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Uptake of dissolved inorganic and organic phosphorus compounds by phytoplankton and bacterioplankton

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Abstract

Potential sources of dissolved P to phytoplankton and bacterioplankton were examined in a small meso-eutrophic lake. Kinetic analyses of whole lake water on three dates demonstrated that the maximal rate (V_{max}) for phosphate uptake was highest (5.2 nM min^{-1}) in spring. On all dates, size fractionation of plankton and kinetic analyses of uptake indicated that most (>50%) uptake of phosphate was by phytoplankton at V_{max} and by bacteria at ambient concentrations. Isotope dilution assays, with either unlabeled phosphate or various dissolved organic P (DOP) compounds, demonstrated that phosphate was the preferred substrate for uptake into both algae and bacteria. Phytoplankton had greater capacity for uptake of P from both phosphate and DOP than bacterioplankton. We concluded that phytoplankton use both phosphate and DOP, particularly at high substrate concentrations, and that bacterial utilization of P may be limited by the availability of organic C or other nutrients.

Phosphorus availability is an important factor controlling phytoplankton productivity and species composition in lakes. Proportionately greater phosphate uptake by bacteria than algae has been demonstrated repeatedly in both marine and freshwaters. Bacteria usually dominated phosphate uptake in the tidal river of the Delaware estuary, but phytoplankton dominated uptake in the lower estuary (Lebo 1990). Most studies in lakes indicate that bacteria sequester most available phosphate at ambient concentrations (Paerl and Lean 1976; Currie and Kalff 1984*b*).

Other studies have shown, however, that predominantly bacterial uptake of phosphate is not universal in aquatic systems. Phytoplankton and bacterioplankton were equally able to sequester phosphate in outer Los Angeles Harbor (Krempin et al. 1981). In Lake Michigan, phytoplankton took up nearly all of the available phosphate (Tarapchak and Moll 1990).

Because bacteria often obtain significant quantities of ambient phosphate, it has been speculated that phytoplankton may use dissolved organic P (DOP) as an alternative source of P. Significant pools of DOP exist in lakes (Wetzel 1983), but much of it may not be readily available. Although ambient phosphate concentrations and fluxes may be insufficient to support phytoplankton growth rates (Currie 1986), little direct evidence exists to suggest that phytoplankton have an advantage for uptake of P from DOP. Autoradiographic methods in whole lake water provided evidence that initial phosphate uptake was by bacteria and that phytoplankton subsequently utilized DOP that had been excreted by bacteria (Paerl and Lean 1976). Uptake of excreted P (presumed to be primarily organic) by phytoplankton was greater than that by bacteria in Lake Memphremagog (Currie and Kalff 1984*b*). Similar methods used in Lake Michigan demonstrated, however, that most of the excreted

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P was taken up by bacteria (Tarapchak and Moll 1990).

Using ^{32}P-labeled substrates, isotope dilution of $^{32}PO_4^{3-}$, and size fractionation of P uptake kinetics, we examined uptake of phosphate and P from organic compounds by phytoplankton and bacterioplankton. We attempted to determine whether phytoplankton take up DOP more effectively than bacterioplankton and whether differential uptake of phosphate and DOP by bacteria and algae is concentration-dependent. We hypothesized that phytoplankton may exploit transient increases in P concentrations to support growth.

Our results support the view that phytoplankton use both phosphate and DOP, particularly at above-ambient concentrations. Additionally, our data suggest that initial uptake of P is not indicative of the relative P acquisition potential of bacteria because their internal P pools are nearly saturated.

Materials and methods

Sampling and site description—Experiments were performed on water collected from Third Sister Lake, which is a small (3.8 ha), moderately eutrophic lake in Washtenaw Co., Michigan (Cotner and Wetzel 1991*a*). Samples were collected into sterile, 1-liter, polyethylene bottles with a Van Dorn water sampler fitted with silicone tubing. Experiments on the lake water began within 1 h of collection. All samples were incubated at ambient lake temperature in 100 μmol quanta m^{-2} s^{-1} of light.

Analytic procedures—Bacterial counts and pigment analyses of filtered (1-μm pore size, Nuclepore) and whole lake water demonstrated that differential filtration usually removed >90% of the Chl *a* and only 15% of the total number of bacteria (Cotner and Wetzel 1991*a*). Although size fractionation did not completely separate phytoplankton and bacterioplankton, 0.2–1- and >1-μm particles will be referred to here as the bacterial and algal size fractions.

Alkaline phosphatase activity was determined fluorometrically with the artificial substrate methylumbelliferyl-phosphate (MUP) in 0.01 M Tris buffer (pH 8.0) at 200 μM final concentration (Cotner and Wetzel 1991*b*). Samples were analyzed with a Turner model 111 fluorometer. Liquid scintillation analyses were performed with a Beckman model 5801 liquid scintillation counter. Nuclepore filters were prepared for radioassay by desiccating and dissolving the filter in a 10 : 1 mixture of methylene chloride : ethanolamine (Lean and White 1983). Dissolved filters were mixed with 7 ml of Redi-Safe liquid scintillation cocktail (Beckman) and analyzed in glass scintillation vials.

Kinetic determinations—Kinetic measurements were performed on duplicate, 100-ml, unfiltered lake water samples at ambient lake temperature. For phosphate uptake measurements, appropriate amounts of 30 μM KH_2PO_4 were added to samples to give nominal concentrations from 0 to 1,500 nM. Ambient concentrations were assumed to be zero. Rigler radiobioassay results indicated that ambient phosphate concentrations were usually <50 nM. Simultaneously with unlabeled phosphate, carrier-free $^{32}PO_4^{3-}$ was added at 400–800 Bq ml^{-1} final concentration. An immediate 5-ml subsample was removed and filtered at 200 mm of Hg pressure differential onto 0.2- or 1.0-μm pore-size Nuclepore filters that had been presoaked in 0.1 M KH_2PO_4 to decrease nonspecific binding. Subsequent subsamples were removed and filtered at 4-min intervals onto 0.2- and 1.0-μm pore-size filters for 20 min. Filters were dried, dissolved, and prepared for radioassay with scintillation cocktail. Total radioactivity was determined by radioassaying 1 ml of the sample.

Uptake rate into particulate size fractions was determined by multiplying the fractional uptake rate of $^{32}PO_4^{3-}$ on each filter type by nominal concentrations. Uptake into particles retained by 1-μm pore-size Nuclepore filters was determined directly and uptake into particles from 0.2 to 1 μm was estimated by subtracting radioactivity on 1-μm filters from radioactivity on 0.2-μm filters. Results are reported as P-specific uptake rates scaled to particulate P (PP) concentrations in each size fraction (Suttle and Harrison 1986). Time-zero samples were used as blanks in all uptake rate determinations. PP concentrations in size fractions

were determined by measuring total P concentrations in whole, 1- and 0.2-μm filtered lake water and subtracting total dissolved P concentrations (Am. Public Health Assoc. 1985).

Kinetic determinations of [^{32}P]ATP hydrolysis and uptake into size fractions were performed similarly with the labeled compound adenosine 5′-(γ-^{32}P)triphosphate instead of $^{32}PO_4^{3-}$. For these experiments, ambient ATP concentrations were assumed to be zero. Unlabeled ATP was prepared at 30 μM and diluted to 0–1,000 nM nominal concentrations in lake water at the start of the experiment. The specific activity of [^{32}P]ATP varied from 0.55 to 1.1 TBq mmol^{-1}, and the final concentration of added [^{32}P]ATP was 1 nM. Duplicate 100-ml samples were subsampled and filtered onto different pore-sized filters as in $^{32}PO_4^{3-}$ uptake experiments.

To determine hydrolysis rates in addition to uptake rates, we collected filtrate and removed an aliquot for radioassay. The remaining filtrate was acidified to 0.44 N with H_2SO_4, treated with 20 mg of activated charcoal, and subsequently filtered through a 0.45-μm pore-size Millipore filter that had been soaked in 0.1 M KH_2PO_4. This procedure discriminates between the proportion of [^{32}P]ATP in the filtrate that remained unhydrolyzed and $^{32}PO_4^{3-}$ (Ammerman and Azam 1985; Cotner and Wetzel 1991*a*). All ^{32}P in particles was assumed to have been hydrolyzed from ATP before being taken up. Therefore, the estimate of hydrolysis rates was made by multiplying the initial slope of the sum of fractional uptake and hydrolyzed ^{32}P that was not taken up by the sum of added [^{32}P]ATP and unlabeled ATP concentration (Ammerman and Azam 1985). Coefficients, estimated from unweighted velocities and substrate concentrations, were fitted to the Michaelis-Menten equation with nonlinear least-squares regression (Wilkinson 1989).

Isotope dilution experiments were designed to examine the effect of different DOP compounds on total community phosphate uptake and size-fractionated community uptake. These experiments were performed similarly to the method of Berman (1988) by adding tracer amounts of $^{32}PO_4^{3-}$ and 1 μM concentrations of unlabeled inorganic P (as P), organic P, or organic C compounds to duplicate samples of lake water. In the control for these treatments, no unlabeled P was added. Plankton was subsampled and size fractionated as in kinetic experiments. In all experiments 1 μM phosphate was added as an additional control. Other treatments were inorganic pyrophosphate (PP_i), adenosine monophosphate (AMP), cyclic AMP (cAMP), adenine (ADN), cyclic cytidine monophosphate (cCMP), phosphoglycollate (PGA), glycollate (GLY), glucose-6-phosphate (G6P), and MUP. All of the organic P compounds are alkaline phosphatase substrates except the cyclic nucleotides (Reid and Wilson 1971). ADN and GLY were added as controls for the organic moiety of the DOP compounds, AMP and PGA, respectively.

Effects on community uptake were esti-

Table 1. Kinetic constants for phosphate uptake in unfiltered and size-fractionated lake water. V_{max} values were normalized relative to lake water volume. SF—Size fraction. Uptake at V_{max} and PP concentrations in each SF were used to calculate PP uptake rates. Values in parentheses are standard errors of the mean.

1989	SF (μm)	K_m (nM)	V_{max} (nM min^{-1})	PP uptake (h^{-1})	PP (μM)	r^2
27 Jan	whole	187.4(25.1)	0.5(0.0)	—		0.98
	>1	225.2(69.9)	0.4(0.1)	0.06	0.46	0.90
	0.2–1	211.9(224.6)	0.09(0.0)	0.06	0.10	0.40
21 Apr	whole	60.2(6.9)	6.5(0.2)	—		0.99
	>1	173.9(18.0)	5.2(0.2)	0.46	0.68	0.99
	0.2–1	19.6(10.2)	2.4(0.2)	0.80	0.18	0.68
18 Oct	whole	52.86(26.0)	1.7(0.2)	—		0.52
	>1	95.7(25.8)	1.0(0.1)	0.14	0.43	0.91
	0.2–1	0.1(14.4)	0.5(0.1)	0.18*	0.17	0.00

* Calculated as the mean of uptake rates measured at ambient phosphate concentration because data did not fit the Michaelis-Menten model.

mated by the percent change in $^{32}PO_4^{3-}$ uptake onto particles on the 0.2-μm pore-sized filter relative to the control (only $^{32}PO_4^{3-}$ added). The importance of the two size fractions in DOP utilization was examined through the uptake of $^{32}PO_4^{3-}$ into each size fraction in the presence of unlabeled DOP relative to the total amount of $^{32}PO_4^{3-}$ taken up into particles in a particular treatment. This comparison assumes that all of the DOP compounds used are hydrolyzed to an organic moiety and phosphate before the phosphate is taken up, and also that hydrolyzed phosphate mixes with bulk-phase water. Although G6P is known to be taken up in entirety by a specific hexose-phosphate transport system in laboratory cultures of *Escherichia coli* (Torriani-Gorini et al. 1987), a similar transport system has not been demonstrated with larger DOP compounds such as AMP (Bengis-Garber and Kushner 1982).

Results

Kinetic analyses—Kinetic analyses of whole lake water uptake of $^{32}PO_4^{3-}$ demonstrated varying results at different times of the year. Results on three dates showed that phosphate uptake velocities fit the Michaelis-Menten model. Of the three dates, the April sample had the highest V_{max} (Table 1). In all three of these experiments, community phosphate uptake was essentially saturated at 1 μM added phosphate.

Analyses of phosphate uptake in January showed that the >1-μm size fraction had a PP uptake rate similar to that of the bacterial size fraction. K_m values for these size fractions were nearly equal as well (Table 1 and Fig. 1). In April the bacterial size fraction had a P-specific uptake rate at V_{max} that was about two times that of the algal size fraction, and the bacterial K_m was about a ninth the K_m of the algal size fraction (Table 1). In October, the bacterial size fraction had an uptake rate at V_{max} about half that of the algal size fraction (Table 1), but the bacterial kinetics did not fit the Michaelis-Menten model (Fig. 1C). Bacterial phosphate uptake decreased substantially at increasing concentrations of phosphate.

Phosphate treatments—The greatest iso-

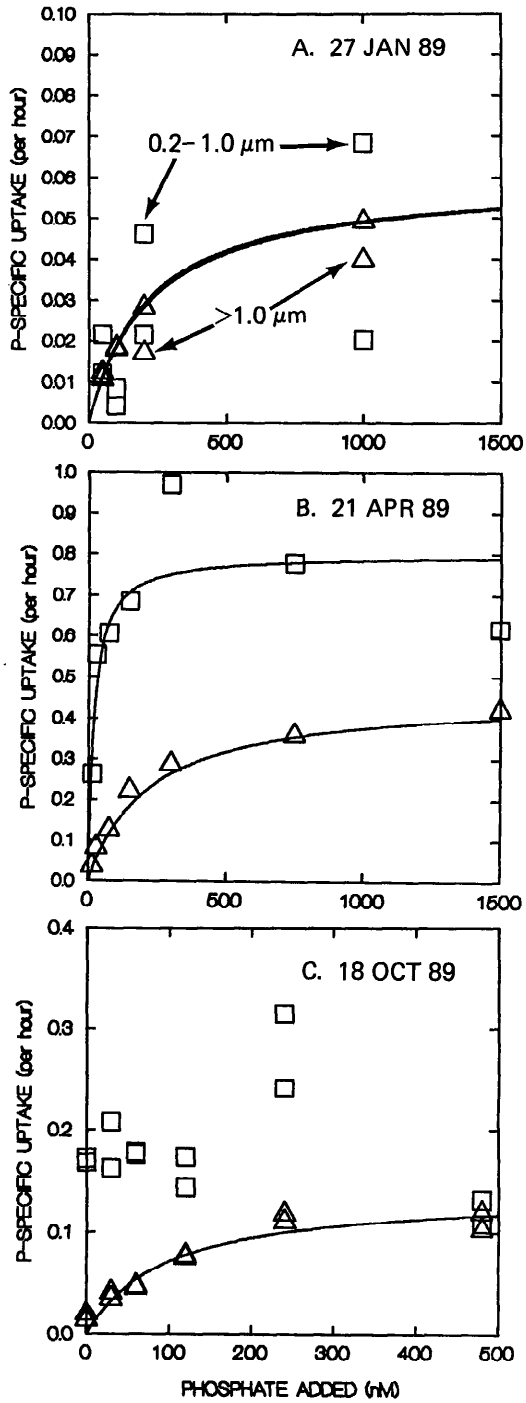

Fig. 1. Rate of phosphate uptake into size-fractionated particles. Data were fitted to the Michaelis-Menten equation with constants in Table 1. Particles >1 μm—△; particles in the 0.2–1-μm size fraction—□.

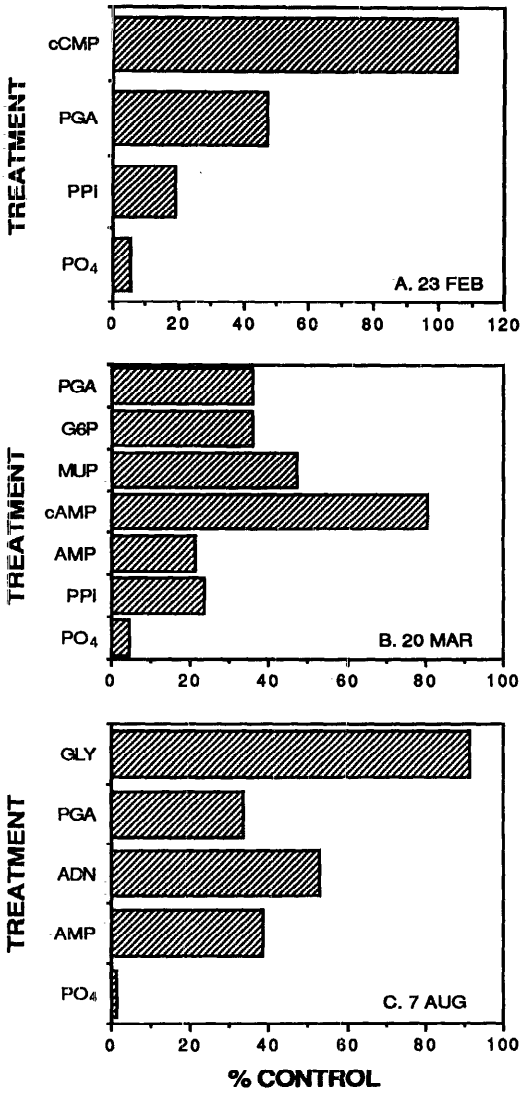

Fig. 2. Effect of 1 μM additions of unlabeled inorganic and organic P compounds and organic C compounds on $^{32}PO_4^{3-}$ uptake relative to controls. Standard errors for duplicate treatments were always <20% of the mean and usually <10% of it. Treatments were cyclic cytidine monophosphate (cCMP), phosphoglycollate (PGA), pyrophosphate (PP_i), glucose-6-phosphate (G6P), methylumbelliferyl phosphate (MUP), cyclic adenosine monophosphate (cAMP), adenosine monophosphate (AMP), glycollate (GLY), and adenine (ADN).

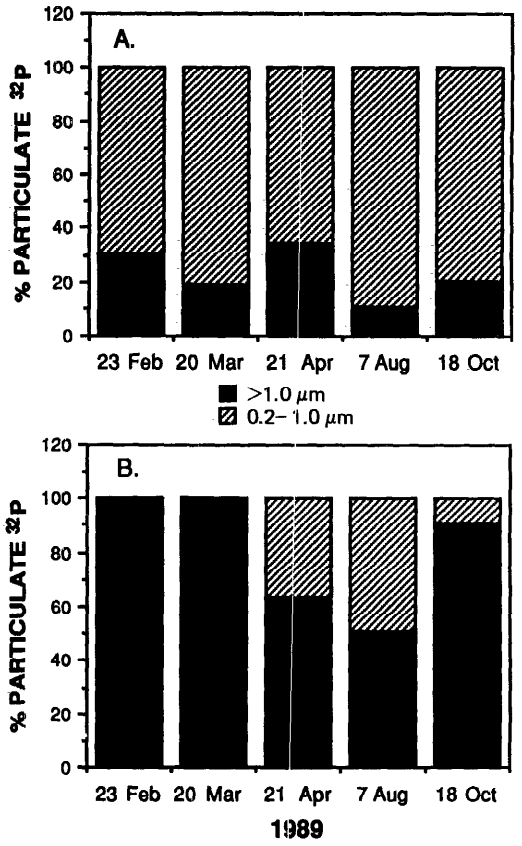

Fig. 3. Effect of 1 μM additions of unlabeled phosphate on total $^{32}PO_4^{3-}$ uptake by different-sized particles. A. No unlabeled phosphate was added to the controls. B. 1 μM phosphate was added on the corresponding dates. Tracer was added as $^{32}PO_4^{3-}$; data represent the percent of total ^{32}P uptake in each size fraction.

tope dilution effect on total community $^{32}PO_4^{3-}$ uptake was by phosphate rather than DOP treatments (Fig. 2). On all dates, the percent of total $^{32}PO_4^{3-}$ uptake into the algal size fraction significantly increased with the addition of 1 μM phosphate (ANOVA; $P < 0.01$) and the effect was most pronounced in early spring and fall samples (Fig. 3). In these samples, the percent of total community $^{32}PO_4^{3-}$ taken up in the algal size fraction increased from <30% at ambient conditions to >90% in the phosphate treatment.

In the April and August experiments, the percent of total community $^{32}PO_4^{3-}$ uptake into the algal size fraction with phosphate addition did not increase as substantially as in spring and fall (Fig. 3). In August, bacteria acquired 51% of total $^{32}PO_4^{3-}$ at 1 μM added phosphate (Fig. 3).

DOP treatments—Isotope dilution of phosphate uptake by added DOP substrates

Table 2. Kinetic constants for ATP hydrolysis and [^{32}P]ATP uptake on 10 October 1989 by size fractions (SF). Rates of PP uptake were determined at V_{max} for the >1-μm SF. For the 0.2–1-μm SF, the mean of the rate measurements at ambient concentrations of ATP was divided by PP in that size fraction to determine PP uptake rate. Values in parentheses are standard errors of the mean.

Parameter	SF (μm)	K_m (nM)	V_{max} (nM min^{-1})	PP uptake (h^{-1})	r^2
Hydrolysis	whole	111.0(65.8)	1.0(0.2)		0.72
Uptake	>1	260.2(127.9)	1.0(0.1)	0.14(0.02)	0.85
	0.2–1	0.91(0.0)	0.0(0.0)	0.00(0.00)	0.85

was used as an index of DOP uptake by the microbial community (Fig. 2). Analyses of these experiments showed that there was a significant isotope dilution effect by added DOP on all dates for all DOP compounds (ANOVA; $P < 0.01$). Additionally, DOP treatment effects differed from control phosphate treatment effects (ANOVA; $P < 0.01$). A significant interaction of P treatments and size fractions occurred on all dates, suggesting that algal size fractions took up different portions of the P pool than did bacterial size fractions.

There were large isotope dilution effects on $^{32}PO_4^{3-}$ uptake by PGA, PP_i, AMP, G6P, and MUP (Fig. 2). The cyclic nucleotides, cCMP and cAMP, had little effect relative to the controls. These results suggest that the compounds with a phosphomonoester bond were more labile than compounds with a phosphodiester bond. Therefore, alkaline phosphatase or other enzymes that hydrolyze phosphomonoesters may be important in making P available from organic P compounds in this lake.

The dissolved organic C compounds, GLY and ADN, were added in the August experiment as controls for the organic moiety of the DOP compounds (Fig. 2C). Results showed that GLY had little effect on the initial community phosphate uptake but that ADN decreased phosphate uptake to 53% of the control. Therefore, the effect of PGA was likely a simple isotope dilution effect, but there may have been additional, nonisotopic dilution effects of AMP.

Additionally, we examined direct uptake of the phosphoryl moiety from [^{32}P]ATP on one date in October. Hydrolysis at V_{max} was less than the phosphate uptake at V_{max} (Tables 1 and 2); this result suggests that planktonic capacity to hydrolyze this organic P compound was less than the capacity to take up phosphate. The initial slope for [^{32}P]ATP uptake (V_{max}/K_m; Healey 1980) was greater than that of phosphate uptake at this time (Tables 1 and 2), suggesting a greater affinity for phosphate than ATP if both are present at low concentrations. Estimates of K_m are influenced by ambient substrate concentrations in lake-water experiments, and therefore differences in ambient ATP and phosphate concentrations would affect this conclusion.

Size fractionations of [^{32}P]ATP uptake indicated that the planktonic community used this compound differently than inorganic phosphate. Similar to phosphate uptake kinetics in October, the bacterial size fraction did not demonstrate Michaelis-Menten uptake kinetics (Fig. 4). P-scaled uptake showed that algal uptake fit the Michaelis-Menten model and also that the algal size fraction was superior to bacteria in uptake at all concentrations above ambient. Comparison of [^{32}P]ATP uptake and $^{32}PO_4^{3-}$ uptake at V_{max}

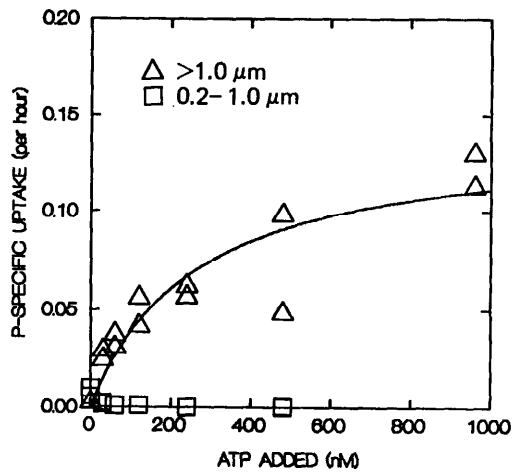

Fig. 4. Rate of [^{32}P]ATP uptake into different-sized particles, 10 October 1989. Data were scaled by P content in each size fraction and fitted to the Michaelis-Menten equation with data in Table 2.

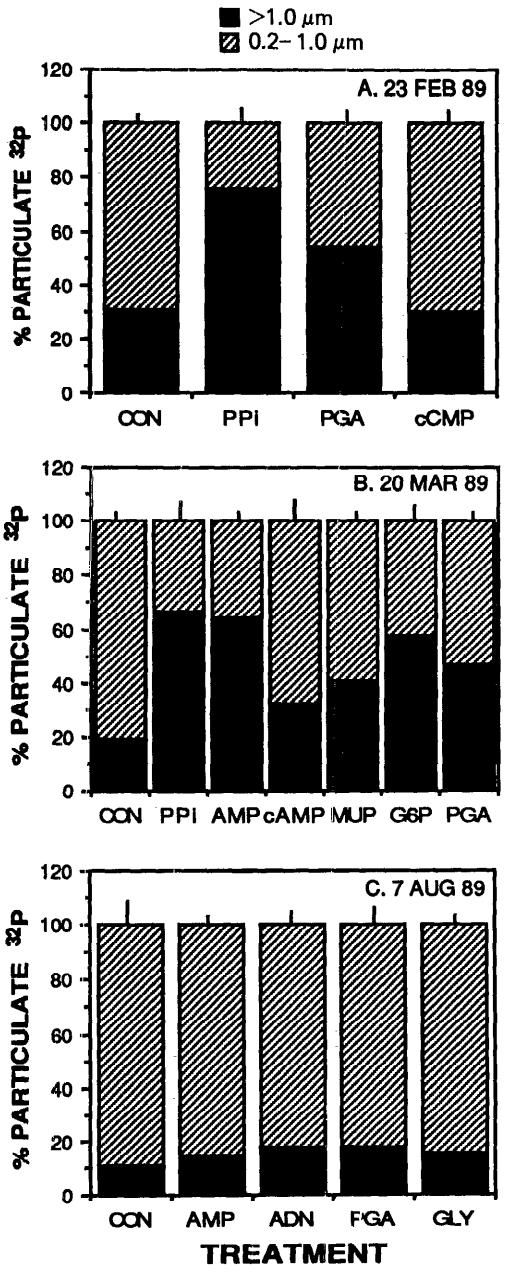

Fig. 5. Effect of 1 μM additions of various DOP and DOC compounds on $^{32}PO_4^{3-}$ uptake rates into different-sized particles. Treatments as in Fig. 2. Bars are standard errors of the mean.

suggests that phytoplankton can use either substrate equally well if both are saturating (Tables 1 and 2).

Size fractions in isotope dilution experiments were examined for the percent of $^{32}PO_4^{3-}$ taken up after treatment with unlabeled DOP compounds. In February and March samples, DOP treatments increased the percent of $^{32}PO_4^{3-}$ taken up into the algal size fraction by 0–50% relative to the control (Fig. 5). In contrast, $^{32}PO_4^{3-}$ uptake into the bacterial size fraction never increased with DOP treatments. No effect was demonstrated in the cCMP treatment in February. If our assumption that hydrolyzed phosphate mixes with the bulk-water phase is correct, increased uptake by the algal size fraction implies that the DOP treatments increased the proportion of the labile P pool taken up into the algal size fraction, just as phosphate treatments did. The compounds that had the least isotope dilution effect on total microbial $^{32}PO_4^{3-}$ uptake, MUP and cyclic nucleotides (Fig. 2), also had the least effect on algal and bacterial $^{32}PO_4^{3-}$ uptake (Fig 5).

In August, the distribution of $^{32}PO_4^{3-}$ in the size fractions changed minimally after addition of unlabeled DOP compounds. There was measurable isotopic dilution, however, of microbial phosphate uptake by unlabeled DOP (Fig. 2). These results indicate that the bacterial size fraction utilized the various DOP compounds to a greater extent than in spring samples, perhaps because the bacteria had greater phosphate uptake capacities at this time (Fig. 3).

24-h size fractionations—To determine whether initial uptake analyses reflected long-term acquisition of P by the algal and bacterial size fractions, we analyzed the distribution of ^{32}P in the March and August samples after ~24 h. These data indicate that initial uptake analyses give a conservative estimate of the long-term acquisition of P compounds by the >1-μm fraction (Fig. 6). On both dates, a larger percentage of the ^{32}P was in the algal size fraction than initial uptake analyses suggested.

This result is consistent with the view that the bacteria were less able to retain the P that was taken up and that it was reassimilated by phytoplankton or that there was transfer of ^{32}P into particles >1 μm through predation by flagellated microheterotrophs. In all treatments the relative relationships between short- and long-term analyses were consistent, i.e. if initial rates showed most

uptake by algal size fractions, then long-term analyses showed the same relationship. The only exception to this result was the AMP treatment in August. Analysis of initial uptake rates suggested that <20% of the P was moving into the algal size fraction, but the 24-h treatment showed >50% of the isotope in this size fraction (Figs. 5, 6).

Discussion

Although bacteria obtained most of the phosphate at ambient concentrations, bacterial phosphate uptake became saturated at low phosphate concentrations and an increasing proportion of phosphate was taken up by the algal size fraction at high concentrations. Only in winter samples, when neither algal or bacterial growth rates would be high and presumably competition for phosphate would be low, were the phytoplankton able to obtain much of the available phosphate at low concentrations.

The bacterial phosphate uptake did not fit Michaelis-Menten kinetics in October, probably because ambient phosphate concentrations saturated bacterial uptake sites. Similar observations have been made in mixed cultures of *Pseudomonas* and *Synedra* from Lake Memphremagog (Currie and Kalff 1984*a*). The decrease in bacterial uptake velocities observed with short-term $^{32}PO_4^{3-}$ and [^{32}P]ATP uptake in the present study may be the result of feedback inhibition of V_{max}. For example, V_{max} decreased 35-fold as intracellular P concentrations increased in cultures of phytoplankton (Gotham and Rhee 1981). Similarly, as pools of bacterial internal P became saturated, P uptake may have decreased through feedback inhibition.

Because bacterial phosphate uptake is saturated at low phosphate concentrations and algal uptake is not, an increasing proportion of phosphate is obtained by phytoplankton when ambient concentrations are increased by means of allochthonous inputs. We have measured a 4-fold increase in soluble reactive P and nearly 10-fold increase in total P after a summer rain event in Third Sister Lake. Microscale patchiness in P distributions in the epilimnion would also increase the proportional uptake of phosphate by phytoplankton relative to bacteria because

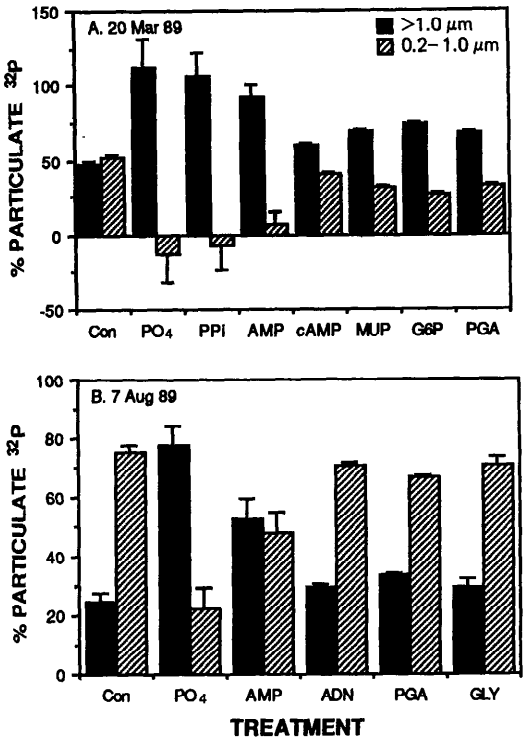

Fig. 6. Percent of particulate ^{32}P in different-sized particles with various treatments 24 h after addition of $^{32}PO_4^{3-}$. Trace amounts of $^{32}PO_4^{3-}$ were added with 1 μM concentrations of either phosphate or various DOP or DOC compounds to lake-water cultures. After ~24 h, samples were analyzed for the percent of particulate ^{32}P in each size fraction. Bars are standard errors of the mean.

of increased concentrations of phosphate in these patches (Lehman and Scavia 1982).

Our data from short- and long-term phosphate uptake suggest that the internal P pools in the phytoplankton were not saturated and much of the P taken up could be incorporated into biomass. Many taxa of phytoplankton can store significant quantities of P as polyphosphate bodies when P supplies are abundant. Because a compromise exists between enzyme-substrate binding and maximal incorporation rates at high substrate concentrations (Fersht 1985), it may not be ecologically advantageous for phytoplankton growing in habitats with fluctuating concentrations of P to have phosphate uptake enzymes that bind P efficiently at low concentrations. Additionally, the apparent detrimental effect of a low affinity for

Table 3. Percent of total alkaline phosphatase (AP) activity in the >1-μm size fraction (SF) relative to unfiltered lake water, 1988–1989.

1988–1989	AP in >1-μm SF (%)
23 Aug	69.9
29 Sep	67.8
27 Oct	78.8
28 Nov	59.4
21 Dec	36.6
23 Feb	33.4
22 Mar	86.1
19 Apr	59.8
31 May	68.9
11 Aug	39.4
13 Oct	50.0

phosphate may be offset by a high cell quota (Gotham and Rhee 1981).

It is possible that much of the phosphate that bacteria take up is not net uptake and is merely replacing losses from internal pools. Button (1991) speculated that oligotrophic bacteria leak nutrients at moderate substrate concentrations because of efficient uptake and accumulation of substrate internally. In Third Sister Lake, most net uptake of phosphate was by cyanobacteria in the <5-μm size fraction and not by heterotrophic bacteria (Lehman and Sandgren 1982). Feedback inhibition of bacterial phosphate uptake as internal P pools become saturated (Gotham and Rhee 1981) could result in increased concentrations of labeled P in algae in long-term experiments relative to short-term experiments, as was noted in this study.

For a net transfer from bacteria to algae, phytoplankton must be able to accumulate P released from bacteria. A small net flux of labeled excreted P into the algal size fraction was measured in Lake Memphremagog (Currie and Kalff 1984*b*), but in Lake Michigan most of this excreted P was taken up into bacterial size fractions (Tarapchak and Moll 1990). Another mechanism for transfer of P from the small size fraction to the >1-μm size fraction in the present study could have been predation on bacteria by microflagellates (Caron et al. 1985) or ciliates (Sherr et al. 1986). These effects would be minimized in the short-term incubations but may have been important in the 24-h incubations. If concentrations and ingestion rates of flagellated microheterotrophs in Third Sister Lake were comparable to those measured in Lake Oglethorpe (Porter 1988), flagellates could consume 3–35% of the biomass of bacteria. These percentages are comparable to the increased percentage of ^{32}P measured in particles >1 μm after 24 h; therefore, this mechanism for transfer of P between size fractions cannot be ignored.

This study supports the view that phytoplankton take up a significant proportion of the labile DOP in Third Sister Lake at certain times. Bacterial uptake of [^{32}P]ATP was greater than algal uptake at ambient concentrations, but the difference between the bacterial size fraction and the algal size fraction at this concentration was not as great as with phosphate uptake. Similar to phosphate, kinetic analyses of [^{32}P]ATP uptake demonstrated that increasing proportions of the ^{32}P were taken up by the algal size fraction at high concentrations, and bacteria did not demonstrate hyperbolic saturation kinetics. Because the ambient concentration of total DOP is greater than ambient phosphate concentration in most lakes (Wetzel 1983), there may be a greater flux of this component of the P pool into phytoplankton. High alkaline phosphatase activity in the algal size fraction lends further support to the view that phytoplankton use DOP as a P source (Table 3). These results support the hypothesis of Curric and Kalff (1984*b*) that DOP may be an important P source for phytoplankton growth.

Two other studies concluded that most P from DOP was taken up by bacteria rather than algae in lakes. Berman (1988) used isotope dilution of $^{32}PO_4^{3-}$ with unlabeled DOP compounds to examine algal and bacterial uptake of P from DOP. Because there was a greater effect of unlabeled DOP on the rate constant of the bacterial size fraction than on the algal size fraction relative to controls where no unlabeled DOP was added, he concluded that bacteria utilized DOP to a greater extent than algae in Lake Kinneret.

However, this interpretation ignored the vast differences in the shape of phosphate uptake curves in algal and bacterial size fractions. Because the initial portion of the bacterial phosphate uptake curve was steeper than that of the phytoplankton size fraction and because bacterial phosphate uptake

became saturated at a lower concentration of phosphate, an increase in phosphate release from DOP would have a much greater proportional effect on bacterial $^{32}PO_4^{3-}$ uptake rates than phytoplankton $^{32}PO_4^{3-}$ uptake rates.

In the present study, we assumed that phosphate hydrolyzed from DOP mixed with the bulk-water phosphate pool and therefore, comparisons were made between algal and bacterial phosphate uptake in the same treatment rather than in controls at ambient concentrations. Isotope dilution data from our study and that of Ammerman and Azam (1985) support the conclusion that phosphate hydrolyzed from DOP mixes with bulk-water phosphate.

The apparently preferential uptake of DOP by bacteria over algae in Lake Michigan (Tarpchak and Moll 1990) differed from our results in Third Sister Lake. This difference may or may not be a consequence of the different methodologies used. Tarapchak and Moll (1990) size fractionated before adding radiolabeled phosphate and unlabeled organic P. Although they concluded that there were no artifacts of prefiltration, this treatment decreased phosphate uptake into the bacterial size fraction by an order of magnitude in our study (data not shown).

Additionally they followed the flux of DOP with ^{32}P that presumably had been excreted by organisms and was organic (E-DOP), rather than with isotope dilution analyses of $^{32}PO_4^{3-}$ uptake by unlabeled DOP and direct analysis of ^{32}P uptake from DOP with [^{32}P]ATP as a model DOP compound. E-DOP likely is a useful tracer of naturally occurring compounds at natural concentrations, but nothing is known about its composition. Isotope dilution of $^{32}PO_4^{3-}$ uptake by unlabeled DOP and [^{32}P]ATP tracer studies have the advantage of following fluxes of specific compounds, but they may not reflect fluxes of compounds that constitute the entire DOP pool in nature.

Alternatively, there may be real biological differences in the utilization of DOP by bacteria in oligotrophic and eutrophic lakes. Bacteria in eutrophic lakes, like Third Sister Lake, may use proportionately more phosphate than bacteria from oligotrophic lakes because of greater availability of dissolved organic C (DOC) in eutrophic lakes (Tarapchak and Moll 1990). Gradients in DOC loading affect uptake of P from DOP as well (Cotner and Wetzel 1991*a*).

Although our data are consistent with the hypothesis that phytoplankton obtain a significant proportion of DOP in Third Sister Lake, one cannot assume that phytoplankton always have an advantage in DOP utilization even when high concentrations of DOP are present. Isotope dilution analyses in August suggested that bacteria took up a significant proportion of P from DOP. This result and evidence that bacterial phosphate uptake capacity was greatest at 1 μM added phosphate in August suggest that bacteria may be able to take up significant quantities of phosphate from DOP when they exhibit phosphate uptake capacities comparable to phytoplankton.

Numerous investigators have suggested that bacteria can outcompete phytoplankton for P because of their higher uptake rates for phosphate at ambient concentrations. Bacteria can outcompete phytoplankton for P in culture experiments when media are supplemented with organic C (Rhee 1972), but this result has not been demonstrated in natural systems. Because organic C can often limit bacterial growth, phytoplankton and bacterioplankton may not compete for P at all.

Results from this study do not suggest that bacteria acquire and retain P more effectively than phytoplankton. Phytoplankton were less saturated with respect to their P pools and therefore were able to garner a larger proportion of inorganic and organic P at high P concentrations. P taken up into storage pools for phosphate can then be used to support phytoplankton growth for several generations (Rhee 1972) and compensate for decreased relative uptake rates at low substrate concentrations.

Competition between algae and bacteria for P is a function of both uptake and storage capacity (Kilham 1978). The phytoplankter, *Asterionella formosa,* can store 80 times the minimum cell quota internally (Tilman and Kilham 1976). Aquatic bacteria have a higher requirement for P than phytoplankton and therefore cannot store proportion-

ately as much P (Vadstein et al. 1988). C doubling rates in the phytoplankton fraction and phosphate uptake suggested that phosphate usually provided a negligible portion of phytoplankton P in Lake Memphremagog (Currie 1986). Therefore, either DOP must have provided the necessary P, or phytoplankton supported their growth by integrating intermittent pulses of phosphate over much longer time intervals than bacteria. Both mechanisms are probably important in meeting phytoplankton P requirements.

Isotope dilution and [^{32}P]ATP uptake data suggest that bacteria took up phosphate more rapidly than P from DOP. To suggest that aquatic bacteria are not using DOP as their primary source of P is not to negate the role of bacteria as remineralizers. Bacteria are likely important in remineralizing P because many DOP compounds must be dephosphorylated before the C moiety can be taken up into the cell (Bengis-Garber and Kushner 1982), and hydrolysis is usually the rate-limiting step for incorporation of organic substrates (Hoppe et al. 1988). Therefore, whether bacteria behave as net remineralizers in lakes is likely less dependent on whether bacteria use phosphate or DOP as their substrate for growth than on the degree of C limitation and growth rate of the bacterial community. Although DOP compounds can be taken up in entirety by aquatic bacteria (Tarapchak and Moll 1990), this process would have little effect on remineralization rates if the bacteria are limited by the availability of organic C and P incorporation is dependent on DOC. As with phosphate, much of the DOP uptake would not result in net P incorporation if another nutrient limits growth.

References

American Public Health Association. 1985. Standard methods for the examination of water and wastewater. APHA.

Ammerman, J. W., and F. Azam. 1985. Bacterial 5′-nucleotidase in aquatic ecosystems: A novel mechanism of phosphorus regeneration. Science **227:** 1338–1340.

Bengis-Garber, C., and D. J. Kushner. 1982. Role of membrane-bound 5′-nucleotidase in nucleotide uptake by the moderate halophile *Vibrio costicola*. J. Bacteriol. **149:** 808–815.

Berman, T. 1988. Differential uptake of orthophosphate and organic phosphorus substrates by bacteria and algae in Lake Kinneret. J. Plankton Res. **10:** 1239–1249.

Button, D. K. 1991. Biochemical basis for whole-cell uptake kinetics: Specific affinity, oligotrophic capacity, and the meaning of the Michaelis constant. Appl. Environ. Microbiol. **57:** 2033–2038.

Caron, D. A., J. C. Goldman, O. K. Andersen, and M. R. Dennett. 1985. Nutrient cycling in a microflagellate food chain. 2. Population dynamics and carbon cycling. Mar. Ecol. Prog. Ser. **24:** 243–254.

Cotner, J. B., Jr., and R. G. Wetzel. 1991*a*. 5′-Nucleotidase activity and inhibition in a eutrophic and an oligotrophic lake. Appl. Environ. Microbiol. **57:** 1306–1312.

———, and ———. 1991*b*. Bacterial phosphatases from different habitats in a small, hardwater lake, p. 187–205. *In* R. J. Chróst [ed.], Microbial enzymes in aquatic environments. Springer.

Currie, D. J. 1986. Does orthophosphate uptake supply sufficient phosphorus to phytoplankton to sustain their growth? Can. J. Fish. Aquat. Sci. **43:** 1482–1487.

———, and J. Kalff. 1984*a*. A comparison of the abilities of freshwater algae and bacteria to acquire and retain phosphorus. Limnol. Oceanogr. **29:** 298–310.

———, and ———. 1984*b*. The relative importance of bacterioplankton and phytoplankton in phosphorus uptake in freshwater. Limnol. Oceanogr. **29:** 311–321.

Fersht, A. 1985. Enzyme structure and mechanism, 2nd ed. Freeman.

Gotham, I., and G-Y. Rhee. 1981. Comparative kinetic studies of phosphate-limited growth and phosphate uptake in phytoplankton in continuous culture. J. Phycol. **17:** 257–265.

Healey, F. P. 1980. Slope of the Monad equation as an indicator of advantage in nutrient competition. Microb. Ecol. **5:** 281–285.

Hoppe, H. G., S. J. Kim, and K. Gocke. 1988. Microbial decomposition in aquatic environments: Combined process of extracellular enzyme activity and substrate uptake. Appl. Environ. Microbiol. **54:** 784–790.

Kilham, S. S. 1978. Nutrient kinetics of freshwater planktonic algae using batch and semicontinuous methods. Mitt. Int. Ver. Theor. Angew. Limnol. **21:** 147–157.

Krempin, D. W., S. M. McGrath, J. B. SooHoo, and C. W. Sullivan. 1981. Orthophosphate uptake by phytoplankton and bacterioplankton from the Los Angeles Harbor and southern California coastal waters. Mar. Biol. **64:** 23–33.

Lean, D. R. S., and E. White. 1983. Chemical and radiotracer measurements of phosphorus uptake by lake plankton. Can. J. Fish. Aquat. Sci. **40:** 147–155.

Lebo, M. E. 1990. Phosphate uptake along a coastal plain estuary. Limnol. Oceanogr. **35:** 1279–1289.

Lehman, J. T., and C. D. Sandgren. 1982. Phosphorus dynamics of the procaryotic nanoplankton

in a Michigan lake. Limnol. Oceanogr. **27:** 828–838.

———, AND D. SCAVIA. 1982. Microscale patchiness of nutrients in plankton communities. Science **216:** 729–730.

PAERL, H. W., AND D. R. S. LEAN. 1976. Visual observations of phosphorus movement between algae, bacteria, and abiotic particles in lake water. J. Fish. Res. Bd. Can. **33:** 2805–2813.

PORTER, K. G. 1988. Phagotrophic phytoflagellates in microbial food webs. Hydrobiologia **159:** 89–97.

REID, T. W., AND I. B. WILSON. 1971. *E. coli* alkaline phosphatase, p. 373–415. *In* P. Boyer [ed.], The enzymes. Academic.

RHEE, G-Y. 1972. Competition between an alga and an aquatic bacterium for phosphate. Limnol. Oceanogr. **17:** 505–514.

SHERR, E. B., B. F. SHERR, R. D. FALLON, AND S. Y. NEWELL. 1986. Small, aloricate ciliates as a major component of the marine heterotrophic nanoplankton. Limnol. Oceanogr. **31:** 177–183.

SUTTLE, C. A., AND P. J. HARRISON. 1986. Phosphate uptake rates of phytoplankton assemblages grown at different dilution rates in semicontinuous culture. Can. J. Fish. Aquat. Sci. **43:** 1474–1481.

TARAPCHAK, S. J., AND R. A. MOLL. 1990. Phosphorus sources for phytoplankton and bacteria in Lake Michigan. J. Plankton Res. **12:** 743–758.

TILMAN, D., AND S. S. KILHAM. 1976. Phosphate and silicate growth and uptake kinetics of the diatoms *Asterionella formosa* and *Cyclotella meneghiniana* in batch and semicontinuous culture. J. Phycol. **12:** 375–383.

TORRIANI-GORINI, A., F. G. ROTHMAN, S. SILVER, A. WRIGHT, AND E. YAGIL [EDS.]. 1987. Phosphate metabolism and cellular regulation in microorganisms. Am. Soc. Microbiol.

VADSTEIN, O., A. JENSEN, Y. OLSEN, AND H. REINERTSEN. 1988. Growth and phosphorus status of limnetic phytoplankton and bacteria. Limnol. Oceanogr. **33:** 489–503.

WETZEL, R. G. 1983. Limnology, 2nd ed. Saunders.

WILKINSON, L. 1989. SYSTAT: The system for statistics. SYSTAT, Inc.

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