LIMNOLOGY AND OCEANOGRAPHY
Letters

ASLO
Open Access

Limnology and Oceanography Letters 2024

doi: 10.1002/lol2.10394

LETTER

Polyphosphate phosphorus in the Great Lakes

Xingyu Yang [1,2] *Rixuan Gao* [1,2] *Audrey Huff* [3] *Sergei Katsev* [3] *Ted Ozersky* [3] *Jiying Li* [1,2]*

[1]Department of Ocean Science, The Hong Kong University of Science and Technology, Hong Kong, China; [2]Center for Ocean Research in Hong Kong and Macau, Hong Kong, China; [3]Large Lakes Observatory, University of Minnesota Duluth, Duluth, Minnesota, USA

Scientific Significance Statement

Polyphosphate (polyP) is a phosphate polymer that many aquatic microorganisms produce as part of their biomass. Microorganisms can accumulate polyP to store phosphorus (P), a key nutrient for their growth and metabolism, and use it as an internal P reserve to survive in low P environments. However, the contribution of polyP to P stocks and its roles in ecosystem-scale P cycling are not well quantified. This work shows that polyP accounts for up to 30% of total P in the Great Lakes. PolyP is decomposed more rapidly compared with other P compounds and thus enhances P recycling. By compiling data within and across diverse systems of various nutrient levels, we show that low P environments have higher polyP and smaller planktons accumulate more polyP compared with larger ones.

Abstract

Polyphosphate (polyP) is important to phytoplankton ecology, but a unified view of its variability and roles in ecosystem-scale phosphorus (P) cycling is lacking. We study polyP in the world's largest freshwater ecosystem, the Laurentian Great Lakes, covering pelagic to nearshore areas across a wide nutrient gradient. We show that polyP (average 10.99 ± 3.90 nmol L^{-1}) constitutes 3.8–30.2% (average 18.1 ± 7.2%) of total particulate P (TPP). PolyP accumulation is higher in low-P pelagic waters compared with more productive nearshore areas. PolyP is preferentially degraded in the water column of the Great Lakes, enhancing P recycling and relieving the nitrogen (N) : P imbalance. Our data enables a coherent large-scale freshwater-to-oceanic comparison. We show that while different plankton groups accumulate different levels of polyP with smaller plankton accumulating more, P availability is the key driver of polyP variability within and across systems.

Phosphorus (P) commonly limits primary productivity in freshwater ecosystems (Hecky and Kilham 1988). The availability of P, therefore, controls the cycles of vital elements such as carbon (C) and nitrogen (N), and determines

*Correspondence: jiyingli@ust.hk

Associate editor: Jill S. Baron

Author Contribution Statement: JL designed the study. JL, AH, SK, and TO collected the samples. XY, RG, and AH analyzed the samples. XY and JL analyzed the data. XY and JL wrote the article with help from all other coauthors.

Data Availability Statement: Data supporting the article are included in Supporting Information and are openly available in BCO-DMO at http://bco-dmo.org/dataset/920878.

Additional Supporting Information may be found in the online version of this article.

the functioning of the ecosystem. Phosphorus availability in water is commonly estimated as the concentration of soluble reactive phosphorus (SRP), consisting of dissolved orthophosphate (PO_4) and easily degradable dissolved organic P (Duhamel et al. 2021). In addition to these ambient dissolved P, which can be readily accessed by microorganisms, an intracellular P pool, in the form of polyphosphate (polyP), is increasingly receiving attention as a dynamic component of the total P pool. It has been demonstrated to be important not only for phytoplankton ecology but also for P recycling on ecosystem scales (Martin et al. 2014; Li and Dittrich 2019; Li et al. 2019; Sanz-Luque et al. 2020).

Polyphosphate is a group of long-chain phosphate polymers that is accumulated by microbes as part of their biomass. PolyP has many cellular functions and is tightly related to nutrient availability (Rao et al. 2009; Li and Dittrich 2019). Phytoplankton can accumulate polyP as P-storage compounds when P is abundant, a process called "luxury uptake" (Li and Dittrich 2019; Jentzsch et al. 2023); they can then use polyP when experiencing acute P stress (Li and Dittrich 2019; Li et al. 2019; Jentzsch et al. 2023). Conditions of chronic P deficiency can also cause the phytoplankton to increase their polyP content to high levels, sometimes higher than those obtained via luxury uptake, a process termed "P deficiency response" (Martin et al. 2014). The mechanism of P deficiency response remains unclear, but it has been hypothesized to enhance P cycling in low-P marine environments: polyP in phytoplankton can be recycled faster compared with other P forms thus keeping bioavailable P in the water column (Diaz et al. 2012, 2016; Martin et al. 2014, 2018; Li et al. 2019).

Despite the potential importance of polyP as a dynamic P stock in aquatic systems, we still don't have a good estimate of the size of this P pool. A comprehensive and unified view of what roles it plays in ecosystem-scale P cycling is lacking. This is largely because of the challenges in quantifying polyP and the corresponding scarcity of data (Martin and Mooy 2013; Bru et al. 2016). PolyP concentrations have been reported in very few marine environments (Orchard et al. 2010; Diaz et al. 2012, 2016; Martin et al. 2014, 2018; Hashihama et al. 2020). Data from freshwaters are even rarer (Li et al. 2019), especially in oligotrophic systems where P is often the limiting nutrient. Here, we present the first study of polyphosphate in the world's largest freshwater ecosystem, the Laurentian Great Lakes. We quantify polyP in Lakes Superior, Michigan, and Huron, covering pelagic (oligotrophic) to nearshore (mesotrophic) regions. We show that polyP is an important P stock in these lakes, and phytoplankton accumulate more polyP in the oligotrophic pelagic waters compared with the productive nearshore. Our data help bridge the existing polyP data gap and compile a global view across systems of a range of trophic states and microbial regimes. We examine the causes of the variability in polyP accumulation, discuss the effects of polyP metabolisms on whole-system P cycling, and propose several critical future directions to explore.

Methods

Samples were collected from 13 locations in Lakes Superior, Michigan, and Huron aboard the R/V *Blue Heron* in the summer of 2018 (Table S1; Fig. 1) (Li et al. 2024). Temperature, oxygen, light transmission, and chlorophyll were measured using a Seabird 911plus conductivity-temperature-depth (CTD)probe. Water samples were collected at the surface (1 m), the chlorophyll maximum (deep chlorophyll layer, DCL), and 1 m above the bottom using Rosette Niskin bottles. Suspended particles were collected on three sizes of filters, including 0.2 and 2.0 μm Millipore PC and 0.7 μm Whatman GF/F filters. The filters can separate picoplankton (0.2–2.0 μm) from larger phytoplankton; picoplankton consists of bacterioplankton (< 0.7 μm), picocyanobacteria (0.7–3 μm), and picoeukaryotes (0.7–2 μm). Filtration was conducted onboard upon sample collection, and particles and the filtrate were stored frozen at −20°C until further analyses.

Particulate phosphorus (PP) was quantified using the molybdenum blue method after persulfate digestion (Grasshoff et al. 1999; Suzumura 2008); the molybdenum blue method was also used to measure SRP in the water (after 0.2 μm filtration). PP on the 0.2 μm filters was considered total PP (TPP). PolyP in the particles was extracted using an enzyme digestion and boiling method (Martin and Van Mooy 2013). The extracted PolyP was fluorometrically quantified using 4′,6-diamidino-2-phenylindole (DAPI) staining (Aschar-Sobbi et al. 2008; Diaz and Ingall 2010). The extraction and quantification methods are commonly used for polyP in natural planktonic samples, and the results can be compared with literature data (see Results). However, the DAPI method only quantifies polyP relatively, thus the results are reported in conventional equivalent units, e.g., nmol eq. L^{-1} (Martin et al. 2014). To quantify polyP more accurately, we used a polyP-specific dye JC-D7 (Angelova et al. 2014), which was tested to produce consistent results compared with those measured by exopolyphosphatase (PPX) (see Data S2), an enzyme that exclusively hydrolyzes polyP to PO_4^{3-} and provides absolute polyP quantification (Bru et al. 2016; Christ et al. 2020). We thus use absolute concentration units (e.g., nmol L^{-1}) for polyP results obtained using JC-D7. Total particulate N (PN) and total particulate carbon (PC) were determined on 0.7 μm GF/F filters using a CHNS Elemental Analyzer (Vario EL Cube, Elementar). Chlorophyll *a* (Chl *a*) was extracted from the particles collected on 0.2 μm filters using 90% acetone and determined fluorometrically (APHA, 1998). Alkaline phosphatase activity (APase), which is a proxy for P stress, was determined using a fluorogenic substrate 3-*O*-methylfluorescein phosphate (Martin et al. 2018).

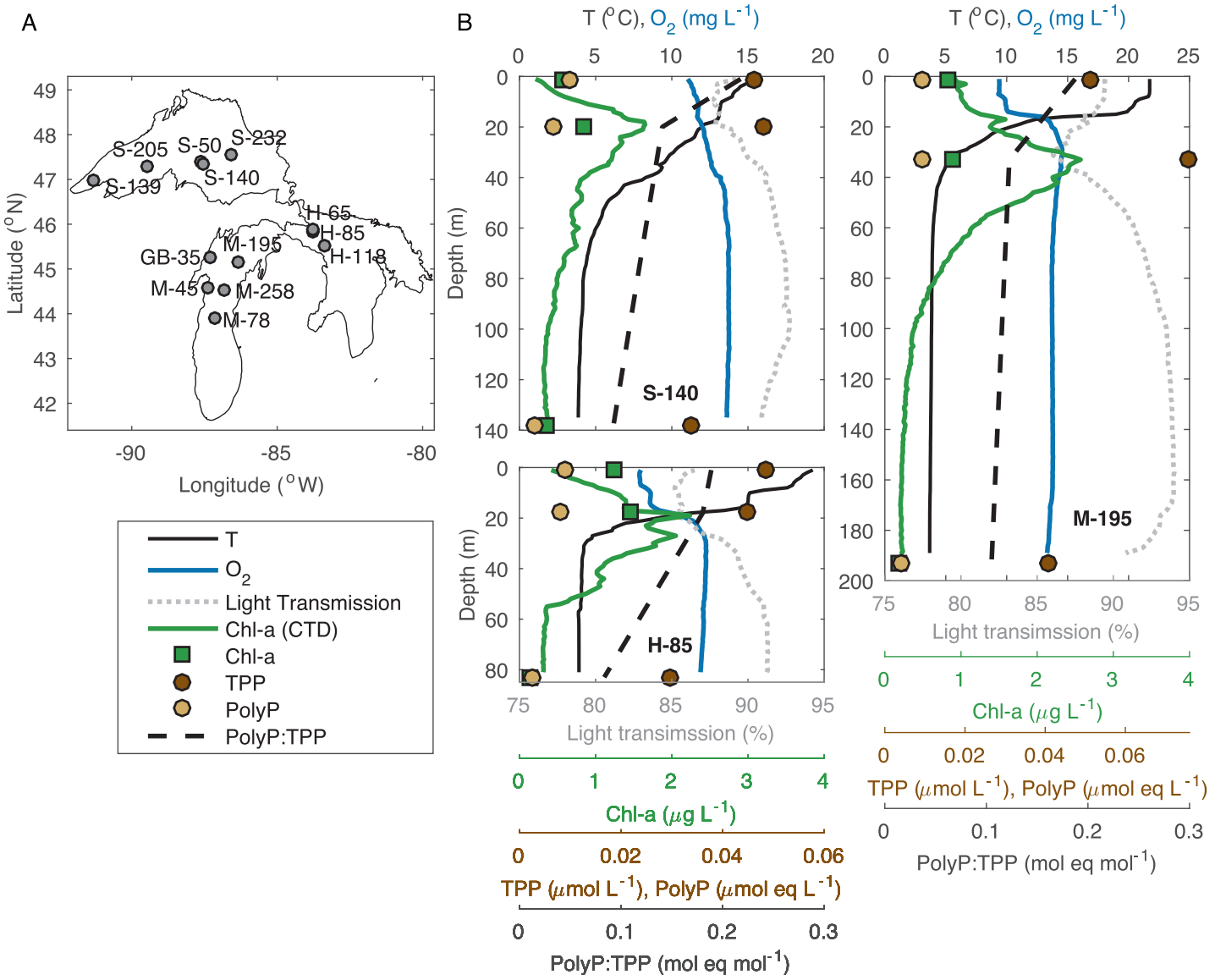

Fig. 1. (**A**) Sampling locations in the upper Great Lakes (Lakes Superior, Michigan, and Huron), (**B**) Vertical distributions of temperature (T), dissolved oxygen (O_2), light transmission, chlorophyll a (Chl *a*), total particulate phosphorus (TPP), polyP, and the ratios of polyP: TPP (quantified by DAPI method) in the three representative stations in Lakes Superior (S-140), Michigan (M-195), and Huron (H-85). Profiles from all sites are shown in Figs. S3–S5. PolyP and TPP concentrations decreased with depth due to their decomposition during the settling of particles.

All methods are described in further detail in supporting materials (S1–S3). Results of replicate samples are averaged and standard deviations from the mean are reported as uncertainties. Linear correlations are conducted between parameters when appropriate (see Results). Nonmetric multidimensional scaling (NMDS) analyss are performed for the phytoplankton community data from the Great Lakes Environmental Database (GLENDA) collected by the US Environmental Protection Agency Great Lakes National Program Office (EPA GLNPO) and their relationships with other parameters (see Data S3 for the description and treatment of the data).

Results and discussion

PolyP is an important P stock in the Great Lakes

PolyP was detected in the water column at all sites in the upper Great Lakes, covering nearshore to pelagic areas from the mesotrophic Green Bay to the most oligotrophic basins in Lake Superior (Fig. 1; Tables S1-2). At the time of sampling, the water column was stratified with DCL located at ~15–50 m except for in shallower Green Bay (Figs. 1, S3–S5). At the surface and DCL where particles are mostly fresh planktonic biomass, polyP ranged f2.02–19.1 nmol L^{-1} (averaged 10.99 ± 3.90 nmol L^{-1}). Previous studies have estimated

polyP in aquatic environments (Martin et al., 2014; Diaz et al. 2016; Rier et al. 2016; Martin et al., 2018; Li et al. 2019; Hashihama et al. 2020), but the DAPI method they used severely overestimated polyP for reasons that we discuss in Data S2. Our JC-D7 method eliminated the interference that causes overestimation by the DAPI method and accurately quantified polyP. Our results show that polyP is a ubiquitous and substantial P stock in the Great Lakes: polyP accounted for 3.8–30.2% of TPP, averaging 18.1 ± 7.2% (Table S2). Comparing our results obtained by using JD-D7 vs. DAPI shows that the DAPI method overestimates polyP concentrations by an average of 35% (Fig. S6).

Increased polyP accumulation in oligotrophic waters

Our results suggest a strong sensitivity of polyP to P availability in the Great Lakes (Fig. 2). PolyP accumulation is enhanced in oligotrophic waters, increasing from ~10% in nutrient-rich productive regions to ~30% in the nutrient-depleted oligotrophic regions (Fig. 2). Consistently, polyP : PP increases with increasing PN : PP and APase, which are indicators of P stress (Fig. 2B,D) (Downing and McCauley 1992; Vandergucht et al. 2013). High PN : PP indicates low P availability and high APase : Chl *a* suggests high P stress (also see correlations between APase : Chl *a*, SRP, and PN : PP in Fig. S7). This phenomenon of storing more polyP under P stress is termed "P deficiency response," which has also been observed in the ocean and has received increasing attention for its potential effects on phytoplankton ecology and ecosystem P cycling (Martin et al. 2014, 2018; Li et al. 2019) (also see discussion later). Yet because of the severe overestimation of polyP by these studies (see Data S2), it is not unreasonable to question whether the observation is true polyP variability or artifact caused by nucleic acid interference. Under P deprivation, the amounts of other P-compounds can decrease, and nucleic acids can become more abundant in proportion because cells must maintain a certain level of nucleic acids to function (Van Mooy et al. 2009). Our results show a strong positive correlation between the DAPI-quantified and the absolute polyP concentrations (Fig. S6), suggesting that the literature data may still provide a good estimate of the trend. Therefore, our data validates the "P deficiency response" theory.

Temporal changes in water column P levels should affect polyP concentrations. PolyP can be more important in the summer when the surface water is more P-limiting due to higher productivity and the stronger stratification restricting the transport of nutrients from the deeper water. PolyP can also become more important over the long term. The Great Lakes have been experiencing drastic changes in their chemistry and ecology over the past century: pelagic primary productivity and total P (TP) in the water columns have declined due to the reduced external P loading and the expansion of invasive mussels (Fig. S8) (Dove and Chapra 2015; Li et al. 2021).

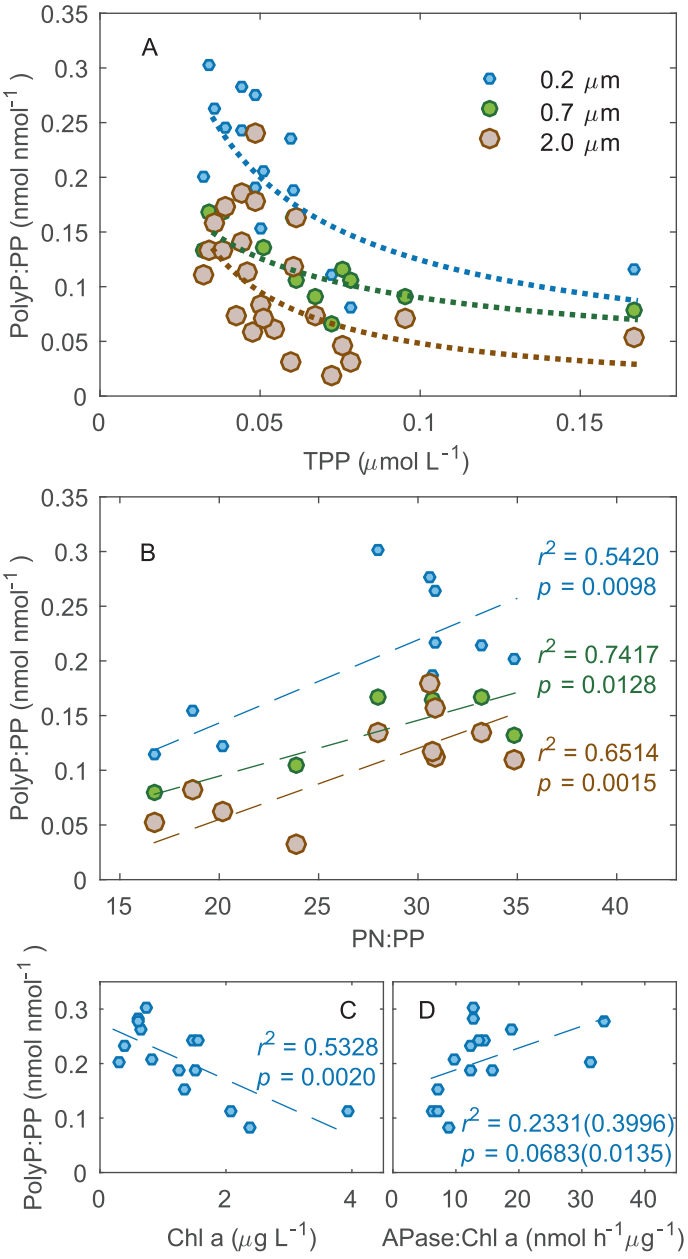

Fig. 2. The ratios of polyP to particulate P (polyP : PP) in the surface and deep chlorophyll maximum layers measured in samples collected on filters of different sizes (0.2, 0.7, and 2.0 μm) vs. (**A**) total particulate phosphorus (TPP), (**B**) ratio of particulate N to particulate P (PN : PP; >0.7 μm fraction), (**C**) chlorophyll a (Chl *a*), and (**D**) the activity of alkaline phosphatase normalized to Chl *a* (APase : Chl *a*). The polyP : PP of the >2.0 μm fractions presented in (A) are results from DAPI methods corrected to absolute values using a factor of 1/1.35 = 0.74 (see Fig. S6) so that they can be comparable to the values of other size fractions (>0.2 and >0.7 μm) measured by the JC-D7 method. The dashed lines in (B–D) represent the linear regressions, with the squared Pearson correlation coefficients r^2 and *p*-values listed in the figures. In (D), the r^2 and *p*-value presented in the brackets are for Spearman's correlation, which is less sensitive to the strong outliers observed in the tail of the samples.

We expect that if the Great Lakes continue to become more oligotrophic in the future, particularly for Lakes Michigan and Huron as quagga mussels continue expanding (Li et al. 2021), polyP accumulation and its relative contribution to the TP pool will continue to increase due to the increasing P stress.

PolyP enhances P recycling and relieves N : P imbalance

The Great Lakes have been experiencing nutrient imbalances: the ratio of dissolved N : P has reached >650 in Lakes Michigan and Huron and >1600 in Lake Superior (Dove and Chapra 2015), about 40–100 times higher than the classic Redfield ratio 16 N : 1P (Redfield 1958) or the PN : PP of ~16–35 in the Great Lakes (Table S3). Interestingly, the ratios of PC : PP and PN : PP decrease with depth (Fig. 3; Table S3; also Sterner 2011). This may be a result of the high dissolved N : P leading to the production of more degradable organic C and N compared with organic P and thus the preferential recycling of C and N over P during the particle settling, providing positive feedback to the high dissolved N : P ratio in the water column (Sterner et al. 2007).

The increase in polyP, on the contrary, enhances P recycling: polyP : TPP decreases with depth in the water column (Fig. 3), suggesting that polyP is preferentially recycled compared with other P forms. Preferential polyP recycling could strongly control productivity dynamics in the Great Lakes as sedimentation is the major sink of the productivity-limiting nutrient P (Katsev 2017; Li et al. 2018). This effect is likely amplified by the invasion of quagga mussels because their feeding on sedimenting materials now dominates the P cycle in the mussel-colonized Great Lakes (Li et al. 2021). We think that the continuing oligotrophication of the pelagic Great Lakes and the projected increase of polyP accumulation will decrease P removal and relieve the water column N : P imbalance by recycling polyP.

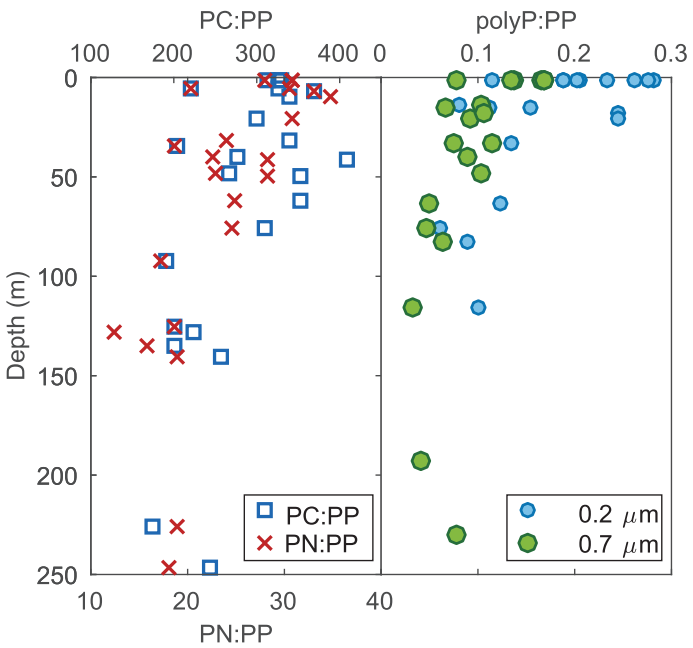

Fig. 3. Vertical distributions of (**A**) the ratio of particulate carbon to particulate phosphorus (PC : PP) and PC to particulate N (PC : PN) and (**B**) the ratio of polyP to particulate P (polyP : PP) in the upper Great Lakes.

A global view: oligotrophic vs. eutrophic systems

We compare polyP levels across ecosystems by compiling literature polyP data together with our Great Lakes results (Fig. 4). Consistently, there is a general trend of higher polyP accumulation in oligotrophic systems (i.e., Sargasso Sea, subtropical N. Pacific Ocean, and Tropical Indian Ocean) compared with mesotrophic and eutrophic systems (e.g., temperate western N. Atlantic Ocean, Green Bay, and Hamilton Harbor) (Fig. 4). Interestingly, this pattern becomes weaker across the meso-to-eutrophic systems (TPP > ~ 0.1 μmol L^{-1}; Fig. 4). This may be a result of other polyP mechanisms in high-nutrient waters, such as overplus and luxury uptake (Li et al. 2019). Overplus uptake is the accumulation of polyP to support recovery when P-stressed microorganisms experience a P resupply, but the effect is transient (Li et al. 2019). Luxury uptake occurs when dissolved P is abundant and plankton take up more P than required for growth to store as polyP (Li et al. 2019). These mechanisms can cause polyP fluctuations, especially in systems with strong temporal variability in nutrient availability, such as shallow bays and nutrient-rich coastal regions experiencing seasonal upwelling (Lin et al. 2016; Li et al. 2019; Mulholland et al. 2019).

In addition, oligotrophic and eutrophic systems possibly differ in their threshold levels of P that can trigger a P deficiency response. In a eutrophic system, P deficiency can occur at a much higher P level (Li et al. 2019), because the communities there may be less tolerant to low P levels than those adapted to low-P conditions in oligotrophic systems. For example, an SRP level of ~0.1 μmol L^{-1} in Hamilton Harbor (Lake Ontario) could trigger P-deficiency accumulation of polyP (Li et al. 2019), whereas this level of P may be sufficient for the communities in the oligotrophic Great Lakes and open oceans.

Physiological response or taxonomic shifts

It is important to understand whether polyP variability is caused by plankton physiological responses to nutrient availability or changes in the community compositions that differ in their polyP levels. Our results show cell-size fractionation of polyP level, suggesting that the latter is possible (Fig. 2). The smallest size plankton (0.2–0.7 μm) seem to accumulate the highest proportion of polyP, with bacterioplankton likely dominating this size fraction because the smallest phytoplankton *Prochlorococcus* (also <0.7 μm) is not an important member of Great Lakes communities. Compared with the smallest plankton (0.2–0.7 μm), the plankton of size 0.7–2 μm accumulate less polyP (Fig. 2). This group may consist of picocyanobacteria (e.g., *Synechococcus*) and picoeukaryotes.

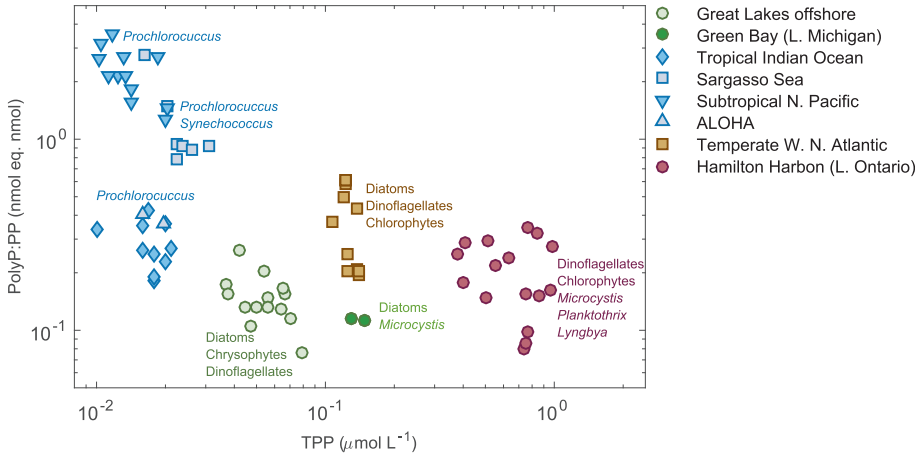

Fig. 4. Comparison of polyP levels and the major taxonomic groups of phytoplankton across ecosystems including the upper Great Lakes open water stations (this study), Green Bay (Lake Michigan; this study and Stasio et al. 2014), Tropical Indian Ocean (Martin et al. 2018), Sargasso Sea (Martin et al. 2014; Mojica et al. 2021; Kramer 2022), Subtropical North Pacific Ocean (Hashihama et al. 2020), ALOHA station (Campbell et al. 1994; Diaz et al. 2016; Karl and Church 2017), Temperate Western North Atlantic Ocean (Martin et al. 2014; Kramer 2022), and Hamilton Harbor (Lake Ontario) (Dermott et al. 2007; Saati 2016; Li et al. 2019). PolyP results shown in the figure are all obtained using the DAPI method for consistency.

The nanoplankton (>2 μm) and larger phytoplankton accumulate even less polyP (Fig. 2). The reasons for polyP variability in plankton of different size ranges are not known. We speculate that smaller cells may respond more rapidly to ambient P variability. For example, bacteria are known to have higher rates of P uptake and more variable P content compared with algae due to their higher surface-to-volume ratios and thus higher P affinity (Currie and Kalff 1984; Mindl et al. 2005). Whether such fractionation occurs in marine ecosystems is not known but is possible. Thus, the common practice of using a filter size of 0.7 μm in polyP studies may have led to sampling bias and obscured polyP variability, especially in regions where small phytoplankton dominate (e.g., *Prochlorococcus* in oligotrophic oceans) (Fig. 4).

The variability of polyP across ecosystems coincides with that of the size of the dominant phytoplankton (Fig. 4): whereas oligotrophic oceans with high polyP are dominated by the picocyanobacteria *Prochlorococcus* and *Synechococcus*, high-P regions are dominated by larger diatoms, dinoflagellates, chlorophytes, and cyanobacteria (e.g., *Microcystis* in Green Bay and filamentous *Planktothrix* and *Lyngbya* in Hamilton Harbor; Fig. 4). Picocyanobacteria are known to better survive low-nutrient environments whereas larger phytoplankton are often associated with relatively nutrient-replete conditions (Sommer et al. 2016). Shifts of the phytoplankton community from picocyanobacteria to diatoms and dinoflagellates often occur during the upwelling of deep nutrient-rich waters to the surface water (Mahaffey et al. 2012; Fujiki et al. 2016). The agreement between phytoplankton distributions and size-fractionated polyP accumulation, however, does not necessarily suggest that polyP variability is caused by taxonomic differences. It is possible that nutrient variability triggers polyP dynamics via both physiologic responses of the phytoplankton and the shifting of groups that can accumulate different levels of polyP. Moreover, polyP can be accumulated by bacterioplankton, and their various contributions to polyP in different systems are not known (Figs. 2, 4).

We analyzed the GLENDA data to investigate possible links between polyP and phytoplankton community structures in the Great Lakes. While correlations between polyP and taxa abundances do not necessarily suggest cause-and-effect relationships, these analyses can show how the different phytoplankton respond to P levels, which is the major determinant of polyP variability. Our analysis shows 8 diatom genera (*Achnanthes*, *Navicula*, *Anomoeoneis*, *Asterionella*, *Tebellaria*, *Gomphonema*, *Cymbella*, and *Fragilaria*) that correlate positively with polyP and negatively with P levels. On the other hand, genera correlating negatively with polyP level spread across various phyla, including diatom (*Stephanodiscus*, *Synedra*, and *Diatoma*), chrysophytes (*Kephyrion* and *Rhizosolenia*), cyanobacteria (*Merismopedia*), dinoflagellate (*Glenodinium*), and cryptophyte (*Rhodomonas*) (Table S4). Analysis at the species level shows consistent results, with most species correlating positively with polyP being diatoms (Table S5). NMDS analyses show that in addition to the taxa identified by the correlation analysis, the presence of some cyanobacteria (*Aphanothece* and *Anabaena*), chlorophytes (*Sphaerocystis* and *Franceia*), and chrysophytes (*Spiniferomonas*, *Chrysosphaerella*, and several species of *Dinobryon*) may be related to P stress (Figs. 5, S9). We suggest that future work should investigate and compare polyP levels and metabolisms in the phytoplankton with different responses to P levels.

In addition to studying phytoplankton polyP, more understanding is needed for polyP in bacterioplankton, an

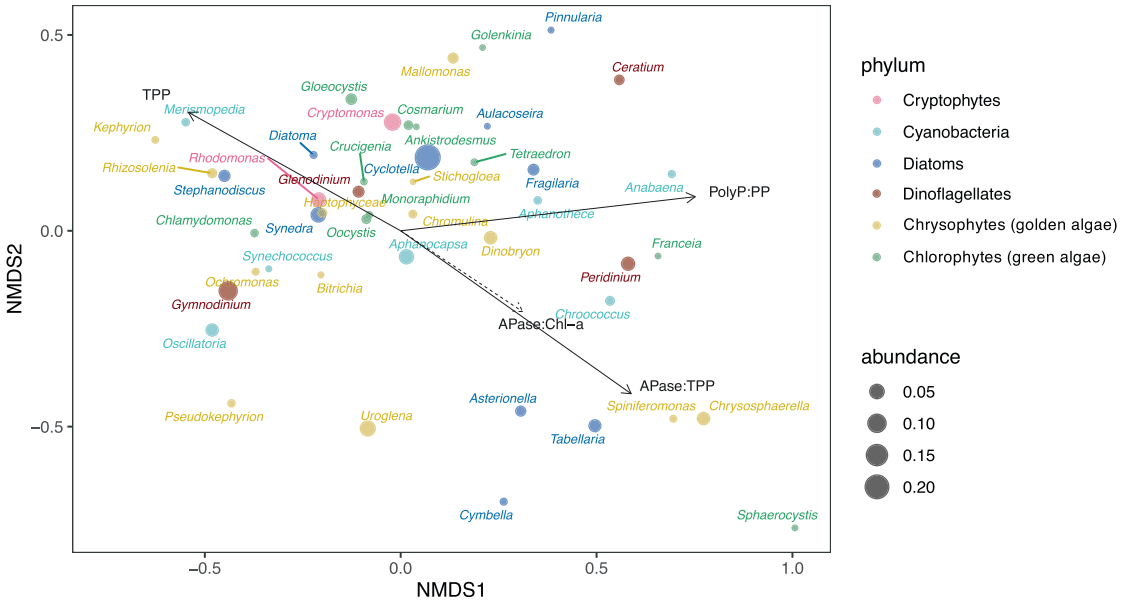

Fig. 5. NMDS analysis of the phytoplankton community (genera of the top 99% abundance) in the water column of Lakes Superior, Michigan, and Huron. The arrows show the directions of the parameters related to P stress and polyP accumulation (TPP, polyP : PP, APase : TPP, and APase : Chl *a*) obtained by fitting them in the ordination space, and the lengths of the arrows indicate the significance of the fits.

important component of picoplankton that is known to accumulate higher levels of polyP than larger-size phytoplankton (Fig. 2). While phytoplankton polyP can be a direct nutrient reserve for primary productivity, polyP in heterotrophic bacteria may have complicated ecological effects. By competing with phytoplankton for P and accumulating polyP, heterotrophic bacteria may increase P stress for the phytoplankton, while at the same time relying in their growth on phytoplankton production of organic matter as carbon and energy sources. It was hypothesized that bacteria can degrade their polyP to benefit phytoplankton via P exudation and get carbon and energy in return (Li et al. 2019). These competing or synergistic interactions remain speculative but may have important impacts on ecosystem P cycling.

In summary, polyP is an important P pool in the Great Lakes, accounting for up to 30.2% of TPP in the water column during the summer. More polyP is accumulated in oligotrophic waters, and polyP is preferentially recycled. Continuing oligotrophication of the Great Lakes thus should lead to increased importance of polyP and enhanced P recycling, as its preferential decomposition over other PP components regenerates bioavailable P, which otherwise would leave the surface water in the settling particles and become unavailable on short time scales. Our data suggest that the "P deficiency response" is the primary mechanism causing polyP variability within the Great Lakes and across ecosystems. Picoplankton, particularly bacterioplankton, respond more strongly to P scarcity by accumulating polyP. Teasing apart the effects of community shifts from individual physiological responses remains a major challenge in understanding polyP variability. In addition to studying bulk polyP concentrations and their spatial variability, we propose that future work should explore temporal polyP variability, e.g., seasonal changes, and study polyP metabolisms in specific plankton groups with various sensitivities to P stress, for example, by using culture studies. Our present knowledge of polyP, particularly the results obtained from DAPI quantification in both natural and cultural studies, needs to be updated by conducting more work using methods that allow accurate polyP quantification. The development of new polyP-specific dyes may allow detecting dynamics of both polyP and community structures at the same time by using microscopy and flow cytometry techniques. These efforts will help understand the ecological importance of polyP, such as whether polyP accumulation affects plankton competing/synergistic interactions, how polyP helps different communities in various systems adapt to P availability, and the roles of polyP dynamics in ecology and ecosystem P cycling.

References

Angelova, P. R., B. K. Agrawalla, P. A. Elustondo, J. Gordon, T. Shiba, A. Y. Abramov, Y.-T. Chang, and E. V. Pavlov. 2014. In situ investigation of mammalian inorganic polyphosphate localization using novel selective fluorescent probes JC-D7 and JC-D8. ACS Chem. Biol. **9**: 2101–2110. doi:10.1021/cb5000696

APHA. 1998. Standard Methods for the Examination of Water and Wastewater. 20th Edition, American Public Health

Association, American Water Works Association and Water Environmental Federation, Washington DC.

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

Bru, S., J. Jiménez, D. Canadell, J. Ariño, and J. Clotet. 2016. Improvement of biochemical methods of polyP quantification. Microb. Cell **4**: 6–15. doi:10.15698/mic2017.01.551

Campbell, L., H. A. Nolla, and D. Vaulot. 1994. The importance of Prochlorococcus to community structure in the central North Pacific Ocean. Limnol. Oceanogr. **39**: 954–961. doi:10.4319/lo.1994.39.4.0954

Christ, J. J., S. Willbold, and L. M. Blank. 2020. Methods for the analysis of polyphosphate in the life sciences. Anal. Chem. **92**: 4167–4176. doi:10.1021/acs.analchem.9b05144

Currie, D. J., and J. Kalff. 1984. A comparison of the abilities of freshwater algae and bacteria to acquire and retain phosphorus1. Limnol. Oceanogr. **29**: 298–310. doi:10.4319/lo.1984.29.2.0298

Dermott, R., O. Johannsson, M. Munawar, R. Bonnell, K. Bowen, M. Burley, M. Fitzpatrick, J. Gerlofsma, and H. Niblock. 2007. Assessment of lower food web in Hamilton Harbour, Lake Ontario, 2002–2004. Canadian Technical Report of Fisheries and Aquatic Sciences 2729, Great Lakes Laboratory for Fisheries and Aquatic Sciences, Bayfield Instituted, Fisheries and Oceans Canada. https://publications.gc.ca/collections/collection_2012/mpo-dfo/Fs97-6-2729-eng.pdf

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

Diaz, J. M., K. M. Björkman, S. T. Haley, E. D. Ingall, D. M. Karl, A. F. Longo, and S. T. Dyhrman. 2016. Polyphosphate dynamics at station ALOHA, North Pacific subtropical gyre. Limnol. Oceanogr. **61**: 227–239. doi:10.1002/lno.10206

Dove, A., and S. C. Chapra. 2015. Long-term trends of nutrients and trophic response variables for the Great Lakes: Great Lakes nutrient trends. Limnol. Oceanogr. **60**: 696–721. doi:10.1002/lno.10055

Downing, J. A., and E. McCauley. 1992. The nitrogen: Phosphorus relationship in lakes. Limnol. Oceanogr. **37**: 936–945. doi:10.4319/lo.1992.37.5.0936

Duhamel, S., J. M. Diaz, J. C. Adams, K. Djaoudi, V. Steck, and E. M. Waggoner. 2021. Phosphorus as an integral component of global marine biogeochemistry. Nat. Geosci. **14**: 359–368. doi:10.1038/s41561-021-00755-8

Fujiki, T., K. Sasaoka, K. Matsumoto, M. Wakita, and Y. Mino. 2016. Seasonal variability of phytoplankton community structure in the subtropical western North Pacific. J. Oceanogr. **72**: 343–358. doi:10.1007/s10872-015-0346-9

Grasshoff K, K. Kremling, and M. Ehrhardt. 1999. *Methods of seawater analysis*. 3rd ed. Wiley-VCH Verlag GmbH, Weinheim. doi:10.1002/9783527613984

Hashihama, F., and others. 2020. Biogeochemical controls of particulate phosphorus distribution across the oligotrophic subtropical Pacific Ocean. Global Biogeochem. Cycles **34**: e2020GB006669. doi:10.1029/2020gb006669

Hecky, R. E., and P. Kilham. 1988. Nutrient limitation of phytoplankton in freshwater and marine environments: A review of recent evidence on the effects of enrichment. Limnol. Oceanogr. **33**: 796–822. doi:10.4319/lo.1988.33.4part2.0796

Jentzsch, L., H.-P. Grossart, S. Plewe, D. Schulze-Makuch, and T. Goldhammer. 2023. Response of cyanobacterial mats to ambient phosphate fluctuations: Phosphorus cycling, polyphosphate accumulation and stoichiometric flexibility. ISME Commun. **3**: 6. doi:10.1038/s43705-023-00215-x

Karl, D. M., and M. J. Church. 2017. Ecosystem structure and dynamics in the North Pacific subtropical gyre: New views of an Old Ocean. Ecosystems **20**: 433–457. doi:10.1007/s10021-017-0117-0

Katsev, S. 2017. When large lakes respond fast: A parsimonious model for phosphorus dynamics. J. Great Lakes Res. **43**: 199–204. doi:10.1016/j.jglr.2016.10.012

Kramer, S. J. 2022. Characterizing global surface ocean phytoplankton community composition from in situ sampling and remote sensing. Ph.D thesis. Univ. of California, Santa Barbara. https://escholarship.org/uc/item/7vt983wz

Li, J., Y. Zhang, and S. Katsev. 2018. Phosphorus recycling in deeply oxygenated sediments in Lake Superior controlled by organic matter mineralization. Limnol. Oceanogr. **63**: 1372–1385. doi:10.1002/lno.10778

Li, J., and M. Dittrich. 2019. Dynamic polyphosphate metabolism in cyanobacteria responding to phosphorus availability. Environ. Microbiol. **21**: 572–583. doi:10.1111/1462-2920.14488

Li, J., D. Plouchart, A. Zastepa, and M. Dittrich. 2019. Picoplankton accumulate and recycle polyphosphate to support high primary productivity in coastal Lake Ontario. Sci. Rep. **9**: 19563. doi:10.1038/s41598-019-56042-5

Li, J., V. Ianaiev, A. Huff, J. Zalusky, T. Ozersky, and S. Katsev. 2021. Benthic invaders control the phosphorus cycle in the world's largest freshwater ecosystem. Proc. Natl. Acad. Sci. U. S. A. **118**: e2008223118. doi:10.1073/pnas.2008223118

Li, J., X. Yang, S. Katsev, T. Ozersky, A. Huff, and R. Gao. 2024. Polyphosphate and associated measurements from water and particulate samples collected from 13 locations in the Great Lakes in July-Aug 2018 on R/V blue heron cruise BH18-09. Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1). Version Date 2024-03-04 doi:10.26008/1912/bco-dmo.920878.1

Lin, P., J. V. Klump, and L. Guo. 2016. Dynamics of dissolved and particulate phosphorus influenced by seasonal hypoxia in Green Bay, Lake Michigan. Sci. Total Environ. **541**: 1070–1082. doi:10.1016/j.scitotenv.2015.09.118

Mahaffey, C., K. Björkman, and D. 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. V. Mooy. 2013. Fluorometric quantification of polyphosphate in environmental plankton samples: Extraction protocols, matrix effects, and nucleic acid interference. Appl. Environ. Microb. **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. U. S. A. **111**: 8089–8094. doi:10.1073/pnas.1321719111

Martin, P., F. M. Lauro, A. Sarkar, N. Goodkin, S. Prakash, and P. N. Vinayachandran. 2018. Particulate polyphosphate and alkaline phosphatase activity across a latitudinal transect in the tropical Indian Ocean. Limnol. Oceanogr. **63**: 1395–1406. doi:10.1002/lno.10780

Mindl, B., B. Sonntag, J. Pernthaler, J. Vrba, R. Psenner, and T. Posch. 2005. Effects of phosphorus loading on interactions of algae and bacteria: Reinvestigation of the 'phytoplankton-bacteria paradox in a continuous cultivation system. Aquat. Microb. Ecol. **38**: 203–213. doi:10.3354/ame038203

Mojica, K. D. A., M. J. Behrenfeld, M. Clay, and C. P. D. Brussaard. 2021. Spring accumulation rates in North Atlantic phytoplankton communities linked to alterations in the balance between division and loss. Front. Microbiol. **12**: 706137. doi:10.3389/fmicb.2021.706137

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

Mulholland, M. R., P. W. Bernhardt, B. N. Widner, C. R. Selden, P. D. Chappell, S. Clayton, A. Mannino, and K. Hyde. 2019. High rates of N2 fixation in temperate, Western North Atlantic coastal waters expand the realm of marine Diazotrophy. Global Biogeochem. Cycles **33**: 826–840. doi:10.1029/2018gb006130

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

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

Redfield, A. C. 1958. The biological control of chemical factors in the environment. Am. Sci. **46**: 205–221 https://www.jstor.org/stable/27827150

Rier, S. T., K. C. Kinek, S. E. Hay, and S. N. Francoeur. 2016. Polyphosphate plays a vital role in the phosphorus dynamics of stream periphyton. Freshw. Sci. **35**: 490–502. doi:10.1086/685859

Saati, R. 2016. *Characterization of the cyanobacterial harmful algal bloom community in Hamilton harbour*. Master's thesis. University of Toronto, https://hdl.handle.net/1807/75984

Sanz-Luque, E., D. Bhaya, and A. R. Grossman. 2020. Polyphosphate: A multifunctional metabolite in cyanobacteria and algae. Front. Plant Sci. **11**: 938. doi:10.3389/fpls.2020.00938

Sommer, U., E. Charalampous, S. Genitsaris, and M. Moustaka-Gouni. 2017. Benefits, costs and taxonomic distribution of marine phytoplankton body size. J. Plankton Res. 39: 494-508. doi:10.1093/plankt/fbw071

Stasio, B. T. D., M. B. Schrimpf, and B. H. Cornwell. 2014. Phytoplankton communities in Green Bay, Lake Michigan after invasion by Dreissenid mussels: Increased dominance by cyanobacteria. Diversity **6**: 681–704. doi:10.3390/d6040681

Sterner, R. W., E. Anagnostou, S. Brovold, G. S. Bullerjahn, J. C. Finlay, S. Kumar, R. M. L. McKay, and R. M. Sherrell. 2007. Increasing stoichiometric imbalance in North America's largest lake: Nitrification in Lake Superior. Geophys. Res. Lett. **34**: L10406. doi:10.1029/2006gl028861

Sterner, R. 2011. C:N:P stoichiometry in Lake Superior: freshwater sea as end member. Inland Waters. **1**: 29–46. doi:10.5268/iw-1.1.365

Suzumura, M. 2008. Persulfate chemical wet oxidation method for the determination of particulate phosphorus in comparison with a high-temperature dry combustion method: Particulate phosphorus determination. Limnol. Oceanogr. Methods **6**: 619–629. doi:10.4319/lom.2008.6.619

Vandergucht, D. M., J. M. Sereda, J.-M. Davies, and J. J. Hudson. 2013. A comparison of phosphorus deficiency indicators with steady state phosphate in lakes. Water Res. **47**: 1816–1826. doi:10.1016/j.watres.2013.01.004

Acknowledgments

The work is substantially supported by grants to JL from the Research Grant Council (RGC) of the Hong Kong Special Administrative Region, China (Project Reference Numbers: 26305621 and 16303022), a startup grant to JL from the Hong Kong University of Science and Technology (HKUST), and a grant from United States National Science Foundation (OCE-1737368) to TO and SK. The work is also funded by the Center for Ocean Research in Hong Kong and Macau (CORE). CORE is a joint research center for ocean research between Laoshan Laboratory and HKUST. We thank the captain and crew of the R/V *Blue Heron* for assisting in sample collection. We thank Euan Reavie (Natural Resources Research Institute, University of Minnesota Duluth) for assistance in obtaining the GLNPO phytoplankton data. The phytoplankton data are from the Great Lakes Environmental Database (GLENDA) collected by the US EPA Great Lakes National Program Office (EPA-GLNPO). Phytoplankton data were collected as part of a grant to Euan Reavie from the U.S. Environmental Protection Agency (EPA) under Cooperative Agreements GL-00E23101 and GL-00E0198. This document

has not been subjected to the EPA's required peer and policy review and does not reflect the view of the Agency, so no official endorsement should be inferred. We thank Maria Dittrich (University of Toronto, Scarborough), Liuqian Yu (HKUST, Guangzhou), and Qiong Zhang (HKUST) for their technical and intellectual support. We thank Editor James Cloern, Associate Editor Jill Baron, and two anonymous reviewers for their comments that helped to improve the manuscript.

Submitted 07 October 2023
Revised 16 March 2024
Accepted 01 April 2024