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Particulate polyphosphate and alkaline phosphatase activity across a latitudinal transect in the tropical Indian Ocean

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Abstract

Polyphosphate (polyP) is an essential chemical constituent of microbial cells, and is hypothesized to play important roles in the marine biogeochemistry of phosphorus. However, polyP has only rarely been measured in the oceans. Here, we present data on the distribution of polyP across a latitudinal transect in the tropical Indian Ocean. PolyP concentrations (quantified as molar equivalents of a synthetic polyP standard) and ratios of polyP to total particulate phosphorus (TPP) along the transect ranged between 3–7 nmol eq. L^{-1} (polyP concentration) and 0.2–0.4 nmol eq. $nmol^{-1}$ (polyP : TPP ratio), which is very similar to values reported from the North Pacific Subtropical Gyre. Yet unlike in the North Pacific, soluble reactive phosphorus was depleted to low concentrations ($\leq$ 0.03 μmol L^{-1}), and alkaline phosphatase activity was relatively high (1–4 nmol P L^{-1} h^{-1}) along our cruise track. We attribute these results to the unique seasonal changes in iron and macronutrient supply in the Indian Ocean, which are caused by the monsoonal reversal in ocean currents. PolyP concentrations and polyP : TPP ratios decreased sharply with depth down to 150 m, suggesting that polyP was preferentially recycled relative to TPP, unlike in the North Pacific Subtropical Gyre. We hypothesize that alkaline phosphatase exerts a significant control over marine polyP biogeochemistry.

Phosphorus (P) is an essential nutrient, and plankton communities in oligotrophic ocean regions rely principally on recycling to provide enough P to support continued primary production (Björkman et al. 2000; Paytan and McLaughlin 2007; Karl 2014). While a lack of P does appear to limit or co-limit primary production in some parts of the ocean, e.g., the eastern Mediterranean Sea (Thingstad et al. 2005) and the Sargasso Sea (Wu et al. 2000), most areas are actually limited by nitrogen or by micronutrients such as iron (Moore et al. 2013). A large fraction of the total dissolved P pool in seawater consists of dissolved organic P (DOP), and it is the recycling of this DOP that helps to sustain phytoplankton production (Mather et al. 2008; Reynolds et al. 2014; Karl and Björkman 2015; Letscher and Moore 2015). Moreover, it has been shown that P is recycled more rapidly than carbon and nitrogen (Copin-Montegut and Copin-Montegut 1983; Loh and Bauer 2000; Schneider et al. 2003; Burkhardt et al. 2014). Nevertheless, our understanding of the controls over P recycling rates is still limited (Karl 2014).

Recent research has begun to examine whether variability in the biochemical composition of plankton biomass affects P recycling rates. Planktonic microbes can substantially alter the carbon-to-nutrient ratios of their biomass depending on nutrient availability (Geider and La Roche 2002; Godwin and Cotner 2015). This variability is caused by specific biochemical changes in cells: for example, phytoplankton (Van Mooy et al. 2006, 2009; Martin et al. 2011) and heterotrophic bacteria (Zavaleta-Pastor et al. 2010; Carini et al. 2015; Sebastian et al. 2016) remodel their cellular membranes by replacing phospholipids with sulphur- or nitrogen-containing lipids upon experiencing P stress, and may even reduce the amount of P allocated to RNA (Zimmerman et al. 2014). Furthermore, planktonic microbes express extracellular alkaline phosphatases to hydrolyse dissolved organic P (Hoppe 2003; Mahaffey

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et al. 2014). Interestingly, however, phytoplankton (and possibly heterotrophic bacteria) appear to retain high levels of polyphosphate (polyP) even under extreme scarcity of dissolved P (Martin et al. 2014). The retention of high cellular polyP levels in the face of P starvation is probably due to the essential role of this phosphate polymer in the microbial stress response (Shiba et al. 1997; Ault-Riché et al. 1998; Jahid et al. 2006; Nikel et al. 2013). Recent transcriptomic and genomic studies of marine plankton (Temperton et al. 2011; Dyhrman et al. 2012; Cruz de Carvalho et al. 2016), and manipulative experiments with freshwater periphyton communities (Rier et al. 2016) have further confirmed the ability of aquatic microbes to maintain significant polyP stores under acute P stress. Moreover, polyP appears to account for a significant fraction of the P pool even in P-replete environments, with quantitative estimates suggesting a contribution of around 10% of total P (Solrzano and Strickland 1968; Diaz et al. 2008, 2016; Orchard et al. 2010; Young and Ingall 2010; Martin and Van Mooy 2013; Saad et al. 2016). Laboratory culture experiments have confirmed that marine phytoplankton can have a high polyP content under a variety of culture conditions (Rhee 1973; Perry 1976). However, because polyP has rarely been measured in the oceans, we still only have a limited understanding of the oceanographic distribution of this molecule: field studies to date have been limited to the temperate and subtropical North Atlantic and North Pacific Oceans.

PolyP has been shown to be important for the biogeochemical cycling of P, yet, intriguingly, previous studies reached apparently contradictory conclusions as to the biogeochemical fate of polyP. On the one hand, Diaz et al. (2008) reported that polyP granules produced by diatoms in coastal waters could be transformed to insoluble apatite upon sinking to the sediment, and these authors therefore suggested that polyP might be responsible for a significant proportion of the global sedimentary P burial. Furthermore, Diaz et al. (2016) observed that particulate polyP and total particulate P showed little decrease with depth in the upper 150 m of the North Pacific Subtropical Gyre, and maintained a steady ratio with depth. Diaz et al. (2016) therefore concluded that polyP was not recycled preferentially relative to total P. In contrast, Martin et al. (2014) found sharp decreases with depth in polyP concentrations and polyP : TPP ratios, and also found that sinking particles collected with sediment traps at 150 m depth contained very little polyP in the P-depleted Sargasso Sea. They therefore concluded that polyP was rapidly recycled relative to total particulate P in that region. Moreover, experimental work has shown that synthetic preparations of polyP are bioavailable to several (albeit not all) marine phytoplankton taxa and can be recycled to soluble reactive P during seawater incubations (Björkman and Karl 1994; Moore et al. 2005; Diaz et al. 2016). PolyP may also play a role in the recycling of P across redox gradients at the boundaries of anoxic zones (Diaz et al. 2012).

Clearly, polyP has the potential to be rapidly recycled, yet also to contribute to P sequestration over geological time-scales. At present, we do not understand how the biogeochemical fate of polyP is controlled. Moreover, because polyP has only been measured in so few locations to date, we need more polyP measurements across larger regions of the oceans and in more diverse biogeochemical settings to properly understand the biogeochemistry of this molecule in the oceans.

The Indian Ocean is unique in that most of its main ocean currents reverse direction due to the seasonal changes in the monsoon winds. This seasonal current reversal causes changes in the patterns of plankton nutrient limitation across large areas of the basin: although aeolian dust supplies significant quantities of iron to the Indian Ocean northward of 10°S, seasonal upwelling along the western boundary provides sufficient macronutrients to exhaust the iron supply at least as far east as 60°E (Wiggert et al. 2006). Large areas of the Indian Ocean are therefore predicted to alternate seasonally between iron and nitrogen limitation (Wiggert et al. 2006), which is supported by the spatial patterns in chlorophyll fluorescence quantum yields across the basin as estimated from satellite data (Behrenfeld et al. 2009). In addition, seasonal iron limitation was recently demonstrated from ship-board measurements along the western Arabian Sea (Naqvi et al. 2010; Moffett et al. 2015). The Indian Ocean therefore differs significantly in its nutrient biogeochemistry from those areas in which polyP has previously been measured, i.e., the persistently P-depleted subtropical North Atlantic, the P-replete temperate North Atlantic, and the persistently N-depleted subtropical North Pacific (Martin et al. 2014; Diaz et al. 2016).

Here, we present the first-ever measurements of polyP and alkaline phosphatase activity from the Indian Ocean. Our results reveal polyP concentrations similar to the subtropical Pacific, but lower dissolved P concentrations, higher alkaline phosphatase activity, and faster polyP recycling.

Methods

Study region and sample collection

Data were collected on a latitudinal transect along 67°E from Goa to Mauritius aboard RV *Sagar Nidhi* in December 2015 as part of the International Indian Ocean Expedition II (cruise SN105, Fig. 1). The majority of samples were collected from a depth of about 4 m using a battery-operated pump (Proactive Mini-Typhoon) deployed by hand over the side of the ship (referred to below as "surface pump" samples). The pump was always run for at least a minute before collecting samples to thoroughly rinse the pump and tubing with sample water, and was rinsed well with ultrapure MilliQ deionized water (18.2 MΩ cm^{-1}, hereafter referred to as DI water) and stored in a ziplock bag after each station. Additional samples were collected from deeper in the water column at six stations with Niskin bottles mounted on a conductivity–temperature–depth (CTD) rosette (2–4 depths per station).

Total particulate phosphorus measurements

Samples of 1–3 L were filtered onto 25-mm diameter Whatman GF/F filters, rinsed with a small volume of DI water from

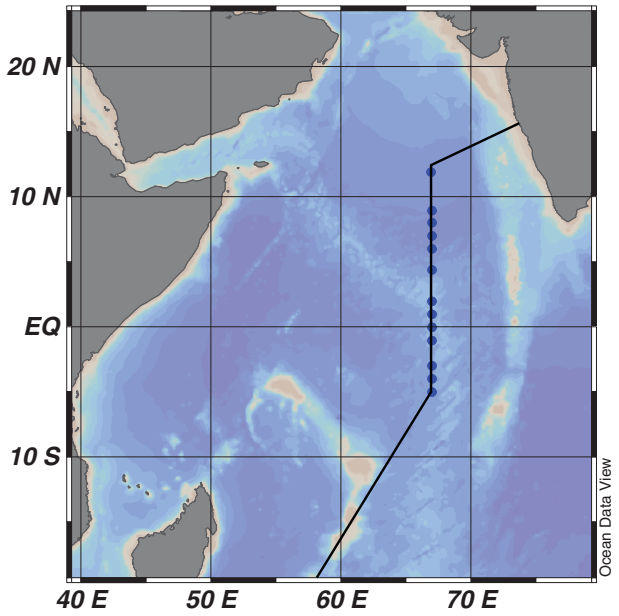

Fig. 1. Ship's track for cruise SN105, showing station locations where polyP samples were taken.

a wash bottle, wrapped in aluminium foil, flash-frozen in liquid nitrogen, and later stored at −20°C until analysis 4 months after collection. Filters were sub-sampled with a cork-borer and subjected to chemical wet-oxidation in an autoclave (121°C, 30 min) in 1 mL 4% $K_2S_2O_8$, diluted with DI water to 8 mL final volume, filtered through 0.2 μm syringe filters, after which dissolved phosphate was measured spectrophotometrically in 10-cm pathlength quartz cuvettes using the molybdenum blue method. Standards (KH_2PO_4) and blank filters were treated in the same manner as the samples. The detection limit was 1.3 nM phosphorus.

Polyphosphate measurements

Samples of 1–3 L were filtered onto 25-mm diameter Whatman GF/F filters, rinsed with DI water, wrapped in aluminium foil, flash-frozen in liquid nitrogen, and later stored at −20°C until analysis around 10 months after collection. PolyP was analyzed using a fluorescence assay based on Aschar-Sobbi et al. (2008) and Diaz and Ingall (2010). Samples were subjected to a sequential extraction protocol using brief heat treatment followed by DNAse, RNAse, and proteinase K digestion, after which polyP was quantified fluorometrically with 2,6-diamidino-2-phenylindole using standard addition to correct for matrix effects (Martin and Van Mooy 2013; Martin et al. 2014). This method yields a relative measure of polyP concentration that is expressed in nano-molar equivalents (nmol eq. L^{-1}) of a synthetic, 45-residue polyP standard (Sigma-Aldrich S4379, Martin et al. (2014)). The limit of detection for polyP depends both on the volume filtered and also on the background fluorescence of the sample, but was typically around 0.1 nmol eq. L^{-1}. Efforts to improve polyP extraction and quantification methods are ongoing in several research disciplines (Bru et al. 2016; Solesio and Pavlov 2016; Lee et al. 2017), suggesting that fully quantitative polyP analysis of environmental samples may soon become feasible.

Alkaline phosphatase measurements

The activity of alkaline phosphatase was measured in particle samples collected onto 25-mm diameter Whatman Nuclepore polycarbonate filters (pore size 0.2 μm) by filtering between 100 mL and 250 mL water. Filters were stored in the dark in 2-mL cryovials at −20°C, except during transport back to the laboratory, when they were in a liquid nitrogen dry shipper for ∼ 24 h. Alkaline phosphatase activity was measured as the increase in fluorescence over time while samples were incubated in 0.2 μm filtered, autoclaved Indian Ocean seawater at room temperature (∼ 23°C) in the dark with the substrate 4-methylumbelliferyl phosphate (MUF-P, Sigma-Aldrich, M8883). A MUF-P stock solution of 1 mM was made with 100% dimethylsulfoxide, of which 20 μL were added to each 2-mL sample (final concentration of MUF-P = 10 μM). Fluorescence was measured on a Tecan Spark 10M microwell plate reader against a methylumbelliferone standard (Sigma-Aldrich, M1381) at excitation/emission wavelengths of 360/460 nm. The detection limit was lower than 0.1 nM h^{-1}. No change in fluorescence was observed in parallel incubations of blank filters, and measurements were made within 1 month of the cruise.

Surface microbial community structure

At a subset of stations, 5–10 L of surface water were collected with the battery-operated pump and filtered through a 0.2-μm pore-size Sterivex filter cartridge. Filters were preserved with RNALater and stored at −20°C. DNA extraction and sequencing were performed as in Jeffries et al. (2015): DNA extraction from the filter cartridges followed the modified sucrose/SDS lysis method of Massana et al. (1997), and Amplicon libraries were generated according to the Illumina 16S Metagenomic Sequencing Library Preparation Protocol, with 20 PCR cycles for the first PCR. Universal primers that target the V6–V8 regions of 16S and 18S ribosomal DNA and have an Illumina-specific overhang were used:

926wF_Illum: 5′-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGAAACTYAAAKGAATTGRCGG 1392R_Illum: 5′-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGACGGGCGGTGTGTRC

After PCR purification with Agencourt Ampure XP beads (Beckman Coulter), a second round of PCR was undertaken. Libraries were sequenced on an Illumina MiSeq V3 at a concentration of 4 pM and a read-length of 301 bp paired-end. Raw sequence data were deposited at the NCBI Sequence Read Archive (BioProject PRJNA399019 with BioSample accessions SAMN07501653-SAMN07501662) and analyzed using QIIME (Caporaso et al. 2010), as described in Jeffries et al. (2015). Taxonomy was assigned against the SILVA database release 128 (Quast et al. 2013) using USEARCH 6.1

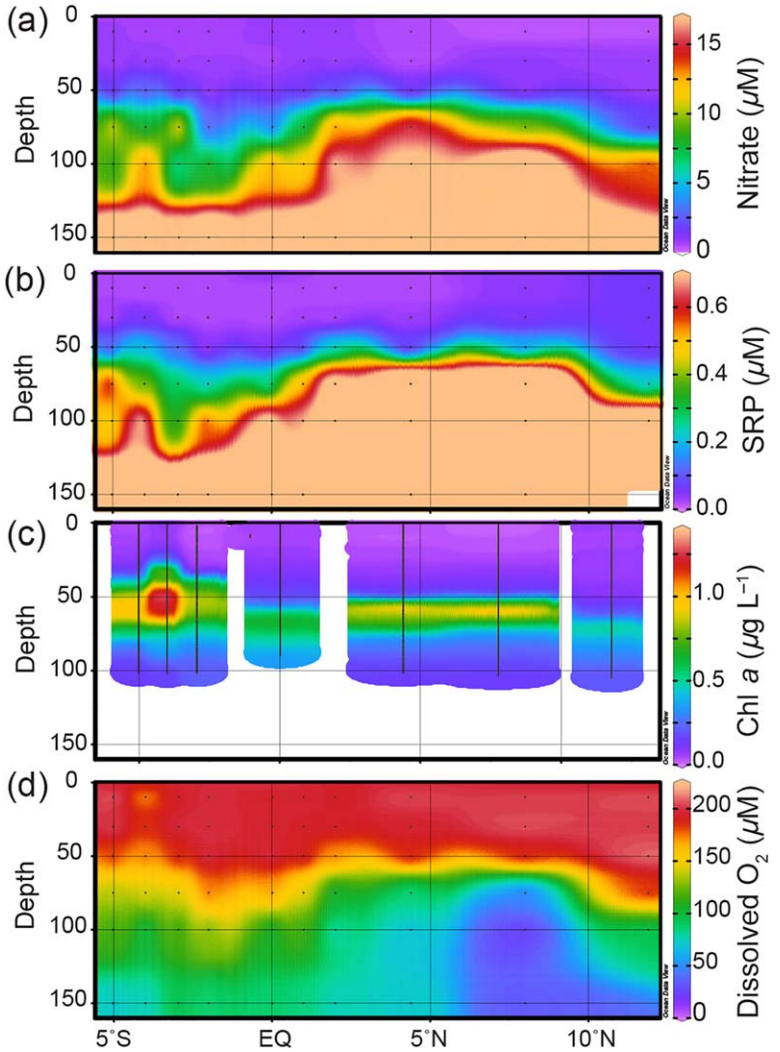

Fig. 2. Vertical sections in the upper 150 m of (**a**) dissolved nitrate; (**b**) soluble reactive phosphorus (SRP); (**c**) chlorophyll *a*; and (**d**) dissolved oxygen. The color scales in panels (**a**, **b**) have been chosen to emphasize the low surface concentrations and the location of the nutricline. Black dots indicate the location of each measurement.

(Edgar 2010). After filtering for chimeras, between 70.2% and 74.2% of sequences were assigned a taxonomic identity. Operational taxonomic unit (OTU) abundance was normalized by rarefaction to 24,300 sequences.

Nutrients, chlorophyll, and dissolved oxygen

Concentrations of dissolved macronutrients were measured on board on unfiltered water samples using a Skalar San++ autoanalyser with standard methods. The detection limit for soluble reactive phosphorus (SRP) was 0.03 μmol L^{-1}, and samples with SRP below the detection limit were assigned a value of 0.03 for the purposes of data analysis and plotting. For four samples, nutrient concentrations were not measured at exactly the same depth as polyP and TPP. In these cases, the SRP concentrations were estimated at the depth of polyP sampling as the average of the measurements immediately above and below. For all of these cases, nutrient measurements were taken not further than 15 m from the depth of polyP sampling, and the SRP concentrations above and below the polyP sampling depth did not differ by more than 0.01 μM.

Chlorophyll *a* concentration was estimated with a fluorometer mounted on a profiling optical sensor package that was deployed pre-dawn between the surface and 100 m depth.

Dissolved oxygen concentrations were determined using Winkler titration on samples collected with the Niskin rosette.

Results

Biogeochemical context

Surface nitrate and SRP concentrations were low throughout the transect, with nitrate concentrations between 0.5 μmol L^{-1} and 0.7 μmol L^{-1} and SRP concentrations mostly

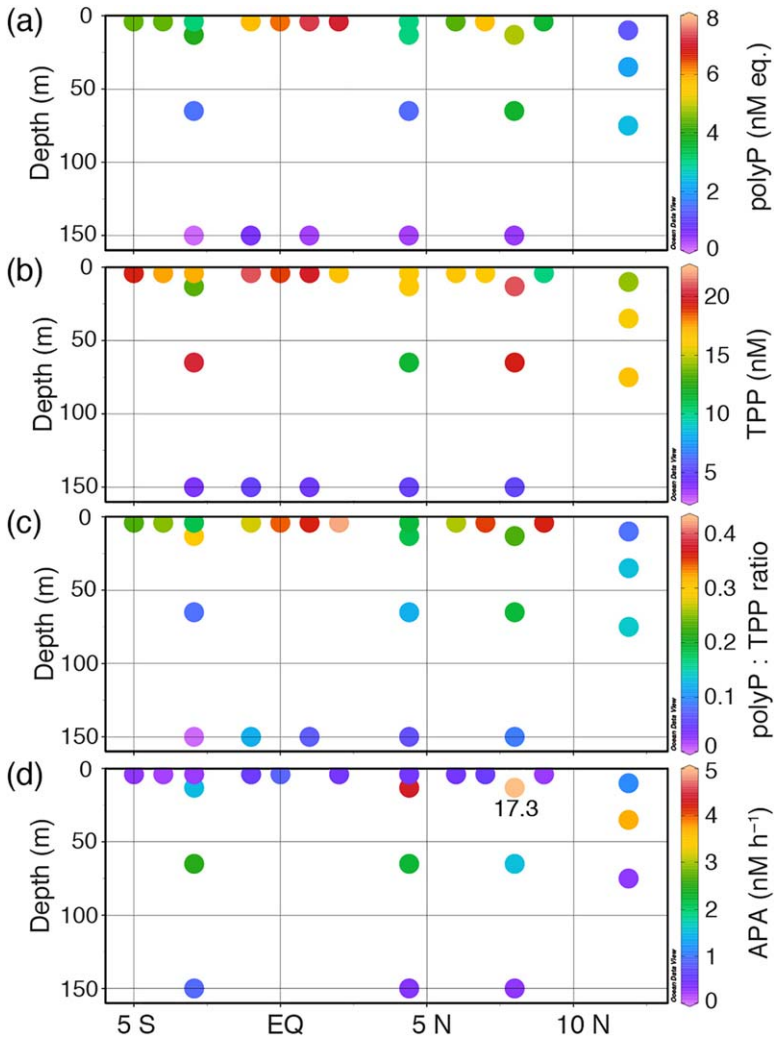

Fig. 3. Vertical sections in the upper 150 m of (**a**) particulate polyphosphate concentration; (**b**) total particulate phosphorus concentration; (**c**) molar ratio of polyphosphate to total particulate phosphorus (in nmol eq. nmol^{-1}); (**d**) activity of extracellular alkaline phosphatase. Owing to the limited depth resolution, all data are shown as individual points, rather than interpolated surfaces.

at or below the detection limit, i.e., ≤ 0.03 μmol L^{-1} (Fig. 2a,b). The nutricline was located at 50–75 m. Chlorophyll concentrations were mostly ≤ 0.05 μg L^{-1} in the upper 20 m, with a chlorophyll maximum of 0.7–1.0 μg L^{-1} located between 50 m and 60 m (Fig. 2c). Thus, despite covering nearly 20° of latitude, the transect did not cross major biogeochemical gradients in surface waters. However, the Arabian Sea oxygen minimum zone was clearly apparent at mid-depths in the northern half of the transect, with dissolved oxygen concentrations between 75 m and 150 m reaching values as low as 20 μmol L^{-1} (Fig. 2d).

Polyphosphate and total particulate phosphorus

Concentrations of particulate polyP (Fig. 3a) in the shallowest samples (< 15 m depth) ranged from 3.0 nmol eq. L^{-1} to 7.2 nmol eq. L^{-1}, except for a very low value of 1.2 nmol eq. L^{-1} at the northern-most station. At intermediate depths close to the chlorophyll maximum (65–75 m), values were lower (1.4–3.7 nmol eq. L^{-1}), and at 150 m concentrations ranged from undetectable to 0.6 nmol eq. L^{-1}. Because deep chlorophyll maxima generally move vertically over the course of a day, the depths sampled during the CTD casts do not necessarily correspond to the exact chlorophyll concentrations shown in Fig. 2c, although the intermediate depth samples were visibly more colored for a given volume of filtered water than the surface or deep samples, indicating that water with elevated chlorophyll concentration was sampled at these depths. Latitudinal variation in polyP concentration was not pronounced, but the highest values were found in surface pump samples between 2°N and the Equator.

Total particulate phosphorus (TPP, Fig. 3b) concentrations between the surface and 75 m depth ranged between 10

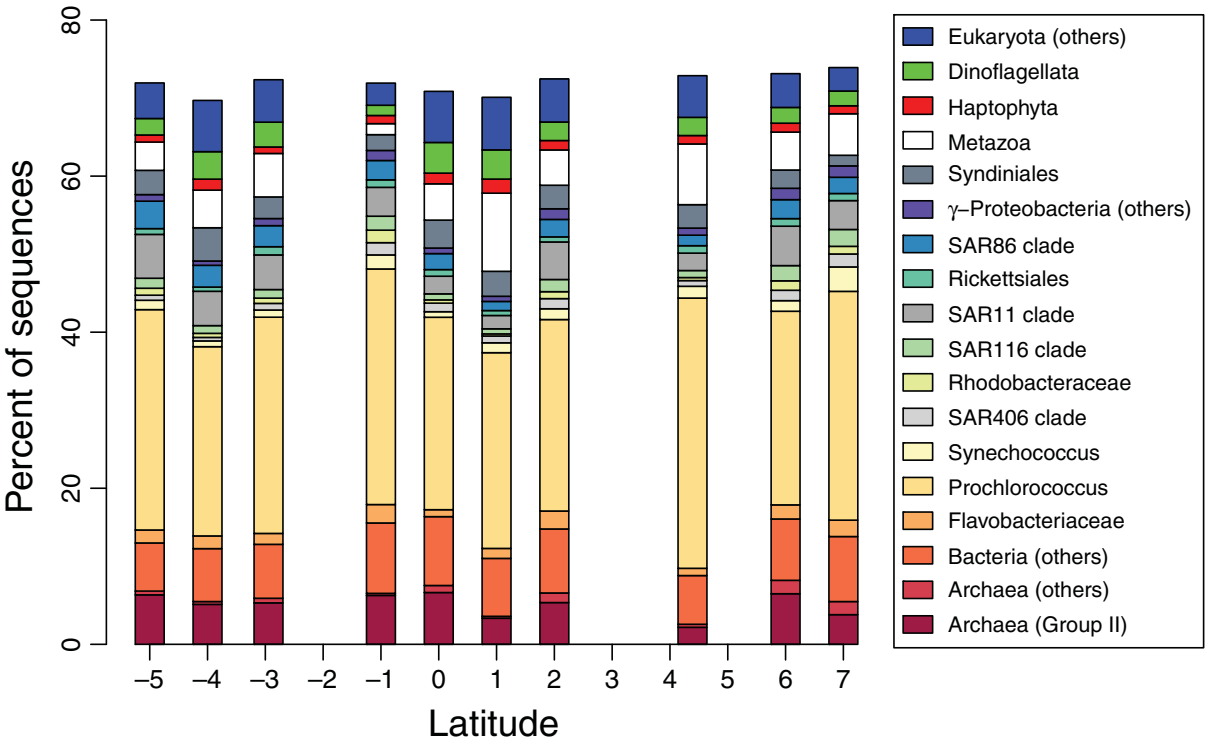

Fig. 4. Microbial community composition of the surface ocean plankton community as revealed by ribosomal DNA sequencing. Bar heights indicate the percentage contribution of each OTU after rarefaction to 24,300 sequences.

nmol L^{-1} and 21 nmol L^{-1} throughout the transect, with highest values recorded in surface pump samples between 2°N and the Equator. TPP at 150 m depth ranged between 4.0 nmol L^{-1} and 4.9 nmol L^{-1}.

The ratio of polyP to TPP (polyP : TPP, Fig. 3c) ranged from 0.18 nmol eq. $nmol^{-1}$ to 0.42 nmol eq. $nmol^{-1}$ at depths shallower than 15 m. Samples from 65 m to 75 m had lower values of 0.08–0.19 nmol eq. $nmol^{-1}$, with even lower ratios of $\leq$ 0.12 nmol eq. $nmol^{-1}$ found at 150 m.

Alkaline phosphatase activity

Alkaline phosphatase activity (APA, Fig. 3d) was moderate in our 4 m surface pump samples throughout the transect (0.18–0.87 nmol P L^{-1} h^{-1}), but higher in all samples collected between 10 m and 65 m (typically 1.1–4.3 nmol P L^{-1} h^{-1}, with one very high value of 17.3 nmol P L^{-1} h^{-1} at 13 m at 8°N). APA below 65 m was lower at 0.29–0.87 nmol P L^{-1} h^{-1}. Measurable APA was thus found in all samples we collected at every depth.

Surface microbial community structure

Ribosomal DNA sequencing revealed only minor variation in the microbial community across the transect (Fig. 4). *Prochlorococcus* was the most abundant OTU (34–47% of assigned sequences), followed by Marine Group II Archaea (3–9%) and SAR11 (2–8%). *Synechococcus* sequences were present at all stations, but at low abundances (<3%). Among the eukaryotes, metazoan sequences were most abundant (2–14%; mostly copepods), followed by dinoflagellates (2–7%) and haptophytes (1–3%).

Discussion

The polyP concentrations in the Indian Ocean are strikingly similar to those reported recently from the North Pacific Subtropical Gyre at Station ALOHA (Diaz et al. 2016). Our Indian Ocean transect was characterized by lower SRP concentrations and higher APA rates above the nutricline than reported by Diaz et al. (2016). Nevertheless, the Indian Ocean is not considered to be a P-limited system, but is instead thought to be limited by iron or nitrogen, depending on season and location (Wiggert et al. 2006), as shown experimentally in the Arabian Sea (Naqvi et al. 2010; Moffett et al. 2015). Owing to a lack of prior observations along this transect, it is not clear whether the SRP concentrations we encountered are typical of this part of the Indian Ocean or whether we sampled during a period of unusually low concentrations. Our transect was located at a longitude where the nitrogen limitation found in the central and eastern Indian Ocean gives way to the iron limitation found in the western Indian Ocean. The location of the boundary between these two biogeochemical regimes varies markedly

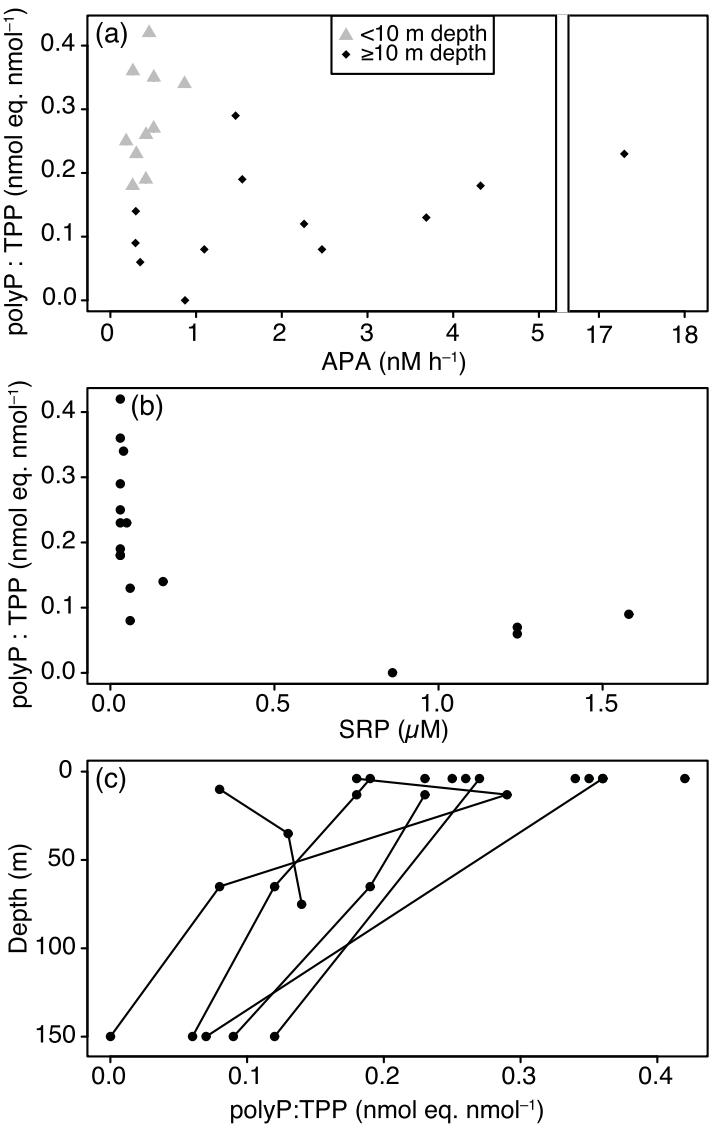

Fig. 5. (**a**) The ratio of polyphosphate to total particulate phosphorus was not significantly correlated with alkaline phosphatase activity, even when only data collected at or below 10 m were considered. (**b**) The polyP : TPP ratio was negatively correlated with the soluble reactive phosphorus concentration, which we attribute to the strong decrease in polyP : TPP with depth seen in panel (**c**) (lines in (**c**) connect points measured at the same station as a vertical profile).

according to the monsoon season (Wiggert et al. 2006). For much of the year, waters at longitude 67°E are thought to receive sufficient iron input to be nitrogen-limited (Wiggert et al. 2006), and this relatively high iron supply might allow SRP to be commonly drawn down to the low levels we encountered. Presumably, it is then the seasonal variability in iron and macronutrient supply at this longitude that prevents the extreme and chronic P depletion found in regions like the subtropical North Atlantic or the eastern Mediterranean (Wu et al. 2000; Thingstad et al. 2005), while still allowing SRP depletion to lower levels than found in the North Pacific Subtropical Gyre. The higher APA values that we measured as compared to the North Pacific Subtropical Gyre (Diaz et al. 2016) are consistent with a greater degree of P stress by the plankton community along our Indian Ocean transect. High APA values were consistently found in our deeper samples. The comparatively low APA rates in our surface samples were probably caused by light inhibition of alkaline phosphatase (Garde and Gustavson 1999), although even our surface values were typically twofold to threefold greater than the values reported by Diaz et al. (2016). The fact that we found high APA rates without the substantial polyP accumulation reported from the subtropical North Atlantic (Martin et al. 2014) suggests that polyP accumulation only takes place upon severe and persistent P stress.

The plankton community structure of our samples clustered closely with samples collected in 2013 across the Indian Monsoon Gyre biogeochemical province of the Indian Ocean (Jeffries et al. 2015) (not shown). However, our results are also broadly similar to other oligotrophic open-ocean sites, with strong dominance by *Prochlorococcus* and lower abundances of *Synechococcus*, as also found in the low-latitude Atlantic and Pacific Oceans (Campbell et al. 1994; Partensky et al. 1999; Brown et al. 2009; Malmstrom et al. 2010). The variation in surface water polyP : TPP ratios across our transect was not significantly correlated with variation in the abundances of any individual taxon in our data. Given that the ability to synthesize and degrade polyP is taxonomically virtually ubiquitous (Kulaev et al. 2004), our data are consistent with the hypothesis that the oceanographic distributions of polyP and polyP : TPP ratios are primarily controlled by nutrient biogeochemistry, rather than by plankton community composition. However, more concomitant measurements of polyP and plankton community composition are needed to fully test this hypothesis.

In the North Atlantic, a strong relationship was reported between polyP : TPP ratios, SRP concentration, and indicators of microbial P stress such as APA (Martin et al. 2014). However, their study spanned two regions that differed substantially in nutrient biogeochemistry, and all measured parameters showed far greater ranges than in the present dataset. Therefore, similar relationships would not necessarily be expected within one biogeochemically more uniform region. Likewise, Diaz et al. (2016) did not report clear relationships between these parameters at Station ALOHA in the North Pacific Subtropical Gyre. In our Indian Ocean dataset, the polyP : TPP ratio was not related to APA (Fig. 5a), likely because of light-inhibition of alkaline phosphatase in our shallowest samples. PolyP : TPP was negatively correlated with SRP in our dataset (Fig. 5b, Spearman's rho $= -0.782$, $p < 0.005$), as found across the North Atlantic transect by Martin et al. (2014). This correlation is clearly driven by the decrease in polyP : TPP with depth across the nutricline in our data (Fig. 5c). Thus, the small amount of variation in polyP : TPP within just our Indian Ocean dataset evidently does

Table 1. Summary of alkaline phosphatase activities measured in the present and in previous studies that have addressed polyphosphate cycling in the ocean.

Site	APA (nM h^{-1})	Evidence for preferential polyP recycling?	Reference
Coastal Pacific fjord (Effingham Inlet)	Not measured (likely no P stress: SRP = 0.5 μM)	No	Diaz et al. (2008)
Temperate western North Atlantic	Mostly not detectable	No	Martin et al. (2014)
North Pacific Subtropical Gyre	0.05–0.15	No	Diaz et al. (2016)
Subtropical North Atlantic	1.2–4.8	Yes	Martin et al. (2014)
Tropical Indian Ocean	0.18–4.0 (one extreme value of 17.3)	Yes	Present study

not reflect physiological responses by the surface plankton community to changing P availability. Strong, physiologically driven correlations between SRP, particulate polyP, and APA can only be expected when comparing data across oceanographic regions that differ significantly in the degree of P stress experienced by the plankton community.

Vertical profiles of polyP and TPP, and estimates of their sinking fluxes in sediment traps, suggested that polyP is preferentially recycled relative to TPP in the upper water column in the subtropical North Atlantic (Martin et al. 2014), but not preferentially recycled in either the temperate North Atlantic (Martin et al. 2014) or in the North Pacific Subtropical Gyre (Diaz et al. 2016). These studies implied that rapid cycling of polyP is likely restricted to regions of acute P stress (or redox gradients, Diaz et al. 2012), but is not a general characteristic of the oligotrophic open ocean. However, the strong decrease in polyP : TPP with depth in the present dataset suggests that polyP produced by the surface plankton community was efficiently recycled within the upper water column throughout our Indian Ocean transect, even though this region does not experience the severe P depletion seen in the subtropical North Atlantic. Overall, polyP : TPP decreased strongly in the deepest samples relative to the shallow samples at five of the six vertical profiles we collected (Fig. 5c). The only exception was the first, northernmost, station, which had an exceptionally low polyP content in surface waters. However, the deepest sample at this station was only at 75 m, so we cannot properly compare this depth profile to the other stations.

Comparing our data to previous marine polyP studies (Diaz et al. 2008, 2016; Martin et al. 2014), we note that the difference in APA rates between studies appears to be correlated with the biogeochemical fate of polyP (Table 1). Extremely low to undetectable rates of APA in the subtropical North Pacific and temperate North Atlantic are associated with little polyP recycling (Martin et al. 2014; Diaz et al. 2016), while elevated APA in both the subtropical North Atlantic (Martin et al. 2014) and the tropical Indian Ocean is apparently associated with preferential polyP recycling. While Diaz et al. (2008) did not measure APA, their samples were taken during a coastal spring diatom bloom with dissolved phosphate of 0.5 μM, well above the level at which P stress-related expression of alkaline phosphatase is expected. Although measurable APA can be found even in high-SRP coastal environments (Dyhrman and Ruttenberg 2006; Davis and Mahaffey 2017), it is not yet clear how common this phenomenon is, and, crucially, whether a large proportion of TPP is recycled by APA in such settings: when normalized to the concentration of particulate organic carbon, the APA rates reported by Davis and Mahaffey (2017) are actually very low relative to P-starved open-ocean regions. Thus, we hypothesize that the recycling of polyP in the water column is controlled to a significant degree by alkaline phosphatases: where APA is elevated, polyP may be more efficiently recycled in the upper water column, while in regions where APA is low, more polyP might be sequestered in sediments.

Enzyme activity rate measurements may be influenced by the choice of assay substrate, and Diaz et al. (2016) used 6,8-difluoro-4-methylumbelliferyl phosphate (DiFMUP), whereas Martin et al. (2014) and the present study used 4-methylumbelliferyl phosphate (MUF-P). However, a recent meta-analysis by Mahaffey et al. (2014) found little evidence that substrate choice affects measured APA rates, and concluded that APA rates can be compared meaningfully across studies (if anything, assays using DiFMUP yield higher rates than assays using MUF-P). Thus, the lower APA rates measured by Diaz et al. (2016) most likely reflect real biogeochemical differences between regions. Higher APA of up to 1.28 nM h^{-1} was found elsewhere in the North Pacific Subtropical Gyre (Duhamel et al. 2010), suggesting that polyP recycling rates might vary within biogeochemical regions.

Whether alkaline phosphatase is directly responsible for polyP recycling in the oceans is unclear. However, alkaline phosphatase from calf intestine displays very strong exopolyphosphatase activity (Lorenz and Schröder 2001), as do bacterial alkaline phosphatase from *Escherichia coli* and acid phosphatase from potato (Huang et al., in press). Given that

each of these different phosphatase enzymes can readily hydrolyse polyP to orthophosphate, it is very plausible that marine alkaline phosphatases might also hydrolyse polyP. Clearly, more work on the mechanisms of polyP recycling in marine environments is warranted.

Conclusion

We have reported the first measurements of polyP and APA from the Indian Ocean. Our estimates of polyP concentration and polyP : TPP ratios are in the same range as measurements from the North Pacific Subtropical Gyre, yet our vertical profiles show a marked decrease in polyP and polyP : TPP with depth. We attribute this to the greater rates of APA along our transect as compared to the North Pacific Subtropical Gyre, and propose that APA may exert an important control over the biogeochemical fate of polyP in the upper ocean. We attribute the combination of moderate polyP : TPP ratios with high APA rates that we observed to the unique seasonal pattern of nutrient limitation that is caused by the monsoonal current reversal in the Indian Ocean.

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Conflict of Interest

None declared.

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