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Competition for phosphorus among co-occurring freshwater phytoplankton[1]

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Abstract

Competition at different levels of phosphorus availability was observed in continuous cultures of freshwater phytoplankton communities. Although dilution rates ranged 10-fold in all, the outcome of competition was usually similar among cultures and resulted in dominance of the small diatom *Synedra acus*. Species growth rates decreased significantly with increasing cell, or colony, size among the 16 species examined. A variable internal stores model of algal growth, combined with functions relating its species-specific parameters to cell size, correctly predicted the observed inverse correlation between cell size and competitive ability. The model's predictions, and the empirical correlation between size and growth, were poor at low dilution rates (<0.2–$0.3 \cdot d^{-1}$), probably due to cell death. The results indicated that variation of phosphorus supply is unlikely to be a major selective influence on the size or species composition of uniformly phosphorus-limited communities.

Interspecific competition for nutrients has long been assigned a central place in controlling phytoplankton succession and species composition (Hutchinson 1967; Dugdale 1967). The classical theory of nutrient-determined succession postulates that one species is superior in growth at low nutrient concentrations, but that some other species is superior at a higher concentration so that the two (or potentially more) species partition natural gradients in the availability of the nutrient (Dugdale 1967; Stewart and Levin 1973). More recently, emphasis has been placed on the demand : supply ratios for multiple, potentially limiting, resources (e.g. Tilman 1981; Rhee and Gotham 1981*a*,*b*; Holm and Armstrong 1981) and a case has been made for the control of some freshwater diatom species distributions by the relative, as well as absolute, supply of silicon and phosphorus (Tilman 1977). Such multiple resource partitioning could account for much of the species diversity and complex successional change seen in nature (Petersen 1975), particularly if underlain by classical, single-resource partitioning. Classical resource partitioning has been demonstrated for nitrogen-limited marine systems by showing that the outcome of competition in continuous cultures depends on the nitrogen supply rate (Dunstan and Tenore 1974; Harrison and Davis 1979), and it continues to attract attention as a possible explanation for species distributions in nature (Mickelson et al. 1979). In many freshwaters, where a variety of evidence points to frequent control of algal abundance by phosphorus supply (Vollenweider 1968; Schindler 1974; Healey and Hendzel 1980), it is especially interesting to examine the potential selective effects of an apparently ubiquitous limiting nutrient.

It is frequently assumed that growth competition among phytoplankton occurs to the advantage of smaller cells (Porter 1977; Margalef 1978); zooplankton grazing (O'Brien 1974), size-dependent respiration (Laws 1975), and photosynthetic light response (Schlesinger et al. 1981) have all been proposed to balance size-dependent nutrient competition in nature. If true, such size dependence implies that species-selective effects of competition should be observed only among species of similar size. Yet there are clear cases of large species outcompeting smaller (Parsons and Takahashi

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1973; Tilman 1977) and differences of taxonomy and habitat sometimes appear more important than simple size differences to competition-related physiological attributes (Chan 1978; Brand and Guillard 1981). The existing direct evidence for a generally significant advantage to small cells derives primarily from the well established decrease of maximum (nonnutrient-limited) growth rate with increasing cell size (Banse 1976).

The evidence for a comparable situation under nutrient limitation is indirect and incomplete. Nitrogen and phosphorus uptake rates are probably more rapid for small cells than large (Eppley et al. 1969; Friebele et al. 1978; Smith and Kalff 1982), but uptake rates alone do not determine growth rate under phosphorus or nitrogen limitation (Droop 1974; Goldman 1977). The variable internal stores model of algal growth, which describes the growth of phosphorus-limited monocultures fairly well (Droop 1974; Goldman 1977), can be combined with empirical functions relating its parameters (maximum growth rate, cell maintenance quota, and uptake kinetics) to cell size (Banse 1976; Shuter 1978; Smith and Kalff 1982). The resulting size-dependent model does predict that small cells will grow faster than large under phosphorus limitation, but this prediction rests on the as yet untested validity of the underlying growth model for describing multispecies systems. Recent results with monocultures (Gotham and Rhee 1981*a*,*b*) indicate that the model requires modification to become generally applicable.

If the magnitude of a size-dependent growth differential varies with phosphorus availability, then competition could contribute to the systematic changes in phytoplankton size composition observed among lakes of differing nutrient supply (Pavoni 1963; Gliwicz 1967; Watson and Kalff 1981). According to the precise assumptions made about the functional relationship between growth and nutrient availability, increased severity of competition has been predicted to maintain or increase the advantage of smaller cells (O'Brien 1974) or, somewhat paradoxically, to decrease it (Laws 1975; Smith and Kalff 1982). One experimental study indicates that the small-size advantage may be reduced under severe light limitation (Schlesinger et al. 1981), but this situation does not seem general (Taguchi 1976; Chan 1978). There has apparently been no systematic experimental study of comparable effects under phosphorus limitation.

We consider here three major hypotheses: that competition for phosphorus favors smaller cells over a range of competitive intensities; that the variable internal stores model is applicable to competition in communities of phytoplankton; and that co-occurring freshwater phytoplankton can partition gradients in the availability of limiting phosphorus. We report experiments with phosphorus-limited continuous cultures of freshwater phytoplankton communities from Lake Memphremagog (Quebec-Vermont) designed to test these hypotheses.

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Materials and methods

Experimental design—To test our hypotheses, we wished to create systems of different limiting phosphorus availability, in which competition in reasonably natural communities could be observed. Continuous culture is a well established technique for manipulating nutrient availability in unialgal cultures (Herbert et al. 1956; Droop 1974); after an initial equilibration period, the population grows at a rate equal to the culture's specific flushing rate (dilution rate, D) and achieves a density proportional to the influent concentration of the limiting nutrient. Population growth rates, and nutrient availability, can then be manipulated by running parallel cultures at different dilution rates. The dynamics of individual species in continuous culture can be described (Herbert et al. 1956) as

$$dN/dt = (\mu - D) \cdot N \qquad (1)$$

(where N is population size, D is dilution

rate, and μ is specific growth rate, per day).

Mixed species continuous cultures are more complicated to analyze than unialgal cultures because true equilibrium is not attained until the superior competitor or competitors have displaced all others. However, theoretical study of mixed species competition in chemostats suggests that the competing species should quickly reach approximately constant growth rates, μ, and that all except the superior competitor then have growth rates $<D$; exclusion accordingly occurs at a roughly constant exponential rate, $\mu - D$ (Powell 1958; Taylor and Williams 1975). Therefore, although not true equilibrium systems, mixed species continuous cultures should permit measurement of exclusion rates under reasonably constant conditions. This conclusion is supported by evidence that both community biomass and nutrient content do quickly equilibrate in mixed cultures, with most species exclusion occurring under an equable nutrient supply (Peterson et al. 1974; Jones et al. 1978). Mixed cultures of two species have previously been used to study interspecific competition of varying intensities (Tilman 1977; Holm and Armstrong 1981; Mickelson et al. 1979).

Species-specific growth rates, μ, can be related to available nutrient concentration and species-specific nutrient kinetics by the variable internal stores model of Droop (1974). The model assumes that growth rate is a saturating function of intracellular phosphorus content (cell quota, q):

$$\mu = \mu'_{\max} \cdot (q - k_q)/q \qquad (2)$$

(where $\mu'_{\max}$ is growth rate at infinitely large cell quota and k_q is maintenance cell quota). Growth rates are indirectly related to available phosphorus through two additional equations:

$$\text{uptake rate, } V = \mu \cdot q, \qquad (3)$$

$$\text{and} \qquad V = V_{\max} \cdot S/(K_m + S). \qquad (4)$$

When Eq. 3–4 are combined, μ can be written as a function of available phosphorus concentration:

$$\mu = \mu'_{\max}/(1 + \mu'_{\max} \cdot k_q/V). \qquad (5)$$

Growth rate at any one phosphorus availability accordingly depends on species-specific values of $\mu'_{\max}$, k_q, $V_{\max}$, and K_m.

More recent work on phosphorus-limited algal growth shows that $V_{\max}$ is not a true constant as used here, but instead increases to a maximum as growth rate, and cell quota, are reduced to their minimum values (Gotham and Rhee 1981*a*). The labor of determining such $V_{\max}$ inhibition functions, which appear species-specific, was prohibitive. We relied on the constant $V_{\max}$ formulation, which has performed quite well in many studies (Droop 1974; Tilman and Kilham 1976; Tilman 1977; Goldman 1977), but we do consider the possible consequences of $V_{\max}$ inhibition to our results (*see below*).

Equation 5 can also be used to predict the effects of size-dependent physiological variation on interspecific competition. Allometric functions of the form ($y = aW^b$) have been previously defined for $\mu_{\max}$ (the actually observable maximum growth rate) and for k_q (Banse 1976; Shuter 1978) and for k_q and $V_{\max}$ with some of the species of the current study (Smith and Kalff 1982). At least for phosphorus limitation, $\mu_{\max}$ and $\mu'_{\max}$ are quite similar (Goldman 1977). Additionally, K_m has been shown not to vary significantly among co-occurring freshwater species (Smith and Kalff 1982), so substitution of these functions in Eq. 5 allows prediction of growth rates as a function of cell size and phosphorus availability:

$$\mu = aW^b/(1 + cW^d * S') \qquad (6)$$

[where S' is $(K_m + S)/S$, aW^b is the allometric function for $\mu_{\max}$, and cW^d is an allometric function combining the covariation with size of $\mu_{\max}$, $V_{\max}$, and k_q]. We used Eq. 6 to generate predictions of how growth rates should vary among species in communities growing at different levels of phosphorus availability.

Our experimental procedure consisted of parallel cultures differing in dilution rate, in which the outcome of competition of different intensities was observed both by the direction of succession and

by the growth rates of the competing species. Our first hypothesis predicted an inverse relationship between growth rates and cell size, our third that the direction of succession should change with dilution rate.

Our second hypothesis could not be critically tested because of difficulties in accurately measuring the minute available phosphorus concentrations in phosphorus-limited systems (Rigler 1966). Lacking, therefore, measurements of phosphorus availability [$S/(K_m + S)$: Eq. 4] we solved the model for a wide range of assumed availabilities to determine whether any choice could produce agreement between the model and the observations. This was not a critical test but could detect failings in the model.

Experimental procedure—Four culture experiments, each consisting of 4–6 cultures operating in parallel, were run with starting dates of May, July, and September 1978 and October 1979. The first experiment consisted of three replicate cultures to assess reproducibility, but dilution rates were varied between 0.055 and 0.93 per day among cultures in the remaining experiments. The cultures were operated for 21–48 days, and samples withdrawn from the reactors at 2–3-day intervals for measurement of total and species-specific phytoplankton biomass.

The cultures comprised a 2.0- (for dilution rates $\leq 0.2 \cdot d^{-1}$) or 1.0- (for higher dilution rates) liter reactor, a reservoir of filter-sterilized lake water medium, and a peristaltic pump delivering medium to the reactor through silicon rubber tubing. The reactors were continuously mixed and the walls scrubbed daily to eliminate growth. Lighting, on a 12:12 LD cycle, was white fluorescent at a culture-surface intensity of 200–300 μEinst$\cdot m^{-2} \cdot s^{-1}$ PAR, depending on the experiment. The entire system was housed in a controlled temperature room matched to the ambient lake temperature (8.5°C in experiment 1, and 18°–20°C in the others).

For each experiment, a 10-m integrated tube sample from the central part of Lake Memphremagog was screened (102-μm mesh) to remove the larger zooplankton, enriched with 10% phosphorus-free synthetic medium (Morgan and Kalff 1979) to ensure phosphorus limitation, and transferred to the reactors as inoculum. The medium was prepared with additional lake water similarly enriched, after filter-sterilization and addition of K_2HPO_4 to approximately match the phosphorus concentration in the inoculum. The inoculum itself received a small (0.06–0.16 μM P) enrichment to stimulate growth. If we assume that soluble, available nitrogen was totally depleted in the lake water and accept the lowest of the existing silicate estimates for central Memphremagog (Cattaneo and Kalff 1980), then the resulting medium had molar N:P and Si:P ratios of 65 and 270. Actual ratios were probably higher, but these conservative estimates seem to preclude Si or N limitation (Tilman 1981; Rhee and Gotham 1980)—a conclusion consistent with signs of strong phosphorus limitation in the cultured communities (Smith and Kalff 1981). Pumping started after 2–3 days of acclimation to culture conditions.

Samples for phytoplankton counts were preserved with Lugol's iodine and counted by the Utermöhl technique. Cell volumes were calculated from geometric approximations from dimensions measured on at least 25, and usually 50 or more, individuals. Colonies were treated as units if the individual cells were closely appressed and of much less exposed surface area than free cells. Biomass was calculated from cell volumes as fresh weight, assuming a specific gravity of unity.

Total phosphorus was measured in samples taken at 2–3-day intervals to monitor phosphorus supply to the cultures. Analysis was by colorimetry after digestion wtih persulfate (Johnson 1971). The inducible enzyme alkaline phosphatase (APA), which increases in activity as phytoplankton become more phosphorus-deficient (Healey and Hendzel 1979), was measured in samples usually also taken at 2–3-day intervals; APA indicated that phosphorus deficiency did equilibrate in the cultures and varied inversely with the dilution rate among cultures (Smith and Kalff 1981).

Table 1. Cell size (Vol, μm^3) and growth rates (μ, day^{-1}) at five dilution rates for 11 species of experiment 3. First six comprise data for Fig. 7B. (n.s.—No significant fit to exponential growth model.)

		Growth rate at dilution rate				
	Vol	0.06	0.06	0.20	0.30	0.43
Synedra acus	69	0.06	0.06	0.20	0.30	0.43
Dinobryon bavaricum	80	0.06	0.06	0.07	0.17	0.33
Asterionella formosa	339	−0.05	n.s.	0.06	0.14	0.29
Fragilaris crotonensis	6,262	−0.05*	−0.06*	0.0	0.13	0.31
Aphanizomenon flos-aquae	1,497	n.s.	n.s.	−0.08	0.16	0.30
Anabaena planctonica	8,980	n.s.	−0.20	−0.03	0.13	0.28
Stephanodiscus astreae	6,761	−0.02	0.0	0.0	0.09	0.17
Rhizosolenia eriensis (L)	851	−0.08*	−0.15*	0.09	0.14	0.32
Monoraphidium setiforme	36	−0.05	−0.03	0.14	0.30	0.43
Oscillatoria limnetica	275	−0.08	−0.06*	0.20	0.30	0.32
Coelosphaerium naegilianum	12,882	−0.06	−0.11*	0.04	0.08	0.16

* Growth rates significantly <0.

Phosphorus uptake rates, at added orthophosphate concentrations of 0–50 μg P·liter^{-1}, were measured by autoradiography in samples collected after 1–3 weeks of culture operation (Smith and Kalff 1982). The cultures used for uptake measurements had dilution rates of 0.06·d^{-1} (experiments 1 and 3) or 0.2·d^{-1} (experiments 2 and 4). Uptake rates were fit to the Michaelis-Menten curve to yield the parameters of uptake. Phosphorus cell quotas were also measured autoradiographically with samples from a fully labelled culture (Smith and Kalff 1982). These measurements provided allometric functions substituted in Eq. 5.

Species-specific growth rates were measured with a linearized version of Eq. 1:

$$\ln(\%N_o) = a + (\mu - D)\cdot t \qquad (7)$$

(where N_o is population size at peak abundance, and t is time, days). If μ and D are constants, then ln ($\%N_o$) should be a linear function of time. Equation 7 was fitted by linear regression to the time-course of species abundance during exclusion from culture, and μ found by adding the known dilution rate to the observed exclusion rate, $\mu - D$.

Regression analysis (least-squares), including interpretation of multiple regressions, followed Draper and Smith (1966). For regressions examining the relationship between competitive ability and cell size, uptake rates, and dilution rates, we used the observed exclusion rates in preference to the more familiar growth rates, μ, to avoid introducing spurious correlation with dilution rate.

Results

Species succession—Three of the four culture experiments underwent succession to a simple, diatom-dominated composition in each culture. Experiment 1, started in May 1978, was initially dominated by diatoms, cryptomonads, and

Table 2. As Table 1, but at six dilution rates for six species of experiment 4.

		Growth rate at dilution rate					
	Vol	0.22	0.21	0.34	0.32	0.76	0.93
Synedra acus	163	0.22	0.21	0.34	0.32	0.76	0.93
Asterionella formosa	295	0.15	0.13	0.28	0.20	0.68	0.75
Aphanizomenon flos-aquae	1,368	0.02	0.02	0.01	0.01	0.47	0.62
Anabaena planctonica	7,674	0.03	0.01	0.05	0.12	0.40	0.61
Oscillatoria tenuis	11,455	−0.01	0.06	0.03	0.05	0.40	0.56
Fragilaria crotonensis	18,113	0.04	−0.1	−0.09	0.03	0.43	0.57

Chrysophyceae. After 27 days of growth at a dilution rate of 0.15 per day, the small diatom *Synedra acus* comprised 62–84% of community biomass among the three replicate cultures. *Asterionella formosa*, the sole other species still abundant, constituted 12–35% of the total biomass. Experiments 3 (September 1978) and 4 (October 1979) each encompassed a range of dilution rates (Tables 1, 2) and were initially dominated by a mixture of diatom and blue-green algae. As in experiment 1, the cultures in both became dominated by *S. acus*, which comprised 75% or more of the community biomass after 48 (experiment 3) or 24 (experiment 4) days of competition (e.g. Fig. 1).

In each of experiments 1, 3, and 4, the community biomass increased to a plateau level (e.g. Fig. 1). Most species exclusion occurred after total biomass had equilibrated (Fig. 1A), although biomass equilibration was occasionally more delayed (Fig. 1B). Total biomass, averaged over the plateau region, was positively and significantly correlated with the total phosphorus concentration of the medium among experiments 1, 3, and 4 ($r = 0.80$, $P < 0.01$).

Experiment 2, run in July 1978, was initially dominated by cryptomonads and diatoms. Succession was again predominantly toward a simple, diatom-dominated community among the cultures of varying dilution rate (Fig. 2, Table 3). *Synedra acus* was once more an important species, but shared dominance with the larger *Synedra ulna* in the two cultures of lowest dilution rate, and was dominated by the Chrysophycean *Dinobryon bavaricum* in one other culture (Fig. 2). Succession was less complete at the end of experiment 2 than in the other experiments; *S. ulna* in particular seemed likely to be excluded from cultures of low dilution rate as it had been from those of higher dilution rate.

Unlike other experiments, total biomass in experiment 2 first increased and then decreased with time to various degrees among the cultures (Fig. 2). Total phosphorus in the reactors was also high initially but then declined, the result of

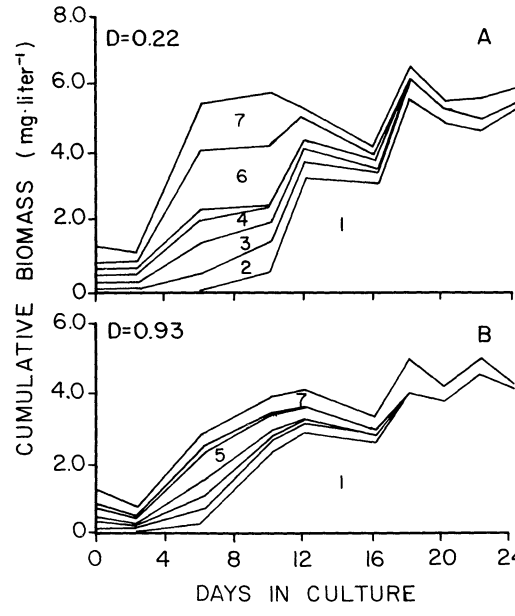

Fig. 1. Cumulative biomass vs. time in two cultures at two dilution rates from experiment 4. Numbers 1–6 are species of Table 2 (*S. acus* = 1), and 7 is the sum of all other species.

an accidental mismatch between the phosphorus concentration in the reservoir and that in the reactor at the outset. Total biomass appeared near to equilibrium with reservoir phosphorus concentrations by the experiment's end, correlating positively with total P ($r = 0.96$, $P < 0.01$).

In all four experiments, most species in the inoculum first increased in abundance before reaching a rough equilibrium or declining again (e.g. Fig. 3A). Notable exceptions were most cryptomonads and small flagellate Chrysophyceae, which comprised the smallest nannoplankton and which disappeared from the cultures within a few days of collection. Their disappearance coincided with transfer to the mixed and bubbled culture vessels, and preceded the increase of community biomass and development of phosphorus deficiency symptoms. This indicates that competition was probably not involved in their disappearance, which may have been caused by turbulence in the cultures. For other species,

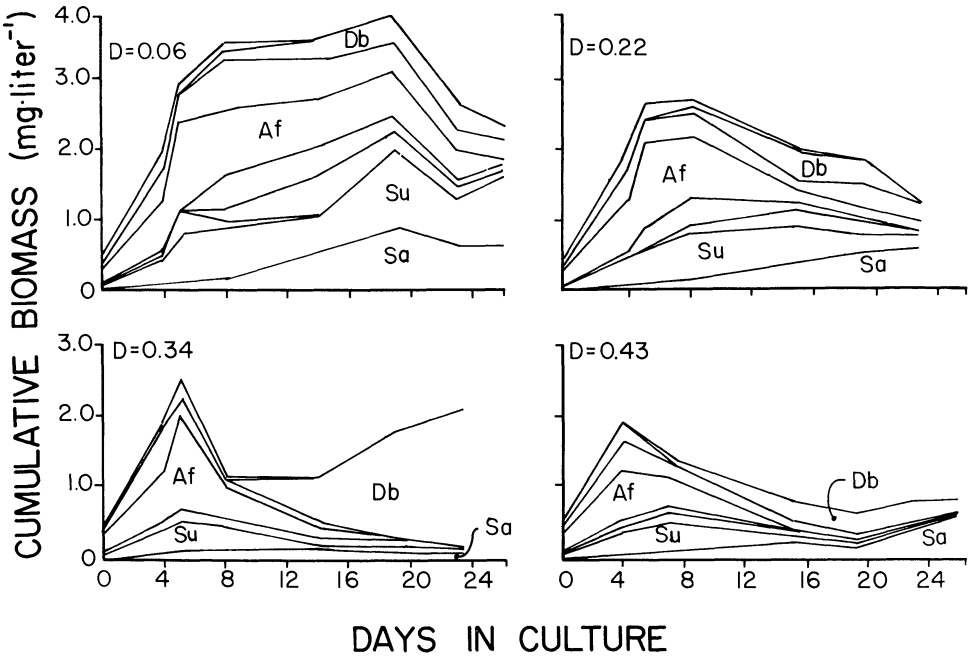

Fig. 2. Cumulative biomass vs. time in four cultures at four dilution rates of experiment 2. Initials refer to first four species of Table 3.

dynamics (not quantified in experiment 1) usually conformed to an exponential decline, after an initial increase in abundance of from 1.5-fold to about 15-fold, depending on the species concerned. Figure 3B illustrates three cases, representing roughly the best, worst, and average goodness-of-fit to the exponential growth model. The measurement period was from about day 6 to the end of the experiment (21–48 days), depending on the species and culture concerned.

Growth rates (the difference between exclusion and dilution rates) of six species in experiment 4 varied from −0.1 to 0.93 per day in all (Table 2). Negative growth rates occurred in cultures of the lowest dilution rate, but were not significantly different from zero. In experiments 2 and 3, significantly negative growth rates did

Table 3. As Table 1, but at five dilution rates for nine species of experiment 2. (n.s.—No significant fit to exponential growth model.)

		Growth rate at dilution rate				
	Vol	0.055	0.06	0.22	0.30	0.43
Synedra acus	158	0.055	0.06	0.22	0.30	0.43
Synedra ulna	5,100	0.055	0.06	0.13	0.05	0.35
Asterionella formosa	402	−0.046	−0.16*	0.0	0.05	0.17
Dinobryon bavaricum	60	0.055	0.06	0.22	0.30	0.43
Stephanodiscus astreae	2,000	−0.175*	n.s.	0.03	−0.024	0.09
Rhizosolenia eriensis (L)	700	−0.068	−0.25*	−0.04	0.0	0.25
Rhizosolenia eriensis (S)	108	−0.086	−0.07*	0.09	0.30	0.34
Stephanodiscus hantzchii	150	0.055	−0.06	0.07	0.14	0.23
Diatoma tenue v. *elongatum*	960	−0.13*	0.07	0.13	0.03	0.31

* Growth rates significantly <0.

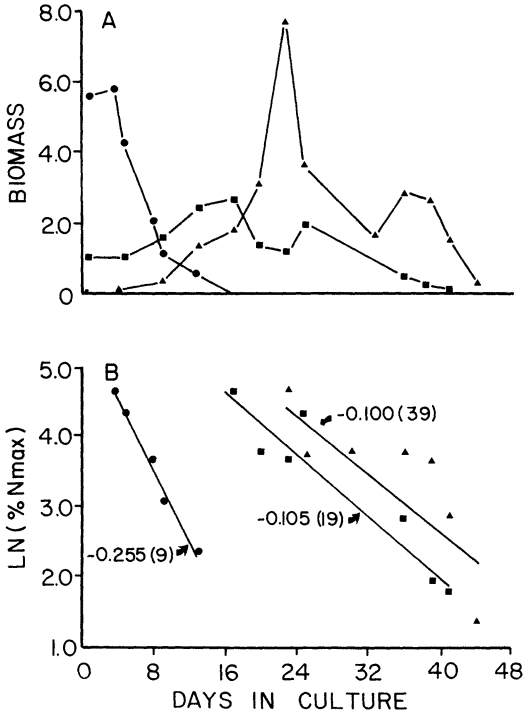

Fig. 3. Population dynamics of *Stephanodiscus astreae* (●), *Oscillatoria limnetica* (■), and *Dinobryon bavaricum* (▲) in a culture (dilution rate = $0.43 \cdot d^{-1}$) of experiment 3. A—Biomass (mg^{-1}) vs. time. B—Log-transformed (% of max abundance) vs. time, with exclusion rates (% SE in parentheses) fitted by linear regression (arrows).

occur in the cultures of lowest dilution rate and some species did not fit the exponential decline model even though they were obviously decreasing in abundance (Tables 1, 3). Because of these problems, the two cultures of lowest dilution rate in each of experiments 2 and 3 were excluded from further statistical analysis.

Growth rates and cell size—In the three experiments for which estimates of species-specific growth rate were available, growth rates varied inversely with cell size. Significant linear regressions showed exclusion rate (the observed difference between growth and dilution rates) to increase with the logarithm of cell size; in experiment 4, the inclusion of dilution rate as an additional independent variable explained significant additional variation of exclusion rate (Table 4). In experiment 2, size explained only 18% of the variation in exclusion rates, although this increased to 50% with the deletion of data for just one species, *S. ulna* (*see below*). Size explained 63–72% of the variation in exclusion rates in experiments 3 and 4 (Table 4); Fig. 4 illustrates the fit of the regression lines (expressed as growth rates) to the growth rates observed in these experiments.

Table 4. Summary statistics of regression analysis of exclusion rates (y, day^{-1}) on biomass-specific V_{max} (10^{-4} pg $P \cdot \mu m^{-3} \cdot h^{-1}$), dilution rate ($D$, day^{-1}), and log cell volume (Vol, μm^3). R^2 (% explained variation) is joint correlation coefficient for multiple regressions. Regressions *a* and *b* in experiment 2 used uptake data from two separate experiments (Smith and Kalff 1982).

Exp	Equation	R^2	F	n
4	$y = -0.294 + 0.011V_{max} - 0.122D$	0.76	79.0	36
	$y = 0.302 - 0.135\text{Vol} - 0.122D$	0.72	71.3	36
3	$y = -0.175 + 0.011V_{max} + 0.14D$	0.89	88.5	18
	$y = 0.085 - 0.070\text{Vol}$	0.63	55.0	33
2	$y = -0.181 + 0.050V_{max}$ (*a*)	0.41	8.7	12
	$y = -0.172 + 0.016V_{max}$ (*b*)	0.49	8.6	9
	$y = 0.109 - 0.094\text{Vol}$	0.18	6.7	27

Substitution of observed uptake parameters for cell size allowed more precise empirical regressions. Using biomass-specific V_{max} as an independent variable, we could explain 41–89%, among experiments, of the variation in exclusion rates (Table 4). In experiments 2 and 3, not all species included in the regressions on size were of known uptake rate. Regressions on size for only the subset of known uptake rates yielded lower percentages of explained variation than the uptake regressions in both experiments; 49% in experiment 3 and 12% in experiment 2 (cf. Table 4). On comparable data sets, uptake rates were therefore better predictors than size alone.

The allometric version of Droop's growth model (Eq. 6) was solved for comparison with the growth rates in experiments 3 and 4, which had shown the strongest relationships between growth and cell size (Table 4). In Fig. 5A, the model was formulated with allometric

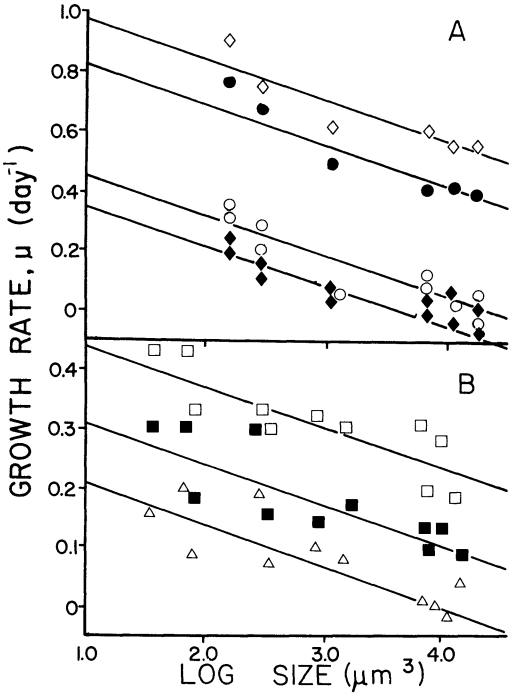

Fig. 4. Observed growth rates in continuous culture vs. cell size, showing lines fit by linear regression (Table 4). A. Experiment 4, with dilution rates of 0.93 (◇), 0.76 (●), 0.32–0.34 (○), and 0.21–0.22 (◆) per day; growth rates from Table 2. B. Experiment 3, with dilution rates of 0.43 (□), 0.30 (■), and 0.20 (△) per day; growth rates from Table 1.

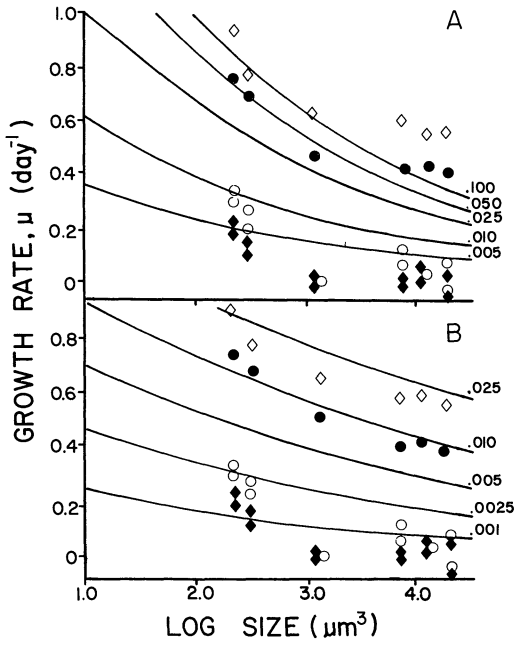

Fig. 5. Observed growth rates in experiment 4 (Table 2) vs. cell size, with rates predicted by Eq. 4 shown as smooth lines for a range of arbitrarily selected availabilities, $S/(K_m + S)$, indicated by numbers next to lines. Dilution rates and symbols as Fig. 4A. A. Assuming $\mu'_{max} = 0.123W^{-0.20}$ (Williams, analyzed by Banse 1976), $V_{max} = 0.336W^{0.522}$, and $k_q = 0.0589W^{0.700}$ (experiment 4: Smith and Kalff 1982): $\mu = 0.123W^{0.20}/(1 + 0.021W^{0.022} \cdot s')$. B. As panel A, but assuming $\mu'_{max} = 0.064W^{-0.022}$ (Parsons et al. *cited in* Banse 1976): $\mu = 0.064W^{-0.06}(1 + 0.003W^{0.108} \cdot s')$.

functions for V_{max} and k_q previously measured for the competing species (Smith and Kalff 1982), and a function for μ_{max} considered best by Banse (1976) for describing nutrient-saturated growth (*see legend*: *Fig.* 5). The model's response was plotted for a number of arbitrarily chosen values of phosphorus availability which gave predicted growth rates similar to those observed. This formulation did not agree with the observations: at the higher phosphorus availabilities, the predicted growth response was steeper and more nonlinear than the observed, while the predicted response at low availabilities was less steep than the response observed (Fig. 5A). The shape of the predicted response curves indicated that no choice of phosphorus availability will bring the model into agreement with the observations.

Agreement was improved by changing the μ_{max} function, choosing one which fits much of the extant data on nutrient-saturated growth rates, but produces a shallower response of growth rate to size (*see legend*: *Fig.* 5). At higher availabilities, the revised model produced approximately linear response, paralleling the observed rates (Fig. 5B); application of nonlinear fitting techniques to find the optimal choice of phosphorus availability would improve the fit further. Predicted response was still too shallow at low availability, however (Fig. 5B). Additional combinations of allometric functions,

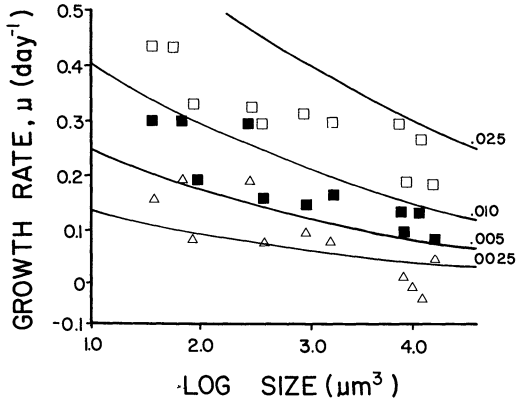

Fig. 6. As Fig. 5, but for experiment 3. Dilution rates and symbols as Fig. 4B. Same allometric functions as Fig. 5B, except $V_{max} = 0.064W^{0.522}$ (experiment 3: Smith and Kalff 1982): $\mu = 0.064W^{-0.06}/(1 + 0.018W^{0.110} \cdot s')$.

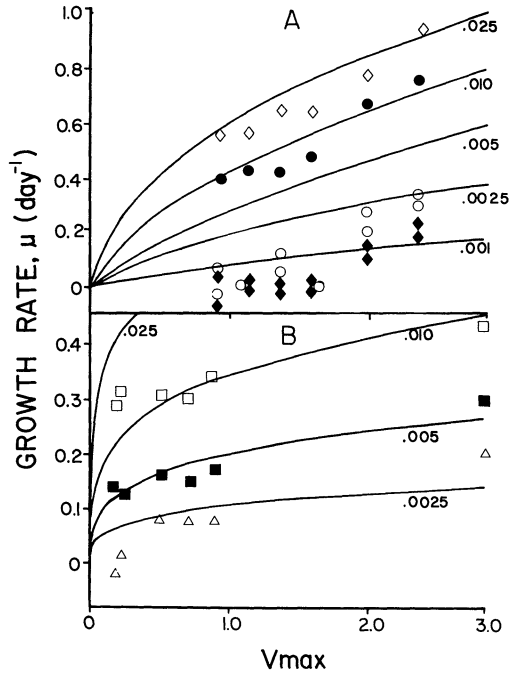

Fig. 7. Observed growth rates vs. uptake parameters, with same dilution rates and symbols as in Fig. 4. A. Growth rate vs. $V_{max}:k_q$ for experiment 4, with Eq. 4, from Fig. 4A, solved as a function of $V_{max}:k_q(=V_m)$: $\mu = 0.0174(V_m)^{0.124}/[1 + 0.0178(V_m)^{0.123} \cdot s']$, V_m in units of (h^{-1}). B. Growth rate vs. $V_{max}:W$ for experiment 3, with Eq. 4, from Fig. 6, solved as a function of $V_{max}:W$: $\mu = 0.088(V_{max}:W)^{0.115}/[1 + 0.010(V_{max}:W)^{-0.211} \cdot s']$, $V_{max}:W$ in units of (10^{-4} pg P·μm^{-3}·h^{-1}).

chosen from among those published for V_{max}, μ_{max}, and k_q, failed to improve upon the response shown in Fig. 5B. By comparison, the empirical linear regressions (Fig. 4) gave reasonable fit over the full range of observed rates.

Comparison of the model to the results of experiment 3 gave similar results. The best formulation is depicted in Fig. 6, using a V_{max} function previously measured on the species of the experiment (Smith and Kalff 1982) and otherwise the same functions as in Fig. 5B. Predicted response once more paralleled that observed at higher availability, but was too shallow at lower availability (Fig. 6). Again, the empirical linear regressions fit comparatively well (Fig. 4).

A possible source of error in the model's predictions is the allometric functions themselves, which contain some error in their representation of species-specific kinetics. To assess the contribution of error in the allometric functions for V_{max} and k_q to discrepancies between observed and predicted growth, we solved the model (Eq. 3) as a function of $V_{max}:k_q$ and $V_{max}:W$ (Fig. 7) to allow it to be tested against directly measured values of these parameters in experiments 4 and 3. The predicted growth rates plotted against the directly observed parameter values were, as with the previous plots against size, in reasonable agreement with the observed growth at higher availability, but formed a much steeper than predicted response at lower dilution rates (Fig. 7).

The subjectively apparent trend to a steeper than predicted response with decreasing phosphorus availability in experiments 3 and 4 was confirmed by allometric regressions of log growth rate on log cell size. The approximate linearity of growth vs. cell size observed (Fig. 4) implies an increase in the absolute magnitude of the allometric exponent. The allometric slopes, b (Table 5), indeed be-

Table 5. Summary statistics of allometric regressions, $\log(\mu) = a + b \cdot \log(\text{cell size})$, with 95% C.I. listed for allometric slope, b.

Exp	D	a	b	95% C.I.	R^2	n	P
4	0.21–0.22	−0.11	−0.400	0.451	0.38	9	0.075
	0.32–0.34	0.259	−0.437	0.411	0.38	11	0.040
	0.76	0.154	−0.135	0.064	0.90	6	0.004
	0.93	0.127	−0.092	0.053	0.86	6	0.008
3	0.20	−0.069	−0.411	0.418	0.44	9	0.053
	0.30	−0.275	−0.177	0.088	0.70	11	0.001
	0.43	−0.201	−0.113	0.068	0.61	11	0.004

came yet more negative, and the goodness-of-fit poorer, as dilution rate decreased in each experiment. Simple scatter in the data did not account for poor fit at lower dilution rate because the corresponding regressions of untransformed rates on cell size were highly significant.

Discussion

Mixed species continuous cultures have been used before to manipulate phytoplankton nutrient status (Peterson et al. 1974; Harrison and Davis 1979; Jones et al. 1978) but the dynamics of individual species have only been quantified once in a two-species system (Mickelson et al. 1979). Our results show that most species reach approximately constant growth rates during exclusion from culture, and that growth rates increase with dilution rate (Tables 1, 2, 3). This reinforces previous evidence at the community level that the intensity of competition can be manipulated by the dilution rate and justifies an assumption of approximate steady state conditions during competitive exclusion.

Our results also show that extremely low dilution rates ($0.055–0.06 \cdot d^{-1}$) are accompanied by negative growth rates in several species (Tables 1, 3). Simple culture stress cannot explain these results because the species concerned grew well at higher dilution rates. Comparison of alkaline phosphatase activity in the cultures and in phosphorus-limited Lake Memphremagog (Smith and Kalff 1981) shows that cultures of dilution rates $<0.2 \cdot d^{-1}$ are much more deficient than in situ populations. The negative growth rates therefore seem to result from phosphorus limitation so severe as to cause population senescence. Negative growth rates were never observed at higher dilution rates, indicating that competition at intensities more typical of Memphremagog produces less drastic effects on algal populations.

The results of our phosphorus competition experiments provide direct support for the hypothesis that small size confers an advantage in competition for inorganic nutrients among phytoplankton. Exclusion rates increased with cell volume in each of three experiments (Table 4) involving 16 different species of freshwater phytoplankton (Tables 1, 2, 3). None of the species was exotic, and some were among the most abundant in Lake Memphremagog (Watson 1979). The advantage of size was evident at all dilution rates between 0.2 and $0.93 \cdot d^{-1}$ (Fig. 4), showing that competition among the naturally co-occurring species was size-dependent throughout a wide range of competitive intensity.

The steady state growth model (Eq. 5) has previously been successfully applied to chemostat monocultures and Jones et al. (1978) demonstrated that it correctly predicted the relationship between cell quota and growth rate, at the community level, in mixed continuous cultures. The model, when combined with known allometric functions governing its parameters (Eq. 6), was consistent with the growth rates observed here except at the lowest dilution rates (Figs. 5, 6), where the response of growth rate to size was steeper than predicted. The disagreement was not the result of error in the

allometric functions for V_{max} or cell quota, because substitution of directly measured parameters for cell size failed to correct the discrepancy (Fig. 7). The two different allometric functions used for μ'_{max} (Fig. 5A, B) represent the extremes in observed steepness of this function (Banse 1976), yet neither produced better predictions at low dilution rate. With at least five independent parameters to be estimated, it may be possible to select values which would, as demanded by the observed results, produce a steeper response at low than high phosphorus availability when substituted in Eq. 5. The present results indicate, however, that such a solution is not to be found with the published allometric parameters (Banse 1976; Shuter 1978; Smith and Kalff 1982), indicating that the assumptions embodied in Eq. 5 are probably insufficient to predict the outcome of multispecies competition at varying intensities.

Even in simpler, monospecific cultures, problems have occurred in applying the Droop model to growth at low dilution rates. "Hookback," an increase of apparent external substrate concentration at low dilution rates in steady state cultures, has been reported several times (Droop 1974; Müller 1972; Goldman 1977) at dilution rates as high as $0.30 \cdot d^{-1}$, the approximate threshold rate found here for departure from the model. Goldman (1977) suggested cell death and nutrient leakage, and Droop (1974) extracellular nutrient complexing, to account for such effects. The chaotic results, including negative growth rates, obtained at very low dilution rate ($0.06 \cdot d^{-1}$) in our study support the hypothesis that cell death, in response to severe nutrient stress, can be significant in continuous culture and is at least partly responsible for departures from predicted growth at somewhat higher dilution rates ($0.2 \cdot d^{-1}$) as well. Light limitation at low dilution rates seems most unlikely, given the strong symptoms of phosphorus deficiency and lack of any dilution-rate-dependent shading in the cultures (Smith and Kalff 1981). Allelo- or autopathic interactions (e.g. Pratt 1966; Keating 1977) may be involved in the response at low dilution rates and may modify the main influence of cell size and P requirements at higher dilution rates. Cell death (and leakage, extracellular complexing, and probiosis) are outside the scope of the internal stores model and will require its modification if such effects turn out to be common in nature. However, the model functioned reasonably well (although ignorance of the ambient P availability prevented a critical test) at dilution rates >0.2–$0.3 \cdot d^{-1}$, the range producing phosphorus deficiency more typical of natural communities (Smith and Kalff 1981).

We have treated our measured V_{max} and K_m as constants over dilution rate. This appears justified for K_m, but not for V_{max} (Gotham and Rhee 1981*a*); although for some species V_{max} does not appear to vary with growth rate, a larger number of species do show a decrease in apparent V_{max} with increasing growth rate. It seems unlikely that such V_{max} inhibition can account for the particular problems with the model noted here because the problems occurred in the low dilution rate cultures in which the uptake measurements were actually made. Also, V_{max} was a better empirical predictor of growth rate than simple cell size, which shows that, despite possible variation with time and dilution rate, the measured V_{max} contained significant information about species physiology. Simulations were nonetheless done to model the effect of a tripling in V_{max} across the approximate range of phosphorus availability suggested to exist in culture by Figs. 5 and 6; as expected, the model continued to perform poorly at low dilution rates, while reasonable agreement obtained at higher rates. Our data and analyses are too imprecise to suggest that V_{max} inhibition did not occur, but they do show that inhibition is not a reasonable explanation for the particular discrepancies observed in our study.

We have also ignored diel periodicity in cell division and nutrient assimilation, despite the documented occurrence of such cycles (e.g. Eppley et al. 1971; Chisholm and Costello 1980). Periodicity is

not likely to have biased our growth rate estimates, which were effectively period-averaged values (Frisch and Gotham 1977) by virtue of the regular daily sampling they were based on. It is possible that uptake kinetics measured at one point in the light cycle (≈"noon"), as ours were, would be nonrepresentative. Given the magnitude of some observed cycles of nutrient uptake rate, and the possibilities of stable coexistence they engender (Frisch and Gotham 1977), it is perhaps remarkable that our uptake rates were significantly related to growth rates. It may well be that considerable suppression of diel uptake cycles by phosphorus limitation, which has been demonstrated for *Euglena gracilis* (Chisholm and Stross 1975), is a common phenomenon in phytoplankton.

Banse (1976) reported allometric exponents for unlimited growth of −0.06 to −0.2 among four different data sets. Our exponents span this range (Table 5). Banse considered that the values of −0.2 best described growth under optimal conditions, and that limitation (by unspecified factors) weakened the apparent size dependence in three of the four studies that actually yielded values closer to −0.1. In contrast, our P-limited cultures showed that the allometric slope increased in absolute magnitude with decreasing dilution rate (Table 5), while the absolute difference of growth rate between size classes was about constant over a wide range of limitation (Fig. 4). Thus, although the allometric exponent may become more negative with increasing phosphorus limitation, the actual advantage to smaller cells does not seem to increase.

Banse (1976), using cell carbon to estimate cell size, explained 90–99% of the variation in allometric regressions of nutrient-saturated growth rates. Our analyses of exclusion rates yielded only 18 (or 50% with the deletion of *S. ulna*) to 72% variation explained by cell volume (Table 4). In all experiments, the explained variation could be increased by using biomass (volume)-specific V_{max} in place of cell volume, showing that volume-independent variation of competitive ability contributed to the lower precision of our results. Cell volume is confounded by interspecific variability of vacuole volume and is therefore not the most precise estimate of size for allometric purposes (Strathmann 1967). Although cell volume is generally well correlated with phosphorus cell quota (Shuter 1978; Smith and Kalff 1982) and has the advantage of being directly observable in natural populations, more precise regressions might have been possible in all experiments with alternative measures of size.

Most previous studies of size-dependent physiology and growth have dealt exclusively or primarily with unicells (e.g. Eppley et al. 1969; Banse 1976; Chan 1978; Friebele et al. 1978), although colony-size-dependent growth rates have been demonstrated for several filamentous blue-green algae (Foy et al. 1976; Foy 1980). We found highly significant relationships governing growth rate (this study) and phosphorus uptake (Smith and Kalff 1982) for mixtures of unicellular and colonial forms. One might expect that size alone is only one aspect of a complex of features that differentiate the ecological strategies of unicells and colonies (Margalef 1978), but the two life forms appear to fall on the same size continuum of uptake and growth rates. This might be explained by the importance of surface area : volume ratio in determining specific uptake rates when marked differences in uptake affinity (K_m) are absent. In the limited sense of area : volume ratio, colonies can often be regarded simply as large unicells, and they appear to behave as such in the present case.

Cases of larger cells outcompeting small for inorganic nutrients have been described before (Parsons and Takahashi 1973; Tilman 1977), and some of the unexplained variation of exclusion rates found here can likewise not be accounted for by any index of size. The notable outlier in the regressions of growth rate on size in experiment 2, the relatively large diatom *S. ulna*, grew unexpectedly well, and no correction for the large vacuoles of diatoms (Strathmann 1967) would place

S. ulna in the same size range as its smaller diatom competitors, *S. acus* and *A. formosa* (Table 3). The unusually high P-limited growth rates of *S. ulna* were correctly predicted by its P uptake kinetics, with a biomass-specific V_{max} of 0.04–0.14 $\times 10^{-10}$ μM P·μ^{-3}·h^{-1} in two different measurements of uptake (Smith and Kalff 1982). Corresponding values for *S. acus* and *A. formosa* were 0.12–0.41 and 0.06–0.12 $\times 10^{-10}$ while K_m did not vary significantly among these species. *Synedra ulna* is therefore inferior in uptake to *S. acus*, but not to *A. formosa*, and its growth was consistent with this ranking (Table 3, Fig. 2). Thus clear exceptions to the general rule that smaller phytoplankton can outcompete larger for phosphorus do occur.

Although members of the genus *Synedra* vary in size by at least two orders of magnitude, the genus appears generally to be remarkably successful in at least some types of exploitative competition. Besides our results with *S. acus* and *S. ulna*, Tilman (1981) reported *Synedra filiformis* to be the superior phosphorus competitor among four different diatom genera common to Lake Michigan. A rather large (195 μm long) *Synedra* sp. showed the greatest population gains of any species when the intensity of competition among phytoplankton was increased by experimental zooplankton reduction in situ (McCauley and Briand 1979). Despite its apparent success in some types of competition, however, *Synedra* is not nearly as common a biomass dominant as some other diatom genera. This may frequently result from a poor ability to compete for silicon (Tilman 1981), but other factors, such as differential susceptibility to allelopathic substances (McCauley and Briand 1979) cannot be ruled out. These observations on *Synedra* perhaps illustrate how the interaction of environmental factors in nature can produce complicated patterns of taxonomic composition while many aspects of resource utilization and metabolism remain strongly and generally size-dependent.

The species used in the size-dependent growth relationships were mainly diatoms and blue-green algae (Tables 1, 2, 3), and the latter are well known for their summertime occurrence in many lakes, when nutrient availability tends to be low (Hutchinson 1967). In Lake Memphremagog, with the phytoplankton deficient in phosphorus at least through summer (Sproule and Kalff 1978; Smith and Kalff 1981), *Oscillatoria tenuis, Anabaena planctonica,* and *Aphanizomenon flos-aquae* are common members of the summer blue-green community (Watson 1979). However, our results do not show these blue-greens to be particularly well adapted to low supplies of P, with their growth rates about as expected for such relatively large forms (Figs. 4, 5). This supports theories attributing blue-green success to minimization of losses rather than growth advantage (Reynolds and Walsby 1975; Kalff and Knoechel 1978) but does not support claims that blue-greens are inherently slow growing (Porter 1977). Rather, their growth rates and phosphorus uptake rates (Smith and Kalff 1982) appear typical of large phytoplankton.

In freshwater, grazing by zooplankton is a major loss factor for nannoplankton (Gliwicz 1977; Porter 1977) and has been assumed to counterbalance their advantage in nutrient competition (O'Brien 1974). Freshwater grazers ingest a very wide variety of particles but show strong preference for particles with maximum dimensions <30 μm (Gliwicz 1977; Porter 1977). Most of the species in our study would therefore not be preferred prey, yet growth rate remained a function of size over the full range of cell sizes observed (Figs. 4, 5). Large and relatively inedible blue-greens display size-dependent growth under optimal culture conditions as well (Foy 1980; Foy et al. 1976), indicating that size advantage persists under nutrient saturation as well as phosphorus limitation. Some size-dependent process or processes other than grazing must compensate for size-dependent competitive advantage among such species not subject to heavy predation.

Dilution rate has been shown to act as a species-selective factor in nitrogen-lim-

ited mixed cultures of marine phytoplankton (Harrison and Davis 1979; Mickelson et al. 1979; Dunstan and Tenore 1974), demonstrating that marine species, at least, can partition gradients of nitrogen availability in mass continuous culture. Our study revealed no convincing evidence for similar phenomena among freshwater species competing for phosphorus. Only experiment 2 provided some sign of partitioning, with *S. acus* and *D. bavaricum* each dominant in different cultures. The two species appeared to have very similar growth requirements, as neither was actually excluded from any of the cultures (Table 3), and the variability of culture conditions in that experiment (reflected in atypical community biomass dynamics) probably determined the dominant. The remaining experiments showed quite clearly that *S. acus* dominated at all dilution rates and maintained its dominance for at least 48 days (experiment 3). This result obtained despite the fact that phosphorus availability varied sufficiently among cultures to generate both order-of-magnitude variation in growth rates and accompanying signs of P deficiency (Smith and Kalff 1981).

The predominant similarity of succession at different dilution rates found here was consistent with predictions from uptake kinetics of some of the competing species (Smith and Kalff 1982), which show none of the variation of half-saturation constants necessary for partitioning to occur through the uptake mechanism (Dugdale 1967). By contrast, interspecific variation of uptake kinetics observed in situ has been proposed to support partitioning of phosphorus (Stross and Pemrick 1974). Such in situ variation may more reflect the environment than ability to compete for P (Hecky and Kilham 1974), especially as uptake kinetics are physiologically plastic. Our results showed that species forced to compete together in a uniform environment did not partition a limiting phosphorus supply.

As in Lake Memphremagog, continuous cultures of Cayuga Lake phytoplankton showed qualitatively similar succession, again toward a small diatom, at different dilution rates (Peterson et al. 1974). The large range of dilution rates spanned by these two studies should have revealed partitioning if it is a common feature in natural communities but did not, although experiments of virtually identical design with nitrogen-limited marine plankton have demonstrated partitioning. This suggests a characteristic difference between freshwater competition for P and marine for N. Comparison of phosphorus uptake kinetics between marine and freshwater species shows that marine species, even those from richer nearshore waters, are also characteristically superior to freshwater species in phosphorus assimilation (Smith and Kalff 1982). These comparisons suggest that marine species are adapted to exploit a more equable, and chronically nutrient-limited, environment than are freshwater forms. Such a conclusion does not necessarily imply that marine phytoplankton are always severely nutrient-limited, but that their evolutionary history does not include the large annual enrichments associated with overturn and littoral inputs.

Simple variation in the rate of supply of limiting phosphorus seems unlikely to influence the selection of taxa or sizes of freshwater phytoplankton by competition because both size advantage and qualitative outcomes were roughly constant over a wide range of competitive intensities in our continuous cultures. In natural systems, change in P availability is accompanied by a panoply of covariates such as alternate resource limitation, change in grazing pressure, and grazing selectivity. We suppressed such complications, so far as possible, in our cultures, and it would seem that they, or their interaction with phosphorus limitation, are responsible for selective changes in community composition with nutrient enrichment. It nonetheless appears that such processes operate in nature on a strong, underlying, size dependence of phosphorus competition.

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