Limnol. Oceanogr., 53(1), 2008, 78–88

Assessing nutrient limitation of *Prochlorococcus* in the North Pacific subtropical gyre by using an RNA capture method

Benjamin A. S. Van Mooy[1]
Department of Marine Chemistry and Geochemistry, Woods Hole Oceanographic Institution, Woods Hole, Massachusetts 02543

Allan H. Devol
School of Oceanography, University of Washington, Seattle, Washington 98195

Abstract

It has been hypothesized that the planktonic community of the North Pacific subtropical gyre (NPSG) underwent a ''domain shift'' in the early 1980s in which phytoplankton of the domain Eukarya were supplanted by phytoplankton of the domain Bacteria, primarily *Prochlorococcus*. P limitation of eukaryotic phytoplankton was implicated as the causative chemical factor in the domain shift, and we sought to investigate the current nutrient limitation status of *Prochlorococcus*, now 2 decades since this event. We measured ribonucleic acid (RNA) synthesis rates by NPSG plankton at Station ALOHA in $^{33}PO_4^{3-}$ tracer incubations and found that RNA synthesis was the single largest biochemical sink for dissolved P, accounting for about half of the total PO_4^{3-} uptake. We also found that NH_4^+ stimulated RNA synthesis but that PO_4^{3-} did not, which suggested N limitation of plankton growth. We developed a new RNA capture procedure, termed radioisotope-based tracking of RNA synthesis by hybridization and capture (RIBOTRACE), to measure RNA synthesis rates by *Prochlorococcus* exclusively. Data from this procedure showed that NH_4^+ stimulated RNA synthesis by *Prochlorococcus* and confirmed that *Prochlorococcus* was N limited and not P limited. Our RIBOTRACE data do not necessarily refute the domain shift hypothesis, but suggest that any critical period of P limitation required for the domain shift must have subsided and given way to the N-limiting conditions that existed previously.

The productivity, abundance, and community composition of plankton in subtropical ocean gyres is inextricably linked to the supply rate, concentration, and chemical composition of dissolved nutrients, and vice versa. But large-scale changes in atmospheric and ocean physics can perturb these linkages. During the last half century the North Pacific subtropical gyre (NPSG) has exhibited major perturbations that, in turn, undoubtedly affected the role of this biome, the world's largest, in the global cycles of carbon and nutrients. Drawing upon long-term observations from the NPSG, Venrick et al. (1987) argued that changes in climate in the 1970s led to a doubling of phytoplankton biomass. Long supposed to be an N-limited system (Perry and Eppley 1981), Karl and colleagues later linked enhanced production in the NPSG during the early 1980s to enhanced N_2 fixation by cyanobacteria (Karl et al. 1995, 1997; Dore et al. 2002). The chemical consequence of this shift was a drawdown in dissolved P (Karl and Tien 1997; Karl et al. 2001*b*), and the primary beneficiaries of this were cyanobacteria of the genus *Prochlorococcus* (Karl et al. 2001*a*). It was suggested that *Prochlorococcus*, though incapable of N_2 fixation, supplanted the previous community of phytoplankton dominated by eukaryotes (e.g., diatoms) because they were better adapted for growth at lower dissolved P concentrations (Karl et al. 2001*a*). Thus it was hypothesized that this ''domain shift'' was the ecological response to the progression of the NPSG from a N-limited to a P-limited ecosystem (Karl et al. 1995).

We sought to better understand the status of this progression, and to assess whether the population of *Prochlorococcus* in the NPSG had itself now—almost 2 decades since the domain shift—become P limited. Our approach was to measure changes in the P-based growth rates of *Prochlorococcus* in incubations supplemented with tracer-level $^{33}PO_4^{3-}$ and amended with either dissolved inorganic N (DIN) or PO_4^{3-}. Although some strains of *Prochlorococcus* can interact directly with the organic reservoir of dissolved P, the preferred form is clearly PO_4^{3-} (Moore et al. 2005) and we expected that if *Prochlorococcus* were truly P limited, then additions of PO_4^{3-} would stimulate growth rates.

[1] Corresponding author (bvanmooy@whoi.edu).

Acknowledgments

We thank D. Karl for providing access to his laboratories after the HOT cruises, for his comments on a draft of this paper, and for his continued encouragement of our studies of planktonic P physiology in the NPSG. K. Björkman, T. Gregory, D. Sadler, and the other HOT program personnel provided essential logistical support and data. We thank D. Stahl, M. Hullar, and V. Armbrust for instruction and advice during the development of the RIBOTRACE method. We particularly acknowledge B. MacGregor for publishing a clear and thorough description of her robust RNA isolation method. Two anonymous reviewers provided exceptionally thorough and helpful reviews of this paper.

A.H.D. was supported by ONR N0014-00-1-0751 and by the Biotechnology Investigations Ocean Margins Program, Office of Biological and Environmental Research, U.S. Department of Energy. B.V.M. was supported by ONR N0014-06-1-0134, the Postdoctoral Program at Woods Hole Oceanographic Institution (WHOI) with funding provided by the Watson Chair to D. Repeta, and the Gryce B. Kerr and Penzance Endowed fund in support of Assistant Scientists at WHOI.

The main challenge to making these measurements was to separate the response of *Prochlorococcus* in the NPSG from that of the more abundant (Campbell et al. 1997; Björkman et al. 2000) PO_4^{3-}-consuming (Van Mooy et al. 2006) heterotrophic bacteria. *Prochlorococcus* are only marginally larger than heterotrophic bacteria and size fractionation by filtration cannot entirely separate $^{33}PO_4^{3-}$ consumption by these two groups (Moutin et al. 2002). As an alternative, flow cytometry could be used to physically separate *Prochlorococcus* from heterotrophic bacteria (Zubkov et al. 2003). The resultant sorted cells could then be analyzed for ^{33}P radioactivity to determine total uptake rates (Zubkov et al. 2007). However, although total PO_4^{3-} uptake includes the synthesis of all classes of P-containing biochemicals required for growth, total PO_4^{3-} uptake also includes processes that are not necessarily related to growth, such as luxury uptake or abiotic sorption (Fu et al. 2005; Thingstad et al. 2005). In amendment experiments these latter processes could easily cause interferences and lead us to draw false conclusions about P-limited planktonic growth.

We developed a hybrid molecular biological method to measure ribonucleic acid (RNA) synthesis rates by NPSG *Prochlorococcus* in incubations supplemented with tracer-level $^{33}PO_4^{3-}$. Our new method is a hybrid of one method used to isolate oligonucleotide probe-defined RNA by hybridization and capture (MacGregor et al. 2002), and another method used to quantify the incorporation of ^{33}P into primer-defined deoxyribonucleic acid (DNA) by hybridization and capture (Van Mooy et al. 2004). This hybrid method, termed radioisotope-based tracking of ribonucleic acid synthesis by hybridization and capture (RIBOTRACE), was used to measure RNA synthesis by *Prochlorococcus* during two Hawaiian ocean time-series (HOT; Karl and Lukas 1996) cruises to Sta. ALOHA in the NPSG. Both of these cruises were during the summer, when N_2 fixation rates are thought to reach their annual peak (Dore et al. 2002).

Methods

Field locations—Seawater samples were collected on HOT cruises (Karl and Lukas 1996) to Sta. ALOHA (22.75°N, 158.00°W) during July 2003 and July 2004. Because of mechanical problems during the 2004 cruise, samples were actually collected south of ALOHA, but still well to windward of the Hawaiian island of Oahu. All samples were collected from a depth of 5 m using a Niskin bottle.

Uptake of $^{33}PO_4^{3-}$—Triplicate incubations were conducted in 1-liter polycarbonate bottles with 1-liter samples of seawater. Incubations were supplemented with 3.7 MBq of carrier-free $^{33}PO_4^{3-}$ (MP Biomedicals), which yielded final concentrations of approximately 33 pmol L^{-1} of $^{33}PO_4^{3-}$ in the incubations. Incubations were then amended with either MilliQ water (Millipore) as a control; KH_2PO_4 to a final concentration of 25 nmol L^{-1} in excess of ambient concentrations, which were 34 and 56 nmol L^{-1} in 2003 and 2004 respectively (see Results); NH_4Cl to a final concentration of 250 nmol L^{-1} in excess of ambient concentrations; or KNO_3 to a final concentration of 250 nmol L^{-1} in excess of ambient concentrations. The MilliQ water that was added to the control incubations was also used to make the amendment stocks. Incubations were placed in an on-deck incubator, and surface seawater flowed through the incubator to maintain in situ temperature. A screen placed over the incubator maintained light at ≈90% of surface irradiance. All incubations were initiated at sunrise and terminated after 24 h; we chose this duration to integrate potential signals of synchronous growth by *Prochlorococcus* (Jacquet et al. 2001) and because uptake rates are generally linear on these timescales (data not shown; Björkman et al. 2000).

Total PO_4^{3-} uptake rates were determined in each of the triplicate incubations by placing 250 μL of seawater from the incubations in a 5-mL syringe and gently pushing the sample through a syringe filter unit (0.2 μm, nylon, Millipore). In the laboratory, nylon membranes retained no measurable $^{33}PO_4^{3-}$ even at picomolar concentrations. A 100-μL subsample of the filtered water was placed in a polyethylene scintillation vial with 20 mL of UltimaGold scintillation cocktail (Perkin Elmer) and ^{33}P radioactivity ($^{33}P_{filtered}$, disintegrations per minute [dpm] L^{-1}) was determined on a Packard TriCab 2770TR/SL high-sensitivity scintillation counter. The ^{33}P radioactivity of a 100-μL aliquot of unfiltered seawater was also determined ($^{33}P_{unfiltered}$, dpm L^{-1}). The difference in ^{33}P radioactivity between the filtered and unfiltered samples ($^{33}P_{unfiltered} - {}^{33}P_{filtered}$) was the amount of ^{33}P taken up by plankton or adsorbed on particles ($^{33}P_{uptake}$, dpm L^{-1}).

Uptake rates of PO_4^{3-} (mol L^{-1} d^{-1}) were calculated from $^{33}P_{uptake}$, the specific activity (SA, dpm mol^{-1}) of the PO_4^{3-}, and the period of incubation (t, d):

$$\text{Uptake rate} = \frac{^{33}P_{uptake}}{SA \times t} \quad (1)$$

The SA was determined from $^{33}P_{unfiltered}$ and the initial concentrations of PO_4^{3-} in the seawater sample ([PO_4^{3-}] mol L^{-1}; data courtesy of K. Björkman and D. Karl, University of Hawai'i, Manoa):

$$SA = \frac{^{33}P_{unfiltered}}{[PO_4^{3-}]} \quad (2)$$

In the incubations amended with PO_4^{3-}, the SA was calculated using the sum of the initial PO_4^{3-} concentration and the PO_4^{3-} amendment of 25 nmol L^{-1}. The steady-state turnover rate of PO_4^{3-} with respect to incorporation into biomass was calculated as the quotient of the uptake rate and [PO_4^{3-}]. The turnover time is the reciprocal of the turnover rate.

Uptake of $^{33}PO_4^{3-}$ into total community RNA—For collection of ^{33}P-labeled RNA, the incubations were terminated by filtering the plankton onto cellulose nitrate membranes (0.2 μm, Whatman) that were then snap-frozen in liquid N_2 and stored in liquid N_2 for transport to the laboratory. RNA was extracted from the filters using an

RNAeasy kit (Qiagen), followed by deoxyribonuclease (Ambion) digestion and two isopropanol precipitations. The quality of ribosomal (r)RNA and the absence of residual DNA was confirmed by gel electrophoresis. This was done by briefly denaturing RNA extracts at 65°C for 15 min in formaldehyde sample buffer (Cambrex) and then running them in a 1.25% agarose gel with 3-[*N*-morpholino]propanesulfonic acid buffer (Cambrex). A high-range RNA ladder (Fermentas) was also loaded on the gel. The gel was stained with ethidium bromide (Fisher Scientific). Residual ribonucleases were eliminated by treating the extracts with RNAsecure reagent (Ambion). The radioactivity of a small aliquot of the RNA extract was determined by scintillation counting as described above. The recovery efficiency of the RNA extraction was determined from parallel extractions of ALOHA plankton (not ^{33}P-labeled) on membranes that were supplemented with ^{3}H-labeled RNA standard (described below). Although this approach does not account for variations in RNA extraction efficiency due to incomplete cell lysis, it does account for losses from liquid handling throughout the extraction procedure, including the isopropanol precipitation step. Furthermore, substantial errors due to incomplete cell lysis are unlikely because RNA synthesis (this study) and phospholipid synthesis (Van Mooy et al. 2006) account for the vast majority of total $^{33}PO_4^{3-}$ uptake (see Results), which limits the possibility of large amounts of ^{33}P-labeled RNA in unlysed cells. As such, we have calculated recovery efficiencies assuming that incomplete cell lysis was negligible. Finally, steady-state RNA synthesis rates were calculated from the extraction efficiency-corrected yields of ^{33}P-labeled RNA ($^{33}P_{RNA}$, dpm L^{-1}), the SA of PO_4^{3-} in the incubtions, and the incubation period (t):

$$\text{RNA synthesis rate} = \frac{^{33}P_{RNA}}{SA \times t} \quad (3)$$

The fraction of the ^{33}P radioactivity in total RNA that was ribosomal (i.e., rRNA) was measured by gel electrophoresis. RNA extracts were run on agarose gels as described above and the 16S rRNA fragment was identified and excised. After dissolution of the excised agarose using buffer QG (Qiagen), the radioactivity of the 16S fragment was determined by scintillation counting as described above. Since the 16S rRNA fragment comprises 34% of the total rRNA in prokaryotes (Noller 1984), the amount of radioactivity in rRNA was calculated by dividing the radioactivity of the 16S rRNA fragment by 0.34 (Pearson et al. 2004). Dividing the rRNA radioactivity by the total RNA activity loaded on the gel yielded the fraction of total RNA radioactivity accounted for by rRNA.

RNA standards—RNA was extracted from *Prochlorococcus* (strain MED4; courtesy of L. Moore, University of Southern Maine) and α-proteobacteria (*Caulobacter sp.* strain CB37; courtesy of J. Staley, University of Washington) using the same techniques described above for the environmental samples. The RNA from these organisms was 5′ end-labeled with ^{32}P using a KinaseMax kit (Ambion) and [α-^{32}P]adenosine triphosphate (ATP) (ICN Radiochemicals). We also prepared ^{3}H-labeled RNA by growing *Caulobacter sp.* strain CB37 in the presence of 2 μmol L^{-1} [5, 6 ^{3}H]uridine (ICN Radiochemicals); we used this strain because we had shown previously that it was capable of taking up nucleosides (Van Mooy et al. 2004). The ^{32}P or ^{3}H radioactivity of these rRNA standards was determined via agarose gel electrophoresis as described above. Samples were diluted so that 10 μL of ^{32}P-labeled standard contained about as much radioactivity as 10 μL of ^{33}P-labeled environmental RNA (usually on the order of 10^4 dpm).

RIBOTRACE—We developed modifications to the RNA hybridization and capture method described by MacGregor et al. (2002) to accommodate the use of radioactive internal recovery standards as described by Van Mooy et al. (2004). We have termed this method RIBOTRACE. MacGregor et al. (2002) demonstrated the ability of their hybridization capture method to isolate rRNA using biotinylated oligonucleotide probes and streptavidin-coated magnetic beads. Although MacGregor et al (2006) have subsequently updated their method, here we have applied the method as originally described (MacGregor et al. 2002) with almost no changes, save for the addition of an internal recovery standard to make the method quantitative. Briefly, a hybridization mixture was created by mixing 10 μl of the ^{33}P-labeled sample RNA, 10 μL of ^{32}P-labeled standard RNA, 20 of μL formamide (discussed below), and 60 μL of hybridization buffer. The hybridization buffer consisted of 5× saline–sodium citrate (SSC) (Cambrex), 0.1% *N*-laurylsarcosine (Fisher Scientific), 1.0% blocking solution (Roche), and 0.02% sodium dodecyl sulfate (Cambrex). The hybridization mixture was incubated at 70°C for 10 min, and then allowed to cool to room temperature for 30 min. Next a 10-μL aliquot of the hybridization mixture was taken to determine the initial ^{33}P and ^{32}P radioactivity by scintillation counting as described above. Then 10 μL of a 1 μmol L^{-1} solution of biotinylated oligonucleotide probe (Operon) was added to the hybridization mixture, and the mixture was gently rotated overnight in the dark at room temperature to form biotinlyated probe : rRNA complexes. The following day, 10 μL of streptavidin-coated superparamagnetic beads (Dynal) were prepared by thrice washing with 1× SSC, resuspending with a mixture of 0.5× SSC and 0.1% blocking solution, and gently rotating the mixture for 60 min at room temperature. After magnetically concentrating the beads, the resuspension mixture was then decanted from the beads. Next the 100-μL hybridization mixture containing the biotinlyated probe : rRNA complexes was added to the beads. The biotinlylated probe : rRNA complexes became bound to the streptavidin-coated magnetic beads during a 2-h incubation at room temperature with gentle rotation. Next the beads were washed thrice with 0.5× SSC. The beads were resuspended in water and denatured at 98°C for 5 min to separate the RNA from the beads. Working very quickly, beads were magnetically separated from the solution containing the RNA, which was then pipetted into a polyethylene vial for scintillation counting. RIBOTRACE analyses were conducted in

batches immediately after each cruise with RNA extracted from triplicate incubations.

Probes, hybridization conditions, and method validation—Three oligonucleotide probes were used in this study: Eub338, targeting all organisms in the domain Bacteria (Amann et al. 1990); Pro444, targeting most *Prochlorococcus* (West and Scanlan 1999); and the nonbinding control probe Non338 (Manz et al. 1992). Our application of these probes draws heavily from previous examinations of their specificity in natural communities, and a more rigorous assessment of their specificity would involve target RNA with known mismatches to probe sequences: there are no sequence mismatches between the Eub338 and the target 16S rRNA sequences in either *Caulobacter sp.* strain CB37 or *Prochlorococcus* MED 4, and there are no mismatches between Pro444 and *Prochlorococcus* MED4 (Cole et al. 2007). The three oligonucleotide probes were 5′ end-labeled with biotin and purified via high-performance liquid chromatography (HPLC) by the manufacturer (Operon). Melting curves were determined for each probe to determine optimal formamide concentrations for the RIBOTRACE. As reported by MacGregor et al. (2002), we found that a formamide concentration of 20% was optimal for Eub338 for both *Caulobacter* sp. and *Prochlorococcus* MED4 (data not shown). The recovery of rRNA using Pro444 also varied predictably, and we found that 20% was also optimal for rRNA from *Prochlorococcus* (Fig. 1); recoveries were on the order of 20%, which is typical of RNA hybridization and capture methods (Pearson et al. 2004). At this formamide concentration the recovery of ^{32}P-labeled *Prochorococcus* rRNA (end-labeled) with the Pro444 probe was 19 ± 4 times greater than ^{3}H-labeled rRNA from *Caulobacter* sp. (internally labeled). This information suggested that the background associated with this analysis was quite low. However, we were concerned that there might be some bias between end-labeled and internally labeled rRNA, and to test this we combined these two types of rRNA at set ratios and applied RIBOTRACE with the Eub338 probe. We found that the two types of rRNA were recovered at ratios that were nearly identical to the initial combinations (Fig. 2). It is important to recognize that this particular test is an essential validation of the method since it shows that the internal standard rRNA, which is 5′ end-labeled, is an accurate surrogate for rRNA extracted from an environmental incubation, which is internally labeled.

Calculations—^{32}P-labeled RNA from *Prochlorococcus* strain MED4 was applied as internal recovery standards in the RIBOTRACE protocol in much the same way as described by Van Mooy et al. (2004). As stated above, an aliquot of the RIBOTRACE reaction was taken to measure the initial ^{32}P and ^{33}P radioactivities via a dual-label scintillation-counting protocol. At the end of the RIBOTRACE hybridization and capture, final ^{32}P and ^{33}P radioactivities were determined. In contrast to the DNA hybridization capture method used by Van Mooy et al. (2004), the background radioactivity using the Non338 probe was small and could be ignored; this will be discussed below. Therefore, the ^{32}P-labeled standard RNA was used to constrain the recovery efficiency of RIBOTRACE reactions.

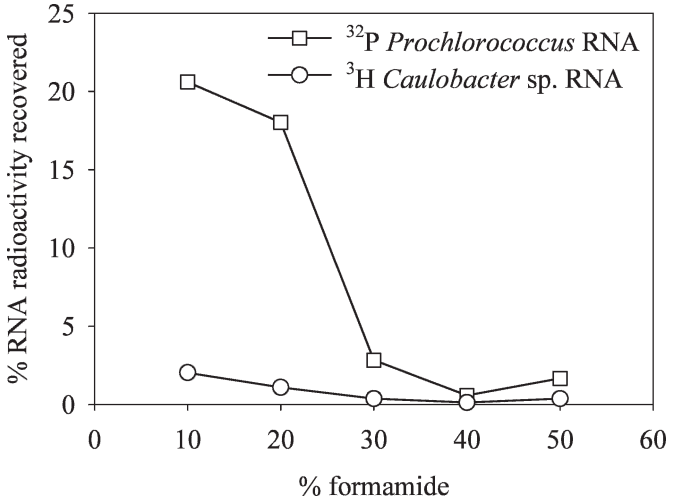

Fig. 1. Recovery of rRNA by using the Pro444 probe at different formamide concentrations.

$$R = \frac{\left(\frac{^{33}P_{RNAfinal}}{^{33}P_{RNAinitial}}\right)_{sample}}{\left(\frac{^{32}P_{RNAfinal}}{^{32}P_{RNAinitial}}\right)_{std}} \times \frac{\left(\frac{^{32}P_{rRNA}}{^{32}P_{totalRNA}}\right)_{std}}{\left(\frac{^{33}P_{rRNA}}{^{33}P_{totalRNA}}\right)_{sample}} \times 100 \quad (4)$$

In Eq. 4, R is the fraction of the ^{33}P radioactivity in total RNA that is complementary to the probe in any given experiment, expressed as a percentage. In the first quotient $^{33}P_{RNAfinal}$ and $^{33}P_{RNAinitial}$ are the initial and final radioactivities of sample RNA as determined by the RIBOTRACE method, whereas $^{32}P_{RNAfinal}$ and $^{32}P_{RNAinitial}$ are the initial and final radioactivities of the standard RNA as determined by the RIBOTRACE method. Thus the first quotient in Eq. 4 is the recovery-corrected capture of the target RNA. The second quotient corrects for the variable fraction of radioactivity in the standard and sample total RNA ($P_{totalRNA}$) that is derived from rRNA (P_{rRNA}) at the beginning of the RIBOTRACE procedure. RNA synthesis rates of the probe-defined phylogenetic groups were determined by multiplying R values (expressed as fractions) by the total RNA synthesis rate.

Results

Total PO_4^{3-} uptake—The total PO_4^{3-} uptake rates observed in the control (no amendment) incubations were 6.8 ± 0.9 and 11.1 ± 2.8 nmol L^{-1} d^{-1} P in 2003 and 2004 respectively (mean ± standard deviation; Fig. 3). Taking the control and amended incubations together, the total PO_4^{3-} uptake rates were lower in 2003 than in 2004 (ANOVA, $p = 0.007$; Fig. 3). PO_4^{3-} concentrations were also lower in 2003 versus 2004: 34 versus 56 nmol L^{-1} (data courtesy of K. Björkman and D. Karl, University of Hawai'i, Manoa). Turnover times of PO_4^{3-} in the control incubations were 5 ± 1 d during both cruises and were within the range of reported values for Sta. ALOHA

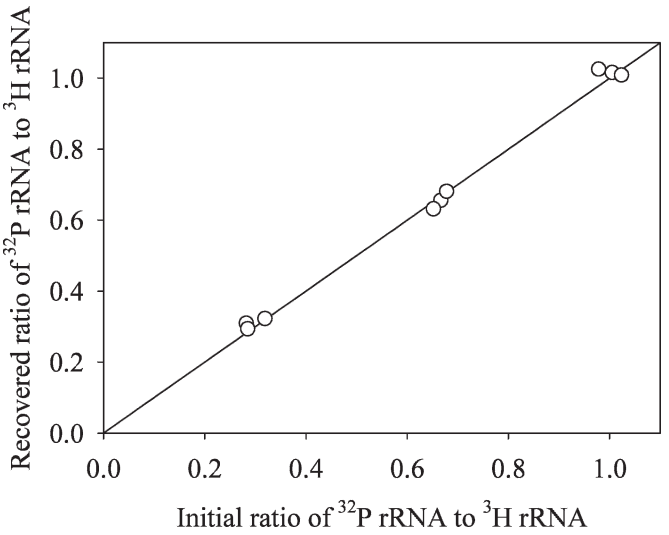

Fig. 2. Comparison of the relative recovery of ^{32}P end-labeled rRNA and ^{3}H internally labeled rRNA by using the Eub338 probe. The line is a 1 : 1 line, and shows that there was little discrimination between the two types of rRNA.

(Björkman et al. 2000; Björkman and Karl 2003). During both cruises, amendments of NH_4^+ significantly enhanced total PO_4^{3-} uptake rates (*t*-test; $p = 0.008$ and 0.026 for 2003 and 2004 respectively). In addition, amendments of PO_4^{3-} actually decreased the uptake rate in 2003 (*t*-test, $p = 0.012$). NO_3^- had no effect on total PO_4^{3-} uptake rates.

Incorporation of $^{33}PO_4^{3-}$ into total community RNA—In the control incubations containing only $^{33}PO_4^{3-}$ without amendments, the total community RNA synthesis rates were significantly lower in 2003 than in 2004 (*t*-test, $p = 0.011$; Fig. 3); the rates were 2.3 ± 0.4 and 6.0 ± 1.7 nmol L^{-1} d^{-1} P respectively. Not surprisingly, RNA synthesis rates from the incubations that were amended with nutrients were also generally higher in 2004 than in 2003 (ANOVA, $p = 0.065$). During both cruises incubations amended with NH_4^+ showed significantly higher RNA synthesis rates than controls (*t*-tests, $p = 0.007$ and $p = 0.010$ for 2003 and 2004 respectively), whereas no other amendments affected any significant change in RNA synthesis rates. RNA synthesis rates accounted for a pooled average of 45% ± 13% of total PO_4^{3-} uptake rates. RNA synthesis rates were exponentially related to total PO_4^{3-} uptake rates ($R^2 = 0.907$; Fig. 4).

A considerable fraction of total RNA synthesis was dedicated to the synthesis of rRNA, which is typical for bacteria (Bremer and Dennis 1987). Among RNA extracts from the incubations conducted during the 2003 cruise, a pooled average of 67% ± 20% of the ^{33}P in RNA was ribosomal, whereas in 2004 the pooled average was 40% ± 7%. During both cruises, the incubations amended with NH_4^+ had the highest fraction of the ^{33}P label in rRNA, with 80% and 48% of the RNA total for 2003 and 2004 respectively.

RIBOTRACE—Results from the RIBOTRACE method showed that different groups of plankton responded

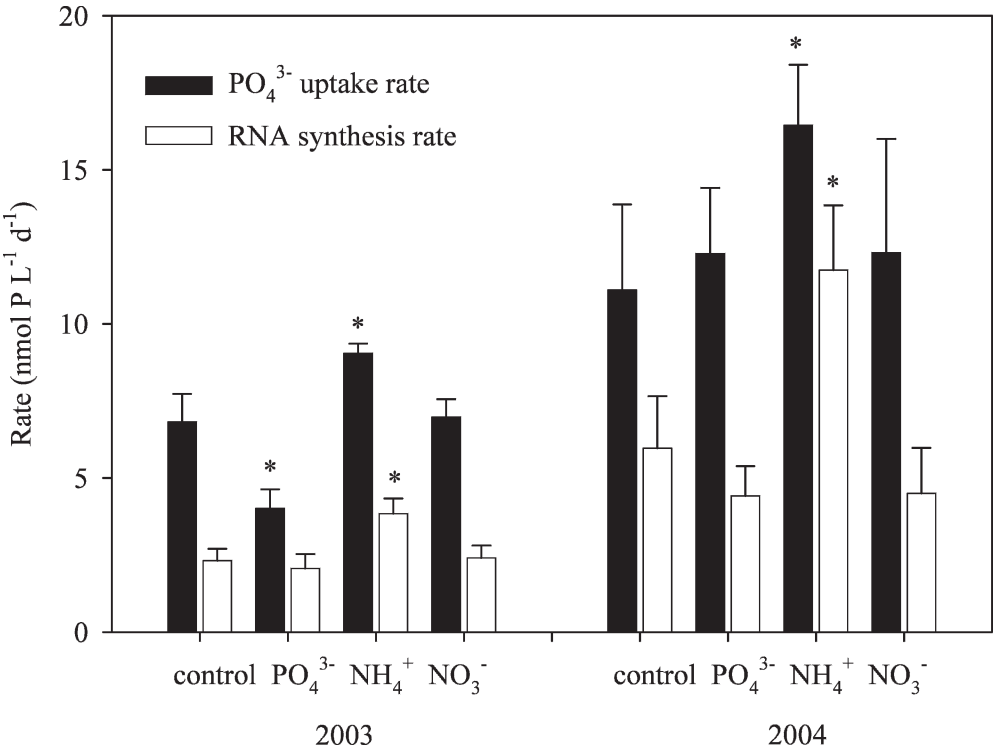

Fig. 3. Rates of total PO_4^{3-} uptake and total RNA synthesis by plankton >0.2 μm during cruises in July 2003 and 2004 to the NPSG. Asterisks denote significant ($p < 0.05$) differences from the control. Error bars are standard deviation of data from triplicate incubations.

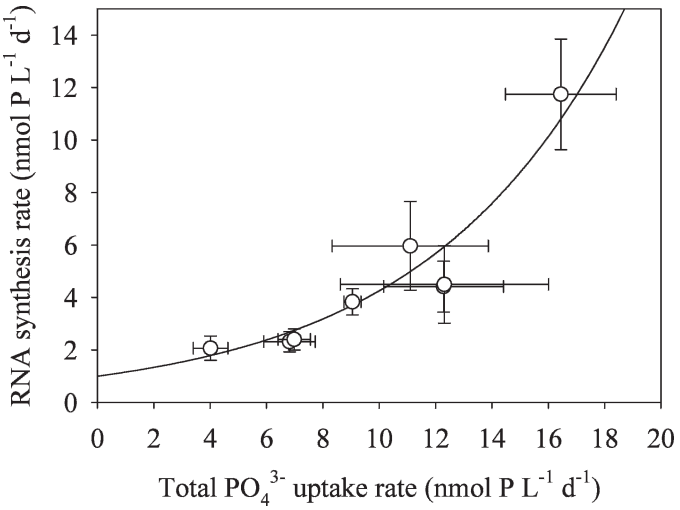

Fig. 4. Relationship between the total PO_4^{3-} uptake rate and total RNA synthesis rate by plankton >0.2 μm during two cruises in July 2003 and 2004 to the NPSG. The line is an exponential fit to the data.

differently to nutrient amendments (Fig. 5). The utilization of PO_4^{3-} for RNA synthesis by plankton of the domain Bacteria was defined by the Eub338 probe, whereas that of plankton of the genus *Prochlorococcus*, which are within the domain Bacteria, was defined by the Pro444 probe. For every sample, RIBOTRACE was also conducted with Non338 (nonbinding control probe), but RNA capture with this probe contributed only 4% ± 10% error in *R* values and, therefore, results obtained using the Non338 probe will not be further discussed.

During both cruises, Bacteria accounted for the majority of RNA synthesis in the control incubations as indicated by R_{Bacteria} values that were generally greater than 50% (Fig. 5); the pooled average across all experiments on both cruises was 63% ± 14%. Unexpectedly, additions of PO_4^{3-} decreased the R_{Bacteria} during the 2004 cruise (*t*-test, $p < 0.001$). We ascribed all remaining ^{33}P radioactivity in RNA not captured by the Eub338 probe synthesis to "eukaryotes" and have therefore defined $R_{\text{eukaryotes}}$ as $1 - R_{\text{Bacteria}}$. The pooled average of $R_{\text{eukaryotes}}$ across all experiments on both cruises was 36% ± 14%. By definition, all responses to amendments that were significant for R_{Bacteria} were also significant for $R_{\text{eukaryotes}}$. Thus $R_{\text{eukaryotes}}$ increased significantly in 2004 in response to PO_4^{3-}.

During both cruises, RNA synthesis by *Prochlorococcus* contributed a substantial fraction of the RNA synthesis by Bacteria under control conditions, 42% ± 19% and 59% ± 11% in 2003 and 2004 respectively. In 2003, $R_{Prochlorococcus}$ increased significantly in response to both PO_4^{3-} and NH_4^+ versus controls (*t*-tests: $p = 0.002$ and $p = 0.001$, for PO_4^{3-} and NH_4^+ respectively; Fig. 5). We defined $R_{\text{other bacteria}}$ as $R_{\text{Bacteria}} - R_{Prochlorococcus}$. Surprisingly, $R_{\text{other bacteria}}$ values in PO_4^{3-}-amended incubations were significantly less than no-amendment controls during both cruises (*t*-test, $p = 0.002$ and $p = 0.001$ for 2003 and 2004 respectively); in fact they were indistinguishable from zero. The error in $R_{\text{other bacteria}}$ is substantial (Fig. 5), but in general $R_{Prochlorococcus} \geq R_{\text{other bacteria}}$ in all of the incubations. Amendments of NO_3^- had no effect on any of the *R* values (Fig. 5).

The RNA synthesis rates of plankton of the domain Bacteria and of *Prochlorococcus* showed substantial variation in their respective contribution to PO_4^{3-} cycling at Sta. ALOHA (Fig. 6). During both cruises, amendments of NH_4^+ led to an increase in the RNA synthesis rate by Bacteria (*t*-test, $p = 0.006$ and $p = 0.021$) and *Prochlorococcus* (*t*-test, $p = 0.001$ and $p = 0.019$, for 2003 and 2004 respectively). In 2004, RNA synthesis by Bacteria decreased in response to PO_4^{3-} (*t*-test, $p = 0.027$). The RNA synthesis rates of eukaryotes and other bacteria were also calculated, but the errors in *R* values and total RNA synthesis rates, once propagated, were too large to reveal any statistically significant changes in RNA synthesis rates by these groups (Fig. 6).

Discussion

Effect of nutrients on PO_4^{3-} uptake and RNA synthesis—The rates of total PO_4^{3-} uptake in control experiments during 2003 and 2004 (Fig. 3) were within the range of 1.40 ± 0.02 to 10.67 ± 1.93 nmol L^{-1} d^{-1} observed by Björkman et al. (2000). This suggests that the planktonic P cycling was not unusual during either of our cruises and that our results are probably broadly representative of the surface waters of the NPSG. The RNA synthesis rates accounted for a variable fraction of the total cellular PO_4^{3-} uptake rate, but on average was about half of the total. During the same two cruises Van Mooy et al. (2006) reported that phospholipid synthesis accounted for about a fifth of total PO_4^{3-} uptake. Thus about 70% of PO_4^{3-} uptake could be unequivocally ascribed to planktonic cellular synthesis of P-containing biochemicals (i.e., growth). DNA synthesis (Van Mooy 2003), the synthesis of transient P-containing biochemicals (e.g., ATP), and abiotic sorption (Fu et al. 2005) accounted for the remaining ≈30% of PO_4^{3-} uptake that could not be accounted for by RNA and phospholipids. Therefore RNA synthesis was the single largest biochemical sink for PO_4^{3-} in the plankton we sampled. Furthermore, RNA synthesis rates increased exponentially as PO_4^{3-} uptake rates increased (Fig. 4). Similar relationships have been observed between concentrations of RNA per cell and growth rates in pure cultures of *Prochlorococcus* (Worden and Binder 2003) and heterotrophic bacteria (Kemp et al. 1993; Kerkhof and Kemp 1999), and it appears that our data reflect this same behavior by plankton in the NPSG. This suggests that RNA would be an even larger biochemical sink for PO_4^{3-} in more productive waters.

Amendments of NH_4^+ to the incubations resulted in greater total PO_4^{3-} uptake rates, which indicated that PO_4^{3-} uptake by the total planktonic community was limited by available NH_4^+. However, NO_3^- amendments had no effect on these rates, and, apparently NO_3^- could not relieve the observed N limitation. This observation is consistent with the fact that cultured strains of *Prochlorococcus* are unable to utilize NO_3^- (Moore et al. 2002). We expected to find some stimulation in PO_4^{3-} uptake rates in response to NO_3^- because eukaryotes are present in

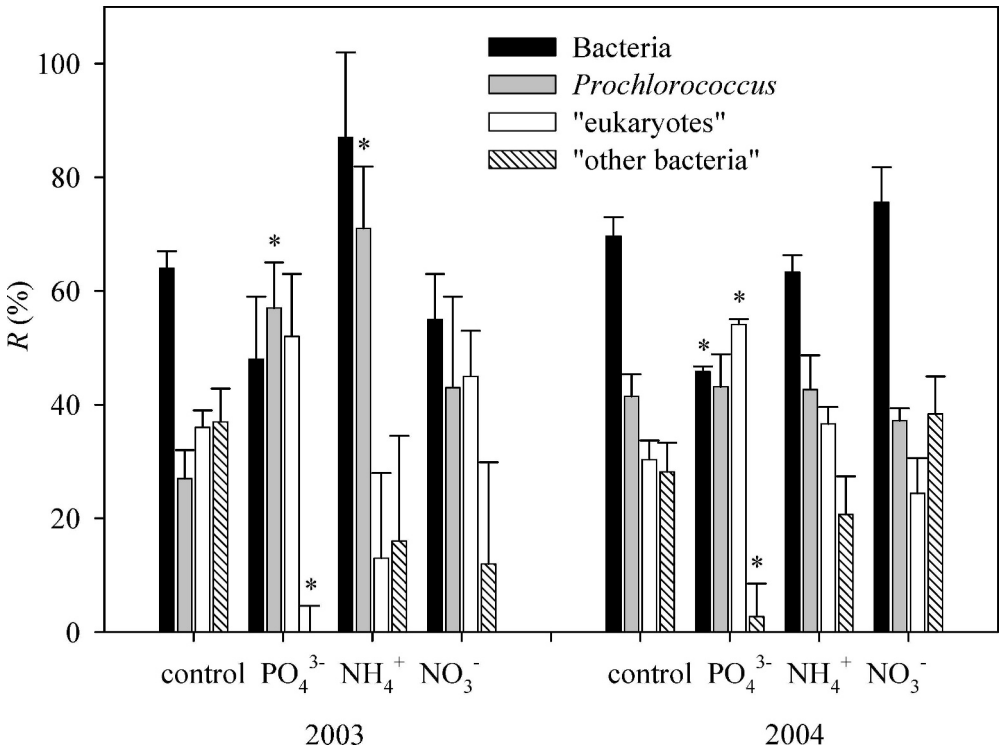

Fig. 5. The fraction of RNA synthesis by groups of plankton as determined by RIBOTRACE. Asterisks and error bars as for Fig. 3.

the NPSG, but this was not observed. Amendments of PO_4^{3-} actually caused a decrease in the average total PO_4^{3-} uptake rate in 2003, and we will discuss this in more detail below.

The increase in the PO_4^{3-} uptake rates in NH_4^+-amended incubations could be entirely accounted for by increased RNA synthesis rates. That is, the difference between the PO_4^{3-} uptake rates and RNA synthesis rates were the same in control and NH_4^+-amended incubations (Fig. 3), which showed that the synthesis rates of other P-containing biochemicals were the same. It stands to reason that NH_4^+ would stimulate RNA synthesis rates because, in addition to being a P-intensive metabolism (Fig. 3), RNA synthesis is also an N-intensive metabolism. Although the N : P ratio of RNA is only 3.8 on average and is considerably less than the N : P of 16 in Redfield plankton, the actual N : P of the demand associated with rRNA synthesis is considerably higher. This is because bacterial ribosomes are about 40% protein (Noller 1984), and thus the synthesis of rRNA demands additional N for the synthesis of protein required to produce whole ribosomes. In the NH_4^+-amended incubations conducted in 2003, we observed that 80% of the RNA synthesis was ribosomal, which is consistent with observations of cultured bacteria (Bremer and Dennis 1987; considerably less RNA synthesis was ribosomal in 2004, but on the basis of gel electrophoresis we attribute this to mild degradation during transport/handling). By applying a stoichiometric relationship for an ideal protein (Anderson 1995), we calculate that the synthesis of ribosomes demands N and P at a ratio of about 6 : 1; i.e., ribosome synthesis demands a nontrivial fraction of Redfield N requirements.

RIBOTRACE results for Prochlorococcus*, eukaryotes, and heterotrophic bacteria under control conditions*—The biological cycling of P is uniquely amenable to interrogation with molecular biological methods. On the basis of the observations we report here, the synthesis of rRNA molecules could be the single largest biochemical sink for PO_4^{3-} in the sea. This is fortuitous because the DNA sequences encoding these rRNA molecules (i.e., rDNA) compose the most comprehensive set of genetic information that has been retrieved from the sea to date. Thus, RIBOTRACE and other related methods that target rRNA (MacGregor et al. 2002, 2006; Pearson et al. 2004) have the potential to directly quantify the chemical and ecological controls on one of the most significant biochemical pathways in the upper ocean.

Since RNA synthesis is such a large component of overall P demand, the fraction of RNA synthesis (R values) as determined by RIBOTRACE is indicative of the partitioning of total PO_4^{3-} uptake between different groups of plankton. Under control conditions, plankton of the domain Bacteria were responsible for about two-thirds of the RNA synthesis (i.e., $R_{\text{Bacteria}} \approx 67\%$; Fig. 5). This fraction was expected since cyanobacteria and heterotrophic bacteria together have been shown to account for 60% to 90% of total microbial carbon in the NPSG (Campbell et al. 1997). The Eub338 probe has known mismatches to rRNA sequences of *Plantomycetales* and *Verrucomicrobia*,

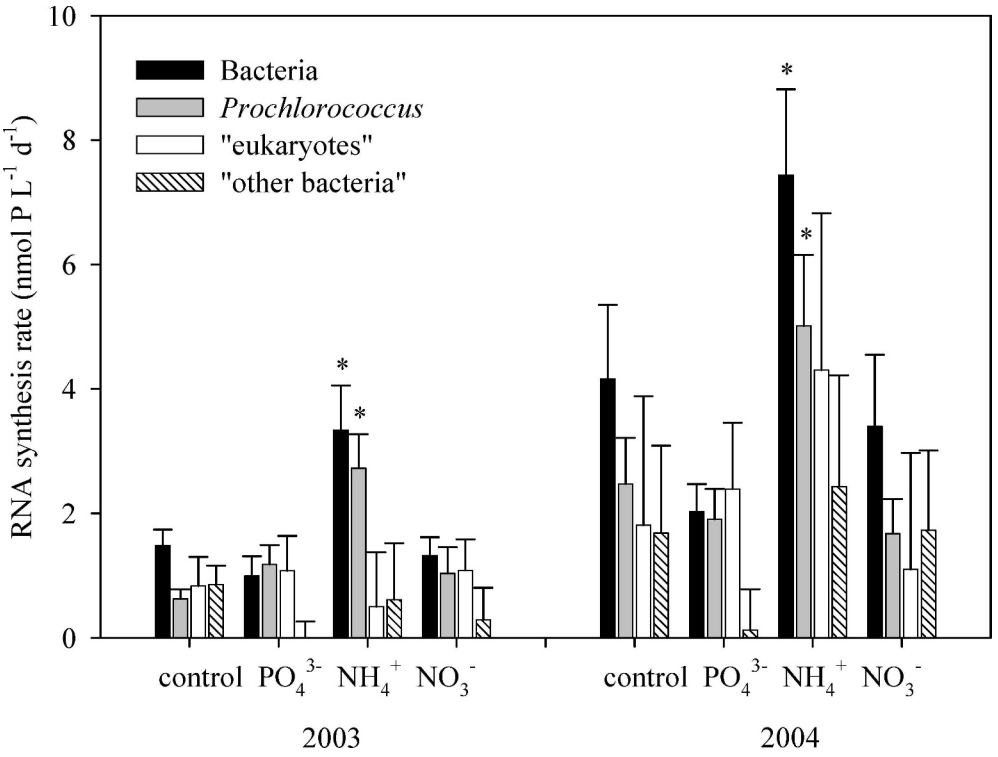

Fig. 6. RNA synthesis rates by groups of plankton as determined by RIBOTRACE. Asterisks and error bars as for Fig. 3.

which are potentially important marine phyla, and thus the Eub338 probe may underestimate the total bacterial contribution to RNA synthesis in this study (Daims et al. 1999). Future studies using RIBOTRACE could overcome this potential problem by using nested probes targeting the domain Bacteria (Daims et al. 1999). Nonetheless, our observation that bacteria account for two-thirds of RNA synthesis underscores the dominant role that bacteria play in the phosphorus cycle at Sta. ALOHA.

Of the RNA synthesis by Bacteria, about half was due to the cyanobacterial phytoplankton of the genus *Prochlorococcus*, which agrees with the observation that *Prochlorococcus* composes about half of prokaryotic biomass at ALOHA (Campbell et al. 1997; Björkman et al. 2000). This comparison also suggests that *Prochlorococcus* and other bacteria turn over at about the same rate. The probe we used to target *Prochlorococcus* rRNA, Pro444, was developed to cover most members of the genus, except for a few low-light strains that were unlikely to be present in the surface waters at ALOHA (West and Scanlan 1999). We reexamined the specificity of this probe using the Ribosomal Database's probe match tool (Cole et al. 2007) and confirmed that none of the strains of *Prochlorococcus marinus* currently in the database, with the exception of low-light strains MIT9303 and MIT9313, had a mismatch with West and Scanlan's (1999) Pro444 probe. So although the Pro444 probe cannot resolve the response of the specific ecotypes that compose the total population of *Prochlorococcus* in subtropical surface waters (Johnson et al. 2006; Zinser et al. 2006), it does provide an integrated view of the genus as a whole. Future applications of RIBOTRACE in the subtropical gyres could benefit from the incorporation of a nested probe strategy to more thoroughly target the behavior of the whole *Prochlorococcus* population as well as specific ecotypes.

RNA synthesis by Bacteria that could not be accounted for by *Prochlorococcus* was due to other bacteria. Admittedly, "other bacteria" rigorously defines neither a phylogenetically nor a functionally cohesive group of organisms. When enumerated by microscopy, small, non-pigmented, DNA-stainable bacterial cells (i.e., other bacteria) are defined as heterotrophic bacteria. A small fraction of these organisms have photoheterotrophic capabilities (Cottrell et al. 2006). Small unicellular N_2-fixing cyanobacteria are also likely to be present, but they appear to be low in abundance (Church et al. 2005). Similarly, the picocyanobacterium *Synechococcus* is generally present only in low abundances at ALOHA (Campbell et al. 1997; Björkman et al. 2000; Cottrell et al. 2006), whereas *Trichodesmium*, larger N_2-fixing cyanobacteria, were absent during our cruises. Therefore we ascribed RNA synthesis by other bacteria to heterotrophic bacteria.

Karner et al. (2001) observed that Archaeal cells were virtually undetectable in surface waters at ALOHA, and during our cruises all major membrane lipids were either bacterial or eukaryotic (Van Mooy et al. 2006). Therefore RNA synthesis not ascribed to the domain Bacteria was almost certainly due to cells of the domain Eukarya (i.e., eukaryotes); clearly this assignment would be unwise in other marine environments where Archaea may be abundant (Herndl et al. 2005). Under control conditions, eukaryotic cells were responsible for the remaining third of

RNA synthesis not accounted for by Bacteria. Andersen et al. (1996) found that about three-quarters of the eukaryotic cells at ALOHA were phototrophic, and 69% to 90% were less than 3 μm (i.e., picoeukaryotes). HPLC pigments from our 2003 and 2004 cruises indicated that prymnesiophytes and diatoms dominated over the less-abundant pelagophytes and dinoflagellates, as indicated by concentrations of 19′-hexanoyloxyfucoxanthin, fucoxanthin + diadinoxanthin, 19′-butanoyloxyfucoxanthin, and peridinin respectively (data courtesy of B. Bidigare, University of Hawai'i, Manoa; http://hahana.soest.hawaii.edu/hot/).

Response of Prochlorococcus, *eukaryotes, and heterotrophic bacteria to nutrients*—Amendments of PO_4^{3-} affected the partitioning of RNA synthesis between the different groups of plankton in unexpected ways (Fig. 5). In 2003, the value of $R_{\text{other bacteria}}$ was indistinguishable from zero in PO_4^{3-}-amended incubations, and we have no additional data to explain this observation. Heterotrophic bacteria such as those of the SAR11 clade (Rappé et al. 2002; Giovannoni et al. 2005) and other ultraoligotrophs (Button et al. 1993) may be very intolerant to changes in their chemical environment (Button 1998), and we speculate that the amendment of PO_4^{3-} was somehow inhibitory to RNA synthesis. We observed this same response by heterotrophic bacteria to PO_4^{3-} again in 2004. In 2003 $R_{Prochlorococcus}$ increased and in 2004 $R_{\text{eukaryotes}}$ increased, but these signals were primarily a result of the very large decreases in $R_{\text{other bacteria}}$. Although the population of eukaryotic phytoplankton at ALOHA is thought to be less dominant than before the domain shift (Karl et al. 2001*a*), the species composition is relatively similar (Venrick 1999). Thus we expected to observe clear signals of P limitation in RNA synthesis rates of eukaryotes, but we did not. To summarize the effects of PO_4^{3-} amendments, aside from unexpectedly inhibiting heterotrophic bacteria, they did not increase RNA synthesis rates by *Prochlorococcus* or eukaryotes (Fig. 6).

In contrast to PO_4^{3-}, amendments of NH_4^+ had a significant effect on *Prochlorococcus*. In 2003, $R_{Prochlorococcus}$ doubled in response to NH_4^+, and the absolute RNA synthesis rate by *Prochlorococcus* also increased significantly (Fig. 5). In fact, the observed increase in total RNA synthesis was due solely to *Prochlorococcus*. These data clearly show that *Prochlorococcus* was N limited during this cruise. In contrast to 2003, the response to NH_4^+ in 2004 was much more balanced. Although the absolute synthesis rate of RNA by *Prochlorococcus* increased significantly, indicating N-limited growth by these organisms (Fig. 6), the value of $R_{Proclorococcus}$ remained unchanged (Fig. 5). This indicates that in addition to *Prochlorococcus*, heterotrophic bacteria and eukaryotic phytoplankton—primarily diatoms as indicated by pigments—also responded to NH_4 in 2004. The lack of a response by eukaryotic phytoplankton to NO_3^- during both cruises and to NH_4^+ in 2003 is somewhat equivocal because the diatoms of the genus *Hemialus* that are frequently observed in the late summer in ALOHA contain N_2-fixing cyanobacterial endosymbionts that would provide NH_4^+ and attenuate the effect of N amendments (Scharek et al. 1999).

Implications of the observed N-limitation of Prochlorococcus *at ALOHA*—The reservoir of PO_4^{3-} in the euphotic zone of the NPSG started to decrease in the 1970s and continued to do so into the 1990s (Karl and Tien 1997; Karl et al. 2001*b*). Primary production rates increased during this same time (Karl et al. 1995; Karl et al. 2001*a*), and this and other evidence led Karl and others to put forth the hypothesis that enhanced N_2 fixation was gradually stripping the euphotic zone of dissolved P (Karl and Tien 1997; Karl et al. 1997; Karl et al. 2001*b*). Analysis of NPSG pigment data from the same time period suggested a domain shift in the phytoplanktonic community where *Prochlorococcus* supplanted eukaryotes as the dominant phytoplankton. The P limitation of eukaryotic phytoplankton was implicated as the cause of this shift in the composition of the phytoplanktonic community (Karl et al. 2001*a*).

Chemical and biological data have unequivocally shown that the NPSG has been on a trajectory toward P limitation (Karl et al. 2001*b*): N-to-P ratios of organic matter have increased, PO_4^{3-} concentrations have decreased, and microbial affinities for PO_4^{3-} have increased. However, the values for these parameters are much more extreme in other P-limited marine environments. For example, PO_4^{3-} concentrations in the surface at ALOHA generally range from 20 to 100 nmol L^{-1} (Karl and Tien 1997; Björkman et al. 2000; Björkman and Karl 2003; this study), but are almost an order of magnitude higher than in the stratified Sargasso Sea (Wu et al. 2000; Cavender-Bares et al. 2001), Mediterranean Sea (Moutin et al. 2002; Thingstad et al. 2005), and Red Sea (Fuller et al. 2005). These environments show clear signs of planktonic P limitation and PO_4^{3-} turnover times are generally on the order of a few hours or less (Dyhrman et al. 2002; Moutin et al. 2002; Lomas et al. 2004; Fuller et al. 2005; Thingstad et al. 2005). In contrast, turnover times of PO_4^{3-} are on the order of 1 week in the NPSG (Björkman et al., 2000; this study), which suggests a far more subdued competition for PO_4^{3-} in the NPSG than in the other aforementioned environments. Furthermore, *Prochlorococcus* appears to be successful in all of these environments (Cavender-Bares et al. 2001; Moutin et al. 2002; Fuller et al. 2005), and thus, it appears that there should be more than sufficient PO_4^{3-} available for *Prochlorococcus* in the NPSG. Indeed, the RIBOTRACE data showed that *Prochlorococcus* were limited not by P but by N during our two summer cruises.

Both of our cruises were in July, and, admittedly, it remains an open question as to whether N limitation of *Prochlorococcus* would be observed during different times of the year. However, N_2-fixation rates typically reach their annual peak in the summer (Dore et al. 2002), and we expect that this would lead to an annual peak in the demand for PO_4^{3-}. On the other hand, DIN inventories are generally at their annual minimum in the summer, whereas PO_4^{3-} inventories do not reach their minimum until the autumn, presumably due the drawdown of PO_4^{3-} by summer N_2 fixation (Karl et al. 2001*b*). So it is possible that the competition for P could intensify in subsequent months. Nonetheless, summer and autumn PO_4^{3-} concentrations in the surface layer at ALOHA have been >30 times higher than DIN on average since the beginning of

this century (http://hahana.soest.hawaii.edu/hot/), and this was also true during our cruises. This is characteristic of the oligotrophic upper ocean and has been repeatedly interpreted as a hallmark of global N limitation of phytoplanktonic production. One of the pitfalls of using DIN and PO_4^{3-} concentrations to assess the relative importance of N and P in limiting production is that dissolved organic N and P may also support phytoplankton production (e.g., Perry and Eppley 1981; Karl et al. 2001*b*). However, since PO_4^{3-} is clearly the preferred form of dissolved P for *Prochlorococcus* and amendments of PO_4^{3-} have been shown to enhance growth by P-limited *Prochlorococcus* cells (Moore et al. 2005), it would be difficult to argue that the observed lack of response by *Prochlorococcus* to PO_4^{3-} was simply due to interference with dissolved organic P.

Despite our data in support of N limitation and our arguments against P limitation of *Prochlorococcus*, we assert that N limitation at this time should not be construed as a refutation of the domain shift hypothesis. Before the domain shift the eukaryotic algae in the NPSG were thought to be N limited (Perry and Eppley 1981), and the hypothesis that N_2 fixers drew down P concentrations to the point where eukaryotic algae became P limited is entirely possible. However, there is mounting molecular evidence to suggest that *Prochlorococcus* is better adapted to low P concentrations than eukaryotes and other plankton (Fuller et al. 2005; Moore et al. 2005; Van Mooy et al. 2006), and, thus, we would not expect the ensuing population of *Prochlorococcus* to also be P limited. The results we present here may simply indicate that the domain shift has progressed past the point where the dominant phytoplankton are P limited. Granted, the chemical and biological data collected as part of the HOT program have unequivocally shown that the NPSG is on a trajectory toward P limitation, but the current population of *Prochlorococcus*—just as with the population of eukaryotic phytoplankton that preceded it—appears to be limited by N, at least for the time being.

References

Amann, R. I., L. Krumholz, and D. A. Stahl. 1990. Fluorescent oligonucleotide probing of whole cells for determinative, phylogenetic, and enviromental studies in microbiology. J. Bacteriol. **172:** 762–770.

Andersen, R. A., R. R. Bidigare, M. D. Keller, and M. Latasa. 1996. A comparison of HPLC pigment signatures and electron microscopic observations for oligotrophic waters of the North Atlantic and Pacific Oceans. Deep-Sea Res. II **43:** 517–537.

Anderson, L. 1995. On the hydrogen and oxygen content of marine phytoplankton. Deep-Sea Res. I **42:** 1675–1680.

Björkman, K., and D. M. Karl. 2003. Bioavailibility of dissolved organic phosphorus in the euphotic zone at station ALOHA, North Pacific Subtropical Gyre. Limnol. Oceanogr. **48:** 1049–1057.

———, A. L. Thomson-Bulldis, and D. M. Karl. 2000. Phosphorus dynamics in the North Pacific subtropical gyre. Aquat. Microb. Ecol. **22:** 185–198.

Bremer, H., and P. P. Dennis. 1987. Modulation of chemical composition and other parameters of the cell by growth rate, p. 1527–1542. *In* F. C. Neidhardt [ed.], *Escherichia coli and Salmonella typhimurium* cellular and molecular biology. American Society for Microbiology.

Button, D. K. 1998. Nutrient uptake by microorganisms according to kinetic parameters from theory as related to cytoarchitecture. Microbiol. Mol. Biol. Rev. **62:** 636–645.

———, F. Schut, P. Quang, R. Martin, and B. R. Robertson. 1993. Viability and isolation of marine bacteria by dilution culture: Theory, procedures and initial results. Appl. Environ. Microbiol. **59:** 881–891.

Campbell, L., H. Liu, H. A. Nolla, and D. Vaulot. 1997. Annual variability of phytoplankton and bacteria in the subtropical North Pacific Ocean at Station ALOHA during the 1991–1994 ENSO event. Deep-Sea Res. I **44:** 167–192.

Cavender-Bares, K. K., D. M. Karl, and S. W. Chisholm. 2001. Nutrient gradients in the western North Atlantic Ocean: Relationship to microbial community stucture and comparison to patterns in the Pacific Ocean. Deep-Sea Res. I **48:** 2373–2395.

Church, M. J., B. D. Jenkins, D. M. Karl, and J. P. Zehr. 2005. Vertical distributions of nitrogen-fixing phylotypes at Stn ALOHA in the Oligatrophic North Pacific Ocean. Aquat. Microb. Ecol. **38:** 3–14.

Cole, J. R., and others. 2007. The ribosomal database project (RDP-II): Introducing myRDP space and quality controlled public data. Nucleic Acids Res. **35**, (Database issue), D169–D172.

Cottrell, M. T., A. Mannino, and D. L. Kirchman. 2006. Aerobic anoxygenic phototrophic bacteria in the Mid-Atlantic Bight and the North Pacific gyre. Appl. Environ. Microbiol. **72:** 557–564.

Daims, H., A. Brühl, R. Amann, K.-H. Schleifer, and M. Wagner. 1999. The domain-specific probe EUB338 is insufficient for the detection of all Bacteria: development and evaluation of a more comprehensive probe set. System. Appl. Microbiol. **22:** 434–444.

Dore, J. E., J. R. Brum, L. M. Tupas, and D. M. Karl. 2002. Seasonal and interannual variability in sources of nitrogen supporting export in the oligotrophic subtropical North Pacific Ocean. Limnol. Oceanogr. **47:** 1595–1607.

Dyhrman, S. T., E. A. Webb, D. M. Anderson, J. W. Moffett, and J. B. Waterbury. 2002. Cell-specific detection of phosphorus stress in *Trichodesmium* from the western North Atlantic. Limnol. Oceanogr. **47:** 1832–1836.

Fu, F. X., Y. H. Zhang, K. Leblanc, S. A. Sanudo-Wilhelmy, and D. A. Hutchins. 2005. The biological and biogeochemical consequences of phosphate scavenging onto phytoplankton cell surfaces. Limnol. Oceanogr. **50:** 1459–1472.

Fuller, N. J., and others. 2005. Dynamics of community structure and phosphate status of picocyanobacterial populations in the Gulf of Aqaba, Red Sea. Limnol. Oceanogr. **50:** 363–375.

Giovannoni, S. J., and others. 2005. Genome streamlining in a cosmopolitan oceanic bacterium. Science **309:** 1242–1245.

Herndl, G. J., and others. 2005. Contribution of archaea to total prokaryotic production in the deep Atlantic Ocean. Appl. Environ. Microbiol. **71:** 2303–2309.

Jacquet, S., F. Partensky, D. Marie, R. Casotti, and D. Vaulot. 2001. Cell cycle regulation by light in *Prochlorococcus* strains. Appl. Environ. Microbiol. **67:** 782–790.

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.

Karl, D., R. Letelier, L. Tupas, J. Dore, J. Christian, and D. Hebel. 1997. The role of nitrogen fixation in biogeochemical cycling in the subtropical North Pacific Ocean. Nature **388:** 533–538.

KARL, D. M., R. R. BIDIGARE, AND R. M. LETELIER. 2001*a*. Long-term changes in plankton community structure and productivity in the North Pacific Subtropical Gyre: The domain shift hypothesis. Deep-Sea Res. II **48:** 1449–1470.

———, AND R. LUKAS. 1996. The Hawaii ocean time-series (HOT) program: Background, rationale, and field implementation. Deep-Sea Res. **43:** 129–156.

———, AND G. TIEN. 1997. Temporal variability in dissolved phosphorus concentrations in the subtropical North Pacific Ocean. Mar. Chem. **56:** 77–96.

———, AND OTHERS. 1995. Ecosystem changes in the North Pacific subtropical gyre attributed to the 1991–92 El-Nino. Nature **373:** 230–234.

———, AND OTHERS. 2001*b*. Ecological nitrogen-to-phosphorus stoichiometry at station ALOHA. Deep-Sea Res. II **48:** 1529–1566.

KARNER, M. B., E. F. DELONG, AND D. M. KARL. 2001. Archaeal dominance in the mesopelagic zone of the Pacific Ocean. Nature **409:** 507–510.

KEMP, P. F., S. LEE, AND J. LAROCHE. 1993. Estimating the growth rate of slowly growing bacteria from RNA content. Appl. Environ. Microbiol. **59:** 2594–2601.

KERKHOF, L., AND P. KEMP. 1999. Small ribosomal RNA content in marine *Proteobacteria* during non-steady-state growth. FEMS Microbiol. Ecol. **30:** 253–260.

LOMAS, M. W., A. SWAIN, R. SHELTON, AND J. W. AMMERMAN. 2004. Taxonomic variability of phosphorus stress in Sargasso Sea phytoplankton. Limnol. Oceanogr. **49:** 2302–2310.

MACGREGOR, B. J., H. T. S. BOSCHKER, AND R. AMANN. 2006. Comparison of rRNA and polar-lipid-derived fatty acid biomarkers for assessment of C-13-substrate incorporation by microorganisms in marine sediments. Appl. Environ. Microbiol. **72:** 5246–5253.

———, V. BRÜCHERT, S. FLEISCHER, AND R. AMANN. 2002. Isolation of small-subunit rRNA for stable isotopic characterization. Environ. Microbiol. **4:** 451–464.

MANZ, W., R. AMANN, W. LUDWIG, M. WAGNER, AND K. SCHLEIFER. 1992. Phylogenetic oligodeoxynucleotide probes for the major subclasses of proteobacteria: Problems and solutions. Syst. Appl. Microbiol. **15:** 593–600.

MOORE, L. R., M. OSTROWSKI, D. J. SCANLAN, K. FEREN, AND T. SWEETSIR. 2005. Ecotypic variation in phosphorus-acquisition mechanisms within marine picocyanobacteria. Aquat. Microb. Ecol. **39:** 257–269.

———, A. F. POST, G. ROCAP, AND S. W. CHISHOLM. 2002. Utilization of different nitrogen sources by the marine cyanobacteria *Prochlorococcus* and *Synechococcus*. Limnol. Oceanogr. **47:** 989–996.

MOUTIN, T., AND OTHERS. 2002. Does competition for nanomolar phosphate supply explain the predominance of the cyanobacterium *Synechococcus*? Limnol. Oceanogr. **47:** 1562–1567.

NOLLER, H. F. 1984. Structure of ribosomal RNA. Ann. Rev. Biochem. **53:** 119–162.

PEARSON, A., A. L. SESSIONS, K. J. EDWARDS, AND J. M. HAYES. 2004. Phylogenetically specific separation of rRNA from prokaryotes for isotopic analysis. Mar. Chem. **92:** 295–306.

PERRY, M. J., AND R. W. EPPLEY. 1981. Phosphate uptake by phytoplankton in the central North Pacific. Deep-Sea Res. **28:** 39–49.

RAPPÉ, M. S., S. A. CONNON, K. L. VERGIN, AND J. GIOVANNONI. 2002. Cultivation of ubiquitous SAR11 marine bacterioplankton clade. Nature **418:** 630–633.

SCHAREK, R., M. LATASA, D. M. KARL, AND R. R. BIDIGARE. 1999. Temporal variations in diatom abundance and downward vertical flux in the oligotrophic North Pacific gyre. Deep-Sea Res. I **46:** 1051–1075.

THINGSTAD, T. F., AND OTHERS. 2005. Nature of phosphorus limitation in the ultraoligotrophic eastern Mediterranean. Science **309:** 1068–1071.

VAN MOOY, B. A. S. 2003. Carbon and phosphorus cycling by phylogenetically-defined groups of bacteria in the North Pacific Ocean. Ph.D. thesis. University of Washington.

———, A. H. DEVOL, AND R. G. KEIL. 2004. Quantifying ^{3}H-thymidine incorporation rates by a phylogenetically-defined group of marine planktonic bacteria (*Bacteriodetes* phylum). Environ. Microbiol. **6:** 1061–1069.

———, G. ROCAP, H. F. FREDRICKS, C. T. EVANS, AND A. H. DEVOL. 2006. Sulfolipids dramatically decrease phosphorus demand by picocyanobacteria in oligotrophic marine environments. Proc. Natl. Acad. Sci. USA **103:** 8607–8612.

VENRICK, E. L. 1999. Phytoplankton species structure in the central North Pacific, 1973–1996: Variability and persistence. J. Plankton Res. **21:** 1029–1042.

———, J. A. MCGOWAN, D. R. CAYAN, AND T. L. HAYWARD. 1987. Climate and chlorophyll *a*: Long-term trends in the central North Pacific Ocean. Science **238:** 70–72.

WEST, N. J., AND D. J. SCANLAN. 1999. Niche-partitioning of *Prochlorococcus* populations in a stratified water column in the eastern North Atlantic Ocean. Appl. Environ. Microbiol. **65:** 2585–2591.

WORDEN, A. Z., AND B. J. BINDER. 2003. Growth regulation of rRNA content in *Prochlorococcus* and *Synechococcus* (marine cyanobacteria) measured by whole-cell hybridization of rRNA-related peptide nucleic acids. J. Phycol. **39:** 527–534.

WU, J., W. SUNDA, E. A. BOYLE, AND D. M. KARL. 2000. Phosphate depletion in the western North Atlantic Ocean. Science **289:** 759–762.

ZINSER, E. R., AND OTHERS. 2006. *Prochlorococcus* ecotype abundances in the North Atlantic Ocean as revealed by an improved quantitative PCR method. Appl. Environ. Microbiol. **72:** 723–732.

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. Microbiol. **69:** 1299–1304.

———, I. MARY, E. M. S. WOODWARD, P. E. WARWICK, B. M. FUCHS, D. J. SCANLAN, AND P. H. BURKILL. 2007. Microbial control of phosphate in the nutrient-depleted North Atlantic subtropical gyre. Environ. Microbiol. **9:** 2079–2089.

Received: 13 January 2007
Accepted: 13 September 2007
Amended: 14 September 2007