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Can Bacteria Outcompete Phytoplankton for Phosphorus? A Chemostat Test

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Abstract. Although the bacterioplankton of lakes are usually considered primarily in terms of mineralization processes, recent studies suggest that they may also strongly compete for phosphorus with the phytoplankton. In the present study, we have tested in chemostat culture, and found support for the hypotheses that (1) a freshwater bacterium (*Pseudomonas paucimobilis*), whose carbon source is excretion from a phosphorus-limited alga (*Synedra ulna* var. *danica*), can outcompete that alga for phosphorus (P) under widely varied P supply rates; (2) exogenously-supplied organic carbon positively influences bacterial biomass and negatively influences algal biomass; (3) the ratio of bacterial to algal phosphorus uptake in short-term ^{32}P orthophosphate uptake experiments is an accurate predictor of their relative long-term phosphorus assimilation (i.e., growth) in mixed culture.

Introduction

The bacterioplankton in epilimnetic waters are usually thought of primarily in terms of recycling dissolved organic matter and detritus [e.g., 29, 37]. While their biomass may be relatively small, the bacteria are metabolically extremely active. As an example, in Saunders' [29] study of pelagic carbon cycling rates, the bacterial carbon pool was estimated to turn over at a rate exceeding 5.0 times per day, compared with a turnover rate of 0.4 per day for the algal pool. Such rapid bacterial carbon metabolism suggests that the bacteria should also be very active in the cycling of other pelagic mineral nutrients. Allometric relationships also predict that bacteria should be disproportionately important in nutrient dynamics [2, 33].

Through the utilization of inorganic nutrients, planktonic bacteria could have a profound influence on the phytoplankton. Phytoplankton growth and production in most temperate freshwater systems has been shown to depend upon the supply of phosphorus [13, 30, 35]. It is therefore reasonable to inquire whether or not planktonic bacteria may be superior competitors for phosphorus, relative to the algae. If they were, what determines the relative utilization of phosphorus by bacteria versus algae? We began to answer this question in earlier culture studies in which the abilities of bacterioplankton and phyto-

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plankton to take up phosphorus were compared. We found that the bacteria have much higher affinity orthophosphate kinetics than co-occurring algal species (expressed in terms of specific uptake rate), and we argued on theoretical grounds that these kinetics should reflect superior competitive ability [7]. Furthermore, in a study of the short-term uptake of ^{32}P orthophosphate in situ, we showed that the bacterioplankton were apparently responsible for nearly all of the epilimnetic orthophosphate uptake [8]. From these studies we concluded that, relative to algae, bacteria have greatly superior abilities to acquire and retain phosphorus.

A major limitation of these earlier studies is that they explicitly examined only nutrient uptake, and not nutrient-limited growth. Growth and uptake are surely related; for example, uptake kinetics, coupled with allometric relationships, have successfully been used to predict the outcome of competition among phytoplankton [34]. However, interspecific variation in maximum growth rate and minimum cell quota can complicate the relationship between growth and uptake [e.g., 6, 10], and these factors have not yet been adequately investigated among bacteria. A second factor that may also affect nutrient kinetics is the nutritional status and limitation of the cells [24, 25]. Our earlier kinetic studies were done using P-limited bacteria and algae. While algal cells in freshwater are likely to be P-limited under most circumstances, there is little evidence concerning bacterial limitation in situ. The bacterial kinetic measurements made under P limitation may not reflect bacterial competitive ability if they were, for example, carbon deficient. A third consideration that could interfere with the relationship between uptake and growth is nutrient excretion or leakage [15, 19]. If bacteria tended to loose nutrient much more readily than did algae, then the pronounced bacterial advantage in uptake might be offset. The relationship between uptake and growth is obviously not unproblematic.

The general purpose of the present study was to determine whether the bacteria are superior competitors of phosphorus in terms of growth as well as uptake. More specifically, we address 3 questions proceeding from the discussion above. First, given that the orthophosphate kinetics of phosphorus-limited bacterioplankton predict that the bacteria should be greatly superior competitors for phosphorus under P-limitation, is this potential realized when the only carbon source to the bacteria is phytoplankton excretion? Second, is the partitioning of P between bacteria and algae, (and thereby their relative biomasses), a function of the supply of organic carbon? Third, given that short-term ^{32}P orthophosphate turnover studies in situ suggest that bacterial uptake of orthophosphate is much greater than algal uptake, is phosphorus uptake by algae and bacteria, as measured in short-term ^{32}P turnover studies, an unbiased indicator of long-term phosphorus assimilation (i.e., growth)?

These questions were addressed in chemostat cultures rather than in situ in order to observe the relationship between P supply and long-term P dynamics, avoiding the complication of unmeasurable losses (e.g., due to predation) in nature. Competitive superiority with respect to a nutrient (first question) is easily tested by establishing a nutrient-limited equilibrium population of 1 species, and then introducing a competitor. A necessary condition for the invasion of the competitor is superior growth kinetics [23]. The second question was addressed by observing equilibrium mixtures of algae and bacteria under

different organic carbon supply regimes. Finally (third question), it is possible in chemostat to follow short-term uptake in the same manner as in situ, and to compare the observed algal and bacterial uptake of P with the long-term utilization of P (i.e., the production of particulate P, which, under these conditions, is equivalent to growth).

Materials and Methods

The experimental organisms used in this study, *Synedra ulna* var. *danica* (Kutz) and *Pseudomonas paucimobilis* were both isolated from the epilimnion of oligo-mesotrophic Lake Memphremagog, Québec [27] and were established in axenic cultures, described earlier [7]. The organisms were grown in a dilute mineral medium [18] prepared with water which had been deionized, distilled, passed through a column of activated charcoal (to remove dissolved organic material), and finally through a high purity deionizing column. The medium contained 0.6 μM K_2HPO_4, and was buffered with $NaHCO_3$. All other inorganic materials were present in sufficient excess to be nonlimiting. The medium was entirely inorganic, except for trace amounts of vitamins, NaEDTA as a chaelator, and whatever organic contaminants were present in the water and reagents. The principal source of organic carbon for bacterial growth was therefore algal exudates. In certain cases (as noted below) D-glucose was added to the medium of some cultures as an additional source of organic carbon for the bacteria.

All experiments were conducted in 1.5–2.0 liter chemostat cultures [18] which were maintained at 20°C under continuous "cool white" fluorescent lighting at an intensity of 100 μEin m^{-2} sec^{-1} photosynthetically active radiation (approximately 0.03 ly min^{-1}). The cultures were bubbled with sterile air, and were gently stirred by a sweep stirrer, which also served to inhibit the attachment of cells to the vessel walls. At the beginning of each chemostat run, a small number of *Synedra* and/or *Pseudomonas* cells were aseptically introduced into the reactor vessels, which were then filled with medium. The chemostats were allowed to stand for 2–3 days, and then the flow of fresh medium was begun.

The abundances of algae and bacteria in the chemostats were expressed in terms of the amount of phosphorus associated with each species, rather than in terms of cell numbers, so that the units of abundance would be strictly comparable. In order to do this, the influent medium was prepared fully labeled with ^{33}P orthophosphate. After 2–3 generation times had elapsed, it was assumed that isotopic equilibrium had been reached, and the radioactivity of any phosphorus fraction was proportional to the amount of P it contained. Algal and bacterial phosphorus were then distinguished by means of size-specific filtration. Samples of chemostat medium were removed directly from the reactor vessel and were filtered sequentially through 3.0 μm and 0.2 μm pore-diameter Nucleopore filters. The isotope activity retained on each filter was determined by liquid scintillation counting in a high efficiency fluor (Aquasol, New England Nuclear). It was assumed that the 0.2 μm filter retained all algal and bacterial cells [cf. 20, 28].

Microscopic examination showed that the 3.0 μm filter retained virtually all the *Synedra* cells. The retention of *Pseudomonas* on the 3.0 μm filters was periodically determined by assaying the uptake of ^{14}C-glucose into particles retained on 0.2 μm and 3.0 μm filters. Assuming that only *Pseudomonas* takes up glucose at low concentrations [4, 38], it was determined that a relatively small fraction of *Pseudomonas* was caught on the 3.0 μm filter (16.7–19.6%). Phosphorus that passed the 0.2 μm filter was regarded as soluble. Since the size-specific separation of algal and bacterial cells was reasonably good, the results presented here are expressed simply in terms of the phosphorus associated with particles larger than 3.0, and with those between 0.2 and 3.0 μm, and are treated as representing the algae and the bacteria, respectively. It is possible to explicitly estimate the algal and bacterial phosphorus in these 2 size fractions based upon certain assumptions [8]. However, this was not done since the final results expressed in this manner are much less precise (due to the propagation of errors), and support the conclusions drawn below.

The approach of the chemostat cultures to equilibrium was determined by monitoring the distribution of phosphorus among size fractions, as well as *in vivo* chlorophyll concentration, and alkaline phosphatase activity. The latter was assayed fluorometrically [21], using 3-*o*-methyl-

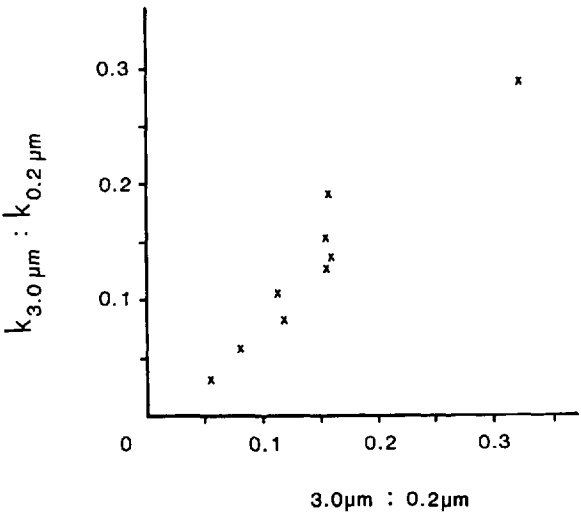

Fig. 1. The ratio of the uptake rate constants on 3.0 μm : 0.2 μm filters as a function of the ratio of ^{32}P activity on 3.0 μm : 0.2 μm filters, using data gathered from Lake Memphremagog, Québec.

fluorescein-phosphate as substrate (see ref. 32 for the fluorometer specifications and calibration). The enzyme activity was taken as the slope of a least squares rectilinear fit to fluorescence as a function of time. *In vivo* chlorophyll was assayed by Lorenzen's [17] technique with minor modifications. The fluorometer specifications were the same as those for alkaline phosphatase, substituting a Corning red CS-260 secondary filter. Cultures were taken to be at equilibrium when these 3 culture characteristics were essentially constant for 3 days.

The long-term rate of phosphorus assimilation in this study was taken as the mean net incorporation of P into particles over a 2–3 day period. It was calculated as

$$D \cdot PP_t + (PP_{t+1} - PP_{t-1})/\Delta t$$

where D = the dilution rate of the chemostat, PP_t = the amount of particulate phosphorus retained by a given filter on day t, PP_{t+1} = the amount of particulate phosphorus 1–2 days later, PP_{t-1} = the particulate phosphorus 1–2 days earlier, and Δt is the time interval. The final results were expressed as the ratio of uptake into particles larger than 3.0 μm as a fraction of the total uptake.

Short-term phosphorus uptake was determined in a manner analagous to the field determinations of ^{32}P orthophosphate turnover rates [e.g., 8, 22, 26]. Triplicate 35 ml samples were withdrawn directly from the chemostat. To each flask, 0.5–1.0 kBq of carrier-free ^{32}P orthophosphate was added. The samples were incubated on a rotating shaker at room temperature. At intervals between 2 and 60 min after spiking, 2.0 ml aliquots were filtered simultaneously through parallel 0.2 and 3.0 μm Nucleopore filters. The filters were counted in a 1:1 mixture of water and Aquasol (New England Nuclear), which quenched nearly all the background ^{33}P counts while efficiently counting the ^{32}P. Total isotope activity was determined by counting a 1:1 mixture of Aquasol and unfiltered medium.

The rate of orthophosphate uptake is usually calculated from the percentage of isotope activity retained by particles as a function of time, and the orthophosphate concentration [e.g., 7, 8]. In this study, it was estimated from the mean ratio of 3.0 μm : 0.2 μm filter activities. This statistic was used in preference to uptake rate constants because it was the more precise with the data obtained in this study. Data collected in a similar size fractionation study conducted in Lake Memphremagog [8] show that the mean ratio of 3.0 μm : 0.2 μm filter activities was a very good estimator of the ratio of uptake constants on 3.0 μm : 0.2 μm filters (Fig. 1).

Results

A series of 2-species chemostat runs served to test the relative competitive abilities of an alga and a bacterium. Four chemostats were inoculated with *S.*

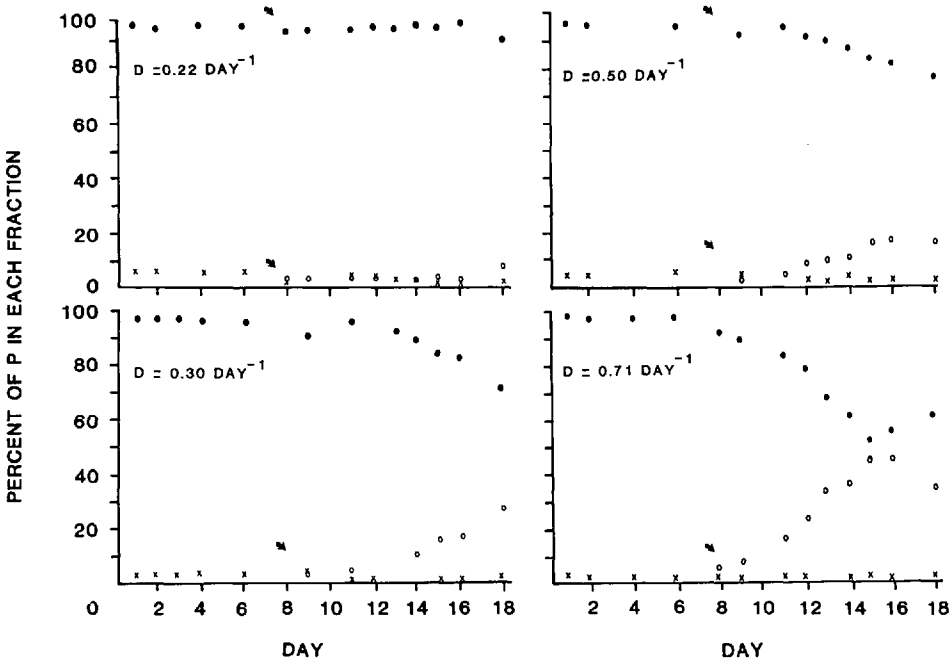

Fig. 2. The percentage of the total phosphorus in fractions larger than 3.0 μm (●), smaller than 3.0 μm but larger than 0.2 μm (○), and smaller than 0.2 μm (x; i.e., soluble) in chemostats that initially contain only *Synedra ulva* var. *danica,* and that were contaminated (*at the arrow*) with *Pseudomonas paucimobilis.* The dilution rate, *D,* is indicated in each case. In every case, the bacterium successfully invaded the equilibrium culture of the alga.

ulna var. *danica,* and were run at dilution rates of 0.22, 0.30, 0.50, and 0.71 day^{-1}. When equilibrium had been reached, each chemostat was contaminated with a small inoculum of *P. paucimobilis.* At all dilution rates, *Pseudomonas* was able to invade the chemostats and cause a decrease in the algal share of the phosphorus (Fig. 2). Direct counts showed that algal cell numbers paralleled measurements of algal P, such that the amount of P per algal cell remained constant, and algal P is proportional to algal numbers. Due to several practical constraints, it was not possible to run the chemostats long enough to reach new equilibria. Therefore, a second set of chemostats was inoculated with both *Pseudomonas* and *Synedra* and run at dilution rates of 0.20, 0.43, and 0.69 day^{-1}. New equilibria were established by day 10 (Fig. 3) with approximately 10% of the phosphorus in the bacteria at all dilution rates. The fact that a 2-species equilibrium was established indicates that the bacteria could not have been limited by phosphorus, and moreover, that the bacteria were superior competitors for P. Further evidence that the bacteria were not P-limited was provided by size fractionation of the alkaline phosphatase activity. Earlier experiments [7] had shown that *P. paucimobilis* produces abundant alkaline phosphatase activity when it is P-limited, whereas in the present mixed chemostat cultures little or no phosphatase activity was associated with the particles of bacterial size at any time. These results support the hypothesis of bacterial competitive superiority with respect to phosphorus.

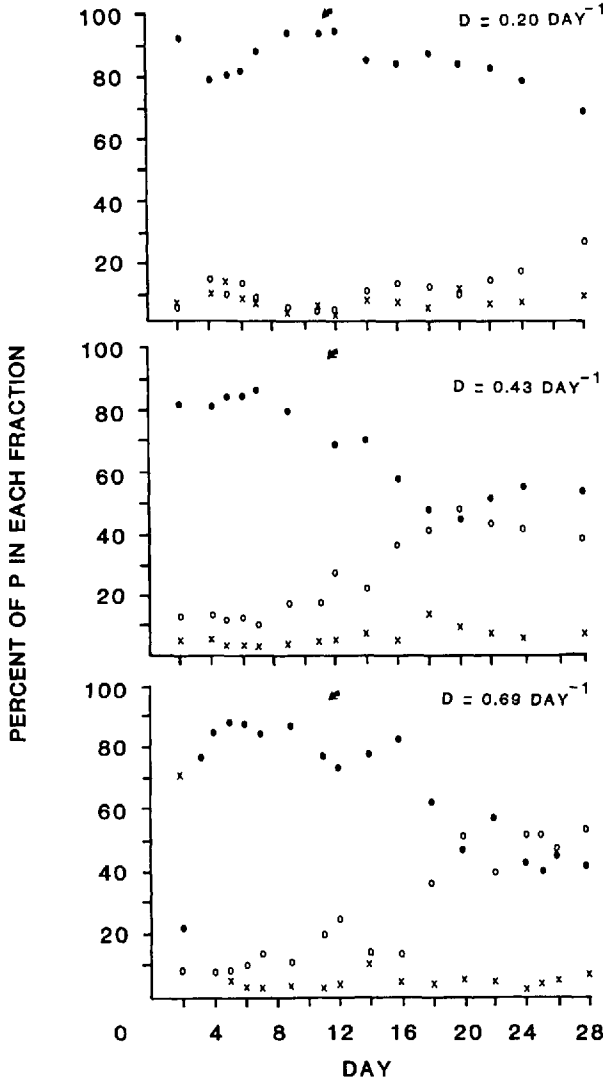

Fig. 3. The variation in the distribution of phosphorus in a chemostat containing both *Synedra* and *Pseudomonas*. At the *arrow*, the medium was changed from one containing no organic carbon to one with 1.25 mg l^{-1} of glucose. Symbols same as in Fig. 2.

If algal growth is P-limited and bacteria are superior competitors for P, then phosphorus partitioning (and thereby biomass) between these organisms should depend upon the supply of the factor that limits bacterial growth. In order to test the proposition that the supply of organic carbon alters the biomass distribution, we switched the equilibrium cultures of *Pseudomonas* and *Synedra* from the inorganic medium to one which was supplemented with 1.25 mg l^{-1} of glucose. In all the chemostats, the proportion of phosphorus in the algal size class (that larger than 3.0 μm) immediately decreased (Fig. 3). In the chemostat at the highest dilution rate (D = 0.69 day^{-1}), a new equilibrium was established in which the phosphorus was partitioned approximately equally between the

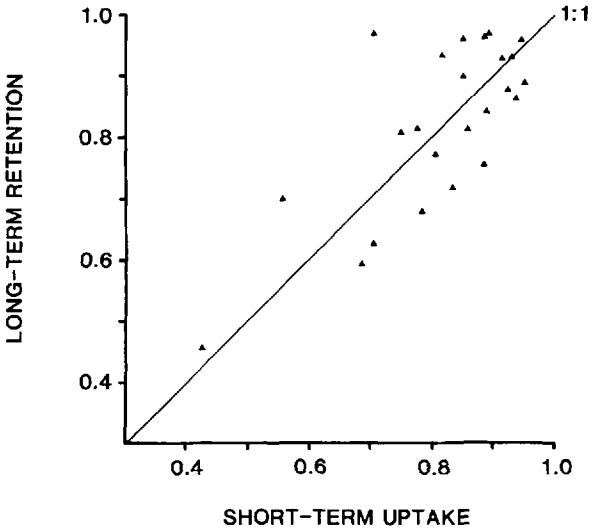

Fig. 4. The fraction of the total phosphorus assimilation which was into particles larger than 3.0 μm, as a function of the fraction of short-term orthophosphate uptake into particles larger than 3.0 μm.

algae and bacteria. In the 2 chemostats at lower D, equilibrium had not yet been attained when it became necessary to terminate the experiments. Nonetheless, it was evident in all these cultures that glucose additions caused the algal population to diminish and the bacterial population to increase.

Finally, the same chemostat cultures were used to determine whether or not short-term P utilization, as it is commonly measured in the field, reflects long-term P utilization. Samples were taken on several occasions during the chemostat runs described above. Orthophosphate uptake (short-term) and phosphorus assimilation (long-term) into the 2 size fractions were simultaneously determined. These processes are compared in Fig. 4 for the larger size class: the fraction of the total long-term phosphorus incorporation by particles larger than 3.0 μm is shown as a function of the fraction of short-term orthophosphate uptake larger than 3.0 μm. Conceptually, this may be regarded as the share of P retained by the algae as a function of their share of P uptake. Although the correlation is not as strong as one might expect (Pearson's $r = 0.753$, H_o: $r = 0$, $P = 0.0001$, $n = 24$; Spearman's $\rho = 0.560$, H_o: $\rho = 0$, $P = 0.0044$), there is no systematic tendency for uptake into a given size class to be either greater or less than the long-term assimilation into that class (sign test, H_o: $E(x_i) = E(Y_i)$, $n = 24$, $P > 0.5$; t test on logged values, H_o: mean $\ln(x_i)$ = mean $\ln(y_i)$, $t = 0.137$, $P > 0.5$). Since the proportion of uptake and assimilation into the bacterial size class are, by definition, 1.0 minus the algal size class, the same patterns hold true for the bacterial size class.

Discussion

While the dynamics of phytoplankton in situ are thought to depend to a large extent upon their ability to obtain and utilize orthophosphate [e.g., 16, 36], studies of orthophosphate uptake in situ show that very little of the uptake can

be traced to phytoplankton [3, 8, 26]. This observation is consistent with studies in culture of the relative abilities of isolated algae and bacteria to take up orthophosphate, which showed a pronounced bacterial superiority in specific at low orthophosphate concentrations [7]. If phytoplankton growth is phosphorus-limited, and if bacteria are physiologically superior competitors for orthophosphate and are responsible for most of its uptake in situ, then how do the phytoplankton manage to survive and obtain their phosphorus? Are orthophosphate uptake and phosphorus-limited growth unrelated?

These questions were addressed by observing patterns of phosphorus uptake and growth (or long-term P dynamics) in algal-bacterial assemblages in chemostats, where population dynamics are easily followed. The pair of species used in this study were arbitrarily chosen from a group of organisms examined earlier [7]. Since the phosphorus kinetics have been shown to be a function of cell size [7, 33], and bacteria and algae differ by approximately two orders of magnitude in cell size, it seems reasonable to assume that, on average, other similarly sized species should behave essentially similarly.

Bacterial competitive superiority in terms of uptake kinetics in culture is considerable [7]. In the present experiments, the bacterium was also able to outcompete [sensu Powell, 23] the phosphorus-limited alga on the basis of growth: at all the dilution rates tested, *Pseudomonas* invaded the equilibrium culture of *Synedra,* and caused the algal abundance to decline from its axenic equilibrium level. Several other experiments, which were formally identical in design to those described above, were accidentally carried out using phosphorus-limited axenic cultures of *Synedra, Cryptomonas obovata,* and *Chlamydomonas planktogloea* at various dilution rates. Single species chemostat cultures of these organisms, initiated for other purposes, were brought to equilibrium. With time, they often became contaminated with bacteria from unknown sources (presumably airborne). In every case, the algal population declined dramatically from its axenic equilibrium as soon as bacterial contamination appeared. As Powell [23] notes, a contaminant that successfully invades an equilibrium population in a chemostat is usually competitively very much superior to that organism. These observations are all consistent with the hypothesis that the bacterial advantage in orthophosphate uptake kinetics is reflected in an advantage in growth.

One possible complication could arise from the attachment of organisms to the vessel walls. The organisms in this study showed a definite tendency to attach to surfaces, which was the prime reason that experiments were deliberately kept short. Attachment of cells to chemostat walls could retard or prevent the elimination of an inferior competitor; however, it should not affect the ability of a superior competitor to invade a steady-state population of a second species. Neither should it affect the response to organic carbon enrichment. The equilibrium distribution of cells may have been affected by attachment, but our conclusion that bacteria are superior competitors should be unaffected.

The experimental conditions in this study were specifically chosen to provide as stringent a test as possible of the hypothesis that bacteria are competitively superior to algae, with respect to phosphorus. That is, we sought the conditions that appeared most likely to falsify this hypothesis. The chemostat cultures

impose strong P-limitation upon the phytoplankton, which would be expected to maximize their competitive ability [25]. On the other hand, by not providing a source of reduced carbon, we forced the bacteria to be C-limited, and thereby minimally efficient competitors for P. Nonetheless, the bacteria prevailed in competition experiments. Our conclusion of bacterial superiority should therefore be conservative.

Bacterial preeminence in steady-state uptake of orthophosphate in situ has also been shown to be considerable [3, 8, 26]. If orthophosphate is indeed the principal source of phosphorus for the plankton, and if uptake and growth are closely related, then this would suggest that the bacteria are growing much faster than the algae. As an example, consider the plankton of Lake Memphremagog. Assume for the moment that (1) orthophosphate is the sole phosphorus source to the plankton, and (2) the steady-state specific uptake rate of phosphorus is equal to the steady-state growth rate (i.e., $dP/Pdt = dN/Ndt$, where P is the amount of phosphorus in a given group of organisms, N is the biomass of those organisms, and t is time). Given these assumptions, one may estimate that, in Lake Memphremagog, the bacteria should have an average growth rate approximately 40 times higher than the phytoplankton, since orthophosphate uptake is 95% bacterial, and phosphorus in the plankton is roughly ⅓ bacterial, or less [8, and unpublished data, A. Vézina, Biology Dept., McGill University]. This seems unreasonably high, given existing estimates of algal and bacterial growth rates [cf. 1, 12, 20, 32], and therefore one or more of the premises above must be incorrect. The comparison of orthophosphate uptake and phosphorus accumulation in chemostat (Fig. 4) was intended to determine if, in a mixture of bacteria and algae, the relative specific phosphorus uptake rates of the organisms are equal to their relative specific growth rates.

The relationship between long-term and short-term phosphorus incorporation (uptake and growth) showed that uptake of orthophosphate into the algae and bacteria was, on average, an unbiased indicator of the long-term, partitioning of phosphorus between the organisms (Fig. 4); that is, there was no systematic tendency for the patterns of growth to deviate from those predicted by orthophosphate uptake. However, the relationship was not tight: only 50% of the variation in the long-term partitioning of phosphorus between the algae and bacteria was statistically related to the patterns of short-term uptake. Measurement error cannot account for all the residual variation in the relationship, since several of the observations differ significantly from the 1:1 line. This indicates that some factor that was not controlled in this study does modify the distribution of phosphorus between algae and bacteria after the initial uptake of orthophosphate. Imbalance between phosphorus uptake and growth (i.e., varying cell quota), a hypothesis readily suggested by the literature [e.g., 6, 11], will not account for the observed deviations, since in the present study growth was defined as increases in the amount of phosphorus associated with cells (as opposed to increases in cell numbers). The discrepancies observed in Fig. 4 could result from differential excretion of phosphorus after its initial uptake, utilization of nonorthophosphate P, or from differential loss rates. Differential loss rates on 2 populations in a chemostat are unlikely, but not impossible. Differential excretion and differential utilization of dissolved organic phosphorus (DOP) are possibilities suggested by earlier work [7, 8, 14].

However, whatever the mechanism responsible for the observed variation, it did not systematically favor either the bacteria or the algae in the chemostat. If, for example, bacteria excreted proportionally more phosphorus than algae, one would expect the majority of observations in Fig. 4 to fall above the 1:1 line; this was not observed. This finding is therefore consistent with the hypothesis that uptake of orthophosphate is an accurate indicator of relative growth rates in an assemblage of organisms.

What does this result suggest regarding phosphorus cycling in nature? It may indicate that bacteria do indeed have very much higher realized growth rates than do the algae, and that existing estimates of bacterial and/or algal growth rates are in error. Alternatively, it is possible that orthophosphate is not the principal P source to the algae; rather, another form of phosphorus such as dissolved organic phosphorus may be preferentially available to the algae. This would cause the relative growth rates estimated from specific orthophosphate uptake to be in error. There is some limited evidence that this might be the case in nature [8]. In the present chemostat experiments, dissolved organic phosphorus levels were much lower than those in situ (5–10% of the total phosphorus, versus approximately 37%; cf. Figs. 2 and 3 above, and ref. 22). It is possible that the partitioning of orthophosphate uptake between phytoplankton and bacteria is indicative of their relative growth rates in chemostat where very little dissolved organic phosphorus is available, but that the algae use the organic phosphorus extensively when it is available.

The glucose addition experiments (Fig. 3) demonstrate that, in chemostat, bacterial use of phosphorus strongly influences algal dynamics, and that the supply of organic carbon impels the system. Similar results have also been observed in culture with an alga and a yeast [5]. Is this also true in nature? One can hypothesize that bacterial activity is primarily a function of their supply of organic carbon. The bacteria would then utilize a proportional quantity of phosphorus. The remaining phosphorus would determine algal activity and abundance. If this were true, one would expect organic carbon additions in situ to cause a decrease in algal abundance. In at least one case, this has been observed [31]. A second prediction emanating from this hypothesis, which we are presently investigating, is that the variation in observed phosphorus-chlorophyll relationships in situ [9] may be related to bacterial activity or abundance.

To summarize, in a series of chemostat competition experiments between a freshwater bacterium and a co-occurring alga, the bacterium consistently outcompeted the alga for phosphorus. With a fixed, small amount of phosphorus available to the 2 groups of organisms, the supply of organic carbon to the chemostats influenced the distribution of biomass in the 2 groups of organisms. Finally, the specific uptake of orthophosphate by each of these groups was proportional to their specific growth rates. These results suggest that, in situ, either bacteria are growing very rapidly compared with the algae, or algae extensively utilize phosphorus sources other than orthophosphate.

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