Seasonal expression of the picocyanobacterial phosphate transporter gene \( \text{phnD} \) in the Sargasso Sea

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**INTRODUCTION**

Phosphonates, previously thought to be refractory sources of P, are utilized by microbial communities in aquatic environments deprived of inorganic phosphates (Clark et al., 1998; Benitez-Nelson, 2004; Dyhrman et al., 2006; Karl et al., 2008). Phosphonates represent up to 25% of the marine DOP pool (Clark et al., 1998; Kolowith et al., 2001), and sources of naturally occurring phosphonates in the oligotrophic open sea include the nitrogen-fixing cyanobacterium, *Trichodesmium erythraeum* (Dyhrman et al., 2009). Recent work also indicates that at least three distinct mechanisms are widespread in nature that function to cleave the phosphate C–P bond (Dyhrman et al., 2006; Gilbert et al., 2009; Kulakova et al., 2009; Martinez et al., 2010; Thomas et al., 2010). For example, *Trichodesmium* spp. possess a C–P lyase pathway that is distributed widely among bacterial taxa, and exhibits broad substrate specificity (Dyhrman et al., 2006). Marine and freshwater picocyanobacteria typically contain a phosphonatase pathway that exclusively hydrolyzes 2-aminoethylphosphonate (2-AEP; Quinn et al., 2007; Ilikchyan et al., 2009). Last, a novel third mechanism for marine 2-AEP utilization was recently unmasked by a functional assay (Martinez et al., 2010), indicating that additional phosphate utilization pathways may await future discovery. Nonetheless, these pathways appear to share common ABC transporters for phosphonates, encoded by the genes \( \text{phnDCE} \). Earlier, we have demonstrated that picocyanobacteria of the genera *Synechococcus* and *Prochlorococcus* possess and express \( \text{phnD} \), encoding the phosphate binding protein, in a variety of aquatic environments, and that \( \text{phnD} \) expression can be used as a proxy for phosphate utilization. Moreover, preliminary studies suggested that expression of *Synechococcus* spp. \( \text{phnD} \) reflected the bioavailability of dissolved inorganic phosphorus (DIP) in the Sargasso Sea, whereas constitutive expression was observed for *Prochlorococcus* spp. \( \text{phnD} \) (Ilikchyan et al., 2009).

Here, we extended our survey of seasonal environmental expression of \( \text{phnD} \) along a N–S transect through the Sargasso Sea. The seasonal physical, chemical, and biological characteristics of the Sargasso Sea are well studied (Schroeder and Stommel, 1969). In winter, deep (>80 m) mixing transports cold, nutrient rich waters to the surface, whereas throughout summer and fall, surface waters in the Sargasso Sea are stratified with a shallow (~50 m), nutrient poor mixed layer. The physical and chemical changes of the surface waters are reflected in the abundance and productivity of phytoplankton (e.g., Menzel and Ryther, 1960, 1961). Picocyanobacteria constitute the majority of phototrophic organisms in the Sargasso Sea. *Synechococcus* spp. reach maximum abundance in April–May shortly after winter mixing, and *Prochlorococcus* spp. populations peak in summer and fall (DuRand et al., 2001; Casey et al., 2007). The Sargasso Sea is characterized as a phosphorus depleted region with DIP concentrations typically less than 10 nM (e.g., Cotner et al., 1997). Under these conditions, utilization of phosphonates from the DOP pool can be an important survival strategy for picocyanobacteria. Indeed, the expression of phosphate transporter genes was observed by endemic picocyanobacteria in the Sargasso Sea in October (Ilikchyan et al., 2009). In this study, we have assessed seasonal picocyanobacterial \( \text{phnD} \) expression at multiple depths and locations in May and October.

**MATERIALS AND METHODS**

**ENVIRONMENTAL SAMPLES**

Table 1 and Figure 1 provides details on sampling and station locations for cruises aboard the R/V Bank of Bermuda Atlantic Explorer during May and October 2008. The samples for RNA and DNA extraction were processed as described in Ilikchyan et al. (2009). Briefly, suspended particles from environmental samples of 3–4 L was collected onto 0.22 \( \mu \)m Sterivex cartridge filters by using a
At all sites and depths, 4 L were filtered, except for Sta. 2 (BATS) in October, where 2.5 and 3 L were filtered at 100 and 200 m, respectively.

### Table 1 | Summary of samples obtained during May and October 2008 cruises.

| Station | Date       | Lat/long       | Depth (m) | Temp (°C) | Salinity | DIP (nmol L\(^{-1}\)) | Pro (cells mL\(^{-1}\)) | Syn (cells mL\(^{-1}\)) |
|---------|------------|----------------|-----------|-----------|----------|------------------------|-------------------------|------------------------|
| 2 (BATS)| 5/3/08     | 31.649 N 64.170 W | 1         | 20.15     | 36.72    | 0.50                   | n.d.                     | n.d.                   |
| 2       | 5/3/08     | 5/4/08         | 2         | 20.12     | 36.72    | 0.50                   | n.d.                     | n.d.                   |
| 3       | 5/4/08     | 28.565 N 64.633 W | 1         | 22.65     | 36.79    | 0.50                   | n.d.                     | 6.8 × 10\(^4\)         |
| 3       | 5/4/08     | 5/5/08         | 2         | 22.55     | 36.79    | 0.50                   | n.d.                     | 7.9 × 10\(^3\)         |
| 4       | 5/5/08     | 27.167 N 64.862 W | 1         | 22.55     | 36.79    | 0.82                   | 2.7 × 10\(^4\)          | 1.39 × 10\(^4\)        |
| 4       | 5/5/08     | 5/6/08         | 2         | 22.55     | 36.79    | 4.72                   | 5.88 × 10\(^4\)         | 1.49 × 10\(^4\)        |
| 5       | 5/5/08     | 25.669 N 65.101 W | 1         | 23.43     | 36.76    | 0.50                   | n.d.                     | 6.7 × 10\(^3\)         |
| 5       | 5/5/08     | 5/6/08         | 2         | 22.89     | 36.74    | 0.50                   | n.d.                     | 9.4 × 10\(^3\)         |
| 6       | 5/6/08     | 24.152 N 65.300 W | 1         | 24.28     | 36.63    | 0.50                   | n.d.                     | 6.3 × 10\(^3\)         |
| 6       | 5/6/08     | 5/7/08         | 2         | 25.28     | 36.60    | 1.36                   | n.d.                     | 6.6 × 10\(^3\)         |
| 7       | 5/6/08     | 22.673 N 65.512 W | 1         | 25.58     | 36.44    | 0.50                   | n.d.                     | 6.0 × 10\(^3\)         |
| 7       | 5/7/08     | 21.162 N 65.747 W | 1         | 26.04     | 36.65    | 17.64                  | n.d.                     | 5.2 × 10\(^3\)         |
| 8       | 5/7/08     | 19.671 N 66.000 W | 1         | 26.27     | 36.61    | 5.86                   | n.d.                     | 5.7 × 10\(^3\)         |
| 8       | 5/7/08     | 5/8/08         | 2         | 25.83     | 36.59    | 4.96                   | n.d.                     | 5.7 × 10\(^3\)         |
| 9       | 5/7/08     | 31.660 N 64.170 W | 1         | 25.90     | 36.51    | 0.50                   | 3.7 × 10\(^4\)          | 5.3 × 10\(^3\)         |
| 9       | 10/16/08   | 5/3/08         | 2         | 25.80     | 36.51    | 0.50                   | 4.5 × 10\(^4\)          | 5.5 × 10\(^3\)         |
| 9       | 10/16/08   | 5/4/08         | 2         | 25.80     | 36.51    | 0.50                   | 5.7 × 10\(^4\)          | 6.3 × 10\(^3\)         |
| 2 (BATS)| 10/16/08   | 31.670 N 64.170 W | 1         | 25.10     | 36.36    | 0.50                   | 7.4 × 10\(^4\)          | 5.4 × 10\(^3\)         |
| 1       | 10/15/08   | 33.670 N 64.170 W | 1         | 23.61     | 36.61    | 28.85                  | 5.2 × 10\(^3\)          | 2.9 × 10\(^3\)         |
| 1       | 10/15/08   | 10/15/08       | 2         | 18.90     | 36.60    | 98.27                  | 2.7 × 10\(^4\)          | 33                     |

At all sites and depths, 4 L were filtered, except for Sta. 2 (BATS) in October, where 2.5 and 3 L were filtered at 100 and 200 m, respectively.

peristaltic pump, and cartridges were frozen immediately in liquid nitrogen. Flow rates were maintained at 125 mL min\(^{-1}\). The cartridges were transferred to storage at −80°C prior to RNA and DNA extraction. Chlorophyll \(a\) was measured fluorometrically after extraction of filtered seston with 90% acetone (Welschmeyer, 1994).

**NUCLEIC ACID EXTRACTION PROCEDURE AND RT-PCR CONDITIONS**

Extraction of environmental DNA and RNA from a Sterivex filter was performed as described previously (Ilikchyan et al., 2009). All PCR and RT-PCR amplifications with Synechococcus and Prochlorococcus spp. \(phnD\) primers were done as described earlier (Ilikchyan et al., 2009). Specifically, the primers employed were: \(phnD\_syn119F: 5′\)-TGGNGCMATYCCSGATCAGAACCCSG-3′; \(phnD\_syn734R1: 5′\)-TTGGGCTGSGCGASCCAGTGGTARTC-3′; \(phnD\_syn731R2: 5′\)-GGNCNGGCCACCCAGTGTARTC-3′. Both reverse primers were used in a single reaction. For the amplification of the \(phnD\) sequence from Prochlorococcus spp. the following primers were used: \(phnD\_pro307F: 5′\)-GTNATWGCTCAAAGAGATATWGAT-3′; \(phnD\_pro551R: 5′\)-GGTGCATCATGACTNCCRCTATANCC-3′. \(rnpB\) is a single copy gene and encodes for RNase P RNA. The \(rnpB\) primers were designed to amplify a 118 bp region specific for picocyanobacteria, \(rnpB\_F: CCGTGAGGAGAGTGCCACAG; rnpB\_R: CAGCACCCTTCTGATGCTGCTGG. Specificity of the primers was confirmed by the absence of amplification from DNA extracted from *Synechococcus* sp. PCC7942, *Synechocystis* sp. PCC6803 and *Microcystis* sp. M300. All available marine and freshwater picocyanobacterial DNA yielded an amplicon of the correct size. The PCR conditions for amplification with the \(rnpB\) primers were as follows: 95°C for 5 min, 30 cycles of 95°C for 30 s, 65°C for 30 s, and 72°C for 30 s, followed by 72°C for 10 min.

For \(phnD\) amplification, each PCR (25 μL) contained 1× PCR buffer (Promega), 0.2 mM of each deoxynucleotide (Promega), 0.5 μM of each primer, and 1.0 unit of GoTaq DNA polymerase (Promega), and ca. 10 ng template DNA. For *Synechococcus* spp. \(phnD\) amplification, the temperature profile was 95°C for 5 min, 40 cycles of 95°C for 1 min, an initial annealing temperature of 65°C for 1 min decreasing by 0.5°C each cycle until 55°C was reached,
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Phylogenetic analysis
PhnD sequences obtained in this (GenBank accession numbers GU724612–GU724682) and previous (EU362636–EU362729, FJ172179–FJ172204; Ilikchyan et al., 2009) studies were aligned together with PhnD sequences from available genomes of Synechococcus and Prochlorococcus spp. using ClustalW2 at http://www.ebi.ac.uk/Tools/clustalw2/ (Larkin et al., 2007). The amino acid sequences that were further included in phylogenetic analysis differed among each other by at least 2%. Neighbor-joining phylogenetic analysis was done in ARB (Ludwig et al., 2004).

Results
Picocyanobacterial PhnD expression in the Sargasso Sea, May and October 2008
The presence and expression of Synechococcus spp. and Prochlorococcus spp. PhnD was assessed in samples collected during Sargasso Sea cruises in May and October 2008. Samples were analyzed from three depths (surface, 40, and 100 m) in May and from four depths (surface, 40 m; deep chlorophyll maximum, DCM,
and 200 m) in October. Additionally, an on-deck nutrient amendment incubation experiment was conducted on board during the October cruise.

DNA extracted from samples taken at three different depths in the Sargasso Sea in May 2008 all yielded PCR amplicons for both *Synechococcus* spp. *phnD* and *Prochlorococcus* spp. *phnD* (Figure 2A). The mixed layer depth was 80 m. Expression of *Prochlorococcus* spp. *phnD* was observed in almost all 24 samples (Figure 2B). The samples yielding no *Prochlorococcus* RT-PCR *phnD* amplicon included the Bermuda Atlantic Time Series station (BATS, also designated as Station 2) at 40 m, Station 3 at 100 m, and Station 4 at 40 m. These samples also did not yield a picocyanobacterial *rnpB* RT-PCR amplicon (Figure 2B). Low RNA yield in samples from BATS at 40 m, Station 3 at 100 m, and Station 4 at 40 m due to errors in RNA extraction might be a reason for the failure to detect a *Prochlorococcus* spp. RT-PCR *phnD* amplicon. By contrast, no samples from any station and depth from the Sargasso Sea May 2008 cruise revealed expression of *Synechococcus* spp. *phnD* (Figure 2B), despite the fact that all corresponding DNA samples yielded a PCR amplicon with *Synechococcus* spp. specific *phnD* primers (Figure 2A).

Samples from two stations, Station 1 and BATS (Station 2), were processed from the October 2008 cruise. All DNA samples yielded *Prochlorococcus* and *Synechococcus* *phnD* amplicons, although only faint bands were observed in the BATS sample at 200 m (Figure 3A). Flow cytometry confirmed low abundance of both taxa at this depth (<100 cells mL⁻¹). *Synechococcus* spp. *phnD* expression was detected in the samples taken at the surface and 40 m depth, but not at the DCM (100 m), or 200 m, both below the mixed layer depth of 55 m (Figure 3B). *Prochlorococcus* spp. *phnD* was expressed in all samples except BATS 200 m, a sample found to be negative for picocyanobacterial RNA (Figure 3B).

Despite the fact that PCR as performed here is not a quantitative assay, the intensity of bands corresponding to *phnD* and *rnpB* reflects the abundance of picocyanobacteria at depth with highest number of *Prochlorococcus* spp. cells at the DCM and *Synechococcus* spp. cells at the surface (DuRand et al., 2001).

### phnD Expression: Dip and 2-AEP

The patterns of *Synechococcus* spp. *phnD* expression were examined in light of parallel determination of inorganic phosphorus concentration during May and October 2008. In both seasons, a

![Figure 2](image2.png)

**Figure 2** | (A) PCR of *Synechococcus* and *Prochlorococcus* spp. *phnD* from the Sargasso Sea samples taken in at several stations during May 2008. The mixed layer was 80 m, and the DCM was at 100 m. (B), top rows: RT-PCR of environmental RNA extracted from the same stations during the May cruise. (B), bottom: RT-PCR of picocyanobacterial *rnpB* transcripts as a positive control for picocyanobacterial RNA. NTC, no template control.

![Figure 3](image3.png)

**Figure 3** | (A) PCR of *Synechococcus* and *Prochlorococcus* spp. *phnD* from the Sargasso Sea samples taken in at Station 1 and BATS (Station 2) during October 2008. The mixed layer was 50 m, and the DCM was at 100 m. (B) Top rows: RT-PCR of environmental RNA extracted from the same stations. (B) Bottom: RT-PCR of picocyanobacterial *rnpB* transcripts as a positive control for picocyanobacterial RNA. NTC, no template control.
depth-dependent gradient of DIP was observed, with depleted P at the surface, increasing at depth yielding maximum values below the mixed layer. Near the surface, spring P was elevated compared to October measurements, with May samples yielding average DIP of 2.62 nmol L$^{-1}$ in the mixed layer samples assayed by RT-PCR ($n = 16$ samples), ranging from 0.5 (detection limit) to 17.64 nmol L$^{-1}$. By contrast, all October mixed layer values remained at or below the detection limit of 0.5 nmol L$^{-1}$. Since phnD expression was only observed in the October samples, yet spring DIP was at or below detection in 9 of 16 mixed layer samples, expression patterns cannot be ascribed to DIP concentration alone. This raises the possibility that DOP (phosphonates and organic phosphates) may directly influence the accumulation of phn transcripts.

A nutrient amendment incubation experiment was performed with water collected from BATS at the surface in October 2008. The RT-PCR assay revealed that an addition of 500 nmol L$^{-1}$ phosphate quenched expression of the Synechococcus spp. phnD in 48 h while not affecting expression of the Prochlorococcus spp. phnD (Figure 4). The amendments with 0.5 μM 2-AEP yielded both Synechococcus spp. and Prochlorococcus spp. phnD amplicons. The control sample failed to amplify Synechococcus spp. phnD and yielded weak bands for Prochlorococcus spp. phnD and picocyanobacterial rnpB (Figure 4). This suggests overall low picocyanobacterial cell abundance in the control at 48 h. Analysis of POP in the control, as well as in the phosphate and 2-AEP-amended bottles revealed a significant increase in both the amended samples after 48 h. (Table 2). Bottles amended with phosphate or with 2-AEP accumulated nearly threefold more POP than did unamended control bottles (one-way ANOVA; $p < 0.005$). There was no treatment effect discernible between the phosphate or phosphonate treatments (Tukey’s honest significance test). Addition of nutrients stimulated the phytoplankton community, as chlorophyll increased from 0.51 to 0.69 μg L$^{-1}$ in phosphate amended and 0.74 μg L$^{-1}$ in the 2-AEP treatments. Overall, these data suggest that phnD expression in Synechococcus spp. was regulated by phosphorus availability, and that 2-AEP was assimilated by the microbial community. Additionally, the experiment raises the possibility that the presence of 2-AEP stimulates phnD expression among the endemic Synechococcus spp. By contrast, Prochlorococcus spp. phnD expression was not influenced by either 2-AEP or phosphate availability. The activity of alkaline phosphatase was measured before and 48 h after amendments with activities ranging from 0.7 to 1.31 pmol h$^{-1}$ ml$^{-1}$, with no significant difference between the samples. Since eubacteria may contribute the largest component of total APA in environmental samples (Hoppe, 2003), this assay was likely not an accurate indication of picocyanobacterial P status.

**PHYLLOGENETIC ANALYSIS OF PICOCYANOBACTERIAL PHnD FROM SARGASSO SEA SAMPLES**

The majority of *Synechococcus* PhnD sequences from May and October 2008 clustered within clade III similar to our previous study (Figure 3; Ilikchyan et al., 2009). *Synechococcus* Clade III is relatively abundant throughout the open oceans between 40N and 40S latitudes (Zwirglmaier et al., 2008). Ahlgren and Rocap (2006) showed that *Synechococcus* clades II, IV, and a novel clade XV (closely related to III) dominated in the western Sargasso Sea in March. However, a comprehensive study on diversity and temporal variation (Tai and Palenik, 2009) of *Synechococcus* groups has not yet been performed for the Sargasso Sea. *Synechococcus* sequences from the Sargasso Sea May and October cruises formed an additional cluster (sequence ADE58334) closely related to the cluster III (Figure 3A). *Synechococcus* cluster IV also includes sequences from Monterey Bay obtained from the previous study (Ilikchyan et al., 2009).

Most *Prochlorococcus* PhnD sequences were clustered within the High Light II clade similar to what has been observed with primers for 16S rRNA and 16S–23S ITS region (Moore et al., 1998; Ahlgren et al., 2006; Zinser et al., 2006). A DNA sample taken at depth (200 m) at BATS in October 2008 yielded *Prochlorococcus* PhnD sequences closely related to Low Light clade IV (Figure 3B). Considering the observed diversity among *PhnD* sequences obtained in this and previous studies, we are confident that the specificity of the *phnD* primers is broad enough to capture representative members of endemic picocyanobacterial community worldwide. This will enable more detailed studies of phosphonate utilization genes in diverse oceanic regions.

**DISCUSSION**

P limitation in the surface waters of the Sargasso Sea surface waters is well documented (Cotner et al., 1997; Karl, 2000; Wu et al., 2000), with a significant depletion in P occurring during late summer and early fall when the Sargasso Sea is stably stratified (DuRand et al., 2001; Ahlgren et al., 2006). In this study, we have assessed spatiotemporal expression of *phnD* in the photic zone of the Sargasso Sea. *Prochlorococcus* spp. *phnD* expression was observed in all samples from the Sargasso Sea drawn in May.
and October 2008, except BATS at 40 m, St. 3 at 100 m, and St. 4 at 40 m in May and BATS 200 m in October. These negative samples likely contained prohibitively low total picocyanobacterial RNA. The constitutive presence of the Prochlorococcus spp. phnD RNA is consistent with the data obtained earlier for the Sargasso Sea samples collected in October 2007 (Ilikchyan et al., 2009). In contrast, whereas a PCR amplicon was detected for Synechococcus spp. phnD in all samples at all depths, expression of this gene was not observed in May when the concentration of phosphate was generally higher throughout the water column due to recent winter mixing (DuRand et al., 2001). In October 2007 and 2008, Synechococcus spp. expressed phnD in the mixed water layer but not at the DCM (ca. 100 m) or at 200 m. This is consistent with lower DIP levels typically observed in the mixed layer, which spans only 50 m in depth in the fall. Overall, the lack of springtime phosphonate gene expression can be partially explained by increased inorganic phosphate availability below the mixed layer depth, but the absence of phnD expression in samples lacking detectable DIP suggests that organic phosphate or phosphonate availability may also be important in regulating phn encoded functions. Furthermore, higher competition for DIP and organic phosphates in the fall may lead to phnD expression, so both P flux and total P concentration need to be considered.

In a nutrient amendment experiment, addition of phosphate yielded no Synechococcus spp. phnD expression at 48 h, whereas the biomass increased as suggested by chlorophyll a measurements and RT-PCR with the rnpB primers. An addition of 2-AEP resulted in increased picocyanobacterial biomass as well, although Synechococcus spp. phnD expression was evident in this sample. The control showed no detectable expression for Synechococcus spp. phnD in 48 h; Prochlorococcus spp. phnD and picocyanobacterial mbp expression was barely detectable as well suggesting an overall decrease in picocyanobacterial biomass. Synechococcus spp. expressed phnD at time zero because the water for the experiment was taken from the surface at the BATS station where in an identical sample, expression of the Synechococcus spp. phnD was detected in the mixed layer (Figure 4). Additionally, amendment with DIP repressed phnD expression. The results confirmed that expression of phnD is influenced by P availability in Synechococcus, but not in Prochlorococcus spp. The experiment also raises the possibility that 2-AEP can induce phnD within the endemic Synechococcus spp. similar to what is observed in some heterotrophic bacteria (Quinn, 2002). However, experiments with cultured Synechococcus sp. WH8102 suggest otherwise, because added 2-AEP does not yield enhanced phnD transcription. Additionally, BLAST searches (http://blast.ncbi.nlm.nih.gov) reveal that the gene for LysR type transcriptional regulator, which is adjacent to the genes for phosphonate utilization in these heterotrophic bacteria (Kulakova et al., 2001, 2003; Quinn, 2002), is lacking in picocyanobacterial genomes (data not shown). The fact that DIP repressed the level of phnD transcripts likely differs from what has been observed for the N-fixing cyanobacterium Trichodesmium IMS101, as DIP addition...
did not inhibit phosphate utilization in culture. This strain is capable of utilizing multiple P sources simultaneously (Beversdorf et al., 2010).

Complicating our understanding of expression of phosphate utilization genes is a recent study describing phn gene expression in thermophilic Synechococcus spp. (Gomez-Garcia et al., 2010). The authors compare two related strains, one of which (OS-B) harbors both the phnCDE transporter and C–P lyase genes. The OS-A strain lacks genes for the C–P lyase, but instead harbors phosphonatase genes that are expressed in the presence of phosphonates (Gomez-Garcia et al., 2010). These data suggest that multiple strategies for phosphate utilization exist in picocyanobacteria and that P deficiency, DIP/DOP ratios and the presence or absence of phosphonates may influence phn transcription in different picocyanobacterial ecotypes. This could explain the pattern of Synechococcus spp. phnD expression observed in this paper, in which the DIP concentration alone does not predict the community-wide phnD transcription detected in our RT-PCR assay.

Prochlorococcus spp. detected via this assay express phnD independently from P limitation. This corresponds to a reduced genome size of Prochlorococcus HL clade species targeted primarily by our primer set (Ilikchyan et al., 2009). The HL clade genomes indicate the loss of many functional and regulatory genes in comparison to the LL clade and to Synechococcus spp. (Moore et al., 2002, 2005; Scanlan and West, 2002; Dufresne et al., 2003; Palenik et al., 2003; Rocap et al., 2003). Nonetheless, the number and distribution of P assimilation genes in picocyanobacteria varies depending on the marine environment (Moore et al., 2005; Martiny et al., 2006; Scanlan et al., 2009).

CONCLUSION

The fact that picocyanobacteria are utilizing phosphonates in marine environments underscores the significance of phosphonates to the bioavailable DOP pool. Synechococcus spp. expressed phnD under seasonal (autumn) P-limited conditions in samples from the Sargasso Sea, and Prochlorococcus spp. phnD expression was constitutive independent of season. Constitutive expression may be a consequence of genomic reduction in Prochlorococcus spp. as some regulatory functions may have been lost through evolution (e.g., Rocap et al., 2003; Moore et al., 2005). In both taxa, the same patterns of phnD expression were observed previously during October 2007 (Ilikchyan et al., 2009). Development of clade specific molecular probes for phosphate utilization genes in picocyanobacteria will bring insight into differences in regulation of phosphate assimilation among the clades and improve our overall understanding of P physiology in picocyanobacteria. Overall, the pattern of Synechococcus spp. phnD expression observed in natural water samples herein and earlier (Ilikchyan et al., 2009) together with the results of a nutrient amendment experiment conducted in the field suggest applicability of the developed RT-PCR method in assessing the P status of Synechococcus spp. populations.

It is clear that much remains to be known about how cyanobacteria and bacterioplankton recruit phosphonates as a P source. Multiple pathways for phosphate degradation and phn gene activation have been documented in diverse taxa, and many more mechanisms may yet to be described. Future work should focus in the distribution of phosphate degradation pathways and their regulatory networks in all taxa capable of recruiting phosphonates from the DOP pool, while simultaneously monitoring DIP and the speciation of the DOP.

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