LIMNOLOGY
and
OCEANOGRAPHY

ASLO

Limnol. Oceanogr. 61, 2016, 227–239

doi: 10.1002/lno.10206

Polyphosphate dynamics at Station ALOHA, North Pacific subtropical gyre

Julia M. Diaz,[†*1,2] Karin M. Björkman,[3] Sheean T. Haley,[2] Ellery D. Ingall,[4] David M. Karl,[3] Amelia F. Longo,[4] Sonya T. Dyhrman*[2]

[1]Department of Marine Chemistry and Geochemistry, Woods Hole Oceanographic Institution, Woods Hole, Massachusetts
[2]Department of Earth and Environmental Science, Lamont-Doherty Earth Observatory, Columbia University, Palisades, New York
[3]Department of Oceanography, Daniel K. Inouye Center for Microbial Oceanography: Research and Education, University of Hawaii, Honolulu, Hawaii
[4]School of Earth and Atmospheric Sciences, Georgia Institute of Technology, Atlanta, Georgia

Abstract

Polyphosphate (polyP) was examined within the upper water column ($\leq$ 150 m) of Station ALOHA (22° 45′N, 158° 00′W) during two cruises conducted in May–June 2013 and September 2013. Phosphorus molar ratios of particulate polyP to total particulate phosphorus (TPP) were relatively low, similar to previously reported values from the temperate western North Atlantic, and did not exhibit strong vertical gradients, reflecting a lack of polyP recycling relative to other forms of TPP with depth. Furthermore, relationships among polyP:TPP, soluble reactive phosphorus (SRP), and alkaline phosphatase activity (APA) were also consistent with previous observations from the Atlantic Ocean. To ascertain potential mechanisms of biological polyP production and utilization, surface seawater was incubated following nutrient additions. Results were consistent with polyP:TPP enrichment under opposite extremes of APA, suggesting diverse polyP accumulation/retention mechanisms. Addition of exogenous polyP (45 ± 5 P atoms) to field incubations did not increase chlorophyll content relative to controls, suggesting that polyP was not bioavailable to phytoplankton at Station ALOHA. To clarify this result, phytoplankton cultures were screened for the ability to utilize exogenous polyP. PolyP bioavailability was variable among model diatoms of the genus *Thalassiosira*, yet chain length did not influence polyP bioavailability. Thus, microbial community composition may influence polyP dynamics in the ocean, and vice versa.

Phosphorus (P) is a vital nutrient present in major biomolecules such as nucleic acids, phospholipids, and adenosine 5′-triphosphate (ATP). P is considered the ultimate limiting nutrient in the ocean over geologic timescales (hundreds to millions of years) because its abundance is regulated by relatively slow processes such as continental weathering and the burial of authigenic calcium phosphate minerals in marine sediments. However, revised estimates of marine P removal have decreased the residence time of P in the ocean (Ruttenberg 1993; Benitez-Nelson 2000; Ruttenberg 2014), and recent discoveries have revealed surprising aspects of microbial P metabolism (Yang and Metcalf 2004; Dyhrman et al. 2006; Karl et al. 2008; Van Mooy et al. 2009; Martinez et al. 2012; Van Mooy et al. 2015), which highlights a need and growing interest to better understand the cycling of marine P on a wide range of timescales (Karl 2014).

Polyphosphate (polyP) is a biopolymer consisting of at least three and up to thousands of phosphate molecules joined by phosphoanhydride (P—O—P) bonds. PolyP has historically been under-recognized in the study of ocean biogeochemistry (Björkman 2014). However, recent evidence suggests that polyP may play a key role in the marine P cycle over both modern and geologic timescales. For example, in sediments polyP may participate in long-term P sequestration by facilitating the formation of authigenic apatite phases (Schulz and Schulz 2005; Diaz et al. 2008). Over shorter timescales, polyP in surface waters may represent a source of bioavailable P capable of supporting marine primary productivity (Moore et al. 2005; Martin et al. 2014).

PolyP can be organic or inorganic, but it is typically detected, if not completely recovered, in operationally-

[†]Present address: Department of Marine Sciences, Skidaway Institute of Oceanography, University of Georgia, Savannah, Georgia
*Correspondence: julia.diaz@skio.uga.edu; sdyhrman@ldeo.columbia.edu

defined organic P pools (Armstrong et al. 1966; Karl and Tien 1992; Thomson-Bulldis and Karl 1998; White et al. 2006). PolyP can bind to organic moieties via P-ester (P—O—C) bonds. ATP and other nucleotide triphosphates (NTPs), which contain two phosphoanhydride bonds consistent with tripolyphosphate (tripolyP), are therefore considered herein to be organic forms of polyP. Indeed, the phosphoanhydride bonds found in NTPs are indistinguishable from other forms of polyP using ^{31}P Nuclear Magnetic Resonance (NMR) spectroscopy (Young and Ingall 2010).

In addition to NTPs, all major groups of life also synthesize inorganic polyP (Kornberg et al. 1999). Inorganic polyP can occur as microscopically visible cell inclusions (Lieberman 1888; Meyer 1904; Wiame 1947) and exist in a range of chain lengths (Kornberg et al. 1999). PolyP is utilized in many biological processes, including energy storage, gene expression, metal chelation, and stress responses (Kornberg 1995; Kornberg et al. 1999; Brown and Kornberg 2004; Rao et al. 2009). In marine systems, inorganic polyP is primarily considered to be a P storage molecule [*see* Solórzano and Strickland (1968), and references therein], and P availability is thought to drive biological cycling of inorganic polyP in the ocean. For example, two models of polyP production by marine microorganisms are luxury synthesis and polyP overplus (Karl and Björkman 2015). PolyP overplus involves the synthesis of polyP from P that has become temporarily available after a period of P stress. P stress is a cellular metabolic state caused by low P availability in the environment. P-stressed cells typically exhibit elevated alkaline phosphatase activities (APAs) and low cellular P quotas but are not necessarily growth limited. In contrast to polyP overplus, luxury synthesis of polyP occurs in the absence of any P stress, and organisms are thought to synthesize and store polyP for future utilization should P become limiting. However, the presence of free phosphate in many surface waters of the ocean (Benitez-Nelson 2000), suggests that there are controls on luxury polyP synthesis that prevent depletion of dissolved phosphate, even when P is in excess supply. Recent studies have also found that marine microbes increase the ratio of polyP to total particulate phosphorus (polyP:TPP) under persistent P stress (Orchard et al. 2010; Dyhrman et al. 2012; Martin et al. 2014), which may be dependent on the presence of excess available energy. Although cellular polyP quotas may decline in these cases, the decrease in cellular total P quotas is more extreme, reflecting the preferential retention of polyP relative to other P forms, such as P-lipids (Van Mooy et al. 2009; Orchard et al. 2010; Dyhrman et al. 2012; Martin et al. 2014).

In addition to being a vital endogenous biomolecule, polyP may also be an important exogenous source of bioavailable P, as suggested by numerous studies. For example, in the Sargasso Sea, particulate polyP is preferentially recycled relative to TPP (Martin et al. 2014), which comprises P in living and non-living material and thus likely reflects a combination of exogenous and endogenous polyP utilization by microorganisms. Furthermore, in culture studies, Moore et al. (2005) found that inorganic tripolyP supported ample growth of picocyanobacteria, including *Synechococcus* WH8102 and several strains of *Prochlorococcus*. Inorganic tripolyP was also bioavailable to coastal phytoplankton communities examined in one field study (Björkman and Karl 1994). PolyP from NTPs appear to be widely bioavailable and may be the most labile form of polyP (Moore et al. 2005; Michelou et al. 2011; Mazard et al. 2012; Duhamel et al. 2014; Evans et al. 2015). Overall, previous studies suggest that organic and inorganic short-chain polyP (tripolyP) are bioavailable to laboratory-grown cyanobacteria and field populations of coastal phytoplankton. However, the bioavailability of exogenous polyP consisting of longer chain lengths is largely unexplored. This knowledge gap may be particularly important if long-chain polyP forms are common in the ocean. For example, long-chain polyP ($\geq$ 15 P atoms) could account for the total polyP content in a variety of marine plankton, bacteria, and sinking particle samples analyzed in one study (Diaz and Ingall 2010).

Despite the potential physiological and biogeochemical importance of marine polyP, the composition, distribution, and dynamics of polyP in the marine environment are not well understood. An improved knowledge of polyP is crucial to determine the potential role of polyP as a biological P source capable of supporting marine primary production and to advance the current understanding of potential mechanisms regulating polyP-driven geologic P sequestration. In this study, the biogeochemistry of long-chain polyP was studied during two cruises conducted in May–June 2013 and September 2013 at Station ALOHA, a long-term oceanographic study site in the North Pacific subtropical gyre (Karl and Church 2014). Laboratory-based experiments with model phytoplankton cultures were also conducted as a complement to field studies to further investigate potential mechanisms of microbial polyP cycling.

Methods

Cruise sampling and field incubations

Sampling was conducted at Station ALOHA (22° 45′N, 158° 00′W) during two cruises in spring 2013 (May–June) and fall 2013 (September) aboard the R/V *Kilo Moana*. In situ seawater sampling was conducted on both cruises using a Niskin rosette sampler equipped with a SBE 911plus CTD (Sea-Bird Electronics). Mixed layer depths were determined for each cast and were defined as the depth corresponding to a density offset of 0.125 kg m^{-3} from the surface (1 dbar). Large-volume incubations were performed during the spring cruise. 20-L polycarbonate carboys ($n = 15$) were filled with surface seawater (~20 m) from the Niskin rosette. Three whole carboys were immediately sampled for the time-zero condition, and the remaining 12 carboys were spiked with

one of the following nutrients (0.2 μm sterile filtered): nitrate (+N; 4 μmol L^{-1} as $NaNO_3$; $n = 9$), or phosphate (+P; 0.3 μmol L^{-1} as $NaH_2PO_4 \cdot H_2O$; $n = 3$). Bottles were incubated on-deck in circulated seawater from the ship's underway intake to maintain in situ temperature. To mimic in situ light conditions (i.e., spectrum and intensity) at the sample collection depth, incubators were covered with blue plastic sheeting (30% light level, Rohm and Haas #2069). After 5 d, whole carboys were sacrificed (+N: $n = 3$; +P: $n = 3$) by sampling as described below, to track particulate polyP:TPP dynamics. The remaining +N bottles ($n = 6$), having been preconditioned to P stress by incubation with nitrate, were spiked at the same time with one of the following nutrients (0.2 μm sterile filtered) to test the bioavailability of exogenous polyP: no addition ($n = 2$), phosphate (0.3 μmol L^{-1} $NaH_2PO_4 \cdot H_2O$; $n = 2$), or polyP (0.3 μmol L^{-1} P; average chain length = 45 ± 5 P atoms; Sigma-Aldrich S4379; $n = 2$). These bottles were incubated for an additional 3 d before being sampled, as detailed below.

Particulate polyP

Four or eight liters of seawater were filtered onto replicate 25 mm or 47 mm GF/F filters, respectively (Whatman). The GF/F filters (0.7 μm nominal pore size) had been previously soaked overnight in 10% HCl, rinsed three times in nanopure water, and muffled (550°C, overnight). Samples were flash frozen in liquid nitrogen and stored at −80°C until analyzed. Samples were analyzed using previously described fluorometric protocols. First, polyP was extracted using the enzymatic protocol of Martin and Van Mooy (2013). Briefly, each filter was subjected to a core extraction protocol consisting of the following steps: (1) sonicate in HEPES buffer (20 mmol L^{-1}, pH = 7), 15 s; (2) boil, 5 min; (3) sonicate, 15 s; (4) add DNase (20 U mL^{-1}; Ambion AM2238) and RNase (205 U mL^{-1}; Ambion AM2286), incubate 10 min, 37°C; (5) add proteinase K (0.4 mg mL^{-1}; Fisher BP1700), incubate 30 min, 37°C while shaking at 300 rpm, (6) centrifuge (16,100 × *g*) and decant. The core extraction protocol was repeated on each sample a total of four times. For each sample, the supernatants generated from first three rounds of the core extraction protocol were pooled to generate the analytical extract, while the forth supernatant was used as the background extract. Next, samples were diluted in HEPES buffer, then DAPI (4′; 6-Diamidino-2-phenylindole; dihydrochloride, Anaspec 83210, 10 μmol L^{-1} final) was added. Samples were reacted for 7 min to allow complete complexation with shorter polyP chain lengths, and fluorescence was measured (ex: 415 nm, em: 550 nm) (Aschar-Sobbi et al. 2008) using a Shimadzu RF-5301PC spectrofluorometer. Sample signals were individually calibrated with the addition of a synthetic polyP standard (chain length = 45 ± 5 P atoms; Sigma-Aldrich S4379). Signals were corrected using several blanks, including the background extract + DAPI, as well as DAPI-free fluorescence readings of the samples. PolyP measured using the enzyme-based extraction approach (Martin and Van Mooy 2013) can yield P molar ratios of polyP:TPP > 1 because natural polyP is thought to fluoresce brighter than the synthetic polyP standard under the conditions of the method (Martin et al. 2014). Thus, polyP content measured with this technique is appropriate for measuring relative, rather than absolute, quantities of polyP [*see* Martin et al. (2014) for full discussion]. A similar fluorometric method used to quantify polyP is insensitive to polyP with chain lengths less than or equal to five P atoms (including NTPs such as ATP) yet detects chain lengths greater than or equal to fifteen P atoms (Diaz and Ingall 2010). Thus, polyP data presented here do not include contributions from NTPs. Rather, our polyP data indicate relative quantities of polyP with chain lengths greater than or equal to approximately 15 P atoms. The in situ detection limits of polyP were different for each sample, depending on the volume filtered as well as the sample's individual calibration, and were typically ~ 0.4 nanomole equivalents of P per liter (neq L^{-1}).

Total particulate phosphorus

Samples for TPP analysis (4 L or 8 L) were collected and stored in the same manner as polyP samples. Samples were analyzed according to the ash-hydrolysis method of Aspila et al. (1976). Briefly, filters were ashed (550°C, 2 h), acidified (1N HCl, room temperature, overnight), and subsequently analyzed as soluble reactive phosphorus (SRP) using the colorimetric molybdate method (Hansen and Koroleff 1999). Duplicate samples typically agreed to within < 5%, and the detection limit was approximately 3 nmol L^{-1}.

Alkaline phosphatase activity

In situ samples (500 mL of seawater) or incubation samples (250 mL) were filtered onto 47 mm polycarbonate membranes (0.2 μm) and stored at −20°C until analysis. APA was assayed after Dyhrman and Ruttenberg (2006) using the fluorogenic phosphatase substrate 6,8-difluoro-4-methylumbelliferyl phosphate.

Soluble reactive phosphorus

Seawater samples were collected from the Niskin rosette bottles into acid cleaned, high density polyethylene bottles and stored upright at −20°C until analysis. The samples were thawed and processed using the MAGnesium Induced Coprecipitation (MAGIC) protocol (Karl and Tien 1992) on triplicate 50 mL subsamples. The analytical precision was ± 1 nmol L^{-1}, with a detection limit of 3 nmol L^{-1}.

Chlorophyll

In the dark, 250 mL of seawater was filtered onto each GF/F filter (25 mm). Samples were stored in the dark at −20°C until analyzed according to protocols adapted from Strickland and Parsons (1972). Briefly, samples were extracted in 90% acetone in the dark (4°C, 9 h) and measured using an AquaFluor fluorometer (Turner). Sample signals were calibrated using a chlorophyll-*a* standard (Sigma-

Aldrich C6144) and were corrected for pheopigments by accounting for the fluorescence of extracts before and after acidification to 0.003 M HCl.

Laboratory bioavailability experiments

Thalassiosira oceanica CCMP1005, *Thalassiosira weissflogii* CCMP1336, *Thalassiosira pseudonana* CCMP1335, and *Emiliania huxleyi* CCMP374 were obtained from the National Center for Marine Algae and Microbiota (NCMA, and formerly the CCMP), Bigelow Laboratories, East Boothbay, Maine. Organisms were grown in f/2 media (Guillard and Ryther 1962; Guillard 1975) prepared with silicic acid (*Thalassiosira*) or without (*E. huxleyi*), using filtered (0.2 μm) Sargasso surface seawater as a base. Sargasso seawater typically has very low total dissolved P concentrations in surface depths ($< \sim$100 nmol L^{-1}; Lomas et al. 2010) and was chosen on that basis to minimize undefined P sources present in the media. Media were prepared in three variations for each organism: no P addition (−P), phosphate (+P; 36 μM P), or inorganic polyP (+polyP; 36 μM P). These replete nutrient concentrations were chosen to examine biological polyP dynamics in a controlled system that yields ample biomass for analysis, rather than to mimic in situ dynamics at Station ALOHA, where natural nutrient levels are much lower. Media were autoclaved (121°C, 20 min), but all vitamins (Guillard and Ryther 1962; Guillard 1975) and polyP sources were filter-sterilized (0.2 μm) and added after autoclaving to avoid potential degradation at high temperatures. PolyP sources had average chain lengths of 130, 60, 45, 15, and 3 P atoms (130polyP, 60polyP, 45polyP, 15polyP, and 3polyP, respectively). 45polyP (S4379) and 3polyP (T5883) were purchased from Sigma-Aldrich, while 130polyP, 60polyP, and 15polyP were generously provided by Dr. Toshikazu Shiba and Dr. Yumi Kawazoe (Regenetiss, Tokyo, Japan).

For every phytoplankton species tested, each media variation was inoculated, in triplicate, with an equal volume of the same parent culture. This transfer was performed using low culture volumes ($\sim$ 100–200 μL in 25 mL media) when the parent culture was in late log or early stationary phase to minimize carryover of dissolved P. Triplicate aliquots of sterile +polyP media were incubated in parallel with each experiment to quantify phosphate impurities in the polyP standards, as well as the abiotic breakdown of polyP to phosphate over time. Subsequent to screening for polyP utilization, a control experiment (+/−P) was performed by cultivating each organism with the total phosphate concentration resulting from the phosphate impurity and abiotic hydrolysis of polyP (+/−P:4 or 5 μM P).

T. oceanica was incubated at 23°C, while *E. huxleyi*, *T. pseudonana*, and *T. weissflogii* were incubated at 18°C. All cultures were grown using 140 μmol photons m^{-2} s^{-1} on a 14:10 h light:dark cycle, and growth was tracked daily by monitoring the relative fluorescence of chlorophyll with a Turner fluorometer (10AU, AquaFluor®, or TD-700). Direct cell counts were performed under transmitted light using a counting chamber. Cultures were confirmed axenic in stationary phase by inoculating $\sim$200 μL into $\sim$10 mL heterotrophic media, incubating in the dark at room temperature, and monitoring for at least one week. Cultures were considered axenic if the media remained visibly clear throughout this timeframe. Heterotrophic media consisted of an artificial seawater base (van Waasbergen et al. 1993), 2 g L^{-1} tryptone (Fisher BP1421), and 0.5 g L^{-1} yeast extract (Fisher BP1422).

Statistical analysis

The effects of sampling season and depth on the water column profiles of particulate polyP:TPP content were analyzed in IBM SPSS Statistics (Version 22) using two-way between groups ANOVA. To identify potential patterns of polyP production and utilization in shipboard incubations, chlorophyll (chl), APA, polyP, TPP, polyP:TPP, polyP:chl, and TPP:chl comparisons were performed with unpaired two-sample *T*-tests assuming equal variance. To examine the bioavailability of polyP in laboratory cultures, cell counts in +polyP media were compared with +P, −P, and +/−P controls using one-way between groups ANOVA (for *T. oceanica* and *T. pseudonana*, since these organisms were grown on multiple polyP chain lengths) or unpaired two-sample *T*-tests assuming equal variance (for *T. weissflogii* and *E. huxleyi*, which were grown on a single polyP chain length).

Results

Water column APA, SRP, polyP, and TPP

Mixed layer depths during the spring and fall cruises were 34 ± 10 m (average ± standard deviation; $n = 52$) and 57 ± 7 m ($n = 27$), respectively. In the upper water column ($\leq$ 150 m), APA was similar during both cruises, ranging from 0.07–0.13 nmol P L^{-1} h^{-1} in the spring and 0.05–0.12 nmol P L^{-1} h^{-1} in the fall (Fig. 1). SRP concentrations ranged from 0.03 μmol L^{-1} to 0.18 μmol L^{-1}; however the depth trends in SRP were opposite between cruises (Fig. 1). In the spring, SRP concentrations in the upper water column (0–100 m) were unusually high (Karl and Tien 1997), whereas the fall SRP profile was more typical, based on long-term observations of SRP at Station ALOHA by the Hawaii Ocean Time-series (HOT) program. Thus, these results do not necessarily reflect a seasonal trend in SRP and are likely a product of shorter-term variability.

Similar to other studies that employed the same polyP extraction technique [e.g., Martin et al. (2014)], polyP content is expressed in relative rather than absolute units (*see* Methods). Specifically, polyP is presented as nanomole equivalents of phosphorus per liter (neq L^{-1}), and polyP:TPP is expressed as mole equivalents of phosphorus per mole of TPP (eq mol^{-1}). PolyP data presented here indicate relative quantities of polyP with chain lengths greater than or equal to approximately 15 P atoms (Diaz and Ingall 2010). Particulate polyP content in the upper water column

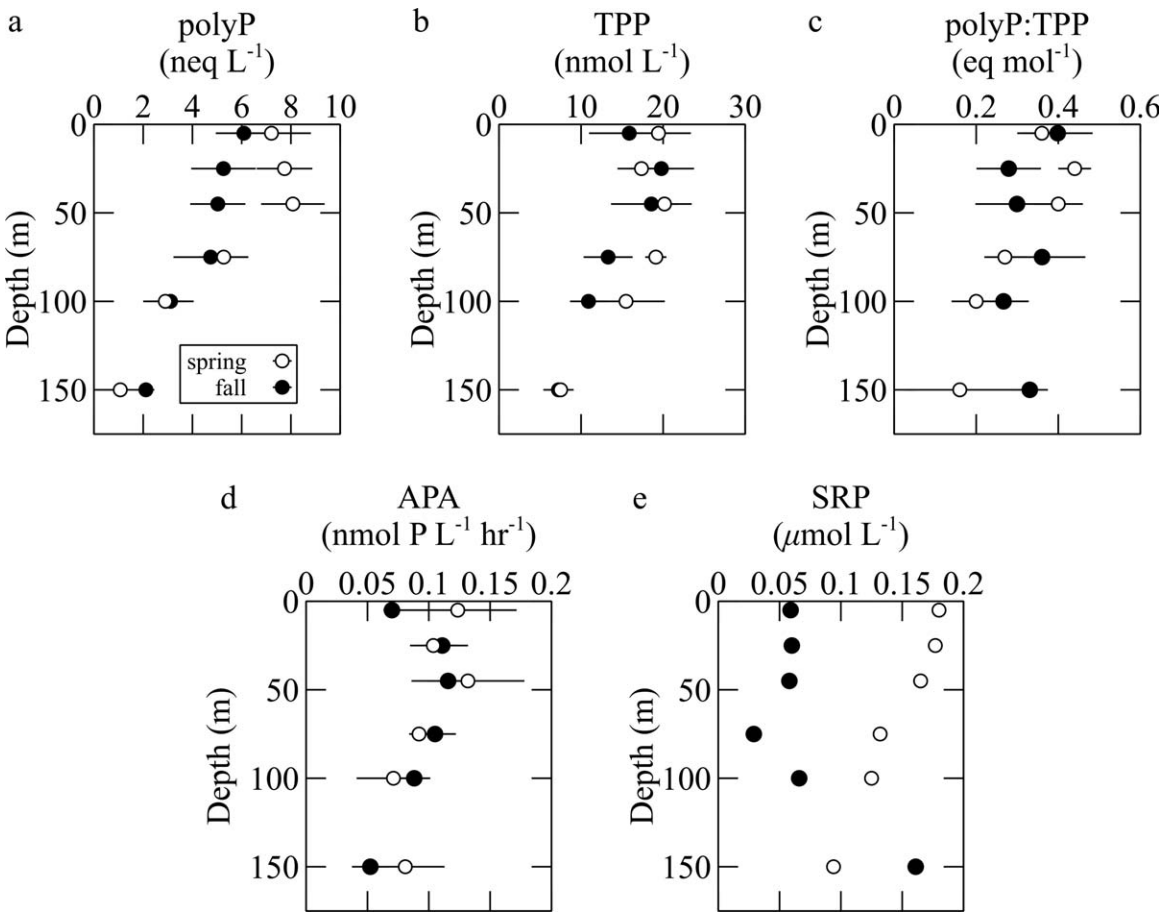

Fig. 1. Water column profiles of (**a**) particulate polyphosphate (polyP), (**b**) total particulate phosphorus (TPP), (**c**) polyP:TPP, (**d**) alkaline phosphatase activity (APA), and (**e**) soluble reactive phosphorus (SRP) in the upper 150 m of Station ALOHA during cruises conducted in spring and fall 2013. Error bars represent one standard error of the mean. Sample sizes: polyP, $n = 3$–6; TPP, $n = 3$–4; APA, $n = 2$; SRP, $n = 2$–4.

($\leq$ 150 m) was similar in both seasons and decreased with depth: 1.1–8.1 neq L^{-1} during the spring cruise and 2.1–6.1 neq L^{-1} during fall (Fig. 1). TPP also exhibited decreasing concentration with depth, which was similar in both seasons: 7.5–20.2 nmol L^{-1} during spring and 7.3–19.8 nmol L^{-1} during fall (Fig. 1).

Although not indicative of absolute quantities, P molar ratios of polyP:TPP were 0.16–0.44 eq mol^{-1} during spring and 0.27–0.40 eq mol^{-1} during fall (Fig. 1). PolyP:TPP content did not vary significantly with depth when data from each cruise were considered together or separately ($F_{5, 55} = 1.32$; $p = 0.28$). PolyP:TPP values fell within the range of expectation, given the APA and SRP at Station ALOHA and the previous relationships among polyP:TPP, APA, and SRP described for the Sargasso Sea and temperate western North Atlantic (TWNA) (Fig. 2).

PolyP production in shipboard incubations

PolyP production mechanisms were examined in shipboard incubations of surface seawater amended with nitrate (+N) or phosphate (+P). In +P incubations, TPP and polyP increased after 5 d by approximately 1.5- and 2-fold, respectively, whereas chlorophyll decreased by roughly 40% (Fig. 3). PolyP:chl and polyP:TPP increased by a factor of ~4.0 and ~1.4, respectively. Although not statistically significant ($p > 0.05$), these average trends are consistent with a rise in cellular polyP quotas driven by increased P availability.

In the +N treatment, polyP and TPP continually increased (Fig. 3). On day five, polyP:TPP dropped by ~25% compared with in situ levels, which was accompanied by a ~7-fold increase in APA. These results are consistent with utilization of polyP stores with increased P stress. By day eight, however, average polyP:TPP in +N treatments had increased by a factor of ~1.7 above day five. This rise in polyP:TPP also coincided with a dramatic increase in APA (~4-fold) and chlorophyll (~6-fold). These trends are consistent with observations of polyP:TPP enrichment under P stress (Orchard et al. 2010; Dyhrman et al. 2012; Martin et al. 2014). In such cases, cellular polyP concentrations may decline, but there is typically a larger decrease in cellular TPP, reflecting the preferential retention of polyP relative to other P-containing biochemicals (Martin et al. 2014), such as

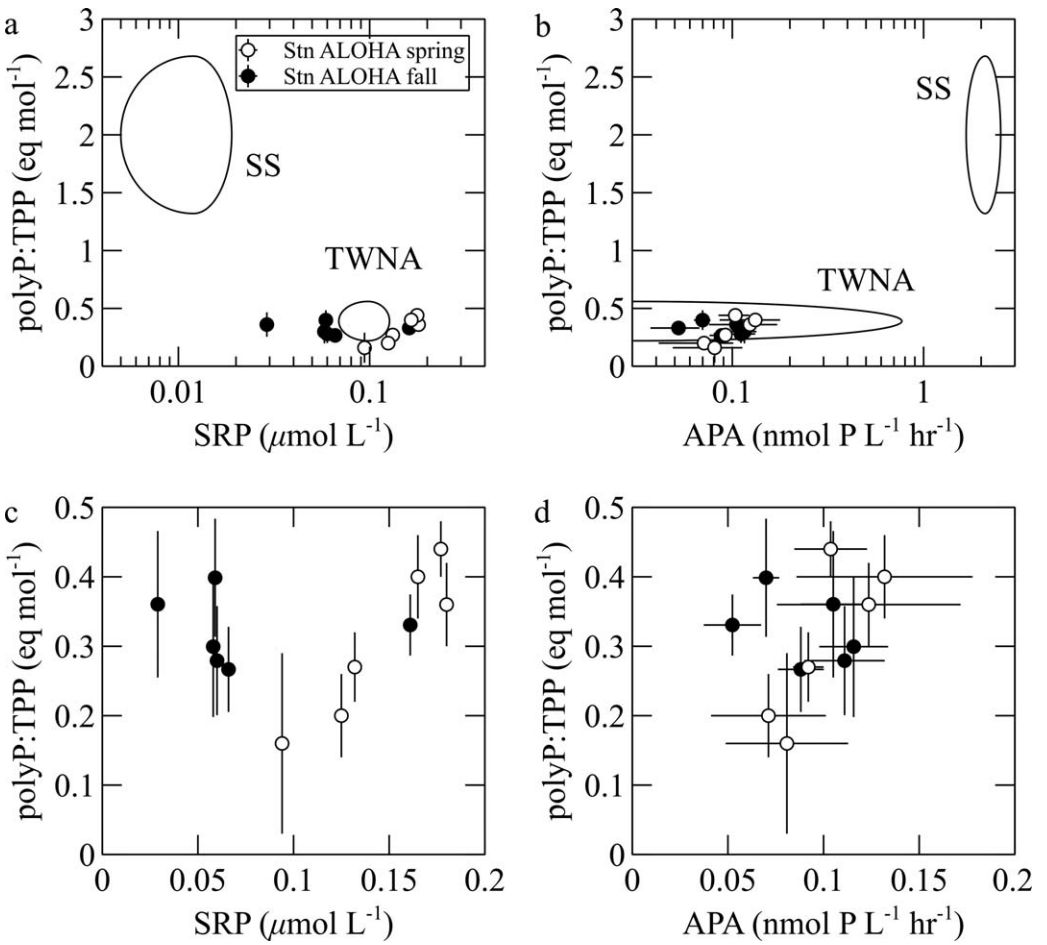

Fig. 2. Ratios of particulate polyphosphate to total particulate phosphorus (polyP:TPP), soluble reactive phosphorus (SRP), and alkaline phosphatase activity (APA) from Station ALOHA (**a**–**d**) and previously reported from Sargasso Sea (SS) and temperate western North Atlantic (TWNA) (**a** and **b**) (Martin et al. 2014). Error bars represent one standard error of the mean. Sample sizes for Station ALOHA data: polyP, $n = 3$–6; TPP, $n = 3$–4; APA, $n = 2$; SRP, $n = 2$–4.

RNA and P-lipids, the latter of which can be substituted with non-P-containing forms to reduce cellular total P quotas (Van Mooy et al. 2009). For example, although not statistically significant ($p > 0.05$), polyP:chl decreased by ~75%, whereas TPP:chl decreased by ~83% between days five and eight, suggesting that polyP:TPP enrichment could be explained by a relatively more dramatic drop in cellular TPP than polyP. The increase in chlorophyll observed throughout the +N incubations (Fig. 3) is consistent with an increasing abundance of phytoplankton yet may also involve some degree of chlorophyll storage (i.e., increasing cellular chlorophyll quotas).

PolyP utilization in shipboard incubations

To examine the potential of the photosynthetic community at Station ALOHA to utilize polyP as a P source for growth, seawater was preincubated with nitrate to drive the community to P stress. Indeed, nitrate amendments increased APA above in situ levels after 5 d (Fig. 3). Following this preconditioning step, the addition of exogenous polyP (average chain length = 45 ± 5 P atoms) only supported ~40% as much photosynthetic growth as experiments that received the phosphate addition. In fact, chlorophyll content in polyP-amended carboys was even lower than in carboys that received no P addition (Fig. 4). PolyP addition also did not lower APA. For example, APA in polyP-amended bottles increased by a factor of approximately 3.0 after 3 d, similar to the unamended controls, in which APA increased ~3.7 times (Fig. 4). APA also increased after 3 d in response to phosphate addition, but only by ~1.7 times.

Bioavailability of exogenous polyP to lab cultures

Growth of *T. oceanica* and *E. huxleyi* cultivated using polyP as a sole source of P was consistent with growth observed using an equivalent P molar quantity of phosphate (*T. oceanica:* $F_{5,\ 11} = 1.27$; $p = 0.34$; *E. huxleyi*: $T = 1.26$, df = 4, $p = 0.28$; Fig. 5; Table 1). In contrast, growth by

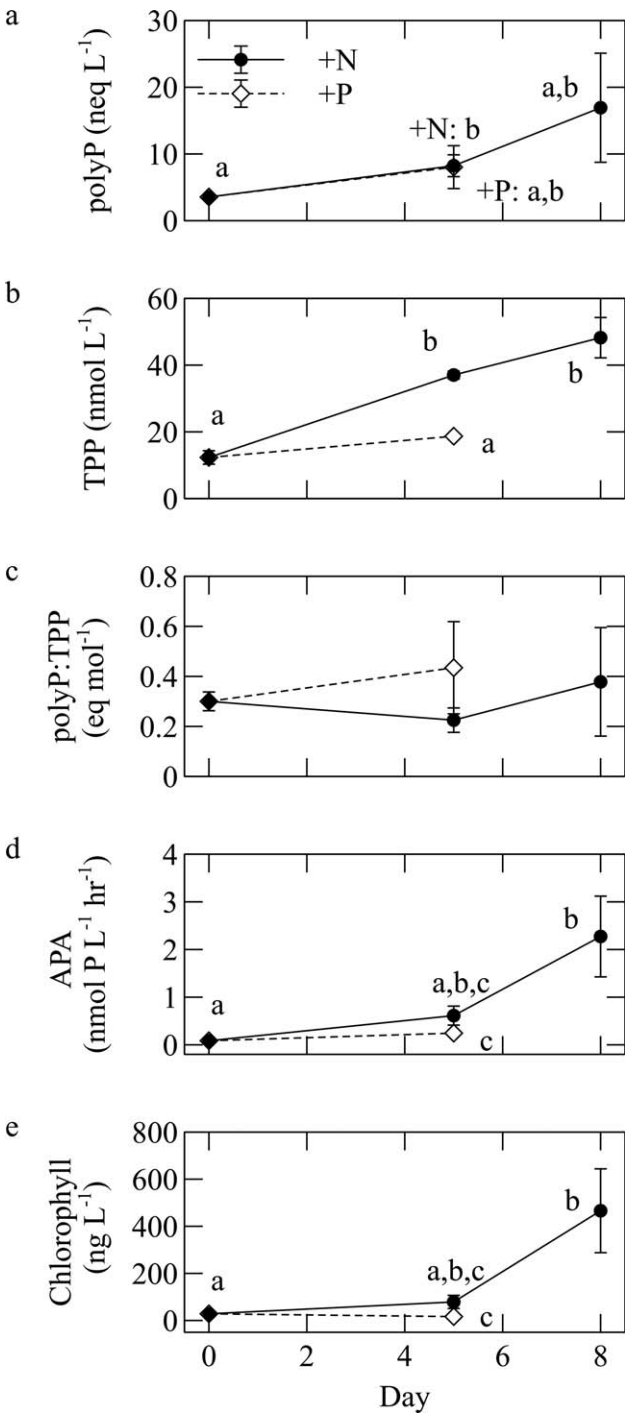

Fig. 3. Polyphosphate (polyP) (**a**), total particulate phosphorus (TPP) (**b**), polyP:TPP (**c**), alkaline phosphatase activity (APA) (**d**), and chlorophyll (**e**) in shipboard incubations amended with either nitrate (+N) or phosphate (+P). Any two samples in the same panel are significantly different ($p < 0.05$) only if they lack a common letter. Error bars represent one standard error of the mean, $n = 2$–3.

T. pseudonana and *T. weissflogii* was significantly lower on polyP than phosphate (*T. pseudonana*: $F_{5,12} = 8.97$, $p = 1.00 \times 10^{-5}$; *T. weissflogii*: $T = 16.0$, df = 4, $p = 8.0 \times 10^{-5}$; Fig. 5;

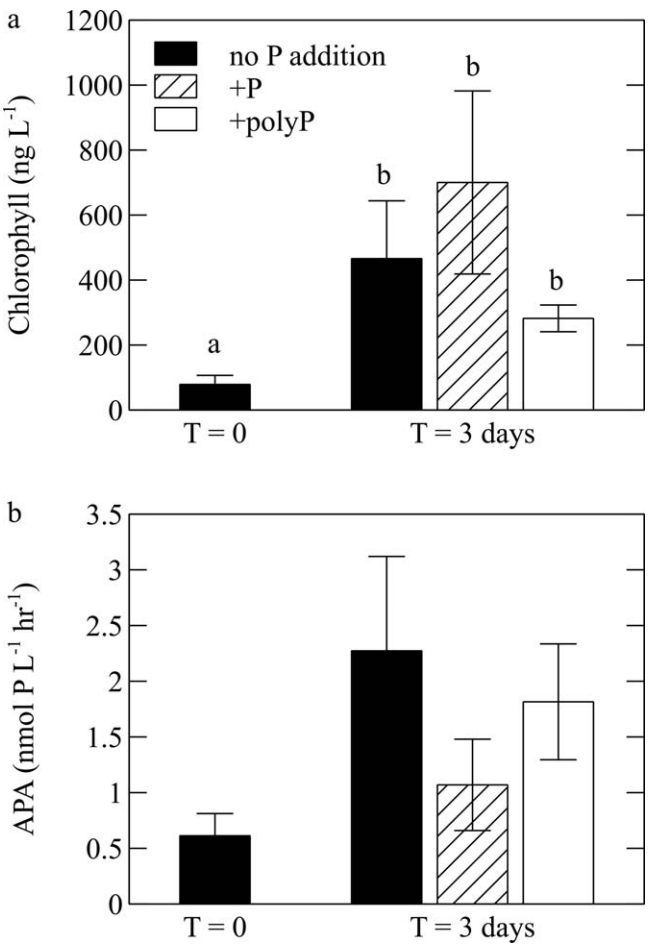

Fig. 4. Chlorophyll (**a**) and alkaline phosphatase activity (APA) (**b**) in shipboard incubations preconditioned to P stress with nitrate for 5 d and subsequently amended with phosphate (+P), polyP (+polyP; average chain length of 45 ± 5 P atoms), or no P addition. Any two samples in the same panel are significantly different ($p < 0.05$) only if they lack a common letter. Error bars represent one standard error of the mean, $n = 2$–3.

Table 1). Cell counts confirmed that growth in −P media was significantly less than growth observed in all polyP cultures (*T. oceanica*: $F_{5,11} = 9.21$, $p = 1.0 \times 10^{-3}$; *T. pseudonana*: $F_{5,12} = 8.65$; $p = 1.0 \times 10^{-3}$; *T. weissflogii*: $T = 5.26$, df = 4, $p = 6.20 \times 10^{-3}$; *E. huxleyi*: $T = 8.70$, df = 4, $p = 1.00 \times 10^{-3}$), demonstrating that growth in +polyP media is attributable to the polyP amendment rather than P in the media base or carried over from the parent culture. In abiotic control experiments with sterile +polyP media, SRP concentrations at time-zero were 0.7 ± 0.4 μmol L^{-1} (all chain lengths), reflecting a phosphate impurity in the polyP standards. However, at the conclusion of culture experiments (15–26 d), SRP concentrations in sterile +polyP media had increased to 3.5 ± 1.0 μmol L^{-1}, which did not vary systematically with chain length. This result indicated that ~8% of P originally present as polyP had hydrolyzed to SRP by the end of the culture experiments. Combined with the phosphate impurity

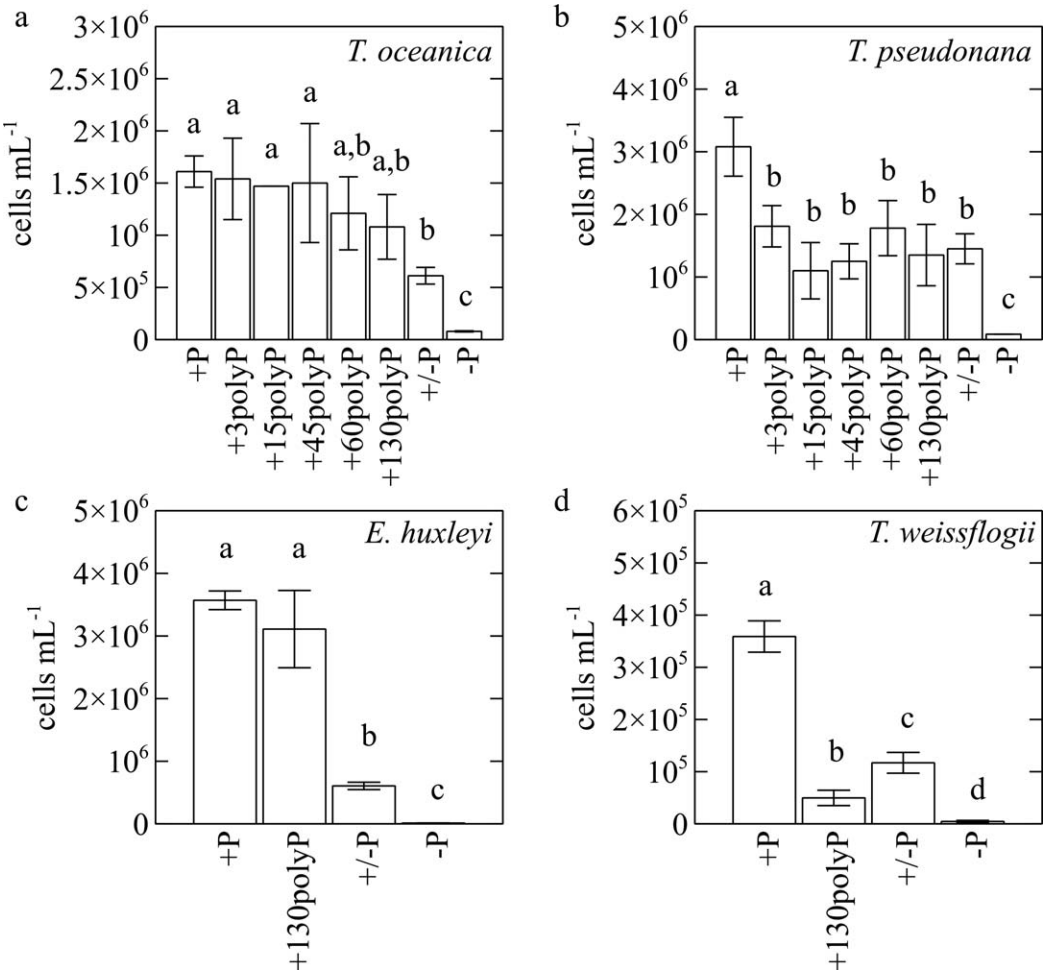

Fig. 5. Growth yields of *Thalassiosira* spp. (**a**, **b**, and **d**) and *E. huxleyi* (**c**) cultivated with various polyphosphate (polyP) chain lengths ranging from 3–130 P atoms (36 μmol L^{-1} P), phosphate (+P = 36 μmol L^{-1} P; +/−P = 5 μmol L^{-1} P for *Thalassiosira* spp.; +/−P = 4 μmol L^{-1} P for *E. huxleyi*), or no P amendment (−P). Any two samples in the same panel are significantly different ($p < 0.05$) only if they lack a common letter. Error bars represent one standard deviation of the mean, $n = 3$.

Table 1. Growth capability of representative marine phytoplankton on various inorganic polyphosphate (polyP) sources. +, growth; -, no growth; nt, not tested.

	polyP chain length (number of P atoms)				
	3	**15**	**45**	**60**	**130**
Prochlorococcus (MED4, MIT9312, MIT9313)*	+	nt	nt	nt	nt
Synechococcus WH8102*	+	nt	nt	nt	nt
Emiliania huxleyi CCMP374	nt	nt	nt	nt	+
Thalassiosira oceanica CCMP1005	+	+	+	+	+
Thalassiosira pseudonana CCMP1335	-	-	-	-	-
Thalassiosira weissflogii CCMP1336	nt	nt	nt	nt	-

*From Moore et al. (2005).

in the polyP standards, this hydrolysis likely supported some growth. Indeed, final cell counts revealed that a comparable level of phosphate (5 μmol L^{-1}) could support growth similar to that observed in all +polyP cultures of *T. pseudonana* and *T. weissflogii* (*T. pseudonana*: $F_{5,11} = 1.59$, $p = 0.24$; *T. weissflogii*: $T = -3.93$, df = 3, $p = 0.99$). However, abiotic polyP degradation was unlikely to explain growth of *T. oceanica* ($F_{5,\ 10} = 2.66$; $p = 0.09$) and *E. huxleyi* ($T = 5.43$, df = 3, $p = 0.01$) on exogenous polyP (Fig. 5). Overall, chain length had no impact on growth (Table 1). In addition to *T. oceanica* and *E. huxleyi*, polyP (in the form of tripolyP) was also bioavailable to picocyanobacteria, based on results from a previous study (Table 1).

Discussion

PolyP is a key component of the marine P cycle, both as a potentially bioavailable P source (Moore et al. 2005; Martin

et al. 2014) and as a possible intermediate in geologic P sequestration (Schulz and Schulz 2005; Diaz et al. 2008). Despite the importance of marine polyP, its distributions and dynamics are not completely understood. Much of what is currently known about marine polyP has been learned from ^{31}P-NMR spectroscopy. For example, based on ^{31}P-NMR, polyP (inorganic + organic) represents ~1–11% of total P in plankton, dissolved matter, particles, and sediments in a range of ocean environments (Paytan et al. 2003; Sannigrahi and Ingall 2005; Diaz et al. 2008; Young and Ingall 2010; Diaz et al. 2012). However, the detection of natural polyP using ^{31}P-NMR is difficult due to high background signals from more abundant P forms, and large sample requirements (e.g., ~mg's of dry material for solid state ^{31}P-NMR) are often impractical for high-resolution sampling. Indeed, polyP was not detected in dissolved or particulate matter at Station ALOHA using ^{31}P-NMR (Sannigrahi et al. 2006), despite the presence of dissolved and particulate NTPs such as ATP (~1–100 pmol L^{-1}; Björkman and Karl 2005) and the presence of particulate long-chain polyP described herein.

Recently developed fluorometric protocols have made the detection of inorganic, long-chain polyP ($\geq$ 15 P atoms) more tractable (Aschar-Sobbi et al. 2008; Diaz and Ingall 2010; Martin and Van Mooy 2013). However, only relative quantities of polyP can be measured using the most sensitive extraction approach currently available (Martin and Van Mooy 2013; Martin et al. 2014). Here, the application of this technique revealed relative levels of particulate polyP:TPP that ranged from 0.16 eq mol^{-1} to 0.44 eq mol^{-1} within the upper water column ($\leq$ 150 m) of Station ALOHA during spring and fall 2013. These bulk measurements likely include contributions from living and non-living particulate material. For example, more than half of particulate carbon at Station ALOHA is probably present as non-living detritus, which may also account for a substantial amount of particulate P. If so, our results are likely indicative of combined intracellular (living) and extracellular (non-living or detrital) pools of polyP. Although these polyP:TPP levels do not reflect absolute quantities, particulate inorganic polyP may represent as much as ~10% of TPP at Station ALOHA, based on the observation that NTPs represent ~0.9–2.4% of TPP (Björkman and Karl 2005) and assuming that total polyP is ~1–11% of TPP, as observed in a number of other ocean environments (Paytan et al. 2003; Diaz et al. 2008).

Profound differences in the relative quantities and dynamics of particulate, long-chain polyP were recently reported along a transect from the eutrophic TWNA to the oligotrophic Sargasso Sea (Martin et al. 2014). PolyP:TPP was much higher in the Sargasso Sea than the TWNA, tracking directly with conventional indicators of P stress in the microbial community (e.g., APA) and inversely with SRP (Martin et al. 2014). PolyP:TPP levels at Station ALOHA are consistent with the trends observed in the Atlantic. In particular, polyP:TPP values at Station ALOHA are similar to values reported from the eutrophic TWNA, yet they are distinct from the ultra-oligotrophic (i.e., P-stressed) Sargasso Sea, which exhibits particulate polyP:TPP values ~6-fold higher than Station ALOHA (Martin et al. 2014). Furthermore, the lack of chlorophyll stimulation by addition of phosphate to shipboard incubations is consistent with a lack of in situ P stress and thus relatively low polyP:TPP content at Station ALOHA. Dramatic increases in polyP:TPP were previously observed below the SRP threshold of 25 nmol L^{-1} in the Atlantic Ocean (Martin et al. 2014). SRP was never this low during either cruise in this study (minimum SRP = 29 ± 1 nmol L^{-1}). Moreover, historical data collected at Station ALOHA over 27 yr of the HOT program indicate that only ~15% of SRP measurements within the upper 150 m (the maximum depth sampled in this study) fall below 25 nmol L^{-1} (http://hahana.soest.hawaii.edu/hot/hot-dogs/13 Feb 2015). Thus, if this SRP threshold is truly an indicator of polyP content, then polyP:TPP levels at Station ALOHA may only occasionally reach levels comparable to the Sargasso Sea. Recent anthropogenic increases in nitrate at Station ALOHA, however, may ultimately drive the system to P limitation (Kim et al. 2014), which could eventually lead to elevated polyP:TPP levels similar to the Sargasso Sea.

Depth distributions of particulate polyP:TPP at Station ALOHA did not change significantly within the top 150 m, suggesting that long-chain polyP is preserved with depth relative to other forms of TPP. This result is in contrast to depth-dependent decreases in bulk particulate polyP:TPP observed in the Sargasso Sea, which were interpreted to indicate net biological polyP utilization in this P-stressed region primed to scavenge P (Martin et al. 2014). Similarly, the absence of strong depth-dependent polyP:TPP attenuation at Station ALOHA may be an imprint of the lack of P stress in that system, as other P sources (e.g., phosphate and NTPs) may be sufficient to repress long-chain polyP utilization in situ. Consistent with this possibility, results from shipboard incubations presented here and in previous studies (Duhamel et al. 2010) suggest that the in situ microbial community at Station ALOHA is not P-stressed. However, these observations cannot rule out the possibility that some portion of the microbial population at Station ALOHA may be producing polyP while others are hydrolyzing it at a similar rate. Indeed, the net concentration of NTPs does not change substantially with depth within the upper 150 m, although these P sources are biologically utilized (Björkman and Karl 2005).

To help clarify potential pathways of biological polyP cycling, shipboard incubations were performed. In bioavailability experiments, the addition of dissolved polyP (average chain length = 45 ± 5 P atoms) did not stimulate photosynthetic growth, despite preconditioning the community to levels of P stress that are associated with polyP utilization in the Sargasso Sea (Martin et al. 2014). This result suggests that long-chain polyP is not bioavailable to phytoplankton at Station ALOHA. Long-chain polyP is likely degraded to phosphate extracellularly

by surface-associated or cell-free enzymes (Young and Ingall 2010), potentially including alkaline phosphatases (Karl and Craven 1980). The bulk APA measured in these experiments includes contributions from the entire microbial community (phytoplankton + heterotrophs), whereas polyP bioavailability was only examined for phytoplankton. Yet regardless of the ultimate microbial source of APA and its potential role in polyP degradation, the biological utilization of polyP would have led to the reduction of APA. However, polyP did not lower the APA induced in these incubations, reinforcing the conclusion that polyP is not bioavailable to the microorganisms making the enzyme. Furthermore, phytoplankton species composition in these incubations may have been different than the in situ microbial community, as seen in previous studies (McAndrew et al. 2007; Mahaffey et al. 2012), which may have influenced the bioavailability of polyP.

The lack of polyP utilization by phytoplankton in field incubations is surprising because tripolyP is bioavailable to various strains of *Procholorococcus marinus* and *Synechococcus* WH8102 (Moore et al. 2005) as well as coastal phytoplankton communities (Björkman and Karl 1994). To broaden the diversity of model phytoplankton examined for polyP bioavailability and to test the possible effects of polyP chain length on bioavailability, several eukaryotic phytoplankton cultures were screened for the ability to utilize multiple polyP sources. Although less abundant than *Prochlorococcus* and *Synechococcus*, diverse eukaryotic phytoplankton including diatoms and haptophytes are ubiquitous and dynamic members of the phytoplankton community at Station ALOHA (Li et al. 2013). Furthermore, these groups are known to dominate incubations enriched in nitrogen such as those performed here (McAndrew et al. 2007; Mahaffey et al. 2012). Laboratory cultivation experiments revealed that the diatoms *T. pseudonana* CCMP1335 and *T. weissflogii* CCMP1336 could not access exogenous polyP as a sole P source, whereas the closely related diatom *T. oceanica* CCMP1005 and the coccolithophore *E. huxleyi* CCMP374 can utilize polyP as a sole P source. Results also indicated that polyP chain length does not affect its bioavailability. The growth of *T. oceanica* and *E. huxleyi* in media prepared with polyP probably cannot be explained by accelerated abiotic polyP hydrolysis through secondary reactions unaccounted for in abiotic controls. For example, the main factors determining the stability of polyP solutions are temperature, pH, and polyP concentration (Rashchi and Finch 2000). Initial polyP concentration was identical in cultures and abiotic controls, as was temperature. The pH of starting media was in the typical range of natural seawater (~8–8.5) and batch cultures of these organisms can reach pH ~9 by stationary phase. However, abiotic polyP hydrolysis is at a minimum at pH 9 (Rashchi and Finch 2000). Overall, these data indicate that utilization of exogenous polyP may be species-specific and that the biological recycling of exogenous polyP, when present, may be dependent on microbial community composition. In turn, polyP may help determine microbial community composition in areas where polyP utilization is more likely to be an important mode of P acquisition due to higher levels of P stress, such as in the Sargasso Sea.

In addition to potential pathways of exogenous polyP utilization, shipboard incubations were conducted to investigate polyP production dynamics at Station ALOHA. A constitutive level of polyP is likely to be present in all cells, given its broad and vital functions [*see* Martin et al. (2014) and references therein], and this baseline state may change as a function of physiological and biogeochemical conditions. For instance, in marine systems such as the Sargasso Sea, as well as the phytoplankton *Trichodesmium* and *T. pseudonana*, strong polyP:TPP enrichment has been observed in response to P-depleted status (Orchard et al. 2010; Dyhrman et al. 2012; Martin et al. 2014). In these scenarios, reductions in P-rich biochemicals like lipids and RNA decrease cellular TPP relative to cellular polyP in response to P stress, resulting in elevated polyP:TPP (Orchard et al. 2010; Dyhrman et al. 2012; Martin et al. 2014). Indeed, incubation results are consistent with P stress mechanisms of polyP:TPP enrichment at Station ALOHA, as well as shifting cellular P quotas observed in P-stressed phytoplankton cultures. Although APA levels in shipboard incubations were driven to Sargasso-like values (~2 nmol P L^{-1} h^{-1}), the average polyP:TPP was still a factor of ~5 lower than the average levels observed in the Sargasso Sea. This discrepancy in polyP:TPP levels, despite similar APA, may reflect differences in community composition and/or bottle effects associated with nitrate addition and subsequent P stress in Station ALOHA incubations, which is likely to be different than the long term in situ response of a different microbial community to P stress in the Sargasso Sea.

Beyond P stress mechanisms, other modes of polyP:TPP enrichment include luxury and overplus strategies, which are associated with P-replete status and the transition from P-deplete to P-replete conditions, respectively. Incubation results were also consistent with such polyP accumulation responses at Station ALOHA. In fact, luxury/overplus polyP accumulation has been hypothesized to occur in the North Pacific subtropical gyre (Karl et al. 1992) when phytoplankton access nutrient-rich deep waters during vertical migration through the water column (Villareal et al. 1993, 1999). Although polyP accumulation under increased phosphate availability has been discounted in the Sargasso Sea (Martin et al. 2014), these pathways cannot be discounted here. Taken together, these data suggest that increases in polyP:TPP at Station ALOHA may be driven by P stress-induced changes in cellular P composition, as previously observed in the Sargasso Sea, as well as luxury/overplus synthesis.

In addition to polyP distributions and dynamics, results from this study also have broad implications for polyP composition, which may affect the function and reactivity of intra- and extracellular polyP. Microorganisms synthesize a wide variety of polyP chain lengths, which range from three

to several hundred P atoms in yeast and *E. coli* (Kornberg et al. 1999), depending on the cellular localization and function of polyP (Rao et al. 2009), as well as external nutrient availability (Vagabov et al. 2000). However, very little is currently known about the chain length distribution of polyP and the biogeochemical processes that may alter polyP chain lengths in marine systems. The polyP detection technique used in this study is only sensitive to chain lengths greater than 15 P atoms, thus indicating the presence of medium- to long-chain polyP in particulate matter at Station ALOHA. Shorter polyP chain lengths could be comparatively abundant, but these forms are unlikely to alter the relative distribution of polyP. The concentration of polyP as NTPs is relatively low (< 3% TPP) and although NTPs are cycled rapidly (i.e., complete turnover in hours to days), the net concentration of NTPs does not change substantially with depth within the upper 150 m (Björkman and Karl 2005). Furthermore, because polyP chain length does not affect its bioavailability in the test cultures herein, the chain length distribution of exogenous polyP is unlikely to be heavily influenced by biological utilization. Rather, biological production and perhaps currently unknown abiotic polyP degradation mechanisms are more likely to influence the chain length distributions of particulate and dissolved polyP in the ocean.

Conclusions

Results from this study support the emerging paradigm that bulk P availability affects the content and dynamics (biological production and utilization) of particulate polyP in the ocean, with lower polyP:TPP levels and less intense production and utilization of polyP being characteristic of systems with relatively higher P availability, including Station ALOHA. PolyP is likely to support more primary productivity in areas with overall lower P availability compared to areas with higher P availability, not only due to the absence of more labile P sources (e.g., phosphate) but perhaps also because of a higher bioavailability to the resident microbial community. In the same way, polyP is more likely to be preserved in areas with overall higher P availability, due to the presence of more labile P phases and perhaps a lower inherent polyP bioavailability. Thus, polyP-driven mechanisms of P sequestration [i.e., polyP-mediated apatite formation (Diaz et al. 2008)] may be more prevalent in systems with higher P content.

References

Armstrong, F. A. J., P. M. Williams, and J. D. H. Strickland. 1966. Photo-oxidation of organic matter in sea water by ultra-violet radiation, analytical and other applications. Nature **211**: 481–483. doi:10.1038/211481a0

Aschar-Sobbi, R., A. Y. Abramov, C. Diao, M. E. Kargacin, G. J. Kargacin, R. J. French, and E. Pavlov. 2008. High sensitivity, quantitative measurements of polyphosphate using a new DAPI-based approach. J. Fluoresc. **18**: 859–866. doi:10.1007/s10895-008-0315-4

Aspila, K. I., H. Agemian, and A. S. Y. Chau. 1976. A semiautomated method for determination of inorganic, organic and total phosphate in sediments. Analyst **101**: 187–197. doi:10.1039/an9760100187

Benitez-Nelson, C. R. 2000. The biogeochemical cycling of phosphorus in marine systems. Earth Sci. Rev. **51**: 109–135. doi:10.1016/S0012-8252(00)00018-0

Björkman, K. M. 2014. Polyphosphate goes from pedestrian to prominent in the marine P-cycle. Proc. Natl. Acad. Sci. USA **111**: 7890–7891. doi:10.1073/pnas.1407195111

Björkman, K., and D. M. Karl. 1994. Bioavailability of inorganic and organic phosphorus compounds to natural assemblages of microorganisms in Hawaiian coastal waters. Mar. Ecol. Prog. Ser. **111**: 265–273. doi:10.3354/meps111265

Björkman, K. M., and D. M. Karl. 2005. Presence of dissolved nucleotides in the North Pacific Subtropical Gyre and their role in cycling of dissolved organic phosphorus. Aquat. Microb. Ecol. **39**: 193–203. doi:10.3354/ame039193

Brown, M. R. W., and A. Kornberg. 2004. Inorganic polyphosphate in the origin and survival of species. Proc. Natl. Acad. Sci. USA **101**: 16085–16087. doi:10.1073/pnas.0406909101

Diaz, J., E. Ingall, C. Benitez-Nelson, D. Paterson, M. D. de Jonge, I. McNulty, and J. A. Brandes. 2008. Marine polyphosphate: A key player in geologic phosphorus sequestration. Science **320**: 652–655. doi:10.1126/science.1151751

Diaz, J. M., and E. D. Ingall. 2010. Fluorometric quantification of natural inorganic polyphosphate. Environ. Sci. Technol. **44**: 4665–4671. doi:10.1021/es100191h

Diaz, J. M., E. D. Ingall, S. D. Snow, C. R. Benitez-Nelson, M. Taillefert, and J. A. Brandes. 2012. Potential role of inorganic polyphosphate in the cycling of phosphorus within the hypoxic water column of Effingham Inlet, British Columbia. Global Biogeochem. Cycles **26**: GB2040. doi:10.1029/2011GB004226

Duhamel, S., S. T. Dyhrman, and D. M. Karl. 2010. Alkaline phosphatase activity and regulation in the North Pacific Subtropical Gyre. Limnol. Oceanogr. **55**: 1414–1425. doi:10.4319/lo.2010.55.3.1414

Duhamel, S., K. M. Björkman, J. K. Doggett, and D. M. Karl. 2014. Microbial response to enhanced phosphorus cycling in the North Pacific Subtropical Gyre. Mar. Ecol. Prog. Ser. **504**: 43–58. doi:10.3354/meps10757

Dyhrman, S. T., and K. C. Ruttenberg. 2006. Presence and regulation of alkaline phosphatase activity in eukaryotic phytoplankton from the coastal ocean: Implications for dissolved organic phosphorus remineralization. Limnol. Oceanogr. **51**: 1381–1390. doi:10.4319/lo.2006.51.3.1381

Dyhrman, S. T., P. D. Chappell, S. T. Haley, J. W. Moffett, E. D. Orchard, J. B. Waterbury, and E. A. Webb. 2006. Phosphonate utilization by the globally important marine diazotroph *Trichodesmium*. Nature **439**: 68–71. doi:10.1038/nature04203

Dyhrman, S. T., and others. 2012. The transcriptome and proteome of the diatom *Thalassiosira pseudonana* reveal a diverse phosphorus stress response. PLoS ONE **7**: e33768. doi:10.1371/journal.pone.0033768

Evans, C., P. R. Gómez-Pereira, A. P. Martin, D. J. Scanlan, and M. V. Zubkov. 2015. Photoheterotrophy of bacterioplankton is ubiquitous in the surface oligotrophic ocean. Prog. Oceanogr. **135**: 139–145. doi:10.1016/j.pocean.2015.04.014

Guillard, R. R. L. 1975. Culture of phytoplankton for feeding marine invertebrates, p. 29–60. In W. L. Smith and M. H. Chanley [eds.], Culture of marine invertebrate animals. Plenum Press.

Guillard, R. R. L., and J. H. Ryther. 1962. Studies of marine planktonic diatoms: I. *Cyclotella nana* Hustedt and *Detonula confervacea* (Cleve) Gran. Can. J. Microbiol. **8**: 229–239. doi:10.1139/m62-029

Hansen, H. P., and F. Koroleff. 1999. Determination of dissolved inorganic phosphate, p. 170–174. In K. Grasshoff, K. Kremling and M. Ehrhardt [eds.], Methods of seawater analysis. Wiley.

Karl, D. M. 2014. Microbially mediated transformations of phosphorus in the sea: New views of an old cycle. Annu. Rev. Mar. Sci. **6**: 279–337. doi:10.1146/annurev-marine-010213-135046

Karl, D. M., and D. B. Craven. 1980. Effects of alkaline phosphatase activity on nucleotide measurements in aquatic microbial communities. Appl. Environ. Microbiol. **40**: 549–561.

Karl, D. M., and G. Tien. 1992. MAGIC: A sensitive and precise method for measuring dissolved phosphorus in aquatic environments. Limnol. Oceanogr. **37**: 105–116. doi:10.4319/lo.1992.37.1.0105

Karl, D. M., R. Letelier, D. V. Hebel, D. F. Bird, and C. D. Winn. 1992. *Trichodesmium* blooms and new nitrogen in the North Pacific Gyre, p. 219–237. In E. J. Carpenter, D. G. Capone, and J. G. Rueter [eds.], Marine pelagic cyanobacteria: *Trichodesmium* and other diazotrophs. Springer Netherlands.

Karl, D. M., and G. Tien. 1997. Temporal variability in dissolved phosphorus concentrations in the subtropical North Pacific Ocean. Mar. Chem. **56**: 77–96. doi:10.1016/s0304-4203(96)00081-3

Karl, D. M., L. Beversdorf, K. M. Björkman, M. J. Church, A. Martinez, and E. F. DeLong. 2008. Aerobic production of methane in the sea. Nat. Geosci. **1**: 473–478. doi:10.1038/ngeo234

Karl, D. M., and M. J. Church. 2014. Microbial oceanography and the Hawaii Ocean Time-series programme. Nat. Rev. Microbiol. **12**: 699–713. doi:10.1038/nrmicro3333

Karl, D. M., and K. M. Björkman. 2015. Dynamics of dissolved organic phosphorus, p. 233–334. In D. A. Hansell and C. A. Carlson [eds.], Biogeochemistry of marine dissolved organic matter. Academic Press.

Kim, I.-N., K. Lee, N. Gruber, D. M. Karl, J. L. Bullister, S. Yang, and T.-W. Kim. 2014. Increasing anthropogenic nitrogen in the North Pacific Ocean. Science **346**: 1102–1106. doi:10.1126/science.1258396

Kornberg, A. 1995. Inorganic polyphosphate: Toward making a forgotten polymer unforgettable. J. Bacteriol. **177**: 491–496.

Kornberg, A., N. N. Rao, and D. Ault-Riché. 1999. Inorganic polyphosphate: A molecule of many functions. Annu. Rev. Biochem. **68**: 89–125. doi:10.1146/annurev.biochem.68.1.89

Li, B., D. M. Karl, R. M. Letelier, R. R. Bidigare, and M. J. Church. 2013. Variability of chromophytic phytoplankton in the North Pacific Subtropical Gyre. Deep-Sea Res. Part II **93**: 84–95. doi:10.1016/j.dsr2.2013.03.007

Lieberman, L. 1888. Uber das nuclein der hefe und kunstliche darstellung eines nucleus eiweiss und metaphosphatsaure. Ber. Dtsch. Chem. Ges. **21**: 598–607. doi:10.1002/cber.188802101109

Lomas, M. W., A. L. Burke, D. A. Lomas, D. W. Bell, C. Shen, S. T. Dyhrman, and J. W. Ammerman. 2010. Sargasso Sea phosphorus biogeochemistry: An important role for dissolved organic phosphorus (DOP). Biogeosciences **7**: 695–710. doi:10.5194/bg-7-695-2010

Mahaffey, C., K. M. Björkman, and D. M. Karl. 2012. Phytoplankton response to deep seawater nutrient addition in the North Pacific Subtropical Gyre. Mar. Ecol. Prog. Ser. **460**: 13–34. doi:10.3354/meps09699

Martin, P., and B. A. S. Van Mooy. 2013. Fluorometric quantification of polyphosphate in environmental plankton samples: Extraction protocols, matrix effects, and nucleic acid interference. Appl. Environ. Microbiol. **79**: 273–281. doi:10.1128/AEM.02592-12

Martin, P., S. T. Dyhrman, M. W. Lomas, N. J. Poulton, and B. A. S. Van Mooy. 2014. Accumulation and enhanced cycling of polyphosphate by Sargasso Sea plankton in response to low phosphorus. Proc. Natl. Acad. Sci. USA **111**: 8089–8094. doi:10.1073/pnas.1321719111

Martínez, A., M. S. Osburne, A. K. Sharma, E. F. DeLong, and S. W. Chisholm. 2012. Phosphite utilization by the marine picocyanobacterium *Prochlorococcus* MIT9301. Environ. Microbiol. **14**: 1363–1377. doi:10.1111/j.1462-2920.2011.02612.x

Mazard, S., W. H. Wilson, and D. J. Scanlan. 2012. Dissecting the physiological response to phosphorus stress in marine *Synechococcus* isolates. J. Phycol. **48**: 94–105. doi:10.1111/j.1529-8817.2011.01089.x

McAndrew, P. M., K. M. Björkman, M. J. Church, P. J. Morris, N. Jachowski, P. J. le B. Williams, and D. M. Karl. 2007. Metabolic response of oligotrophic plankton communities to deep water nutrient enrichment. Mar. Ecol. Prog. Ser. **332**: 63–75. doi:10.3354/meps332063

Meyer, A. 1904. Orientierende untersuchungen über verbreitung, morphologie, und chemie des volutins. Bot. Zeitung **62**: 113–152.

Michelou, V. K., M. W. Lomas, and D. L. Kirchman. 2011. Phosphate and adenosine-5 ′-triphosphate uptake by cyanobacteria and heterotrophic bacteria in the Sargasso Sea.

Limnol. Oceanogr. **56**: 323–332. doi:10.4319/lo.2011.56.1.0323

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. doi:10.3354/ame039257

Orchard, E. D., C. R. Benitez-Nelson, P. J. Pellechia, M. W. Lomas, and S. T. Dyhrman. 2010. Polyphosphate in *Trichodesmium* from the low-phosphorus Sargasso Sea. Limnol. Oceanogr. **55**: 2161–2169. doi:10.4319/lo.2010.55.5.2161

Paytan, A., B. J. Cade-Menun, K. McLaughlin, and K. L. Faul. 2003. Selective phosphorus regeneration of sinking marine particles: Evidence from ^{31}P-NMR. Mar. Chem. **82**: 55–70. doi:10.1016/S0304-4203(03)00052-5

Rao, N. N., M. R. Gómez-García, and A. Kornberg. 2009. Inorganic polyphosphate: Essential for growth and survival. Annu. Rev. Biochem. **78**: 605–647. doi:10.1146/annurev.biochem.77.083007.093039

Rashchi, F., and J. A. Finch. 2000. Polyphosphates: A review their chemistry and application with particular reference to mineral processing. Miner. Eng. **13**: 1019–1035. doi:10.1016/S0892-6875(00)00087-X

Ruttenberg, K. C. 1993. Reassessment of the oceanic residence time of phosphorus. Chem. Geol. **107**: 405–409. doi:10.1016/0009-2541(93)90220-D

Ruttenberg, K. C. 2014. The global phosphorus cycle, p. 499–588. In D. M. Karl and W. H. Schlesinger [eds.], Treatise on geochemistry, v. 10: Biogeochemistry. Elsevier.

Sannigrahi, P., and E. Ingall. 2005. Polyphosphates as a source of enhanced P fluxes in marine sediments overlain by anoxic waters: Evidence from ^{31}P-NMR. Geochem. Trans. **6**: 52–59. doi:10.1063/1.1946447

Sannigrahi, P., E. D. Ingall, and R. Benner. 2006. Nature and dynamics of phosphorus-containing components of marine dissolved and particulate organic matter. Geochim. Cosmochim. Acta **70**: 5868–5882. doi:10.1016/j.gca.2006.08.037

Schulz, H. N., and H. D. Schulz. 2005. Large sulfur bacteria and the formation of phosphorite. Science **307**: 416–418. doi:10.1126/science.1103096

Solórzano, L., and J. D. H. Strickland. 1968. Polyphosphate in seawater. Limnol. Oceanogr. **13**: 515–518. doi:10.4319/lo.1968.13.3.0515

Strickland, J. D. H., and T. R. Parsons. 1972. A practical handbook of seawater analysis. Fisheries Research Board of Canada.

Thomson-Bulldis, A., and D. Karl. 1998. Application of a novel method for phosphorus determinations in the oligotrophic North Pacific Ocean. Limnol. Oceanogr. **43**: 1565–1577. doi:10.4319/lo.1998.43.7.1565

Vagabov, V. M., L. V. Trilisenko, and I. S. Kulaev. 2000. Dependence of inorganic polyphosphate chain length on the orthophosphate content in the culture medium of the yeast *Saccharomyces cerevisiae*. Biochem. Mosc. **65**: 349–354.

Van Mooy, B. A. S., and others. 2009. Phytoplankton in the ocean use non-phosphorus lipids in response to phosphorus scarcity. Nature **458**: 69–72. doi:10.1038/nature07659

Van Mooy, B. A. S., and others. 2015. Major role of planktonic phosphate reduction in the marine phosphorus redox cycle. Science **348**: 783–785. doi:10.1126/science.aaa8181

van Waasbergen, L. G., J. A. Hoch, and B. M. Tebo. 1993. Genetic analysis of the marine manganese-oxidizing *Bacillus* sp. strain SG-1: protoplast transformation, Tn917 mutagenesis, and identification of chromosomal loci involved in manganese oxidation. J. Bacteriol. **175**: 7594–7603.

Villareal, T. A., M. A. Altabet, and K. Culver-Rymsza. 1993. Nitrogen transport by vertically migrating diatom mats in the North Pacific Ocean. Nature **363**: 709–712. doi:10.1038/363709a0

Villareal, T. A., C. Pilskaln, M. Brzezinski, F. Lipschultz, M. Dennett, and G. B. Gardner. 1999. Upward transport of oceanic nitrate by migrating diatom mats. Nature **397**: 423–425. doi:10.1038/17103

White, A. E., Y. H. Spitz, D. M. Karl, and R. M. Letelier. 2006. Flexible elemental stoichiometry in *Trichodesmium* spp. and its ecological implications. Limnol. Oceanogr. **51**: 1777–1790. doi:10.4319/lo.2006.51.4.1777

Wiame, J.-M. 1947. Yeast metaphosphate. Fed. Proc. **6**: 302.

Yang, K., and W. W. Metcalf. 2004. A new activity for an old enzyme: *Escherichia coli* bacterial alkaline phosphatase is a phosphite-dependent hydrogenase. Proc. Natl. Acad. Sci. USA **101**: 7919–7924. doi:10.1073/pnas.0400664101

Young, C. L., and E. D. Ingall. 2010. Marine dissolved organic phosphorus composition: Insights from samples recovered using combined electrodialysis/reverse osmosis. Aquat. Geochem. **16**: 563–574. doi:10.1007/s10498-009-9087-y

Acknowledgments

We thank Patrick Martin for providing access to published data. The authors also thank the captain, crew and science party aboard the HOE PhoR-I and -II cruises, as well as Dave Kulis, Dawn Moran, Elizabeth Harvey, and Matt Johnson for facilitating access to laboratory instrumentation. This work was supported by a Postdoctoral Fellowship from the Ford Foundation (JMD), the National Science Foundation under grants OCE 1225801 (JMD), OCE 1316036 (STD), EF 04-24599 (DMK), the Woods Hole Oceanographic Coastal Ocean Institute, the Center for Microbial Oceanography: Research and Education, and the Gordon and Betty Moore Foundation (DMK). Additional support was provided by grants from the Simons Foundation to DMK and STD and is a contribution of the Simons Collaboration on Ocean Processes and Ecology (SCOPE).

Submitted 23 June 2015
Revised 26 August 2015, 2 September 2015
Accepted 15 September 2015

Associate editor: M. Dileep Kumar