). This would imply that, unlike *Synechococcus*, *Prochlorococcus* populations are virtually incapable of engaging in any form of so-called "new" primary production. In addition, the *Prochlorococcus* strain SS120 lacks the genes needed for urea utilization (Dufresne et al., 2003), thus pointing to ammonium as possibly being the major N-source for this abundant phytoplankter. At the molecular level, *Prochlorococcus* strain

PCC9511 (genetically nearly identical to strain MED4) expresses *ntcA* in response to N-stress (Lindell et al., 2002). However, replenishment of N-deprived cultures with fresh ammonium failed to induce an immediate reduction of *ntcA* transcript levels as was observed in *Synechococcus* WH7803 (Lindell et al., 1998; Lindell et al., 2002). The expression of *ntcA* showed some correlation with the N:C quota of the cells. The expression pattern observed for *ntcA* was not mirrored by that of the *amt* gene, which encodes the ammonium transporter. *amt* transcripts were high in N-replete and N-limited cells, but they declined sharply in N- and P-deprived cells (Lindell et al., 2002). Moreover, *amt* transcript levels did not correlate with N:C quota, but showed positive correlation with photosynthetic efficiencies of the cells.

The third marine cyanobacterium, globally important and abundantly present in the open waters of the northern Red Sea and the Gulf of Aqaba, is the filamentous, non-heterocystous *Trichodesmium* spp. Its presence is most pronounced in marine waters that are N-deplete in which it contributes significant input of "new" nitrogen due to its nitrogen fixation (Carpenter and Romans, 1991; Capone et al., 1997). Often *Trichodesmium* is considered as an obligate diazotroph, although it has the potential for utilizing combined N-sources. Cultures of *Trichodesmium* can be maintained with ammonium as the N-source (Kustka et al., 2003). *Trichodesmium* strains IMS101 and NIBB 11067 are capable of nitrate utilization (Ohki et al., 1991; Wang et al., 2000). The involvement of *ntcA* in N-stress responses of diazotrophic cyanobacteria is more complex. The *nirA* operon, encoding nitrate utilization, of strain IMS101 was found to have distinct promoter elements but lacks a clearly defined NtcA binding sequence (Wang et al., 2000). The promoter region of the *nifHDK* operon, which encodes nitrogenase, in *Trichodesmium* IMS101 shows sequence features with similarity to NtcA binding sites (Dominic et al., 1998). However, the fact that nitrogen fixation by *Trichodesmium* is accompanied by a release of ammonium from the filaments to the environment (Mulholland and Capone, 1999), predicts that *ntcA* expression is not simply controlled by ammonium levels. Indeed, it was found that *ntcA* transcript levels were not negatively affected by ammonium. *Trichodesmium* IMS101 cultures supplied with ammonium contained levels of *ntcA* mRNA just like those accumulated in N-deprived, nitrogen-fixing cultures (Wang and Post, unpublished results). Thus nitrogen fixation may require *ntcA* to be expressed, but *ntcA* and *nif* expression are not repressed by ammonium. On the other hand, transcript levels of the nitrate transport gene decreased with increasing external ammonium concentrations (Wang and Post, unpublished results). This decrease does not correlate with the *ntcA* transcription patterns, suggesting that nitrate utilization is ammonium repressed but not *ntcA* controlled.

In conclusion, the three abundant cyanobacterial taxa that often coexist in the same nutrient-deplete ocean waters, each have a distinctly different genetic potential for nitrogen acquisition and different nitrogen stress responses. One can thus propose a hypothesis regarding the coexistence of these taxa based on their location in the photic zone, available N-sources, the N-acquisition potential and the N-stress responses. Although *Trichodesmium* has a potential to acquire combined N-sources, the chemical composition of its natural environment, the very surface layer of stratified water bodies, suggests that it rarely employs this potential. Its nitrogen

fixation produces intracellular ammonium, part of which is leaked to the environment (Mulholland and Capone, 1999), by which it may form a source of direct N-supply. *Trichodesmium* populations are mostly found to express their nitrogen fixation potential and thus require continued NtcA-mediated expression of the *nif* genes. The uncoupling between *ntcA* expression and ammonium repression is consistent with this strategy. *Trichodesmium* thus avoids N-limiting conditions by nitrogen fixation. Whole genome analyses of *Prochlorococus* spp. show that they are restricted in the N-sources they can acquire. They depend mostly on ammonium and urea, which are supplied through nutrient regeneration in the surface ocean. The *Prochlorococcus* types that utilize nitrite are indeed found at depths near the primary nitrite maximum (Rocap et al., 2002). *ntcA* expression in *Prochlorococcus* appears to respond both slowly and moderately to changes in N-nutrition. This is in good agreement with the stratified environments it thrives in, which are characterized by a predictably stable availability of N-sources at any depth. Moreover, *Prochlorococcus*, by means of its small cell size (< 1 μm), may successfully compete for nutrients at the low end of the concentration range of any nutrient (Chisholm, 1992). Possibly, *Prochlorococcus* encounters limited N-supply only rarely, if at all. Findings so far indicate that *Synechococcus* spp. are the most versatile of the three as they have the potential to utilize a wide range of N-sources. Of the three cyanobacteria, *Synechococcus* is the only one to thrive and bloom in water bodies that have recently been subjected to upwelling or deep convective mixing. Such water bodies may show thermal stratification, but they are still enriched with nutrients. The nature of such environments, reflected in dynamic changes in the distribution and concentration of inorganic and organic N-species, requires a genetic capability to meet these changes. *Synechococcus* can express its adaptive capability by means of a rapid modulation of *ntcA* transcription (Lindell et al., 1998). Since *Synechococcus* cells (> 1 μm) are larger than those of *Prochlorococcus*, it is less suited in the competition for nutrients at extremely low concentrations. *Synechococcus* may thus actually meet N-limiting conditions and need to express its adaptive potential. Therefore, this non-nitrogen-fixing, unicellular cyanobacterium forms the candidate group for N-stress studies among natural phytoplankton communities.

6 Probing the N-status of natural populations

Once sufficient information has been gathered on the ability of marine cyanobacteria to adapt to changes in their N-nutrition in culture, the true challenge lies in determining their N-status in their natural environment. Given the multitude of N-compounds and the little known order of preference in their utilization, one ideally would choose a gene that is both informative for the N-status irrespective of the N-source(s) being utilized and specific to cyanobacteria.

The global nitrogen regulator gene *ntcA* is such a gene. The responsiveness of *ntcA* to nitrogen availability and the pivotal role the gene plays in the adaptation of *Synechococcus* cells to ammonium- and nitrogen-depleted conditions suggests that different levels of *ntcA* expression may be useful indicators of the N-status of

Synechococcus populations among marine phytoplankton. In order to develop molecular tools based on *ntcA* expression, a few more characteristics of *ntcA* need to be established:

1. Ideally, *ntcA* is present on the cyanobacterial genome as a single copy gene. Multiple copies of the gene would likely be expressed differently as they may have different promoter regions, and respond to different input signals, including different environmental stresses. Southern analysis of *Synechococcus* WH7803 genomic DNA indicated that only a single copy of the *ntcA* gene was present (Lindell et al., 1998). Whole genome sequences for oceanic *Synechococcus* and *Prochlorococcus* strains (Dufresne et al., 2003; Palenik et al., 2003; Rocap et al., 2003) also contained a single *ntcA* copy suggesting that nitrogen stress responses, conveyed by the global nitrogen regulator NtcA, are expressed from a single gene copy.
2. Equally important, *ntcA* expression is required to respond to nitrogen stress and not be activated under other stresses. Since virtually all studies of *ntcA* were performed as part of laboratory experiments of nitrogen acquisition and metabolism by cyanobacterial cultures, little information is available on this subject. However, the only reported promoter element of cyanobacterial genes involved in nitrogen stress response, including *ntcA* itself, is the binding site for NtcA (Flores and Herrero, 1994). This binding site was also found upstream of nitrogen stress genes in marine cyanobacteria (Lindell et al., 1998; Wang et al., 2000; Lindell et al., 2002). In addition, *ntcA* expression in *Synechococcus* WH7803 remained at basal level when cells were subjected to either iron or phosphate stress (Lindell and Post, 2001).
3. Furthermore, *ntcA* expression needs to reflect rapid changes in the availability of nitrogenous compounds, especially the down regulation of *ntcA* expression by increased ammonium levels. As discussed above, this condition is not met by either *Prochlorococcus* PCC9511 nor by *Trichodesmium* IMS101 (Lindell et al., 2002; Wang and Post, unpublished results) and may extend to related genotypes of these marine cyanobacteria. However, marine *Synechococcus* WH7803 was shown to respond rapidly to both upward and downward changes in ammonium availability (Lindell and Post, 2001).
4. Natural levels of ammonium in the marine environment should permit induction and repression of *ntcA* transcription. *Synechococcus* WH7803 cultures express *ntcA* at < 1 μM, a threshold concentration that falls in the range of ammonium concentrations in the sea. This range spans nanomolar concentrations in the oligotrophic surface ocean and concentrations of > 1 μM in more productive coastal regions. Marine phytoplankton reportedly utilize ammonium > 1 μM preferentially over other N-compounds irrespective of their concentrations. The alternative compounds are utilized when ammonium concentrations drop below 0.5-1 μM (Glibert and Ray, 1990; McCarthy et al., 1999). Therefore, the ammonium concentration range of *ntcA* expression, and thus utilization of alternative N-sources by *Synechococcus*, matches that of the natural environment. Moreover, the immediate down regulation of *ntcA* transcript levels upon ammonium addition has potential for the identification of point sources of

ammonium in the marine environment: exudation by grazer communities, fish, benthic systems including coral reef communities, but also release resulting from anthropogenic activity like wastewater discharge and mariculture activities.

5. Lastly, probing of the N-status of natural phytoplankton communities based on *ntcA* expression should be informative and specific of the targeted group, namely the marine cyanobacteria. *ntcA* presence in other marine planktonic organisms or unrelated RNA sequences with a close similarity to that of *ntcA* would obviously yield inaccurate information on the cyanobacterial N-status. A PCR protocol based on degenerate primers targeting conserved regions of the *ntcA* gene identified *ntcA* in cyanobacterial templates, but not in DNA templates provided by other organisms, be it from heterotrophic and photosynthetic bacteria or eukaryotic algae (Lindell and Post, unpublished results). PCR amplification of environmental templates yielded specific products, the sequences of which - without exception – showed high similarity to *ntcA* sequences of *Prochlorococcus* and *Synechococcus* culture isolates (Fig. 5). The differences in *ntcA* sequence between these two closely related groups were very distinct and allow for specific probing of the *Synechococcus* genotypes.

The general fulfillment of the five criteria listed above is a requirement for any molecular probe designed to study a given environmental stress response. The next steps towards probing of natural communities are the design of a specific probe and the establishment of a protocol for probing. Foremost, genes of which the transcription does not produce high cellular levels of mRNA cannot be studied by standard northern blots or RNAse protection assays. Expression of such genes in lowly populated oligotrophic waters is quantified by reverse transcriptase PCR (RT-PCR) protocols, The expression of *ntcA* among natural *Synechococcus* populations was thus studied by RT-PCR and primers were designed which amplify *Synechococcus ntcA* mRNA but not that of *Prochlorococcus* or other cyanobacteria (Lindell and Post, 2001). Since *Synechococcus* population size, RNA extraction efficiency, sample quality, etc., may fluctuate with depth and time, one should judge actual *ntcA* expression of each sample relative to a minimal and maximal standard to be determined. Minimal or basal expression can be rapidly induced by addition of 1 mM NH_4^+, whereas maximal expression is obtained by the addition of methionine-sulfoximine, a glutamine synthetase inhibitor (Lindell and Post, 2001). This protocol has been applied to surface samples from the Gulf of Aqaba that contained ammonium (0.6 µM), nitrite (0.2 µM) and nitrate (0.6 µM) and *Synechococcus* expressed ntcA at basal levels (Lindell and Post, 2001). Subsequent analysis of surface samples over a yearly cycle revealed that *Synechococcus* populations expressed *ntcA* at levels that were significantly lower than those for induced N-stress (Fig. 6). These populations were thus nitrogen sufficient, both during periods of deep winter mixing and of stable summer stratification. Whether these populations utilize ammonium or alternative N-sources was determined in a separate study.

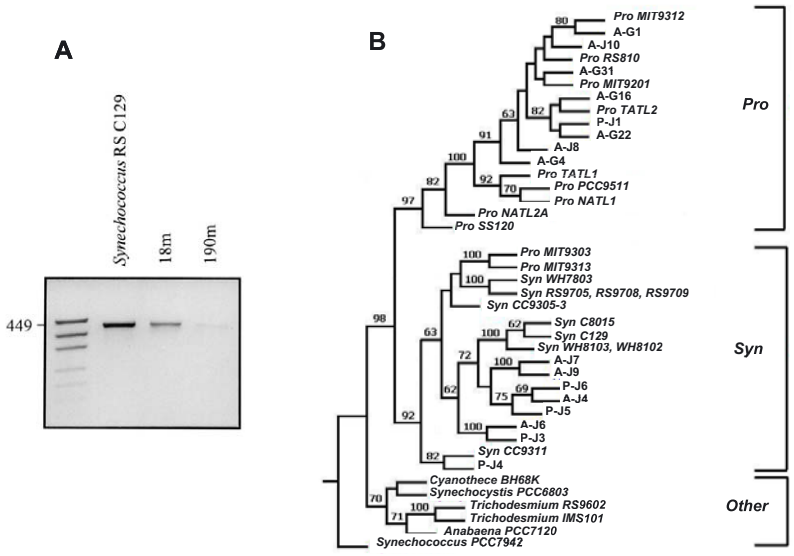

Figure 5. (A) PCR amplification of a 449 bp ntcA *product from stratified, nutrient depleted waters of the Gulf of Aqaba. DNA was obtained from 18 and 190 m depth, within and just below the photic zone. Genomic DNA of the* Synechococcus *isolate C129 from the Red Sea was used for the positive control. (B) PCR products were cloned into the pGEM-T vector (Promega) and sequenced. All products (A- and P- codes) yielded* ntcA *sequence and fell into the* Synechococcus *(Syn) and* Prochlorococcus *(Pro) clusters of the* ntcA *gene tree (Lindell, 2000).*

Throughout the year, with exception of the final stages of winter mixing, *Synechococcus* was shown to be ammonium sufficient (Lindell, 2000) in surface waters with ambient concentrations fluctuating between 20-300 nM (David and Post, unpublished results). However, *Synechococcus* expressed *ntcA* at intermediate levels in nitrate enriched waters, towards the end of the deep mixing event (Lindell, 2000). The conclusion of these findings is that *Synechococcus* and – based on the arguments discussed above – *Prochlorococcus* populations in the Gulf of Aqaba are nitrogen sufficient, even in surface waters which have been nutrient deplete for an extended period. This conclusion does not necessarily extend to eukaryotic algae and thus not to the phytoplankton community as a whole. The question thus arises whether the marine cyanobacteria in the Gulf of Aqaba are nutrient limited at all or whether their populations are controlled by other factors, e.g. mortality due to viral infection and grazing. Based on a number of additional observations a hypothesis has been developed which couples the ecology of the unicellular picoplanktonic cyanobacteria to that of filamentous microplanktonic *Trichodesmium* populations. Firstly, an underlying aspect of cyanobacterial N-stress studies, largely ignored as mentioned above, is the change in the genotypic make-up of the *Synechococcus* and

Prochlorococcus population in response to changes in nitrogen availability. Clear evidence was found that *Prochlorococcus* genotypes occupied different niches. Genotypic diversity of *Prochlorococcus* populations has been studied from *in situ* hybridization using 16S rDNA targeted oligonucleotides as well as from functional genes like *ntcA*, *cpeB*, *fstZ* and *psbA* (Lindell, 2000; West et al., 2001; Holtzendorff et al., 2002; Steglich et al., 2003; Zeidner et al., 2003). These studies showed that one distinct genotypic group occupies the upper layers of the photic zone (0-70 m) and may extend below that but at much lower abundances. The second genotypic group is found in the bottom half of the photic layer (60-150 m). Representatives of these two groups were shown to belong to distinct ecotypes (Moore and Chisholm, 1999; West and Scanlan, 1999) that differ in their utilization capability of nitrogen sources (Moore et al., 2002). Surface populations of the high-light adapted *Prochlorococcus* ecotype maintain their genotypic make-up over seasons despite strong changes in nitrogen availability (Zandbank and Post, unpublished results). Globally, the genotypic diversity of *Synechococcus* is extensive: 6-10 distinct clusters belonging to the abundant group A of marine *Synechococcus* have been identified (Rocap et al., 2002; Fuller et al., 2003). These clusters are coherent in their characteristics like pigmentation, motility, utilization of N-compounds, etc. Cultured isolates of *Synechococcus* from the Gulf of Aqaba belong to clusters II, III, VII, VIII and IX, but none of the other clusters (Fuller et al., 2003). Cluster II was found to be by far the most abundant among the *Synechococcus* populations of the Gulf of Aqaba year-round (Fuller et al., 2003). In conclusion, the genotypic composition - and by consequence the physiology - of the *Prochlorococcus* and *Synechococcus* are maintained over the year despite the extreme changes in water column properties in the Gulf of Aqaba.

The last point to consider in this chapter is whether another nutrient might be limiting cyanobacterial, and possibly phytoplankton, productivity in the Gulf of Aqaba. Of the inorganic nutrients phytoplankton have the highest demand for phosphate in waters where carbon and nitrogen supplies are replete. Phosphate is found as various species of inorganic and organic phosphate, but only orthophosphate is being taken up by phytoplankton (Björkman and Karl, 1994). Organic phosphate can be utilized by phytoplankton following hydrolysis of these compounds, a process mediated by extracellular enzyme activity, e.g. that of alkaline phosphatase, a mono-esterase with a broad range of organic phosphate substrates (see Dignum et al., Chapter 4 in this volume). Both transcription and activity of alkaline phosphatase are rapidly induced upon phosphate depletion of *Synechococcus* cells (Gillor et al., 2002). The plankton community of the Gulf of Aqaba showed elevated levels of alkaline phosphatase activity in early summer, approximately one month after the cessation of the deep mixing event (Li et al., 1998). The bulk of this activity was associated with picoplankton (Li et al., 1998). The spatial distribution of alkaline phosphatase activity showed the highest correlation with that of *Synechococcus* populations (Li et al., 1998). *Trichodesmium* populations, which appear during the same period, were also characterized by strong alkaline phosphatase activities (Stihl et al., 2001). Moreover, populations of the picoplanktonic cyanobacteria and *Trichodesmium* were shown to have elevated levels of the phosphate transport protein PstS (Fuller et al., 2005; Scanlan, pers.

comm.). Thus, the invasion of stratified waters, recently depleted of combined nitrogen, by populations of nitrogen-fixing cyanobacteria like *Trichodesmium* spp. injects new nitrogen into the surface layers. Hence, an imbalance is created between N and P-supply to the phytoplankton community leading to a draw down of the inorganic phosphate pool. *Trichodesmium* has been implied in P-depletion in open oceanic systems as well (Wu et al., 2000; Sanudo-Wilhelmy et al., 2001; Dyhrman et al., 2002). This imbalance then causes a P-stress response in the cyanobacterial species which is detected by immunoassays (Scanlan et al., 1997; Fuller et al., 2005) and enzyme activities (Li et al., 1998; Stihl et al., 2001; Dyhrman et al., 2002). Possibly, this scenario has application for the ocean as a whole. Recent findings that both *Prochlorococcus* and *Synechococcus* have lower P quota than expected from the Redfield ratio (Bertilsson et al., 2003; Heldal et al., 2003), suggest that these phytoplankters are adapted to environments with low phosphate availability.

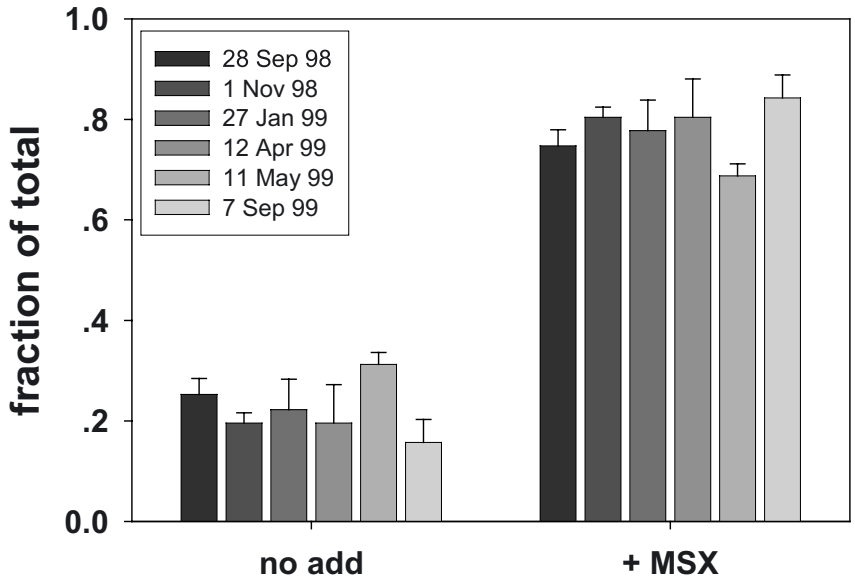

Figure 6. Actual (no add) and maximal (+ Methionine-Sulfoximine) levels of ntcA *expression for* Synechococcus *genotypes in surface samples (5 m) at sampling station A in the northern Gulf of Aqaba over a yearly cycle (1998-1999).*

7 Concluding remarks

In contrast to their freshwater counterparts, marine cyanobacteria are indicators of pristine oligotrophic conditions. Hence, their biomass is likely controlled by a limited supply of nutrients. The recent development and use of molecular tools allows the probing of nutrient stress responses in marine cyanobacterial species within plankton communities. This approach has provided an extra dimension to techniques that are common to biological oceanography, e.g. determination of nutrient levels and tracer studies of stable isotopes. For the Gulf of Aqaba it was shown from molecular studies that cyanobacterial phytoplankton are N-replete, but show P-stress responses, especially in periods when *Trichodesmium* is abundant. A potential risk is formed by the fact that the Gulf of Aqaba receives additional phosphate through anthropogenic activities, specifically due to fertilizer shipping via the ports of Aqaba and Eilat, but also by mariculture, tourist activities and wastewater disposal. This phosphate loading could potentially stimulate and enhance *Trichodesmium* blooms. Since *Trichodesmium* has a distinct toxic potential and forms an ecological nuisance due to its limited interaction in the marine food web, such blooms are highly undesired. Conversely, a combined phosphorus and nitrogen loading may suppress cyanobacterial abundance near pollution sources (as is observed during deep winter mixing events in the Gulf) and provide favorable conditions for the development of harmful algal populations (e.g., dinoflagellates). The questions pertaining to the latter scenario require the application of molecular techniques to eukaryotic phytoplankton (studies of nutrient stress, toxin production, allelopathy). This field provides exciting challenges for the development of novel molecular tools in marine ecology.

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9 References

Berman-Frank, I., Cullen, J.T., Shaked, Y., Sherrel, R.M. and Falkowski, P.G. (2001) Iron availability, cellular iron quotas and nitrogen fixation in *Trichodesmium*, *Limnology and Oceanography* **46**, 1249-1260.

Berman-Frank, I., Lundgren, P., Chen, Y.B., Kuepper, H., Kolber, Z., Bergman, B. and Falkowski, P.G. (2001) Segregation of nitrogen fixation and oxygenic photosynthesis in the marine cyanobacterium *Trichodesmium*, *Science* **294**, 1534-1537.

Bertilsson, S., Berglund, O., Karl, D.M. and Chisholm, S.W. (2003) Elemental composition of marine *Prochlorococcus* and *Synechococcus*: implications for the ecological stochiometry of the sea, *Limnology and Oceanography* **48**, 1721-1731.

Björkman, K. and Karl, D.M. (1994) Bioavailability of inorganic and organic phosphorus compounds to natural assemblages of microorganisms in Hawaiian coastal waters, *Marine Ecology Progress Series* **111**, 265-273.

Capone, D.G., Ferrier, M.D. and Carpenter, E.J. (1994) Amino-acid cycling in colonies of the planktonic marine cyanobacterium *Trichodesmium thiebautii*, *Applied and Environmental Microbiology* **60**, 3989-3995.

Capone, D.G., Zehr, J.P., Paerl, H.W., Bergman, B. and Carpenter, E.J. (1997) *Trichodesmium*, a globally significant marine cyanobacterium, *Science* **276**, 1221-1229.

Carpenter, E.J. and Price, C.C. (1977) Nitrogen fixation, distribution and production of *Oscillatoria (Trichodesmium) thiebautii* in the eastern Sargasso Sea, *Limnology and Oceanography* **20**, 381-401.

Carpenter, E.J. and Romans, K. (1991) Major role of the cyanobacterium *Trichodesmium* in nutrient cycling in the North Atlantic Ocean, *Science* **254**, 1356-1358.

Chavez, F.P., Buck, K.R., Coale, K.H., Martin, J.H., DiTullio, G.R., Welschmeyer, N.A., Jacobson, A.C. and Barber, R.T. (1991) Growth rates, sinking, and iron limitation of equatorial Pacific phytoplankton, *Limnology and Oceanography* **36**, 1816-1833.

Chisholm, S.W. (1992) Phytoplankton size, in P.G. Falkowski and A.D. Woodhead (eds.), *Primary Productivity and Biogeochemical Cycles in the Sea*, Plenum Press, New York, USA**,** pp. 213-237.

Coale, K.H., Johnson, K.S., Fitzwater, S.E., Gordon, R.M., Tanner, S., Chavez, F.P., Ferioli, L., Sakamoto, C., Rogers, P., Millero, F., Steinberg, P., Nightingale, P., Cooper, D., Cochlan, W.P., Landry, J., Constantinou, J., Rollwagen, G., Trasvina, A. and Kudela, R. (1996) A massive phytoplankton bloom induced by an ecosystem-scale iron fertilization experiment in the equatorial Pacific Ocean, *Nature* **383**, 495-501.

Collier, J., Brahamsha, B. and Palenik, B. (1999) The marine cyanobacterium *Synechococcus* sp. WH7805 requires urease (urea amido hydrolase, E.C. 3.5.1.5) to utilize urea as a nitrogen source: molecular-genetic and biochemical analysis of the enzyme, *Microbiology* **145**, 447-459.

Dominic, B., Chen, Y.B. and Zehr, J.P. (1998) Cloning and transcriptional analysis of the *nifUHDK* genes of *Trichodesmium* sp. IMS101 reveals stable *nifD, nifDK* and *nifK* transcripts, *Microbiology* **144**, 3359-3368.

Dufresne, A., Salanoubat, M., Partensky, F., Artiguenave, F., Axmann, I.M., Barbe, V., Duprat, S., Galperin, M.Y., Koonin, E.V., Le Gall, F., Makarova, K.S., Ostrowskii, M., Oztas, S., Robert, C., Rogozin, I.B., Scanlan, D.J., Tandeau de Marsac, N., Weissenbach, J., Wincker, P., Wolf, Y.I. and Hess, W.R. (2003) Genome sequence of the cyanobacterium *Prochlorococcus marinus* SS120, a nearly minimal oxyphototrophic genome, *Proceedings of the National Academy of Sciences USA* **100**, 10020-10025.

Dyhrman, S.T., Webb, E.A., Anderson, D.M., Moffett, J.W. and Waterbury, J.B. (2002) Cell specific detection of phosphate stress in *Trichodesmium* from the Western North Atlantic, *Limnology and Oceanography* **47**, 1832-1836.

Falkowski, P.G. (1997) Evolution of the nitrogen cycle and its influence on the biological sequestration of CO_2 in the ocean, *Nature* **387**, 272-275.

Fanning, K.A. (1992) Nutrient provinces in the sea: Concentration ratios, reaction rate ratios, and ideal covariation, *Journal of Geophysical Research* **97**, 5693-5712.

Flores, E. and Herrero, A. (1994) Assimilatory nitrogen metabolism and its regulation, in D.A. Bryant (ed.), *The Molecular Biology of Cyanobacteria*, Kluwer Academic Publishers, Dordrecht, the Netherlands, pp. 487-517.

Fuller, N.J., Marie, D., Partensky, F., Vaulot, D., Post, A.F. and Scanlan, D.J. (2003) Clade-specific 16S ribosomal DNA oligonucleotides reveal the predominance of a single marine *Synechococcus* clade throughout a stratified water column in the Red Sea, *Applied and Environmental Microbiology* **69**, 2430–2443.

Fuller, N.J., Marie, D., Yallop, M., Rivlin, T., West, N.J., Post, A.F. and Scanlan, D.J. (2005) Dynamics of community structure and P status of picocyanobacterial populations in the Gulf of Aqaba, Red Sea, during 1999-2000, *Limnology and Oceanography* (in press).

Genin, A., Lazar, B. and Brenner, S. (1995) Vertical mixing and coral death in the Red Sea following the eruption of mount Pinatubo, *Nature* **377**, 507-510.

Gillor, O., Hadas, O., Post, A.F. and Belkin, S. (2002) Phosphorus bioavailability monitoring by a bioluminescent cyanobacterial sensor strain, *Journal of Phycology* **38**, 107-105.

Glibert, P.M. and Ray, R.T. (1990) Different patterns of growth and nitrogen uptake in two clones of marine *Synechococcus* spp., *Marine Biology* **107**, 273-280.

Gordon, N., Angel, D.L., Neori, A., Kress, N. and Kimor, B. (1994) Heterotrophic dinoflagellates with symbiotic cyanobacteria and nitrogen limitation in the Gulf of Aqaba, *Marine Ecology Progress Series* **107**, 83-88.

Gorl, M., Sauer, J., Baier, K. and Forchhammer, K. (1998) Nitrogen-starvation induced chlorosis in *Synechococcus* PCC 7942: adaptation to long-term survival, *Microbiology* **144**, 2449-2458.

Grossman, A.R., Schaefer, M.R., Chiang, G.G. and Collier, J.L. (1994) The responses of cyanobacteria to environmental conditions: light and nutrients, in D.A. Bryant (ed.), *The Molecular Biology of Cyanobacteria*, Kluwer Academic Publishers, Dordrecht, the Netherlands, pp. 641-675.

Guerrero, M.G. and Lara, C. (1987) Assimilation of inorganic nitrogen, in P. Fay and C. Van Baalen (eds.), *The Cyanobacteria,* Elsevier Science Publishers, Amsterdam, the Netherlands, pp. 165-186.

Heldal, M., Scanlan, D.J., Norland, S., Thingstad, F. and Mann, N.H. (2003) Elemental composition of single cells of various strains of marine *Prochlorococcus* and *Synechococcus* using X-ray microanalysis, *Limnology and Oceanography* **48**, 1732-1743.

Herrero, A., Muro-Pastor, A.M. and Flores, E. (2001) Nitrogen control in cyanobacteria, *Journal of Bacteriology* **183**, 411-425.

Holmes, R.M., Aminot, A., Kerouel, R., Hooker, B.A. and Peterson, B.J. (1999) A simple and precise method for measuring ammonium in marine and freshwater ecosystems, *Canadian Journal of Fisheries and Aquatic Sciences* **56**, 1801-1808.

Holtzendorff, J., Marie, D., Post, A.F., Partensky, F., Rivlin, A. and Hess,W.R. (2002) Synchronized expression of *ftsZ* in natural *Prochlorococcus* populations of the Red Sea, *Environmental Microbiology* **4**, 644–653.

Karl, D.M. (1999) A sea of change: biogeochemical variability in the North Pacific subtropical gyre, *Ecosystems* **2**, 181-274.

Karl, D.M., Letelier, R., Hebel, D.V., Bird, D.F. and Winn, C.D. (1992) *Trichodesmium* blooms and new nitrogen in the north Pacific gyre, in E.J. Carpenter, D.G. Capone and J.G. Reuter (eds.), *Marine Pelagic Cyanobacteria: Trichodesmium and other Diazotrophs*, Kluwer Academic Publishers, Dordrecht, the Netherlands, pp. 219-237.

Kilham, P. (1988) Comparative ecology of marine and freshwater phytoplankton, *Limnology and Oceanography* **33**, 776-795.

Klinker, J., Reiss, Z., Kropach, C., Levanon, I., Harpaz, H., Halicz, E. and Assaf, G. (1976) Observations on the circulation pattern in the Gulf of Aqaba (Elat), Red Sea, *Israel Journal Earth Science* **25**, 85-103.

Klinker, J., Reiss, Z., Levanon, I., Harpaz, H. and Shapiro, Y. (1978) Nutrients and biomass distribution in the Gulf of Aqaba (Elat), Red Sea, *Marine Biology* **45**, 53-64.

Kolber, Z.S., Barber, R.T., Coale, K.H., Fitzwater, S.E., Green, R.M., Johnson, K.S., Lindley, S. and Falkowski, P.G. (1994) Iron limitation of phytoplankton photosynthesis in the equatorial Pacific Ocean, *Nature* **371**, 145-149.

Krom, M.D., Kress, N., Brenner, S. and Gordon, L.I. (1991) Phosphorus limitation of primary productivity in the eastern Mediterranean Sea, *Limnology and Oceanography* **36**, 424-432.

Kustka, A.B., Sanudo-Wilhelmy, S.A., Carpenter, E.J., Capone, D., Burns, J. and Sunda, W.J. (2003) Iron requirements for dinitrogen and ammonium supported growth in cultures of *Trichodesmium* (IMS101): comparison with nitrogen fixation rates and iron:carbon ratios of field populations, *Limnology and Oceanography* **48**, 1869-1884.

Li, H., Veldhuis, M.J.W. and Post, A.F. (1998) Alkaline phosphatase activities among planktonic communities in the northern Red Sea, *Marine Ecology Progress Series* **173**, 107-115.

Lindell, D. (2000) Assessing the nitrogen status of marine prokaryotic phytoplankton using molecular methods, *Department of Microbial and Molecular Ecology Report,* Hebrew University**,** Jerusalem, Israel.

Lindell, D., Erdner, D., Marie, D., Claustre, H., Prasil, O., Le Gall, F., Rippka, R., Partensky, F., Scanlan, D.J. and Post, A.F. (2002) Contrasting expression of *ntcA* and *amt* in *Prochlorococcus* sp. strain PCC 9511, *Journal of Phycology* **38**, 1113-1124.

Lindell, D., Padan, E. and Post, A.F. (1998) Regulation of *ntcA* expression and nitrite uptake in the marine *Synechococcus* sp. strain WH 7803, *Journal of Bacteriology* **180**, 1878-1886.

Lindell, D., Padan, E. and Post, A.F. (1999) The effect of ammonium on *ntcA* expression, nitrate and nitrite uptake in marine *Synechococcus* sp. strain WH 7803, in L. Charpy and A.W.D. Larkum (eds.), *Marine Cyanobacteria*, *Bulletin de l'Institut Oceanographique*, *Monaco*, special issue N° 19**,** pp. 273-278.

Lindell, D. and Post, A.F. (1995) Ultraphytoplankton succession is triggered by deep winter mixing in the Gulf of Aqaba (Eilat), Red Sea, *Limnology and Oceanography* **40**, 1130-1141.

Lindell, D. and Post, A.F. (2001) Ecological aspects of *ntcA* gene expression and its use as an indicator of the nitrogen status of marine *Synechococcus* spp., *Applied and Environmental Microbiology* **67**, 3340-3349.

Luque, I., Flores, E. and Herrero, A. (1994) Molecular mechanism for the operation of nitrogen control in cyanobacteria, *EMBO Journal* **13**, 2862-2869.

McCarthy, J.J., Garside, C. and Nevins, J.L. (1999) Nitrogen dynamics during the Arabian Sea northeast monsoon, *Deep-Sea Research* **46**, 1623–1664.

McKay, R.M.L., Geider, R.J. and LaRoche, J. (1997) Physiological and biochemical responses of the photosynthetic apparatus of two marine diatoms to Fe stress, *Plant Physiology* **114**, 615-622.

Montesinos, M.L., Muro-Pastor, A.M., Herrero, A. and Flores, E. (1998) Ammonium/methylammonium permeases of a cyanobacterium: identification and analysis of three nitrogen regulated *amt* genes in *Synechocystis* sp. PCC 6803, *Journal of Biological Chemistry* **273**, 31463-31470.

Moore, L.R. and Chisholm, S.W.(1999) Photophysiology of the marine cyanobacterium *Prochlorococcus*: ecotypic differences among cultured isolates, *Limnology and Oceanography* **44**, 628-638.

Moore, L.R., Post, A.F., Rocap, G. and Chisholm, S.W. (2002) Differential nitrogen utilization of the marine cyanobacteria *Prochlorococcus* and *Synechococcus*, *Limnology and Oceanography* **47**, 989-996.

Mulholland, M.R. and Capone, D.G. (1999) Nitrogen fixation, uptake and metabolism in natural and cultured populations of *Trichodesmium* spp., *Marine Ecology Progress Series* **188**, 33-49.

Ohki, K., Zehr, J.P., Falkowski, P.G. and Fujita, Y. (1991) Regulation of nitrogen-fixation by different nitrogen sources in the marine non-heterocystous cyanobacterium *Trichodesmium* sp. NIBB1067, *Archives of Microbiology* **156**, 335-337.

Olson, R.J., Chisholm, S.W., Zettler, E.R., Altabet, M.A. and Dusenberry, J.A. (1990) Spatial and temporal distribution of prochlorophyte picoplankton in the North Atlantic Ocean, *Deep-Sea Research* **37**, 1033-1051.

Olson, R.J., Chisholm, S.W., Zettler, E.R. and Armbrust, E.V. (1990) Pigments, size, and distribution of *Synechococcus* in the North Atlantic and Pacific Oceans, *Limnology and Oceanography* **35**, 45-58.

Palenik, B., Brahamsha, B., Larimer, F.W., Land, M., Hauser, L., Chain, P., Lamerdin, J., Regala, W., Allen, E.E., McCarren, J., Paulsen, I., Dufresne, A., Partensky, F., Webb, E.A. and Waterbury, J. (2003) The genome of a motile marine *Synechococcus*, *Nature* **424**, 1037-1042.

Palinska, K.A., Jahns, T., Rippka, R. and Tandeau de Marsac, N. (2000) *Prochlorococcus marinus* strain PCC 9511, a picoplanktonic cyanobacterium, synthesizes the smallest urease, *Microbiology* **146**, 3099-3107.

Partensky, F., Hess, W.R. and Vaulot, D. (1999) *Prochlorococcus*, a marine photosynthetic prokaryote of global significance, *Microbiology and Molecular Biology Reviews* **63**, 106-127.

Post, A.F., Dedej, Z., Gottlieb, R., Li, H., Thomas, D., El-Absawy, M., El-Naggar, A., El-Gharabawi, M. and Sommer, U. (2002) Spatial and temporal distribution of *Trichodesmium* spp. in the stratified waters of the Gulf of Aqaba (northern Red Sea), *Marine Ecology Progress Series* **239**, 241-250.

Rocap, G., Distel, D.L., Waterbury, J.B and Chisholm, S.W. (2002) Resolution of *Prochlorococcus* and *Synechococcus* ecotypes by using 16S-23S ribosomal DNA internal transcribed space sequences, *Applied and Environmental Microbiology* **68**, 1180-1191.

Rocap, G., Larimer, F.W., Lamerdin, J., Malfatti, S., Chain, P., Ahlgren, N.A., Arellano, A., Coleman, M., Hauser, L., Hess, W.R., Johnson, Z.I., Land, M., Lindell, D., Post, A.F., Regala, W., Shah, M., Shaw, S.L., Steglich, C., Sullivan, M.B., Ting, C.S., Tolonen, A., Webb, E.A., Zinser, E.R. and Chisholm, S.W. (2003) Genome divergence in two *Prochlorococcus* ecotypes reflects oceanic niche differentiation, *Nature* **424,** 1042-1047.

Sanudo-Wilhelmy, S.A., Kustka, A.B., Gobler, C.J., Hutchins, D.A., Yang, M., Lwiza, K., Burns, J., Capone, D.G., Raven, J.A. and Carpenter, E.J. (2001) Phosphorus limitation of nitrogen fixation by *Trichodesmium* in the central Atlantic Ocean, *Nature* **411**, 66-69.

Sauer, J., Margit,G. and Forchhammer, K. (1999) Nitrogen starvation in *Synechococcus* PCC 7942: involvement of glutamine synthetase and NtcA in phycobiliprotein degradation and survival, *Archives of Microbiology* **172**, 247-255.

Scanlan, D.J., Silman, N.J., Donald, K.M., Wilson, W.H., Carr, N.G., Joint, I. and Mann, N.H. (1997) An immunological approach to detect phosphate stress in populations and single cells of photosynthetic picoplankton, *Applied and Environmental Microbiology* **63**, 2411-2420.

Scanlan, D.J. and Wilson, W.H. (1999) Application of molecular techniques to addressing the role of P as a key effector in marine ecosystems, *Hydrobiologia* **401**, 149-175.

Sommer, U., Berninger, U.G., Bottger-Schnack, R., Cornils, A., Hagen,W., Hansen, T., Al-Najjar, T., Post, A.F., Schnack-Schiel, S.B., Stibor, H., Stubing, D. and Wickham, S. (2002) Grazing during the spring bloom in the Gulf of Aqaba and the Northern Red Sea, *Marine Ecology Progress Series* **239**, 251-261.

Steglich, C., Post, A.F. and Hess, W.R. (2003) Analysis of natural populations of *Prochlorococcus* spp. in the northern Red Sea using phycoerythrin gene sequences, *Environmental Microbiology* **5**, 681–690.

Stihl, A., Sommer, U. and Post, A.F. (2001) Alkaline phosphatase activities among populations of the colony-forming, diazotrophic cyanobacterium *Trichodesmium* (Cyanobacteria) in the Red Sea, *Journal of Phycology* **62**, 310-317.

Suzuki, I., Sugiyama, T. and Omata, T. (1993) Primary structure and transcriptional regulation of the gene for nitrite reductase from the cyanobacterium *Synechococcus* PCC 7942, *Plant and Cell Physiology* **34**, 1311-1320.

Tyrrell, T. and Laws, C.S. (1997) Low nitrate:phosphate ratios in the global ocean, *Nature* **387**, 793-796.

Valladares, A., Montesinos, M.L., Herrero, A. and Flores, E. (2002) An ABC-type, high affinity urea permease identified in cyanobacteria, *Molecular Microbiology* **43**, 703-715.

Vega-Palas, M.A., Madueno, F., Herrero, A. and Flores, E. (1990) Identification and cloning of a regulatory gene for nitrogen assimilation in the cyanobacterium *Synechococcus* sp. strain PCC 7942, *Journal of Bacteriology* **172**, 643-647.

Veldhuis, M.J.W. and Kraay, G.W. (1993) Cell abundance and fluorescence of picoplankton in relation to growth irradiance and nitrogen availability in the Red Sea, *Netherlands Journal of Sea Research* **31**, 135-145.

Wang, Q., Li, H. and Post, A.F. (2000) The nitrate assimilation genes of the marine diazotrophic cyanobacterium *Trichodesmium* sp. strain WH9601, *Journal of Bacteriology* **182**, 1764-1767.

West, N.J. and Scanlan, D.J. (1999) Niche-partitioning of *Prochlorococcus* populations in a stratified water column in the eastern North Atlantic Ocean, *Applied and Environmental Microbiology* **65**, 2585-2591.

West, N.J., Schoenhuber, W.A., Fuller, N.J., Walsby, A.E., Amann, R.I., Rippka, R., Post, A.F. and Scanlan, D.J. (2001) Identification and enumeration of *Prochlorococcus* sp. in natural communities by in situ hybridization using 16S rRNA-targeted oligonucleotides and tyramide signal amplification, *Microbiology* **147**, 1731-1744.

Wolf-Vecht, A., Paldor, N. and Brenner, S. (1992) Hydrographic indications of advection/convection in the Gulf of Eilat, *Deep-Sea Research* **39**, 1393-1401.

Wu, J., Sunda, W., Boyle, E.A. and Karl, D.M. (2000) Phosphate depletion in the western North Atlantic Ocean, *Science* **289**, 759-762.

Wyman, M., Gregory, R.P.F. and Carr, N.G. (1985) Novel role for phycoerythrin in a marine cyanobacterium, *Synechococcus* strain DC2, *Science* **230**, 818-230.

Yahel, G., Post, A.F., Fabricius, K., Marie, D., Vaulot, D. and Genin, A. (1998) Phytoplankton distribution and grazing near coral reefs, *Limnology and Oceanography* **43**, 551-563.

Zeidner, G., Preston, C.M., DeLong, E.F., Massana, R., Post, A.F., Scanlan, D.J. and Béjà, O. (2003) Molecular diversity among marine picophytoplankton as revealed by *psbA* analyses, *Environmental Microbiology* **5**, 212–216.