Limnol. Oceanogr., 58(6), 2013, 1959–1971

doi:10.4319/lo.2013.58.6.1959

Distribution and expression of the cyanate acquisition potential among cyanobacterial populations in oligotrophic marine waters

Nina A. Kamennaya,[1] and Anton F. Post [2,*]

Department of Plant and Environment Sciences, Life Sciences Institute, Hebrew University, Jerusalem, Israel

Abstract

We assessed the significance of cyanate utilization in marine primary productivity from the distribution of a dedicated transporter (encoded by *cynABD*) in different ocean environments. Several lines of evidence indicate that the cyanate utilization potential is associated mainly with surface populations of *Prochlorococcus*. Spatial and temporal dimensions of *cynA*, *cynS*, and *ntcA* expression by picocyanobacteria in the northern Red Sea supported our previous finding that *cynA* transcripts accumulate under more stringent N-limiting conditions. At the same time, cyanate utilization appeared to be more complex than suggested in our earlier publication, as we showed that picocyanobacteria also express their cyanate utilization potential under conditions where labile organic N compounds, such as urea, accumulate. These include N-sufficient transient conditions that result from nutrient upwelling during early mixing events in autumn as well as during spring bloom conditions that follow deep mixing events. Our finding that *cynA* occurrence is common in diverse marine environments suggests that cyanate utilization may be of a more fundamental importance to picophytoplankton productivity than previously considered.

Nitrogen (N) may limit primary production in vast oceanic areas, especially in subequatorial regions (Chisholm et al. 1988). To cope with N limitation, marine picocyanobacteria employ an array of survival strategies, among which are a high surface-to-volume ratio (Chisholm 1992), reduction in genome size (Dufresne et al. 2004), low N content of N-stress proteins (Gilbert and Fagan 2011), rapid N-stress responses (Lindell et al. 1998; Lindell and Post 2001; Lindell et al. 2005*a*), and an ability to utilize a wide range of both organic and inorganic N compounds (Scanlan et al. 2009). Determination of the concentrations of dissolved inorganic N (i.e., ammonia, nitrate and nitrite) is the most common estimate of N availability in the ocean. Deviation of dissolved N and phosphorus concentrations from the 16 : 1 Redfield ratio serves as an indicator of the N status (Deutsch et al. 2007; Koeve and Kahler 2010). Multiple studies employed nutrient enrichments to identify the factors that limit primary production (Hecky and Kilham 1988; Suggett et al. 2009). Comprehensive studies of N fluxes (Dugdale and Goering 1967; Hansell and Goering 1990) are less common, with the majority of the studies typically neglecting to include dissolved organic N species (e.g., urea and amino acids), though these labile nitrogen compounds are ubiquitous in seawater (Remsen 1971) and are readily utilized by diverse marine phytoplankton species (Dugdale and Goering 1967; Mccarthy 1972; Vargo 1979). Marine *Prochlorococcus* were reported to consume amino acids (Zubkov et al. 2003; Mary et al. 2008), and urea is readily assimilated by several *Synechococcus* and *Prochlorococcus* isolates (Moore et al. 2002; Kamennaya et al. 2008; Kamennaya and Post 2010). The latter compound in seawater also spontaneously decomposes to cyanate (Kamennaya et al. 2008). A number of *Synechococcus* and *Prochlorococcus* strains grow on cyanate as the sole N source (Miller and Espie 1994; Kamennaya et al. 2008; Kamennaya and Post 2010). Cyanase (Enzyme Commission [EC] 4.2.1.104), first characterized in *Escherichia coli* (Anderson 1980; Harano et al. 1997), catalyzes a reaction of cyanate with bicarbonate producing ammonium and carbon dioxide. Growth on cyanate as the sole N source requires its transport over the cell membrane followed by its conversion to ammonium. The prokaryotic cyanate transporter is found in a number of marine bacteria, including the cyanobacteria *Prochlorococcus* and *Synechococcus* (Palenik et al. 2003; Rocap et al. 2003). It belongs to the adenosine triphosphate–binding cassette superfamily of permeases (Saier 2000). The periplasmic substrate binding protein CynA is related but phylogenetically distinct from cyanobacterial nitrate and bicarbonate binding proteins (Espie et al. 2007). Transcription of *cynA* is strongly upregulated in both *Prochlorococcus* sp. MED4 and *Synechococcus* sp. WH8102 when grown with N sources other than ammonium (Tolonen et al. 2006; Thomas et al. 2009), indicating that cyanate acquisition is part of the N-adaptive response in cyanobacteria. The transcriptional regulation of the *cynABD* genes is consistent with the action of NtcA (Kamennaya and Post 2010), a global regulator of cyanobacterial N metabolism. The presence of *cynABDS* in *Prochlorococcus* and *Synechococcus* enhances their N-scavenging capacity and suggests adaptation to different N sources in oligotrophic marine waters (Kamennaya et al. 2008); however, the distribution and ecological significance of cyanate utilization in situ has not yet been assessed. Here, we examined the *cynA* diversity to identify the cyanate assimilation

* Corresponding author: apost@mbl.edu

Present addresses:

[1] Earth Sciences Department, Lawrence Berkeley National Laboratory, Berkeley, California

[2] The Josephine Bay Paul Center for Comparative Molecular Biology and Evolution, Marine Biological Laboratory, Woods Hole, Massachusetts

potential of picocyanobacteria in different ocean environments. Furthermore, using a broad-stroke approach, we examined spatial and temporal distribution of picocyanobacterial *cynA* and *cynS* transcripts in the Gulf of Aqaba as a proxy for active cyanate utilization. We observed enhanced *cynA* transcript levels in surface waters during late stratification. Additionally, accumulation of *cynA* and *ntcA* transcripts during transition periods between stable stratification and deep mixing indicates that cyanobacterial cyanate utilization is differentially expressed during periods of water column instability.

Methods

Database searches—Predicted protein sequences of *cynA*, *cynS* (cyanate), *urtA*, *ureC* (urea), *glnA*, *amt* (ammonium), and *ntcA* (N control) of high light (HL)–adapted marine *Prochlorococcus* (strains MED4, MIT9312, MIT9301, AS9601) and *Synechococcus* (WH8102, WH8109, CC9902, BL107, WH8016, CC9311, and RS9916—given presence of the genes in the genome) were retrieved from the National Center for Biotechnology Information (NCBI) database. These sequences were submitted as queries in similarity searches against translated nucleotide databases using a protein query (tblastn) against the NCBI environmental sequences nonredundant database, specifically the marine metagenome database (taxid:408172). For tblastn results with the Expect value (E-value) $< 1 \times 10^{-20}$, the taxonomy was assigned in accordance with the NCBI identifier of the top-scoring pair of tblastn hits.

Study area and sampling—We examined four distinct ocean sites, representative of oligotrophic, mesotrophic, and eutrophic environments. Hydrographic characteristics of the water column were examined alongside cyanate acquisition potential at each site:

(1) The Gulf of Aqaba in the northern tip on the Red Sea is a desert-enclosed 180 km long, 6–25 km wide, and $>$ 1820 m deep waterbody (Wolf-Vecht et al. 1992). It is subject to an annual cycle of stable summer stratification and deep winter mixing (Genin et al. 1995; Post et al. 2011). Early stratification in spring brings about a phytoplankton bloom that is dominated by *Synechococcus*, with typical mid-bloom chlorophyll *a* (Chl *a*) concentration < 0.73 μg L^{-1} (Genin et al. 1995; Lindell et al. 2005*b*). In the summer, a photic layer with a continuous thermocline (surface temperature $>$ 26°C), overlies a thermally homogeneous (20.6°C) deep waters (Wolf-Vecht et al. 1992; Post et al. 2011). During this period, dissolved inorganic N concentrations in the upper 100 m are at or below the detection limit, and *Prochlorococcus* dominates the phytoplankton community (Lindell and Post 1995). Deep convective mixing in winter reaches its maximal depth (350–750 m at Sta. A; *see* below) in February–March (Genin et al. 1995; Lindell et al. 2005*b*; Post et al. 2011).

Monthly sampling was performed during 2005–2008 on board the R/Vs *Queen of Sheba* and *Sea Bell* at sampling Sta. A (29°28′N, 34°55′E) in the northern Gulf of Aqaba. For nucleic acids extraction, discrete 20 liter samples were collected from five depths (0, 20, 50, 80, and 160 m) using a Rosette sampler mounted with 11 Niskin bottles, a SBE19 conductivity, temperature, depth (CTD) profiler (Sea Bird Electronics), a LI-190SA quantum sensor (LI-COR), and a Chl fluorometer (Seapoint Sensors). The samples were filtered onto 0.2 μm Gelman Supor filters (47 mm), flash frozen, and stored at −80°C. Profiles of Chl *a*, nutrients, and cell counts were obtained from a separate CTD-Rosette cast with samples routinely taken at 0, 20, 40, 60, 80, 100, 120, 150, 200, 300, 400, 500, 600, 700, and 730 m depth. All samples were passed over a 20 μm mesh. Chl *a* was extracted from 0.1 liter samples collected on GF/F. Triplicate aliquots of 10 mL each collected in sterile 15 mL screw cap Falcon tubes were stored at 4°C until analysis of nitrate and nitrite. For ammonium determination, 1 mL seawater samples were collected directly from the spigot with a syringe and dispensed into 12 mL sterile polycarbonate tubes and processed immediately.

(2) The South Indian Ocean is characterized by a bathymetric complexity with ridges, oceanic plateaus, and continental islands outlining a considerable number of abyssal plains (Weijer 2007). The Seychelles archipelago forms part of the Mascarene Ridge. It is subjected to nutrient enrichment due to upwelling processes caused by the confluence of the westward-flowing branch of the South Equatorial Current with the Seychelles-Mauritius Ridge. Water samples from the Seychelles archipelago area of the Indian Ocean were collected during the second Interuniversity Institute Seychelles Expedition in January 2002. Collection and handling of samples for deoxyribonucleic acid (DNA), nutrient, and Chl analyses was performed as described above.

(3) The Mediterranean Sea is a semienclosed basin with narrow continental shelf and a western and eastern basin divided by a transverse shallow ridge at the Strait of Sicily. The major inflow of the Atlantic surface water affects mostly its western basin. The eastern Mediterranean is among the most oligotrophic marine environments studied to date (Tyler 2003; Pitta et al. 2005; Siokou-Frangou et al. 2010). The highest productivity occurs along the coasts and in river plumes. Pelagic regions, including those offshore Israel, have low productivity of 1.14 mg ($\pm$ 0.85) C mg Chl^{-1} h^{-1} with a productivity peak in March (Azov 1986). According to Chl *a* fractionation, the picophytoplankton fraction ($<$ 3 μm) is a dominant contributor to primary productivity. Altogether, pelagic waters experience limited influence from continental shelf waters and/or anthropogenic effects (Azov 1986).

Monthly samples were taken from December 2005 to July 2006 (excluding April) on board the R/V *MedEx* at Sta. NH (32°10′N, 34°25′E) 70 km offshore of Israel. The maximum depth at the sampling site was 700 m. At each

station, profiles of salinity, temperature, Chl fluorescence, and irradiance over photosynthetically active radiation were obtained using a Sea-Bird CTD (SBE 19 Plus) profiler fitted with a Submersible fluorometer (Turner Designs) and quantum scalar sensor (QSP-2300, Biospheric Instruments). Sample collection was performed using CTD-Rosette in situ water sampler mounted with 12 Niskin bottles (8 liters). For the molecular analysis, 10 liters of seawater were collected as previously described. Collection and handling of samples for Chl *a* and nutrients was performed as described above.

(4) The Amundsen and Bellingshausen Seas of the Southern Ocean comprise sections of mesotrophic and eutrophic environments with no light limitation during the austral summer. Ice melting in the marginal ice zones leads to transient vertical stability of the water column and supports phytoplankton accumulation. Open waters are often well mixed, and its phytoplankton standing stocks display dramatic spatial and temporal variability (Figueiras et al. 1994). Increase in plankton biomass along off-to-onshore gradients was reported (Figueiras et al. 1994; Montes-Hugo et al. 2009).

Samples were collected from several locations, including coastal and open-water stations, in the Amundsen and Bellingshausen Seas during the expedition ANTARKTIS-XXIII/4 in February–April 2006 on board the R/V *Polarstern* (Gohl 2006). At each station, profiles of salinity, temperature, and fluorescence were obtained using a Sea-Bird CTD (SBE 9) profiler fitted with a Dr. Haardt Fluorometer. Sample collection was performed using a Carousel in situ water sampler mounted with 24 Niskin bottles (12 liters). Discrete water samples spanning the approximate euphotic zone (0–180 m) were obtained at 20 m intervals. For the molecular analysis, 4.5 liters of seawater were collected. Chl *a* concentrations were determined for 0.25 liter of 100 μm mesh prefiltered seawater. For determination of nitrate, nitrite, and phosphate concentrations, 15 mL seawater aliquots were collected and stored at 0°C in the dark prior to analysis. Water collection for ammonium determination was performed as described above.

Chemical analyses—The pigments from samples collected on Whatman GF/F filters were extracted with 90% acetone overnight at 4°C in the dark. Chl *a* concentrations were determined using a Turner Designs model 10AU™ fluorometer or a TBS-380 mini-fluorometer (Turner BioSystems) employing its ultraviolet (UV) channel (Antarctic samples only). The instruments were calibrated with standard Chl *a* solutions, and calculations of Chl *a* concentrations were performed as described in the Bermuda Atlantic Time-Series Methods (Sorensen 1997). Nitrate and nitrite concentrations were determined spectrophotometrically with a QuikChem 8000 flow injection analyzer (Lachat Instruments). Concentrations of ammonium were determined with the orthophthaldialdehyde method (Holmes et al. 1999) and quantified by fluorescence detection on a Hoeffer DyNA Quant TM 200 fluorometer using internal standards as described by (David 2003). For the Antarctic samples, fluorescence determination of ammonium concentrations was performed with a TBS-380 mini-fluorometer. The concentrations were extracted using the standard curve of a wide range.

Nucleic acid extraction—Back in the laboratory, samples intended for phylogenetic studies were thawed on ice. Following disruption of the cells with lysozyme, sodium dodecyl sulfate (SDS), and proteinase K, DNA extraction was performed by the phenol–chloroform method as described by Penno et al. (2006).

For dot blot analysis, the Supor-450 membranes were thawed on ice, cut in halves using sterile razor blades. DNA and ribonucleic acids (RNA) were extracted from the opposite halves. DNA extraction was performed as described in the preceding paragraph, and the resulting DNA was added with 20 μg mL^{-1} final concentration of RNase A (United States Biochemical Corporation) for DNA purification. For RNA extraction, the membranes were immersed in 0.5 mL of Total RNA Isolation (TRI) Reagent (Ambion), and the RNA isolation was performed according to standard procedure recommended by the manufacturer. RNA samples were treated with the DNA-*free*™ kit (Ambion) to eliminate DNA contamination. Nucleic acid concentrations were determined photometrically (NanoDrop).

DNA analysis—To amplify the cyanobacterial *cynA* gene, polymerase chain reaction (PCR) was performed using degenerate primers cAdc-F (5′-SCHKCRGAYAT-GAAAGGMTTY-3′) and cAdc-long-R (5′-GYT-TAAARTCDATTCKGTCDGG-3′). The reaction contained 1–2 ng of environmental DNA, 0.5 μmol L^{-1} of each primer, 0.25 mmol L^{-1} of each dNTP, 1.5 mmol L^{-1} $MgCl_2$, and 1.25 units of Taq DNA polymerase (PEQLAB) in a final volume of 50 μL. The PCR reaction mix was first submitted to denaturing conditions at 94°C for 5 min followed by 40 cycles of denaturation at 94°C for 45 s, primer annealing at 47–55°C for 30 s, and elongation at 72°C for 45 s. A final elongation step at 72°C was performed for 5 min. The bands of expected size (516 base pairs [bp]) were excised and purified from 1% agarose gels with the Wizard® SV Gel and PCR Clean-Up System (Promega). The products were cloned into the pGEM®-T Easy Vector System II (Promega) and transformed into *E. coli* DH5α, with the blue-white selection applied. To eliminate the false-positive clones, white colonies were screened by colony PCR using cAdc-F, cAd-mid-F (5′-GGNCAYCCDTGYTGYGCHTTYG-3′), and cAdc-long-R primers producing two amplification products of 516 and 259 bp. The reaction conditions were the same as in the primary PCR reaction. Plasmids extracted from the positive clones with Wizard® SV Mimiprep DNA Purification System (Promega) were sent out for Sanger sequencing with T7 and SP6 primers. Sequences of the *cynA* gene-clones were submitted to GenBank under the accession numbers GU810427-32, GU810434-38, GU810465-472, and JX483815-51.

Table 1. Target organism, target gene, primer identity, hybridization temperature (Tm, °C), and length (base pairs [bp]) of DNA fragments that were PCR amplified and radioactively labeled for use as probes in the dot blot hybridization analyses. Nucleotide abbreviations are according to the International Union of Pure and Applied Chemistry code.

Target gene	Primer	Sequence (5′–3′)	Tm	Length
Prochlorococcus sp. MED4				
cynA	cynA F*	GARYTNGAYGCNTAYCAYATGC	50	705
	PcynA R*	YTGWATCCAATGWSWAAAWSWYTGCC		
cynS	RTcS-Med4 F	CCTACGGATCCTCTTATCTA	52	163
	RTcS-Med4 R	CTAGAACCCTATCTCCCTTT		
ntcA	1F†	AGAGGAGCAGTAAGGTTATC	53	449
	4R†	TCAGACCTATGTCCTGTTAG		
Synechococcus sp. WH8102				
cynA	cynA F*	GARYTNGAYGCNTAYCAYATGC	54	705
	ScynA R*	YTGRATCCAATGSSWAAAWSWYTGCC		
cynS	cS-8102 F	AGGTTTGGGTTGCATCTTTG	52.5	235
	cS-8102 R	TCTCCGAAATGCTCCTGAAT		
ntcA	1F†	AGAGGAGCAGTAAGGTTATC	59	449
	4R†	TCAGACCTATGTCCTGTTAG		
Synechococcus sp. WH7803				
cynS	RTcS-2 F	GGCCACAGCATCAGCGGAGG	63	283
	RTcS-2 R	GGTGATCTTCACGCGATCGCC		
ntcA	1F†	AGAGGAGCAGTAAGGTTATC	63	449
	4R†	TCAGACCTATGTCCTGTTAG		

* The cynA F, PcynA R, and ScynA R primers were previously published in (Kamennaya et al. 2008).
† The 1F and 4R primers were previously published in (Lindell et al. 1998).

Nucleic acid blotting—The application of nucleic acids to the nitrocellulose membranes was performed using Bio-Dot® Microfiltration Apparatus (Bio-Rad) according to the instruction manual. Equal DNA and RNA amounts (300 and 200 ng, respectively) were applied at each dot. Genomic DNA of *Prochlorococcus* sp. strains MED4 and AS9601 and *Synechococcus* sp. strains WH8102 and WH7803 were used as controls.

Probes preparation and labeling—Probes for DNA-DNA and DNA-RNA hybridizations were obtained by PCR amplification of *ntcA*, *cynA*, and *cynS* from genomic DNA templates with degenerate primer sets (*see* Table 1). PCR products were purified with the PCR Clean-Up System. The identity of individual amplicon sequences was confirmed by Sanger sequencing. Each gene was probed discreetly by specific amplicon mix. For *ntcA* and *cynS* detection, equal amounts of appropriate amplicon DNA from *Prochlorococcus* sp. MED4 and *Synechococcus* sp. strains WH8102 and WH7803 were mixed prior to labeling. For *cynA*, the probe mix contained equal amounts of amplicons from the first two strains only. Probe mixes (120 ng) were radioactively labeled with ^{32}P-dCTP (Institute of Isotopes) by random oligonucleotide-primed synthesis following standard protocols for the HexaLabel™ DNA Labeling Kit (Fermentas). Labeled probes were purified of unincorporated nucleotides by gel filtration on Sephadex™ G-50 (GE Healthcare).

DNA-DNA and DNA-RNA hybridization—Membranes were immersed in 2× saline-sodium phosphate-ethylenediaminetetraacetic acid (SSPE) and prehybridized in Ambion ULTRAhyb® Ultrasensitive Hybridization Buffer (150 μL for each cm^2 of membrane) following the manufacturer's instructions for DNA and RNA hybridization. Following heat denaturation for 5 min at 95°C and rapid cooling on ice, labeled probe was added directly to the ULTRAhyb® buffer. Following overnight hybridization at 65°C for DNA and 42°C for RNA, membranes were washed twice with 2× SSPE + 0.1% SDS for 5 min and then with 0.1× SSPE + 0.1% SDS for 15 min at the temperature of hybridization. The high DNA-DNA hybridization temperature was required to eliminate nonspecific signal for the negative controls. Washed membranes were exposed to UV crosslinking of nucleic acids to the membrane by exposing it to 120,000 μJ cm^{-2} for 10 s (UVC-515 Ultraviolet Multilinker, Ultra-Lum) and then sealed. The membranes were visualized with Fluorescent Image Analyzer FLA-3000 (Fuji), and image analysis was performed with Science Lab 99 Image Gauge 3.4X software. A typical example of a hybridized sample is given in Fig. 1. For repeat probing, immediately after the autoradiography, the membranes were stripped by boiling twice in 250 mL of 0.1× Saline-Sodium Citrate (SSC) + 0.5% SDS for 30 min. The probe removal efficiency was checked by overnight exposure of membranes to an X-ray film. Stock solutions used for wash buffers were 20× SSPE (3.6 mol L^{-1} NaCl, 0.2 mol L^{-1} $Na_2HPO_4 \times 7H_2O$, 0.02 mol L^{-1} EDTA) and 20× SSC (3 mmol L^{-1} NaCl, 0.3 mol L^{-1} trisodium citrate; TE: 10 mmol L^{-1} Tris-HCl, pH 8.0, 1 mmol L^{-1} EDTA, pH 8.0).

Results

Cyanate utilization potential in diverse marine environments—Kamennaya et al. (2008) proposed that urea is an important source of cyanate in the ocean. It was also shown

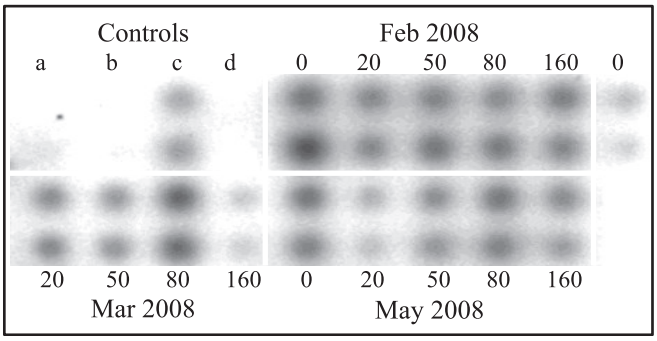

Fig. 1. Typical example of the autoradiography image of the nitrocellulose membrane blots following hybridization of DNA with picocyanobacterial ^{32}P-labeled *cynA* probe mix. Negative control: blank (a), *Synechococcus* WH7803 (b), and *Prochlorococcus* sp. AS6901 DNA (d) that both lack the *cynA* gene. Positive control was the genomic DNA of *Prochlorococcus* sp. MED4 (c). For each month, presence of *cynA* was evaluated in duplicate at five depths: 0, 20, 50, 80, and 160 m.

that cyanate is a labile compound that decomposes slowly to ammonium (Kamennaya et al. 2008) and (presumably) carbon dioxide. Thus, the three N compounds form a sequence of sources and sinks of organic N each of which can be utilized by marine picocyanobacteria. As a first approximation of cyanate utilization in the surface ocean, we mined marine metagenomes (GenBank, taxid 408172, formerly the Global Ocean Survey [GOS]) for the presence of *urtA*, *ureC* (urea); *cynA*, *cynS* (cyanate); and *glnA*, *amt* (ammonium) genes and used the *ntcA* (N control) gene as a basis to normalize incidence rates for each gene query (Table 2). The near 1 : 1 ratio of *ureC* and *urtA* incidence suggests that most urea utilizing picocyanobacteria contain both the urea transporter and the urease, so that either gene may be used as a trustable marker for this functional trait. By contrast, *cynA* : *cynS* ratios for *Prochlorococcus* (0.31) and *Synechococcus* (0.04) deviated significantly from 1 : 1, implying that the majority of picocyanobacteria do not couple the capability of cyanase metabolism to that of cyanate acquisition. Incidence of ammonium assimilating genes was invariably the highest. Relative to *ntcA* incidences normalized to gene length (100%), the data suggest that ~ 90% (not significantly different from 100%) of the cyanobacteria in the surface ocean utilize ammonium, ~ 70% utilize urea, and < 5% utilize cyanate. Naturally, this survey does not cover the full temporal and spatial dimensions of cyanate utilization in the ocean. The following sections aim at providing a more detailed analysis of *cynA* and *cynS* distributions and their expression over seasonal cycles.

To assess the broader distribution of cyanate utilization in marine cyanobacteria, we constructed *cynA* amplicon libraries on environmental samples from Mediterranean Sea, Indian Ocean, and Southern Ocean, representing oligotrophic (the first two) and meso- to eutrophic environments. After exclusion of redundant sequences from individual samples, we arrived at 23 unique sequences for the Mediterranean Sea, 21 for the tropical Indian Ocean, and 12 for the Southern Ocean. This data set was complemented with 40 *cynA* clones previously obtained from the oligotrophic Gulf of Aqaba, northern Red Sea, and eight GOS sequences of > 470 bp length that spanned our *cynA* amplicon sequences. The *cynA* was readily amplified from stratified surface waters of the Gulf of Aqaba, northern Red Sea (Kamennaya et al. 2008). The bulk of these *cynA* sequences clustered with *Prochlorococcus* HLII, and only a single sequence (cyn6-0-11) aligned with *Synechococcus* (Fig. 2).

Cyanobacterial *cynA* was successfully amplified from 0–50 m samples collected in the eastern Mediterranean off the coast of Israel (Table 3) during the decline of the

Table 2. The incidence (tblastn, E-value < 10^{-20}) of cyanobacterial genes for urea, cyanate, ammonium assimilation, and the nitrogen assimilation regulation in the GOS data set. Ratio values (bold) indicate the incidence of the transport gene (*urtA*, *cynA*, *amt*) relative to the assimilating enzyme (*ureC*, *cynS*, *glnA*), both corrected for gene length (*urtA*, 1317 bp; *ureC*, 1707 bp; *cynA*, 1683 bp; *cynS*, 441 bp; *amt*, 1461 bp; *glnA*, 1419 bp; *ntcA*, 732 bp) as a proxy for the respective chance that shotgun cloned fragments carried (part of) either gene.

	Prochlorococcus		*Synechococcus*		Total cyanobacteria	
Gene	Hits	Hits : length	Hits	Hits : length	Hits	Hits : length
Urea						
urtA	147	0.112	49	0.037	196	0.149
ureC	184	0.108	62	0.036	246	0.144
ratio		**1.04**		**1.02**		**1.03**
Cyanate						
cynA	12	0.007	4	0.002	16	0.010
cynS	10	0.023	28	0.063	38	0.086
ratio		**0.31**		**0.04**		**0.11**
Ammonium						
amt	220	0.151	52	0.036	272	0.186
glnA	170	0.120	61	0.043	231	0.163
ratio		**1.26**		**0.83**		**1.14**
N regulator						
ntcA	115	0.157	38	0.052	153	0.210

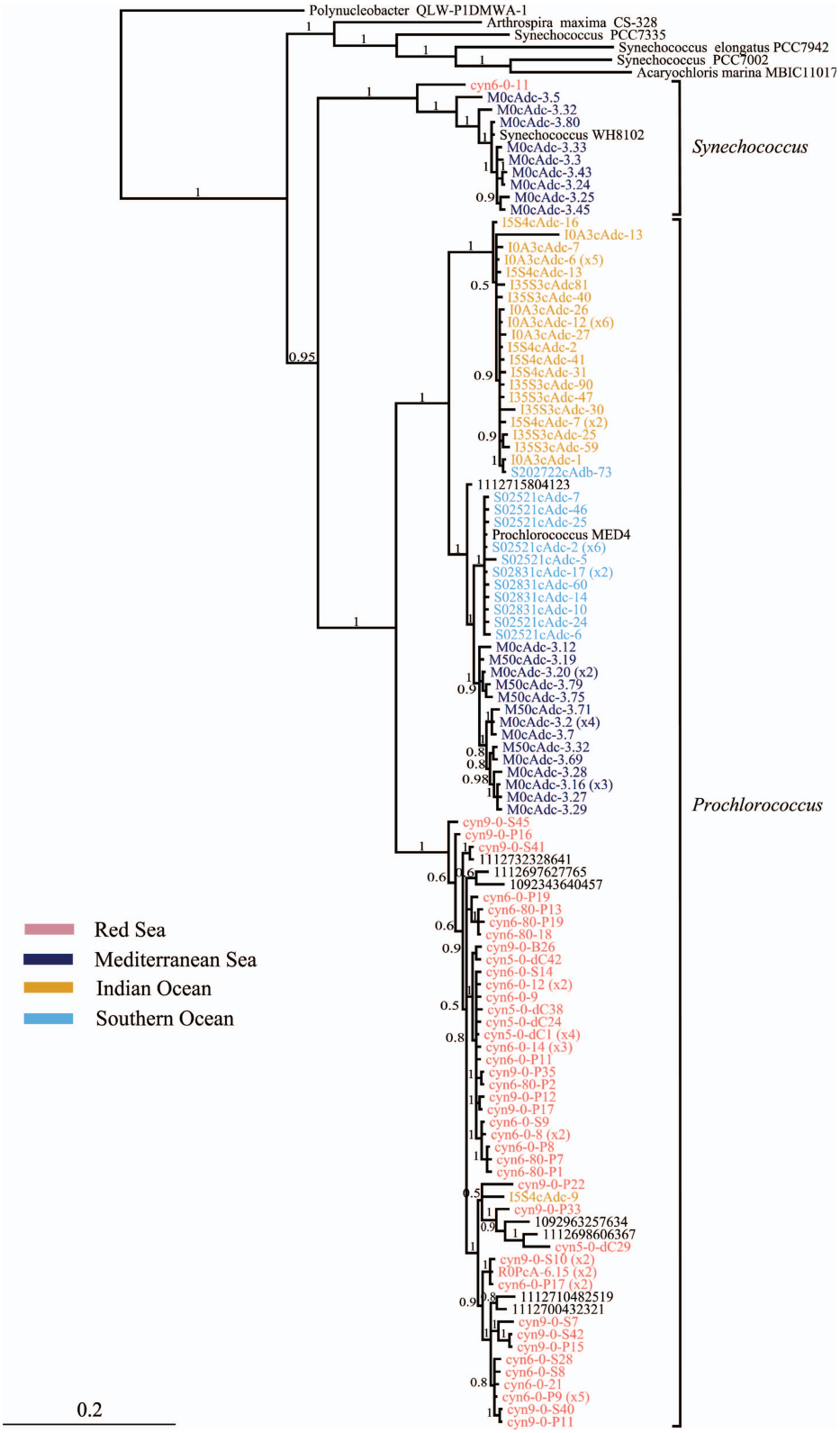

Fig. 2. Bayesian phylogeny for environmental 471 bp *cynA* sequences obtained in this study, complemented with previously published sequences from the Northern Red Sea (Kamennaya et al. 2008) and *cynA* clones identified in a tblastn search of the GOS. The scale bar provides a distance measure of two substitutions per 100 nucleotides between sequences. Posterior probabilities are given at the nodes using a 0–1 scale. The taxa names are colored according to their geographic origin. The GOS-derived samples are in black and labeled with their accession number with omission of the preceding J. Craig Venter Institute, open reading frame (JCVI ORF). The total number of redundant clones obtained for some sequences is given in parentheses.

Table 3. Sampling date, geographic position, maximum depth (MD, m), and surface water temperature (SWT, °C) at the open-water stations sampled in the Southern Ocean, Indian Ocean, Mediterranean Sea, and Red Sea and the depths (m) at which *cynA* clones were obtained.

	Station	Date	Latitude	Longitude	MD	SWT	Depth
Southern Ocean	PS69.252.1	25 Feb 06	71°51′S	106°19′W	563	−1.8	0
	PS69.272.2	06 Mar 06	73°54′S	118°30′W	1545	−1.5	20
	PS69.283.1	10 Mar 06	72°46′S	115°19′W	600	−1.7	0
Indian Ocean	A3	09 Jan 02	7°0′S	52°43′E	12	27.6	0
	S3	11 Jan 02	5°34′S	54°19′E	>2000	27.6	35
	S4	11 Jan 02	5°09′S	54°45′E	>2000	27.6	5
Mediterranean Sea	NH	19 Mar 06	32°10′N	34°25′E	700	17.2	0 50
Red Sea	A	12 Jun 03	29°28′N	34°55′E	700	25.4	0 80
	A	15 May 05	29°28′N	34°55′E	700	22.9	0
	A	06 Sep 05	29°28′N	34°55′E	700	24	0

Synechococcus-dominated spring bloom in March, after surface Chl *a* concentrations had decreased (data not shown). Almost half of the Mediterranean *cynA* clones ($n = 9$) clustered with *Synechococcus*, and the remaining 14 sequences clustered within *Prochlorococcus* HLI (Fig. 2). Mediterranean samples collected during winter mixing or summer stratification failed to yield *cynA* amplicons. Sequences of *cynA* from the Indian Ocean originated from warm open waters but not from lagoons, at different locales in the Seychelles archipelago (Table 3). These sequences ($n = 20$) formed a distinct cluster away from *Prochlorococcus* sp. MED4 *cynA*, suggesting that sequences in this clade are contributed by HL *Prochlorococcus* clade lacking culture representatives, possibly of the type recently identified with other gene markers (Rusch et al. 2010; West et al. 2011; Huang et al. 2012). Interestingly, we obtained *cynA* sequences from the Southern Ocean (Table 3), where presence of marine picocyanobacteria has only recently been predicted from metagenome assemblies (Wilkins et al. 2012). With the exception of a single sequence, *cynA* clones from the Amundsen and Bellingshausen Seas clustered with *Prochlorococcus* sp. MED4 and thus affiliated with the HLI ecotype. Finally, eight *cynA* fragments retrieved from the GOS database clustered with the *Prochlorococcus*. Particularly, the sequences clustered with those of the Red Sea–derived *cynA* clones (Fig. 2), previously identified as HLII *Prochlorococcus* (Kamennaya et al. 2008). Sequences from each of the study sites comprised a significant fraction of singletons, suggesting that *cynA* diversity was not sampled to saturation. Although some identical *cynA* fragments were obtained for clones that originated from different locations, the bulk of cyanobacterial *cynA* sequences showed a geographical pattern.

Distribution of cyanate utilization genes during a seasonal cycle (2004–2005)—The Gulf of Aqaba is a well-studied marine oligotrophic system with a predictable annual cycle of stable summer stratification and deep winter mixing (Fig. 3) determining a seasonal dynamics of phytoplankton populations (Genin et al. 1995; Post et al. 2011). Before the onset of the mixing period in November 2004, the thermocline spanned the entire photic zone down to 160 m, and the upper 125 m were deplete of inorganic N (Fig. 3C). Sea surface cooling in December enhanced convective mixing, and during the ensuing winter the thermocline gradually eroded until it reached > 670 m by the end of March 2005 (data not shown). Elevation of deep-water masses during mixing caused a significant enrichment of the photic zone with combined inorganic nitrogen (Fig. 3C) and other macronutrients (P, Si; data not shown). During development of the phytoplankton spring bloom (Fig. 3B) supported by these nutrients, much of the Chl *a* standing stock in March was contributed by fast-growing picoeukaryotes followed by a distinct *Synechococcus* bloom during the ensuing month, as previously reported (Lindell 1995). During the following summer, due to the extensive phytoplankton growth, levels of inorganic N in euphotic layer were rapidly depleted and fell below the detection limit in the upper 125 m (Fig. 3C). The development of more oligotrophic conditions was followed by a decline of the surface bloom (Fig. 3B) due to slower growth (nutrient limitation) and increased mortality (grazing, sinking, and so on). Further stratification halted nitrate entrainment into the surface layers, restricting primary production to the utilization of regenerated N compounds. Earlier reports (Lindell and Post 1995; Al-Najjar et al. 2007) have established that these oligotrophic waters carry abundant *Prochlorococcus* populations that contribute the bulk of primary production in the Gulf until the onset of mixing in October.

In the absence of sufficiently sensitive assays for cyanate detection in seawater, we studied the genetic capacity for cyanate uptake as a proxy for the distribution of this compound. The ability to acquire cyanate is encoded by *cynABD* (cyanate transporter) and its subsequent metabolism by *cynS* (cyanase). Since cyanate utilization requires the simultaneous expression of *cynABD* (cyanate uptake), *cynS* (cyanate degradation), and *ntcA* (N regulatory), we determined distributions of the latter two genes as a control alongside *cynA*. Since quantitative PCR approaches with different primer combinations consistently yielded nonspecific results, we decided to use dot-blot hybridization, in analogy to previous studies with 16S rDNA (West and Scanlan 1999; Fuller et al. 2005; Zwirglmaier et al. 2008). To estimate vertical and seasonal distributions of this potential, we hybridized picocyanobacteria-specific probes for *cynA*, *cynS*, and *ntcA* with environmental DNA from

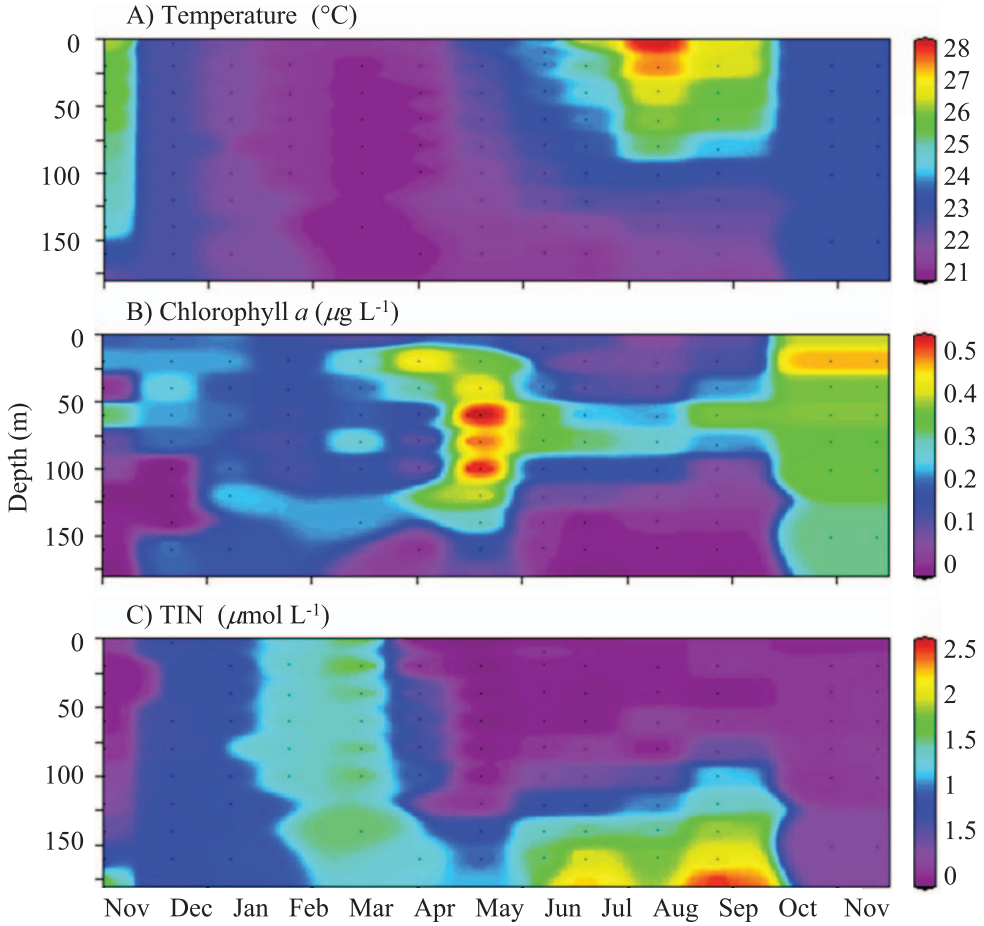

Fig. 3. Seasonal variation of oceanographic and biological parameters in the upper 180 m water layer of the Gulf of Aqaba, northern Red Sea: (A) water temperature, (B) phytoplankton standing stock (Chl *a*), and (C) total inorganic N (TIN), measured between November 2004 and November 2005.

samples collected monthly at five depths over a seasonal cycle (data not shown). Distributions of the cyanate utilization potential were not homogeneous. A strong presence of *cynA*, *cynS*, and *ntcA* was observed during the beginning of mixing event (November–January). In February–March, the cyanate utilization potential was not detected. During this period, following the peak of the winter mixing, the nutrient-rich water column witnessed the onset of the spring bloom with phytoplankton dominated by eukaryotic algae. During the *Synechococcus*-dominated second stage of the spring bloom (April–May), *cynS* was clearly evident in the upper 80 m. Strong *cynA*, *cynS*, and *ntcA* signals were observed in the upper 50 m in May only. Evidence for cyanate utilization was largely lacking during the first half of the summer stratification (June–August). It reappeared only in September–October in the upper 80 m deplete of combined inorganic N.

Expression of cyanate utilization in the Gulf of Aqaba (2007–2008)—Detection of the genetic potential for cyanate utilization was the first step toward asserting actual acquisition of cyanate in marine environments. To confirm our initial findings for 2004–2005, we continued tracing the presence of the cyanate utilization potential in the Gulf of Aqaba along with the appearance of their mRNA transcripts as a proxy for acclimation to utilize cyanate. This study covered a period of pronounced difference between mixing events: the two mild winters in 2004–2005 and 2005–2006 with mixing to intermediate depths only (670 and 540 m, respectively) were followed by relatively cold winters in 2006–2007 and 2007–2008, during which vertical mixing reached below 750 m.

We hybridized the same probe sets to RNA-free DNA (300 ng) and DNA-free total RNA (200 ng) aliquots. On exposure on X-ray film (Fig. 1), we obtained a proxy from gene expression by normalizing the RNA signal over those of DNA (Figs. 4, 5). This approach assumes that the probe hybridization to gene transcripts closely resembles that of the genes from which they derive. Since the genetic diversity of the picocyanobacterial community is low (communities are dominated by a single *Synechococcus* and a single *Prochlorococcus* type), we estimated this to be a fair assumption. Constitutive transcription of *cynS* was evident: samples from all layers within the photic zones and from all dates with only few exceptions showed RNA : DNA signals that exceeded the 1 : 1 ratio, often reaching ratios of 10 : 1

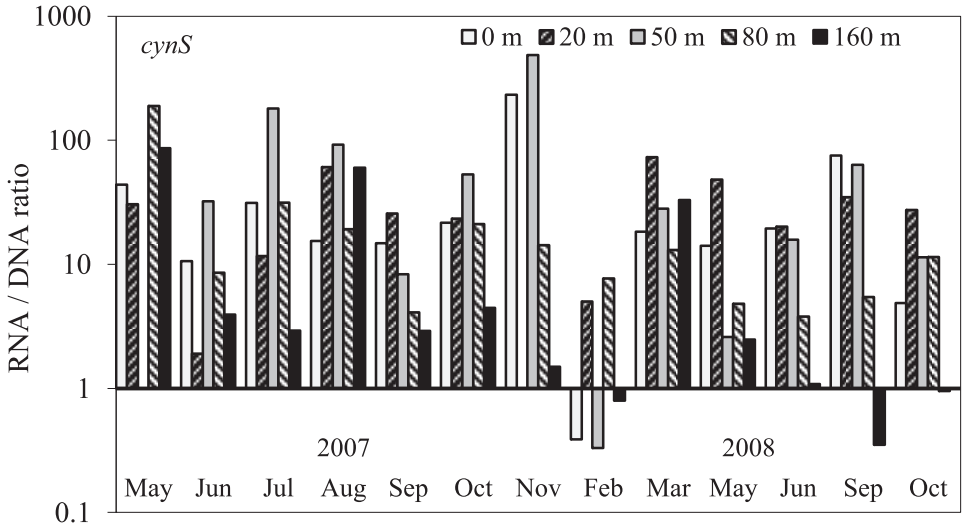

Fig. 4. Temporal and spatial expression patterns of *cynS* in the Gulf of Aqaba, northern Red Sea, from May 2007 to October 2008, except December 2007 and January, April, July, and August 2008. Data represent the RNA : DNA ratios in relative units, with a threshold of 1. Note that transcript levels are plotted using a logarithmic scale for the *y*-axis.

up to 500 : 1 (Fig. 4). In contrast, *ntcA* and *cynA* transcripts showed a more dynamic pattern (Fig. 5). Previously, Lindell et al. (2005*a*) reported that natural *Synechococcus* communities expressed *ntcA* during the spring bloom in 2000, consistent with nitrate availability during that period. Furthermore, *ntcA* transcripts of experimentally N-deprived *Synechococcus* populations in surface waters of the Gulf of Aqaba were $>$ 5 times higher than ammonium treated controls. The current study confirms the latter finding, as RNA : DNA ratios for *ntcA* exceeded a 10 : 1 ratio in most samples from the 2007–2008 mixing period, with *ntcA* transcript particularly abundant in March (Fig. 5A). We further observed *ntcA* transcript accumulation in samples from deeper photic layers during stratification (August 2007 and September and October 2008), suggesting that picocyanobacterial N stress responses are not limited to the surface layers (Fig. 5A). We arbitrarily set a RNA : DNA ratio of 1 : 1 from *cynA* transcript accumulation in the Gulf of Aqaba (Fig. 5B). Using this threshold, we found that *cynA* transcripts in general followed those of *ntcA* and that the highest *cynA* ratios were found during the winter mixing period and the ensuing spring bloom between November 2007 and May 2008. Finally, we observed a simultaneous increase in RNA : DNA ratios for both *ntcA* and *cynA* in stratified waters (July–August 2007 and September 2008), suggesting that picocyanobacterial communities in the upper half of the photic zone adapted to N-limiting conditions by inducing a cyanate scavenging response during this period.

Discussion

Until recently (Rocap et al. 2003; Kamennaya et al. 2008), cyanate was not considered an N source of importance in the surface ocean. However, spontaneous decomposition of urea can form a significant source of cyanate (Kamennaya et al. 2008). In the absence of an accurate method to detect cyanate in seawater, the distribution of cyanate assimilation genes serves the only proxy for cyanate presence (assuming that assimilation is an adaptive trait). We examined the distribution of *cynA* (cyanate transport) and *cynS* (cyanate metabolism) relative to other N-assimilatory genes across ocean biomes by querying the GOS database. Interpretation of the incidence ratios for the genes assumes that they are present as single copies on the picocyanobacterial genome. Genome comparisons show that, whereas ammonium transport (*amt*) and assimilation (*glnA*) are encoded by multiple gene copies in few *Synechococcus*, this holds true for urea and cyanate assimilatory genes (Scanlan et al. 2009). Thus, the finding that cyanate acquisition is present in approximately 10% of the picocyanobacteria (Table 2) appears to be a realistic estimate. Our finding further suggests that cyanate utilization may be limited to a specialized subsection of the marine picocyanobacteria. However, it is important to remember that the GOS database provides only limited insight in depth distributions and temporal patterns of cyanate assimilatory potential in the ocean. Only 10% of our cyanobacterial *cynA* clones were ascribed to members of the genus *Synechococcus*. PCR bias toward AT-rich *Prochlorococcus* does not adequately explain this phenomenon, as *Synechococcus cynA* was readily amplified from Mediterranean samples. Therefore, we conclude that, out of the locations examined, notable cyanate acquisition potential of *Synechococcus* was observed in the Levantine basin of the Mediterranean Sea only. Furthermore, all eight GOS *cynA* sequences of $>$ 470 bp in length clustered within the *Prochlorococcus* clades, implying that the cyanate utilization potential is more characteristic of *Prochlorococcus*.

Spatial partitioning of HL-adapted *Prochlorococcus* ecotypes is determined primarily by the water temperature, with HLII ecotype dominating warm tropical waters (e.g., the Gulf of Aqaba) and HLI being more abundant at cooler areas with temperatures $<$ 24°C (Johnson et al.

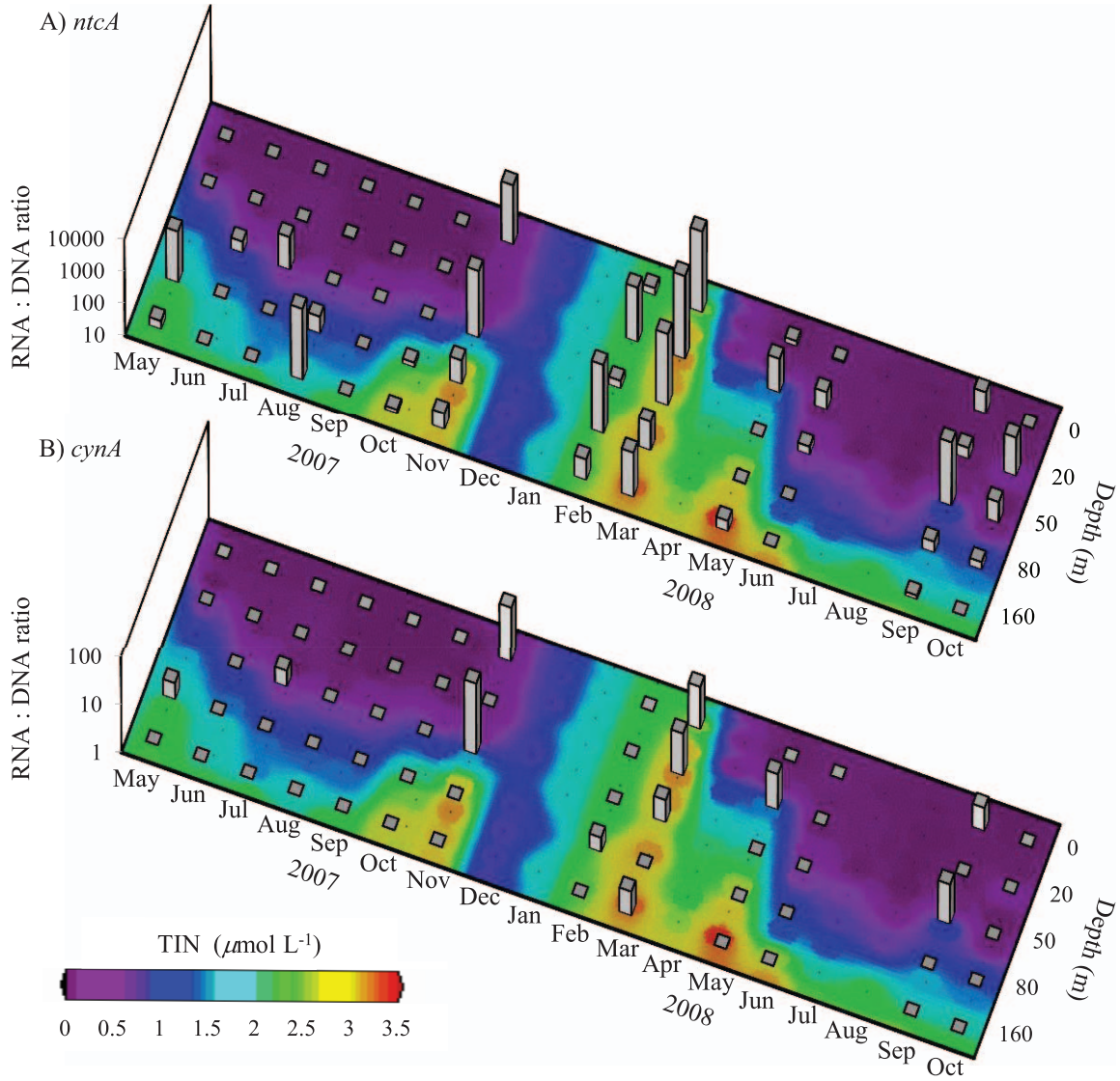

Fig. 5. Temporal and spatial expression patterns of (A) *ntcA* and (B) *cynA* genes in the Gulf of Aqaba, northern Red Sea, monitored over a period from May 2007 to October 2008, except December 2007 and January, April, July, and August 2008. Data represent the RNA : DNA ratios in relative units, with thresholds of 10 and 1 for *ntcA* and *cynA*, respectively, plotted over total inorganic N (TIN) distribution in the upper 160 m of a water column. Note that transcript levels are presented on a logarithmic scale.

2006; Zwirglmaier et al. 2008). The *cynA* phylogeny reflected mainly this distribution pattern. The phylogenetic relationship in Fig. 2 further exemplifies a biogeographic pattern where the different sequence clusters reflect their sample origin. We previously reported (Kamennaya et al. 2008) that *Prochlorococcus cynA* is abundantly present during spring and summer in the Gulf of Aqaba. These *Prochlorococcus* sequences clustered away from a single *cynA* representative for the HLI strain MED4 and were tentatively assigned to HLII genotypes (Kamennaya et al. 2008) dominating the *Prochlorococcus* population of the Gulf of Aqaba (Steglich et al. 2003; Fuller et al. 2005; Penno et al. 2006). Environmental sequences from the Mediterranean Sea, with its cool waters dominated by HLI *Prochlorococcus* populations, form a cluster closely related to the MED4. Notably, a third cluster of *Prochlorococcus cynA* stems from the tropical Indian Ocean. Although based on *cynA* gene phylogeny it is related to HLI, this genotype may be representative of one of the recently defined high-light-adapted genotypes (Rusch et al. 2010; West et al. 2011; Huang et al. 2012). Collected from the Seychelles archipelago experiencing occasional upwelling, (27°C, Chl $a \leq 0.3$ μg L^{-1} at surface), the Indian Ocean clones fall in line with the characteristics defined for the HNLC high-light-adapted clades (West et al. 2011).

A number of sequences from Antarctic waters cluster with MED4. This is surprising, as *Prochlorococcus* was until recently considered to be restricted to 40°S–48°N latitudes (Johnson et al. 2006; Zwirglmaier et al. 2008; Wilkins et al. 2012). HLI *Prochlorococcus* is abundant at the boundaries of the southern Atlantic Ocean (Johnson et al. 2006), and cells may be captured from more northerly water masses by the Antarctic Circumpolar Current. In a separate study of cyanobacterial *ntcA* clone libraries, we

observed that Antarctic waters carry low-abundance populations of *Prochlorococcus* that span all known high-light-adapted genotypes as well as a cluster of low-light-adapted genotypes that seem unique to Antarctic waters (N. A. Kamennaya et al. unpubl.). However, no *cynA* cluster specific to the meso- to eutrophic Southern Ocean was detected in this study.

All GOS *cynA* sequences originated from low-Chl ($\leq$ 0.151 mg m^{-3}) tropical waters of the South Pacific Ocean, the Indian Ocean, and the Caribbean Sea. Seven clones derived from warm surface waters ($\geq 27^{\circ}C$), grouped within the HLII cluster, in agreement with the previously determined distribution of the genotype (Rusch et al. 2010). The only GOS clone affiliated with the HLI cluster was collected from surface waters of 20.2°C. Interestingly, not a single LL *Prochlorococcus cynA* was identified, even though our clone libraries included samples from as deep as 80 m. Similarly, none of the LL-adapted *Prochlorococcus* genomes sequenced to date possess the *cynA* gene. Phylogenetic distribution of *cynA* thus leads us to conclude that cyanate utilization is most common among HL *Prochlorococcus* ecotypes.

The distribution patterns of *cynA* and *cynS* genes in the photic layer (160 m) of the Gulf of Aqaba showed temporal change. During late winter mixing (February–March) and early summer stratification (July–August), *cynA* was detected by neither PCR (Kamennaya et al. 2008) nor community dot-blot hybridization (this study). The cyanate utilization potential (*cynA* and *cynS*) was found during late summer stratification and early winter mixing (September–November) but was also very evident during the final stages of the spring bloom (May–June). In 2007–2008, we observed accumulation of both *cynA* and *cynS* transcripts along with that of *ntcA* during the same water column states, indicating that picocyanobacterial communities indeed adapt to assimilate cyanate during these periods. Due to specific characteristics of each seasonal cycle (e.g., mixing depth, maximal enrichment with total organic nitrogen, maximal Chl *a* concentrations, and so on), some variability in the *cynA* expression patterns between the years was noticeable (Fig. 5). The expression of a cyanate assimilation coincided with a dominant presence of *Prochlorococcus* during late summer stratification and early winter mixing and after a *Synechococcus*-dominated spring bloom period (Lindell and Post 1995; Rusch et al. 2010). We further note that the failure to amplify *Synechococcus cynA* during the spring bloom indicates that in the Gulf of Aqaba, the bulk of cyanate assimilation is carried out by *Prochlorococcus*. The *Prochlorococcus* populations may thus (at least in part) meet their N requirements from the assimilation of cyanate, lending further support for our hypothesis on the role of cyanate as an N source in marine waters (Kamennaya et al. 2008). However, in addition to the N-deplete surface layers proposed to confine the majority of cyanate assimilation (late spring bloom and late summer stratification periods), this study identified the presence and expression of the cyanate utilization potential in a mixed water column. The likely source of cyanate is the decomposition of ambient urea (Kamennaya et al. 2008), which in turn originates from excretion by zooplankton and lysis of cells in the stratified column (Glibert and Terlizzi 1999) and as a result of decomposition of the dissolved organic matter in the mixed water column (Antia et al. 1991).

Transcripts of *cynS* were abundantly and constantly present in the water column (Fig. 4). Based on the genomes of sequenced representatives, 11 out of 12 *Synechococcus* genomes (Scanlan et al. 2009) but only 3 out of 12 *Prochlorococcus* genomes (Kamennaya and Post 2010) carry *cynS*. Whereas the bulk of *cynA* transcripts in the Gulf of Aqaba were ascribed to *Prochlorococcus*, we assume that a significant fraction of *cynS* transcript was contributed by the *Synechococcus* population. Since *cynS* transcripts were abundantly present at all depths over the seasonal cycle, it appears that, as we have previously suggested (Kamennaya and Post 2010), *cynS* transcripts reflect intracellular N cycling via urea and cyanate rather than cyanate assimilation. Despite the lack of arginase (EC 3.5.3.1), *Synechococcus* can intracellularly produce urea by agmatine ureahydrolase (EC 3.5.3.11), along with conversion of arginine to spermidine. Since picocyanobacteria are mostly N sufficient (Lindell et al. 2005*a*), especially during the spring bloom, when nitrate is abundantly present (Lindell and Post 1995; Badran 2001), they may convert excess N to spermidine for storage (Bryant 2003). Metabolism of spermidine may lead to accumulation of intracellular urea and thus cyanate and so select for cyanate detoxification.

We conclude that the natural abundance of cyanate utilization detected in the Gulf of Aqaba and supported by *cynA* presence in the Mediterranean Sea, the Indian Ocean, the Circumpolar Current, and the GOS data collection indicates that cyanate may constitute an essential N source in ocean environments, utilized by a variety of organisms, particularly by the HL-adapted *Prochlorococcus*. Using a "reverse genomics" approach, we were able to study a multitude of ecotypes across different marine environments in a culture-independent manner and thus to reveal novel insights regarding distribution and activity of cyanate-utilizing organisms. This study does not provide enough data to ascribe the cyanate utilization to an environment with the particular nutrient state. Novel analytical methods are now required to assess the occurrence and distribution of cyanate in marine environments.

Acknowledgments

We are grateful to the National Monitoring Program at the Interuniversity Institute for Marine Science in Eilat, which provided us with monthly sampling opportunities as well as free access to ancillary data on physical, chemical, and biological properties of the Gulf of Aqaba at times of sampling. We thank two anonymous reviewers for their constructive criticism and helpful remarks. Financial support for experimental work was provided by Israel Science Foundation grant 135/05; DNA sequencing, phylogenetic analyses, data processing, and manuscript preparation were supported by the National Science Foundation grant 1155566 in Chemical Oceanography.

References

Al-Najjar, T., M. I. Badran, C. Richter, M. Meyerhoefer, and U. Sommer. 2007. Seasonal dynamics of phytoplankton in the Gulf of Aqaba, Red Sea. Hydrobiologia **579:** 69–83, doi:10.1007/s10750-006-0365-z

Anderson, P. M. 1980. Purification and properties of the inducible enzyme cyanase. Biochemistry **19:** 2882–2888, doi:10.1021/bi00554a010

Antia, N. J., P. J. Harrison, and L. Oliveira. 1991. Phycological reviews 2: The role of dissolved organic nitrogen in phytoplankton nutrition, cell biology and ecology. Phycologia **31:** 1–89, doi:10.2216/i0031-8884-30-1-1.1

Azov, Y. 1986. Seasonal patterns of phytoplankton productivity and abundance in nearshore oligotrophic waters of the Levant Basin (Mediterranean). J. Plankton Res. **8:** 41–53, doi:10.1093/plankt/8.1.41

Badran, M. I. 2001. Dissolved oxygen, chlorophyll *a* and nutrients: Seasonal cycles in waters of the Gulf of Aquaba, Red Sea. Aquat. Ecosystem Health Manag. **4:** 139–150, doi:10.1080/14634980127711

Bryant, D. A. 2003. The beauty in small things revealed. Proc. Natl. Acad. Sci. **100:** 9647–9649, doi:10.1073/pnas.1834558100

Chisholm, S. W. 1992. Phytoplankton size, p. 213–237. *In* P. G. Falkowski and A. D. Woodhead [eds.], Primary productivity and biogeochemical cycles in the sea. Plenum Press.

———, R. J. Olson, E. R. Zettler, R. Goericke, J. B. Waterbury, and N. A. Welschmeyer. 1988. A novel free-living prochlorophyte abundant in the oceanic euphotic zone. Nature **224:** 340–343, doi:10.1038/334340a0

David, E. 2003. Dynamics of ammonia and nitrite in the water column of the northern Gulf of Eilat. M.Sc. thesis. The Hebrew University of Jerusalem.

Deutsch, C., J. L. Sarmiento, D. M. Sigman, N. Gruber, and J. P. Dunne. 2007. Spatial coupling of nitrogen inputs and losses in the ocean. Nature **445:** 163–167, doi:10.1038/nature05392

Dufresne, A., L. Garczarek, and F. Partensky. 2004. Accelerated evolution associated with genome reduction in a free-living prokaryote. Genome Biol. **6:** 1–10.

Dugdale, R. C., and J. J. Goering. 1967. Uptake of new and regenerated forms of nitrogen in primary productivity. Limnol. Oceanogr. **12:** 196–206, doi:10.4319/lo.1967.12.2.0196

Espie, G. S., F. Jalali, T. Tong, N. J. Zacal, and A. K.-C. So. 2007. Involvement of the *cynABDS* operon and the CO_2-concentrating mechanism in the light-dependent transport and metabolism of cyanate by cyanobacteria. J. Bacteriol. **189:** 1013–1024, doi:10.1128/JB.01328-06

Figueiras, F. G., F. F. Perez, Y. Pazos, and A. F. Rios. 1994. Light and productivity of Antarctic phytoplankton during austral summer in an ice edge region in the Weddell-Scotia Sea. J. Plankton Res. **16:** 233–253, doi:10.1093/plankt/16.3.233

Fuller, N. J., N. J. West, D. Marie, M. Yallop, T. Rivlin, A. F. Post, and D. J. Scanlan. 2005. Dynamics of community structure and phosphate status of picocyanobacterial populations in the Gulf of Aqaba, Red Sea. Limnol. Oceanogr. **50:** 363–375, doi:10.4319/lo.2005.50.1.0363

Genin, A., B. Lazar, and S. Brenner. 1995. Vertical mixing and coral death in the Red Sea following the eruption of Mount Pinatubo. Nature **377:** 507–510, doi:10.1038/377507a0

Gilbert, J. D., and W. F. Fagan. 2011. Contrasting mechanisms of proteomic nitrogen thrift in *Prochlorococcus*. Mol. Ecol. **20:** 92–104, doi:10.1111/j.1365-294X.2010.04914.x

Glibert, P. M., and D. E. Terlizzi. 1999. Cooccurrence of elevated urea levels and dinoflagellate blooms in temperate estuarine aquaculture ponds. Appl. Environ. Microbiol. **65:** 5594–5596.

Gohl, K. 2006. Expeditionsprogramm Nr. 75, FS Polarstern, ANT-XXIII/4 and ANT-XXIII/5, p. 5–30. *In* E. Fahrbach [ed.], Alfred Wegener Institute for Polar and Marine Research. [program of the expedition 75, R/V *Polarstern*, ANT-XXIII/4 and ANT-XXIII/5], http://hdl.handle.net/10013/epic.37259

Hansell, D. A., and J. J. Goering. 1990. Pelagic nitrogen flux in the northern Bering Sea. Cont. Shelf Res. **10:** 501–519, doi:10.1016/0278-4343(90)90079-2

Harano, Y., I. Suzuki, S.-I. Maeda, T. Kaneko, S. Tabata, and T. Omata. 1997. Identification and nitrogen regulation of the cyanase gene from the cyanobacteria *Synechocystis* sp. strain PCC 6803 and *Synechococcus* sp. strain PCC 7942. J. Bacteriol. **179:** 5744–5750.

Hecky, R. E., and P. Kilham. 1988. Nutrient limitation of phytoplankton in freshwater and marine environments: A review of recent evidence on the effects of enrichment Limnol. Oceanogr. **33:** 796–822.

Holmes, R. M., A. Aminot, R. Kérouel, B. A. Hooker, and B. J. Peterson. 1999. A simple and precise method for measuring ammonium in marine and freshwater ecosystems. Can. J. Fish. Aquat. Sci. **56:** 1801–1808.

Huang, S., S. W. Wilhelm, H. R. Harvey, K. Taylor, N. Jiao, and F. Chen. 2012. Novel lineages of *Prochlorococcus* and *Synechococcus* in the global oceans. ISME J. **6:** 285–297, doi:10.1038/ismej.2011.106

Johnson, Z. I., E. R. Zinser, A. Coe, N. P. Mcnulty, E. M. S. Woodward, and S. W. Chisholm. 2006. Niche partitioning among *Prochlorococcus* ecotypes along ocean-scale environmental gradients. Science **311:** 1737–1740, doi:10.1126/science.1118052

Kamennaya, N. A., M. Chernihovsky, and A. F. Post. 2008. The cyanate utilization capacity of marine unicellular cyanobacteria. Limnol. Oceanogr. **53:** 2485–2494, doi:10.4319/lo.2008.53.6.2485

———, and A. F. Post. 2010. Characterization of cyanate metabolism in marine *Synechococcus* and *Prochlorococcus* spp. Appl. Environ. Microb. **77:** 291–301, doi:10.1128/AEM.01272-10

Koeve, W., and P. Kahler. 2010. Ocean science: Balancing ocean nitrogen. Nat. Geosci. **3:** 383–384, doi:10.1038/ngeo884

Lindell, D., J. D. Jaffe, Z. I. Johnson, G. M. Church, and S. W. Chisholm. 2005*a*. Photosynthesis genes in marine viruses yield proteins during host infection. Nature **438:** 86–89, doi:10.1038/nature04111

———, E. Padan, and A. F. Post. 1998. Regulation of *ntcA* expression and nitrite uptake in the marine *Synechococcus* sp. strain WH 7803. J. Bacteriol. **180:** 1878–1886.

———, S. Penno, M. Al-Qutob, E. David, T. Rivlin, B. Lazar, and A. F. Post. 2005*b*. Expression of the nitrogen stress response gene *ntcA* reveals nitrogen-sufficient *Synechococcus* populations in the oligotrophic Northern Red Sea. Limnol. Oceanogr. **50:** 1932–1944, doi:10.4319/lo.2005.50.6.1932

———, and A. F. Post. 1995. Ultraphytoplankton succession is triggered by deep winter mixing in the Gulf of Aqaba (Eilat), Red Sea. Limnol. Oceanogr. **40:** 1130–1141, doi:10.4319/lo.1995.40.6.1130

———, and ———. 2001. Ecological aspects of *ntcA* gene expression and its use as an indicator of the nitrogen status of marine *Synechococcus* spp. Appl. Environ. Microb. **67:** 3340–3349, doi:10.1128/AEM.67.8.3340-3349.2001

Mary, I., G. A. Tarran, P. E. Warwick, M. J. Terry, D. J. Scanlan, P. H. Burkill, and M. V. Zubkov. 2008. Light enhanced amino acid uptake by dominant bacterioplankton groups in surface waters of the Atlantic Ocean. FEMS Microbiol Ecol. **63:** 36–45, doi:10.1111/j.1574-6941.2007.00414.x

Mccarthy, J. J. 1972. The uptake of urea by natural populations of marine phytoplankton. Limnol. Oceanogr. **17:** 738–748, doi:10.4319/lo.1972.17.5.0738

Miller, A. G., and G. S. Espie. 1994. Photosynthetic metabolism of cyanate by the cyanobacterium *Synechococcus* UTEX 625. Arch. Microbiol. **162:** 151–157, doi:10.1007/BF00314468

Montes-Hugo, M., S. C. Doney, H. W. Ducklow, W. Fraser, D. Martinson, S. E. Stammerjohn, and O. Schofield. 2009. Recent changes in phytoplankton communities associated with rapid regional climate change along the Western Antarctic Peninsula. Science **323:** 1470–1473, doi:10.1126/science.1164533

Moore, L. R., A. F. Post, G. Rocap, and S. W. Chisholm. 2002. Utilization of different nitrogen sources by the marine cyanobacteria. Limnol. Oceanogr. **47:** 989996, doi:10.4319/lo.2002.47.4.0989

Palenik, B., B. Brahamsha, F. W. Larimer, M. Land, L. Hauser, P. Chain, J. Lamerdin, W. Regala, E. E. Allen, J. McCarren, I. Paulsen, A. Dufresne, F. Partensky, E. A. Webb, and J. Waterbury. 2003. The genome of a motile marine *Synechococcus*. Nature **424:** 1037–1042, doi:10.1038/nature01943

Penno, S., D. Lindell, and A. F. Post. 2006. Diversity of *Synechococcus* and *Prochlorococcus* populations determined from DNA sequences of the N-regulatory gene *ntcA*. Environ. Microbiol. **8:** 1200–1211, doi:10.1111/j.1462-2920.2006.01010.x

Pitta, P., N. Stambler, T. Tanaka, T. Zohary, A. Tselepides, and F. Rassoulzadegan. 2005. Biological response to P addition in the Eastern Mediterranean Sea. The microbial race against time. Deep-Sea Res. II **52:** 2961–2974, doi:10.1016/j.dsr2.2005.08.012

Post, A. F., S. Penno, K. Zandbank, A. Paytan, S. Huse, and D. Mark Welch. 2011. Long term seasonal dynamics of *Synechococcus* population structure in the Gulf of Aqaba, Northern Red Sea. Frontiers in Microbiology **2:** 131, doi:10.3389/fmicb.2011.00131

Remsen, C. C. 1971. The distribution of urea in coastal and oceanic waters. Limnol Oceanogr **16:** 732–740, doi:10.4319/lo.1971.16.5.0732

Rocap, G., F. W. Larimer, J. Lamerdin, S. Malfatti, P. Chain, N. A. Ahlgren, A. Arellano, M. Coleman, L. Hauser, W. R. Hess, Z. I. Johnson, M. Land, D. Lindell, A. F. Post, W. Regala, M. Shah, S. L. Shaw, C. Steglich, M. B. Sullivan, C. S. Ting, A. Tolonen, E. A. Webb, E. R. Zinser, and S. W. Chisholm. 2003. Genome divergence in two *Prochlorococcus* ecotypes reflects oceanic niche differentiation. Nature **424:** 1042–1047, doi:10.1038/nature01947

Rusch, D. B., A. C. Martiny, C. L. Dupont, A. L. Halpern, and J. C. Venter. 2010. Characterization of *Prochlorococcus* clades from iron-depleted oceanic regions. Proc. Natl. Acad. Sci. **107:** 16184–16189, doi:10.1073/pnas.1009513107

Saier, M. H., Jr. 2000. A functional-phylogenetic classification system for transmembrane solute transporters. Microbiol. Mol. Biol. Rev. **64:** 354–411, doi:10.1128/MMBR.64.2.354-411.2000

Scanlan, D. J., M. Ostrowski, S. Mazard, A. Dufresne, L. Garczarek, D. E. Hess, A. F. Post, A. Hagemann, I. Paulsen, and F. Partensky. 2009. Ecological genomics of marine picocyanobacteria. Microbiol. Mol. Biol. Rev. **73:** 249–299, doi:10.1128/MMBR.00035-08

Siokou-Frangou, I., U. Christaki, M. G. Mazzocchi, M. Montresor, M. Ribera d'Alcalá, D. Vaqué, and A. Zingone. 2010. Plankton in the open Mediterranean Sea: A review. Biogeosciences **7:** 1543–1586, doi:10.5194/bg-7-1543-2010

Sorensen, J. 1997. Measurement of chlorophyll *a* and phaeopigments by fluorometric analysis, p. 89–93. *In* C. Rathbun and A. Doyle [eds.], BATS methods. Bermuda Biological Station for Research.

Steglich, C., A. F. Post, and W. R. Hess. 2003. Analysis of natural populations of *Prochlorococcus* spp. in the northern Red Sea using phycoerythrin gene sequences. Environ. Microbiol. **5:** 681–690, doi:10.1046/j.1462-2920.2003.00456.x

Suggett, D. J., N. Stambler, O. Prášil, Z. Kolber, A. Quigg, E. Vázquez-Dominguez, T. Zohary, T. Berman, D. Iluz, O. Levitan, T. Lawson, E. Meeder, B. Lazar, E. Bar-Zeev, H. Medova, and I. Berman-Frank. 2009. Nitrogen and phosphorus limitation of oceanic microbial growth during spring in the Gulf of Aqaba. Aquat. Microb. Ecol. **56:** 227–239, doi:10.3354/ame01357

Thomas, E. V., K. H. Phillippy, B. Brahamsha, D. M. Haaland, J. A. Timlin, L. D. H. Elbourne, B. Palenik, and I. T. Paulsen. 2009. Statistical analysis of microarray data with replicated spots: A case study with *Synechococcus* WH8102. Comp. Funct. Genomics **2009:** 950171, doi:10.1155/2009/950171

Tolonen, A. C., J. Aach, D. Lindell, Z. I. Johnson, T. Rector, R. Steen, G. M. Church, and S. W. Chisholm. 2006. Global gene expression of *Prochlorococcus* ecotypes in response to changes in nitrogen availability. Mol. Syst. Biol. **2:** 1–15, doi:10.1038/msb4100087

TylerP. A. [ed.]. 2003. Ecosystems of the world. Elsevier Science & Technology Books.

Vargo, G. A. 1979. The contribution of ammonia excreted by zooplankton to phytoplankton production in Narragansett Bay. J. Plankton Res. **1:** 75–84, doi:10.1093/plankt/1.1.75

Weijer, W. 2007. Normal modes of the Mascarene Basin. Deep-Sea Res. I **55:** 128–136, doi:10.1016/j.dsr.2007.10.005

West, N. J., P. Lebaron, P. G. Strutton, and M. T. Suzuki. 2011. A novel clade of *Prochlorococcus* found in high nutrient low chlorophyll waters in the South and Equatorial Pacific Ocean. The ISME Journal **5:** 933–944, doi:10.1038/ismej.2010.186

———, and D. J. Scanlan. 1999. Niche-partitioning of *Prochlorococcus* populations in a stratified water column in the eastern North Atlantic Ocean. Appl. Environ. Microb. **65:** 2585–2591.

Wilkins, D., F. M. Lauro, T. J. Williams, M. Z. Demaere, Z. Matthew, M. V. Brown, J. M. Hoffman, C. Andrews-Pfannkoch, J. B. McQuaid, M. J. Riddle, S. R. Rintoul, and R. Cavicchioli. 2012. Biogeographic partitioning of Southern Ocean microorganisms revealed by metagenomics. Environ. Microbiol. **15:** 1318–1333, doi:10.1111/1462-2920.12035

Wolf-Vecht, A., N. Paldor, and S. Brenner. 1992. Hydrographic indications of advection/convection effects in the Gulf of Elat. Deep-Sea Res. **39:** 1393–1401, doi:10.1016/0198-0149(92)90075-5

Zubkov, M. V., B. M. Fuchs, G. A. Tarran, P. H. Burkill, and R. Amann. 2003. High rate of uptake of organic nitrogen compounds by *Prochlorococcus* cyanobacteria as a key to their dominance in oligotrophic oceanic waters. Appl. Environ. Microb. **69:** 1299–1304, doi:10.1128/AEM.69.2.1299-1304.2003

Zwirglmaier, K., L. Jardillier, M. Ostrowski, S. Mazard, L. Garczarek, D. Vaulot, F. Not, R. Massana, O. Ulloa, and D. J. Scanlan. 2008. Global phylogeography of marine *Synechococcus* and *Prochlorococcus* reveals a distinct partitioning of lineages among oceanic biomes. Environ. Microbiol. **10:** 147–161.

Associate editor: Wade H. Jeffrey

Received: 21 December 2012
Accepted: 02 July 2013
Amended: 08 July 2013