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ALGAL PERIPHYTON GROWTH ON NUTRIENT-DIFFUSING SUBSTRATES: AN IN SITU BIOASSAY[1]

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Abstract. Differences in nutrient limitation for dominant species within an algal periphyton community were determined using additions of N and P supplied by nutrient-diffusing artificial substrates. Sealed clay flowerpots were filled with 2% agar and one of nine nutrient treatments (all combinations of K_2HPO_4 at 0.0, 0.05, and 0.5 mol/L with $NaNO_3$ at 0.0, 0.05, and 0.5 mol/L). The pots were submerged at 0.5 m depth in Douglas Lake, Michigan, and diffused N and P to their outer surfaces in proportion to internal concentrations. After 51 d the pots were scraped and analyzed for attached algae.

Total algal biomass as chlorophyll *a* on the pots ranged from 0.17 ± 0.02 (SE) $\mu g/cm^2$ for pots without added nutrients to 15.7 ± 2.0 $\mu g/cm^2$ for pots with K_2HPO_4 at 0.05 mol/L and $NaNO_3$ at 0.5 mol/L. Chlorophyll *a* on pots containing just P (0.05, 0.5 mol/L) increased 6- to 10-fold over controls. The diatoms *Epithemia adnata* and *Rhopalodia gibba* and the blue-green alga *Anabaena* increased significantly on the P-only pots; these species are suspected of N-fixing capability. Chlorophyll *a* on pots containing just N (0.05, 0.5 mol/L) increased 1.5- to 2-fold, though this increase was nonsignificant; *Achnanthes minutissima, Gomphonema tenellum,* and *Cocconeis placentula* showed enhanced growth on these pots.

Combinations of N and P caused heavy growths of the filamentous alga *Stigeoclonium tenue.* Naviculoid diatoms were also most abundant on the N + P pots.

Average nutrient levels in Douglas Lake during the study were: NH_3, 2.02 μmol/L; NO_3, 0.44 μmol/L; and PO_4, 0.06 μmol/L. The low ambient concentrations of both N and P, together with results of the periphyton bioassay, indicate that the two nutrients may jointly limit overall growth, and that the form of growth limitation differs by species within the periphyton community.

Key words: algal growth; bioassay; nitrogen; nitrogen fixation; nutrient limitation; periphyton; phosphorus.

INTRODUCTION

Algal species are known to respond to certain nutrients as noninteractive essential resources (Tilman 1982, Tilman et al. 1982), and their growth is thus limited by the nutrient in least supply relative to needs (Droop 1974, Rhee 1978). Phosphate has been demonstrated both on theoretical grounds (Schindler 1977) and through numerous field studies to directly control algal abundance in many lakes. Nitrogen limitation is also common (Storch and Dietrich 1979, Kratzer and Brezonik 1981, Zevenboom et al. 1982).

A variety of bioassay procedures have used algal growth enhancement in response to added nutrients to identify the form of nutrient limitation. Such bioassays have included both laboratory and in situ lake studies (Glass 1973, Middlebrooks et al. 1976, Marvan et al. 1979). In situ methods have most commonly used closed plastic or glass containers suspended in the water column (Schelske et al. 1975, Komarkova 1979) or open tubes reaching from the surface into the lake sediments (Moss 1981).

Bioassay procedures, however, can give varying interpretations of nutrient limitation in a given lake. Gerhart and Likens (1975), who compared four methods of predicting phytoplankton response to N and P in Mirror Lake, New Hampshire, USA, found that short-term C-14 bioassays gave misleading results compared to other methods used. Although increases in algal biomass were consistent for both their chemostat and in situ bag enrichment procedures, species composition was highly sensitive to artificial conditions imposed and thus was of little value for interpreting nutrient limitation results.

Although most studies have involved phytoplankton, algal periphyton growth may also be used to assess the form of nutrient limitation, as shown by Wuhrmann and Eichenberger (1974), Stockner and Shortreed (1978), Marcus (1980), Krewer and Holm (1982)

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and Peterson et al. (1983) for streams, and Sladeckova (1979) for lakes. Periphyton, because they are attached, are especially useful as biological indicators of point sources of nutrients in lakes (Kuhn et al. 1981). Localized supplies of particular ions from natural springs, influent streams, or sewage outfalls, for example, may have little impact upon the water chemistry or phytoplankton of a lake, but may cause identifiable changes in periphyton attached to nearby substrates (Stevenson and Stoermer 1982).

The research described here utilized artificial substrates that act as point sources of nutrients. Clay flowerpots, when filled with specified ions, sealed, and placed in situ, slowly release the ions over many weeks and promote the establishment of periphyton communities on their outer surfaces according to the particular ions supplied. Chapman and Craigie (1977) and Harlin and Thorne-Miller (1981) have used a similar method in studying growth responses of marine algae to added nutrients. We examined the effects upon algal periphyton in Douglas Lake, Michigan, of supplying N and P together in varying relative amounts. We were able to describe overall algal growth and to identify those indicator species that became dominant in response to particular nutrient additions. The method is thus an in situ bioassay of nutrient limitation using periphyton. Our data were further used in an evaluation of Tilman's (1982) model predicting growth response to essential resources.

Materials and Methods

Nutrient diffusion rates

Clay flowerpots (outside diameter = 8.8 cm, height = 8.0 cm) were first soaked in deionized water for 3 d, and dried. A plastic Petri dish was attached to the large opening of each pot with silicon adhesive, creating an internal chamber of volume 245 cm^3. A hot agar solution (2%) containing one of four nutrient treatments (K_2HPO_4, 0.05 and 0.5 mol/L; $NaNO_3$, 0.05 and 0.5 mol/L) was then poured through the smaller aperture of each pot; each treatment was poured into three pots (n = 12). Because the agar was partially absorbed into the clay walls, 300 cm^3 could be poured into each pot. Therefore, estimated nutrient contents per pot for the four treatments were 15 mmol and 150 mmol K_2HPO_4, and 15 mmol and 150 mmol $NaNO_3$, respectively. The smaller aperture of each pot was closed with a number 000 neoprene stopper.

After the agar had gelled, each pot was placed in a widemouth 4-L glass jar with 1.5 L of deionized water. The water was sampled for released nutrients and replaced with 1.5 L new deionized water every 24 h for 23 d. Room temperatures during this time were recorded daily using a max-min thermometer. Daily mean estimates (=[maximum + minimum]/2) fluctuated irregularly between 17.8° and 25.3°C, averaging 20.8° (SD = 2.0°). Each day's 12 nutrient samples were stored frozen in acid-washed polyethylene bottles. Daily release rates of NO_3 and PO_4 were determined for both N and P pots on days 1, 2, 3, 6, 9, 12, 15, 18, 21, and 23 using a Technicon II Autoanalyzer.

At the end of the experiment, the Petri dish was removed from each pot and the agar scraped from the inner chamber. These agar samples were heated, immediately diluted 1000× with deionized water, and frozen. Concentrations of NO_3-N were determined for N pots. Both PO_4-P and total P were measured for P pots. Amounts remaining in the walls of the pots were not measured.

In situ periphyton study

Thirty-six pots were sealed with glass Petri dishes, and a single-holed number 10 neoprene stopper with a 15-cm length of wooden dowel was glued to the bottom of each Petri dish. The pots were filled with 2% agar and one of nine nutrient treatments, representing combinations of P (as K_2HPO_4 at 0.0, 0.05, or 0.5 mol/L) and N (as $NaNO_3$ at 0.0, 0.05, or 0.5 mol/L) in a complete factorial design. Each of the nine nutrient treatments was administered to four replicate pots.

The smaller aperture of each pot was then closed with a number 000 neoprene stopper containing a thumbtack, color-coded to denote treatment type. After the agar had gelled, the 36 substrates (pots) were placed 0.5 m apart in a grid at 0.45–0.55 m depth in a uniformly sandy portion of South Fishtail Bay in Douglas Lake, Michigan. The dowel of each substrate was pushed into the sand to the base of the large stopper, the flowerpot itself remaining perched 2–3 cm above the sand. One pot of each treatment was assigned to each of the four rows of the grid. Placement location within rows was determined using a random numbers table. Rows were situated parallel to the shore, 10–15 m from the water's edge.

The substrates were set out on 7 June 1982 and collected 51 d later for determination of chlorophyll *a* and enumeration of algal periphyton. In sampling, a plastic 400-mL beaker was placed over each substrate, fitting snugly against the expanded lip of the flowerpot. The beaker thus enclosed 138 cm^2 of pot surface and 255 mL of surrounding water. The substrate and beaker were then pulled from the sand, inverted underwater, and transported to a nearby rowboat. Surrounding water in each sample and any organisms dislodged from the enclosed pot surface were placed in a 1-L widemouth jar. The pot surface was then cleaned with a knife blade and a hard-bristled toothbrush, and rinsed with deionized water, which was also added to the sample jar.

The sample was transferred to a 1-L graduated cylinder and adjusted to 500 mL with deionized water. The cylinder was shaken well, and a 20-mL (4%) aliquot immediately removed using a wide-bore pipet for chlorophyll *a* analysis. A second 20-mL aliquot was preserved for periphyton counts by adding 2 ml glutaraldehyde.

Chlorophyll *a* samples were filtered (Millipore, 0.45

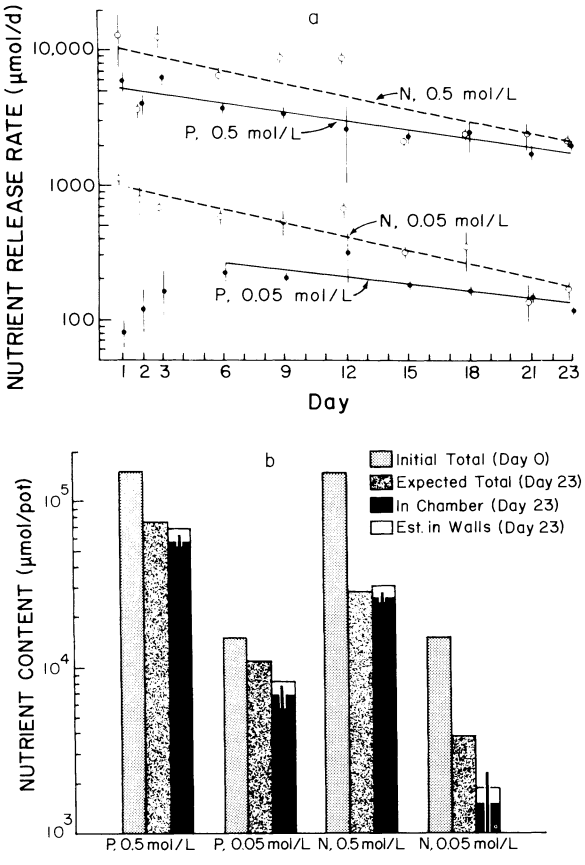


FIG. 1. (a) Average release rates (RR) ($\bar{x}$ + SE) from three pots of each of four nutrient treatments. The data were fit with the function $\ln(RR) = a - b(\text{DAYS})$. The regression for pots with phosphorus at 0.05 mol/L used data beginning on day 6 ($n = 7$ dates); for the other three treatments, average values for all 10 sampling dates were used. For N at 0.5 mol/L: $\ln(RR) = 9.31 - 0.07(\text{DAYS})$, $r^2 = 0.59$, $s_b = 0.02$, $F_{1,8} = 11.7$; for P at 0.5 mol/L: $\ln(RR) = 8.63 - 0.05(\text{DAYS})$, $r^2 = 0.87$, $s_b = 0.01$, $F_{1,8} = 53.3$; for N at 0.05 mol/L: $\ln(RR) = 7.01 - 0.08(\text{DAYS})$, $r^2 = 0.86$, $s_b = 0.01$, $F_{1,8} = 49.3$; for P at 0.05 mol/L: $\ln(RR) = 5.83 - 0.04(\text{DAYS})$, $r^2 = 0.62$, $s_b = 0.01$, $F_{1,5} = 8.3$. (b) Mean (±SE) amounts of N (as NO_3) and total P measured on days 0 and 23 in the 245-cm³ internal chamber and amounts estimated for the clay walls (55 cm³), compared to the expected amounts remaining as determined by summing the daily losses predicted by the four equations in (a).

μm), extracted in 90% buffered acetone, and analyzed after 24 h using a Turner 111 fluorometer. Chlorophyll *a* values were corrected for the presence of phaeopigments (Holm-Hansen et al. 1965) and expressed as micrograms per square centimetre of pot surface.

Algal densities were estimated and generic determinations made using a Palmer-Maloney (0.1 mL) nannoplankton counting chamber at 450×. Species identifications of the dominant taxa were made from wet mounts for the soft-bodied algae or from permanent Hyrax® mounts (Patrick and Reimer 1966) for the diatoms. The volume of subsample analyzed was

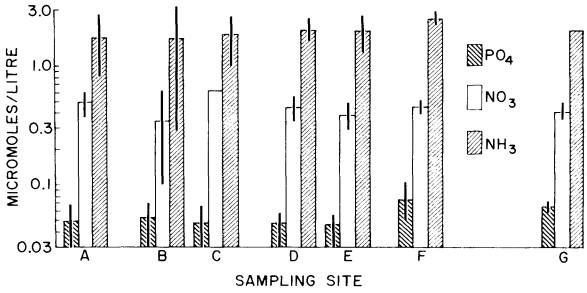


FIG. 2. Mean (±SE) ambient concentrations of PO_4, NO_3, and NH_3 at each of seven sites (A–G) along a 40-m transect through the grid of artificial substrates. Site B was in the flowerpot grid. The bar diagrams for each site are spaced to approximate the relative distances between sampling locations.

dependent upon the concentration of cells. No fewer than 450 cells were identified from each subsample and in many instances 1000–3000 cells were examined per subsample.

Biovolume (corrected for cell wall volume but not for vacuole volume) was estimated for 10 individuals of each taxon by assuming their shapes to be geometrically simple and regular. The biovolumes of cells with more complicated shapes (e.g., *Epithemia, Gomphonema,* and *Navicula*) were determined from scale drawings. For each taxon, the biovolume per surface area of substrate was calculated with the formula $B = bN(V/v)/S$, where b = biovolume per cell, N = number of cells counted, V/v = the portion of the sample analyzed, and S = surface area of the substrate.

In addition to the 36 samples described, sestonic algae were sampled at four locations within the grid by placing the plastic beaker over a clean pot and retaining the 255 mL of water collected. Mean values for chlorophyll *a* and abundances of taxa from these samples were subtracted from each of the 36 sample estimates to eliminate effects of the surrounding water from the data. The corrected values for chlorophyll *a* presented here were very slightly less than uncorrected values, but correcting did serve to reduce the importance of plankton found in the samples.

Statistical analyses comparing chlorophyll *a* or algal biovolumes by nutrient treatment were organized according to a two-way ANOVA model, with three levels of each of the stratifying variables N and P. All comparisons of specific treatments, however, utilized one-way ANOVA. Data were log-transformed where necessary to meet the assumption of homogeneous variances.

Nutrient analysis of Douglas Lake water

On 13 and 27 July, during the periphyton study, concentrations of NH_3, NO_3, and PO_4 were determined from samples collected at seven sites designated A–G along a 40-m transect through the flowerpot grid and perpendicular to the shoreline. Samples were taken from

TABLE 1. Mass of chlorophyll *a* ($\mu g/cm^2$) and total algal biovolume (10^6 $\mu m^3/cm^2$) that accumulated on submerged pots containing nine combinations of N and P ($\bar{x} \pm$ SE).

Phosphorus concentration (mol/L)	Measure	Nitrogen concentration (mol/L)		
		0.0	0.05	0.5
0.0	Chlorophyll *a* ($\mu g/cm^2$)	0.171 ± 0.020	0.272 ± 0.046	0.340 ± 0.055
	Alg. vol. (10^6 $\mu m^3/cm^2$)	9.26 ± 2.10	14.68 ± 1.29	15.08 ± 2.18
0.05	Chlorophyll *a* ($\mu g/cm^2$)	1.636 ± 0.528	2.229 ± 0.228	15.71 ± 2.033
	Alg. vol. (10^6 $\mu m^3/cm^2$)	29.87 ± 4.83	419.4 ± 92.2	1332. ± 106.5
0.5	Chlorophyll *a* ($\mu g/cm^2$)	1.054 ± 0.303	1.497 ± 0.096	12.65 ± 1.292
	Alg. vol. (10^6 $\mu m^3/cm^2$)	22.49 ± 5.65	159.8 ± 19.2	694.9 ± 137.5

both 0.5 m and 1.0 m depth at the deeper sites (E–G). Single samples just above the sandy bottom were collected from sites A–D. Site B was located within the flowerpot grid. The weather on both days was calm. Samples were refrigerated, then analyzed within 2–3 d using a Technicon II Autoanalyzer.

RESULTS

Diffusion rates from the substrates under laboratory conditions varied according to both the type of ion (NO_3 vs. PO_4) and internal concentration (0.05 vs. 0.5 mol/L). Replicate pots showed similar release patterns, with no consistent differences between pots. Mean release rates generally declined during the 23-d study, showing linear trends on a semi-log plot (Fig. 1a). Diffusion rates from N pots were initially higher than from P pots of the same concentration. Unlike the other treatments, the P pots with 0.05 mol/L showed increasing leaching rates during days 1–3. Release rates of NO_3-N from P pots and of PO_4-P from N pots were not detectable at the dilution levels used, and ions supplied by the pots themselves must have been extremely low, if present, in comparison to ions introduced in the agar solution.

Regression lines were fit to the data from days 1 to 23 using mean daily release rates (RR) for one P treatment (0.5/mol/L) and two N treatments (0.05 and 0.5 mol/L). The regression used for the pots with P at 0.05 mol/L used data beginning on day 6. The proportion of variation explained (r^2), the standard error of the slope (s_b) and the significance level of the regression are given with each equation (see Fig. 1a).

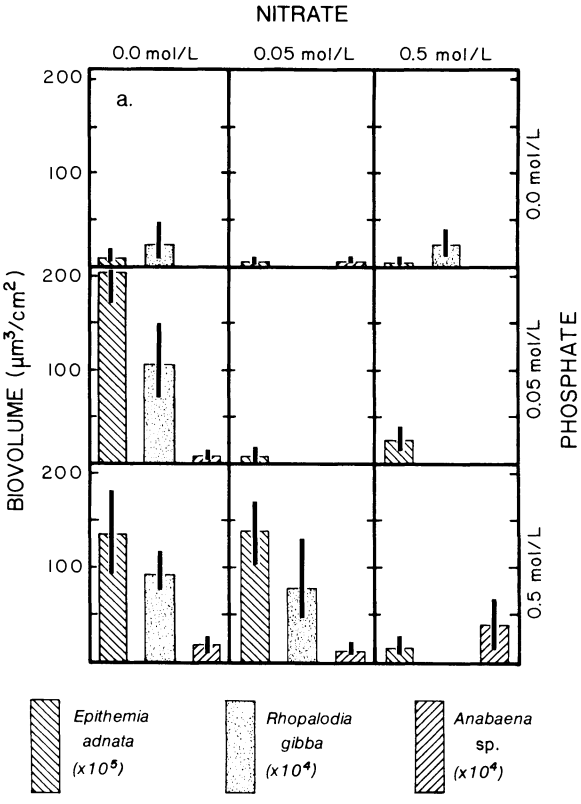
Nutrients remaining in the agar on day 23 (Fig. 1b) were consistently below amounts predicted by summing daily losses using the equations in Fig. 1a. If the nutrient content of agar absorbed into the clay walls (55 cm^3) is assumed to be the same as that of agar in the internal chamber (245 cm^3), however, the agreement of their combined values with expected contents is quite good, except for the pots with N at 0.05 mol/L, one of which produced an anomalously low estimate. Whereas all N measured in the agar on day 23 was present as NO_3-N, most of the P was organically bound, and PO_4-P represented only 9.9% and 2.1% of total P in the pots with P at 0.05 and 0.5 mol/L, respectively. The slower release of PO_4-P may therefore be in part a result of the apparent complexing of the phosphate with the agar.

Differences between the laboratory and Douglas Lake with respect to conditions known to affect diffusion rates (e.g., temperature, current velocity, pH, and external ion concentrations) do not permit precise estimation of release rates in situ. The results indicate, however, that both nutrients were probably available in at least small amounts throughout the field study, and that substrates with higher initial internal nutrient concentrations supplied proportionally higher amounts of these nutrients to their external surfaces.

Concentrations of NO_3, NH_3, and PO_4 found along the transect through the grid of nutrient-rich substrates differed little by either depth or sampling date. Nutrient release from substrates within the grid apparently did not affect ambient concentrations of either NO_3 or PO_4 at site B (Fig. 2). There was thus no evidence of pots chemically affecting neighboring pots within the grid. Concentrations of PO_4 along the transect averaged 0.06 μmol/L while those of NO_3 averaged 0.44 μmol/L. Concentrations of NH_3 were relatively high in comparison, averaging 2.02 μmol/L. Values obtained were consistent with past data for the epilimnion of Douglas Lake (R. Glover, *personal communication*) in that both NO_3 and PO_4 concentrations were very low during midsummer.

Algal biomass, assessed either as chlorophyll *a* or as total biovolume of all algal taxa (Table 1), showed approximately 100-fold variation according to nutrient treatment. Mean chlorophyll *a* values ranged from 0.17 $\mu g/cm^2$ for pots without added nutrients to 15.7 $\mu g/cm^2$ for pots with P at 0.05 mol/L + N at 0.5 mol/L. Addition of P alone (0.05 or 0.5 mol/L) caused a significant increase in chlorophyll *a* ($P < .001$). Addition of N alone produced a (nonsignificant) 1.5- to twofold increase in chlorophyll *a* over control pots. Chlorophyll *a* on all four N + P treatments showed significant ($P < .001$) increases over control levels; the increase was maximal on pots containing N at 0.5 mol/L. Chlorophyll *a* values on N + P pots with P at 0.5 mol/L were significantly lower than on N + P pots with P at 0.05 mol/L ($P < .001$).

Similar results were obtained for total periphyton



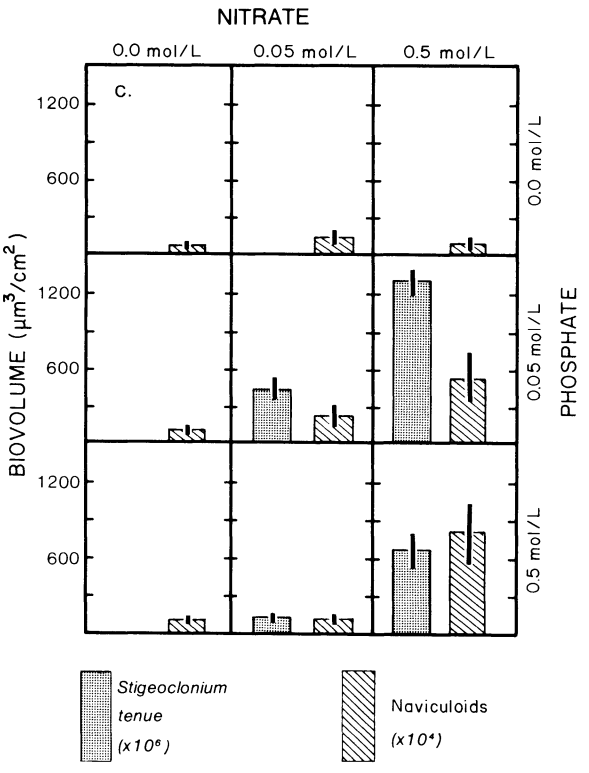
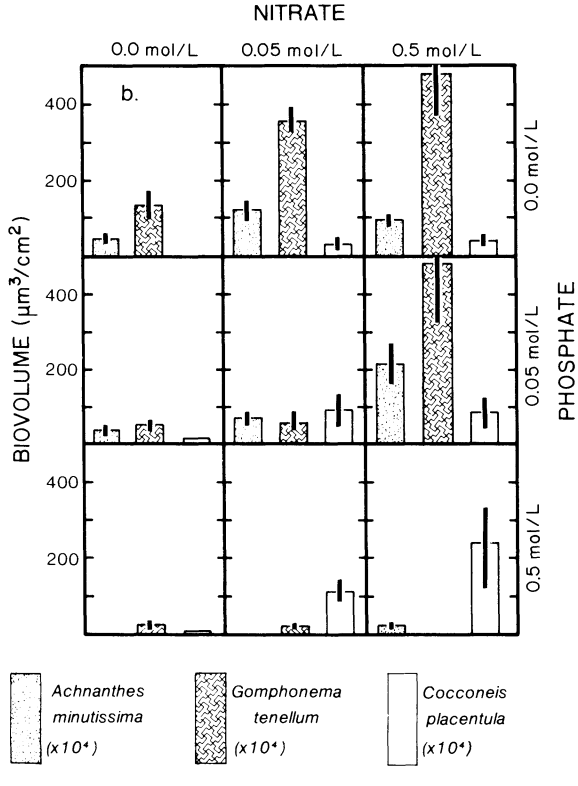


FIG. 3. Biovolumes of eight taxa on pots containing nine combinations of N and P ($\bar{x} \pm$ SE, $n = 4$). Taxa with significant growth responses to P alone and to N alone are shown in (a) and (b), respectively. Two taxa (c) responded significantly only when N and P were supplied together.



biovolume. Reduced growth at N + P at 0.5 mol/L relative to N + P at 0.05 mol/L was again evident ($P < .001$). A slight increase in biovolume again accompanied the addition of N alone ($P < .05$).

Algal taxa that experienced significant growth enhancement in response to particular nutrient additions are shown in Fig. 3a–c. Three general types of growth responses were distinguished. Three taxa were capable of significantly enhanced growth with P alone added (Fig. 3a). This was determined by comparing the two P treatments ($n = 8$) with the control pots ($n = 4$). Three species experienced significant growth in response to N addition ($n = 8$) as compared to controls ($n = 4$) (Fig. 3b). Finally, two taxa (Fig. 3c) responded significantly to additions of N + P ($n = 16$) compared to growth when either nutrient was added alone ($n = 8$).

Of the three taxa that responded to P alone, two were large diatoms (*Epithemia adnata* [Kütz.] Bréb. and *Rhopalodia gibba* [Ehr.] O. Müll.), and one was a filamentous blue-green (*Anabaena* sp.). *Epithemia* experienced a 15- to 25-fold increase on pots with P at 0.05 or 0.5 mol/L compared to control pots ($P < .01$),

and was clearly the dominant species present, comprising 68.6 and 58.8% of total biovolume, respectively. Although the growth of *Epithemia* on pots containing P at 0.5 mol/L + N at 0.05 mol/L remained high, its contribution to total biovolume declined to 8.6%. *Rhopalodia* also responded significantly to P addition ($P < .05$), but was less abundant than *Epithemia*, representing 3.5 and 4.7% of total biovolume on the pots with P at 0.05 and 0.5 mol/L, respectively. *Anabaena* increased significantly ($P < .001$) from 7.8×10^3 $\mu m^3/cm^2$ on control pots to 1.0×10^5 $\mu m^3/cm^2$ and 1.8×10^6 μ^3/cm^2 on pots with P at 0.05 and 0.5 mol/L, respectively.

Achnanthes minutissima Kütz., a diminutive diatom, increased two- to threefold in biovolume when N was added alone ($P < .01$), with a maximum abundance of 1.3×10^6 $\mu m^3/cm^2$ (8.6% of total biovolume) on pots with N at 0.05 mol/L. *Gomphonema tenellum* Kütz. also achieved significantly higher growth on pots with N added alone ($P < .01$), with 4.9×10^6 $\mu m^3/cm^2$ (36.3% of total biovolume) on the 0.5 mol/L substrates. The diatom *Cocconeis placentula* Ehr., found very infrequently on substrates without N, increased significantly ($P < .01$) in response to N addition, but achieved a maximum relative abundance of only 2.5% of total biovolume on the substrates with N at 0.5 mol/L.

The three taxa that experienced increased growth with P addition (*Epithemia, Rhopalodia, Anabaena*) experienced no further growth when N was also added. Of the three taxa that responded to N addition, only *Cocconeis* showed significant further growth ($P < .01$) when P was also supplied. Both *Achnanthes* and *Gomphonema* were significantly inhibited on the pots with P at 0.5 mol/L relative to those with P at 0.05 mol/L ($P < .001$).

The increase in total biovolume on the N + P pots was due almost entirely to the growth response of the filamentous green alga *Stigeoclonium tenue* (Ag.) Kütz. *Stigeoclonium* represented 1.7×10^6 $\mu m^3/cm^2$ on control pots (7.7% of total biovolume) and reached its maximum growth of 1.3×10^9 $\mu m^3/cm^2$ (98.7% of total biovolume) on pots with P at 0.05 mol/L + N at 0.5 mol/L. *Stigeoclonium* clearly dominated the other P + N treatments as well, with 98.1, 96.4, and 87.3% of total biovolume on pots with P + N at 0.05 mol/L + 0.05 mol/L, 0.5 mol/L + 0.5 mol/L, and 0.5 mol/L + 0.05 mol/L, respectively. Whereas *Stigeoclonium* did not respond to either N or P added alone, its growth on the N + P additions was significant for both N ($P < .001$) and P ($P < .01$). Naviculoid diatoms (not identified to species), increased 10-fold on pots with N at 0.5 mol/L + P at 0.5 mol/L, compared to control pots, but reached maximum relative abundances of just 1.3% of total biovolume. The naviculoids, like *Stigeoclonium*, did not experience significant growth enhancement when either N or P was added alone, but did respond to both N ($P < .01$) and P ($P < .05$) when the two nutrients were added together. The greater the amount of N contained in the N + P pots, the more pronounced was the growth response for both taxa.

Centric diatoms (chiefly *Cyclotella* sp.) were abundant on all pots, ranging between 1.1 and 3.8×10^5 $\mu m^3/cm^2$, but showed no significant differences among treatments. Other taxa among the 46 enumerated were found sporadically, and in generally lower densities than those named above.

Discussion

Algal response to P addition

Of the three taxa that responded positively to the addition of P alone, *Anabaena* sp. possessed heterocysts and is presumed to be capable of fixing nitrogen (Rhee and Lederman 1983); *Rhopalodia gibba* was first described to possess endosymbiotic blue-green algae by Drum and Pankratz (1965), and the ability of this endosymbiont to fix nitrogen was shown later by Floener and Bothe (1980); and Geitler (1977) described spherical bodies in *Epithemia zebra* (= *E. adnata*) and suspected that they were symbiotic blue-green algae. Observations with transmission electron microscopy confirm the presence of endosymbiotic blue-green algae in specimens of *E. adnata* from the P pots (Lowe et al. 1984).

The increase in algal biovolume on the P pots relative to control pots is contributed by these three taxa alone. If they are removed from the analysis, the adjusted biovolumes for the P at 0.05 and 0.5 mol/L and control pots are 8.2×10^6 $\mu m^3/cm^2$, 7.7×10^6 $\mu m^3/cm^2$, and 8.0×10^6 $\mu m^3/cm^2$, respectively, with no significant growth stimulation of taxa not suspected of fixing elemental nitrogen.

The decline in overall algal biovolume on pots with P at 0.5 compared to 0.05 mol/L indicates an apparent inhibition of growth, particularly of some taxa (e.g., *Stigeoclonium, Epithemia, Achnanthes, Gomphonema*). This inhibition is most reasonably ascribed either to an excess of phosphate (Rodhe 1948) or possibly to concurrent release from the pots of large amounts of potassium, which accompanied the phosphate as K_2HPO_4. Lehman's (1976) findings of significant growth inhibition of the planktonic chrysophytes *Dinobryon cylindricum* and *D. sociale* var. *americanum* when cultured at high levels of K_2HPO_4 compared with Na_2HPO_4 are consistent with the second possibility.

Algal response to N addition

The low NO_3 values for Douglas Lake and the growth response of *Achnanthes, Gomphonema,* and *Cocconeis* when N was supplied indicate that these taxa may have been N limited. *A. minutissima* is a common and frequently dominant member of the periphyton in a wide variety of situations (Allanson 1973, Brown and Austin 1973, Kuhn et al. 1981), and was inferred by Williams et al. (1973) to be nitrate limited relative to other species in Lake George, New York. In Stockner and Short-

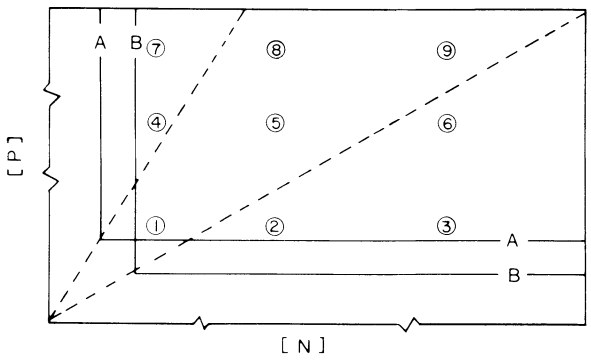


FIG. 4. Zero net-growth isoclines (ZNGI) are provided for two hypothetical periphyton species (A and B) as described in Discussion: Periphyton Species as Nutrient Specialists. The dotted lines denote their optimal N:P ratios. The placement of each of the nine nutrient treatments indicates its relative supplies of N and P (see also Fig. 1). 1: Control; 2: N at 0.05 mol/L; 3: N at 0.5 mol/L; 4: P at 0.05 mol/L; 5: P at 0.05 mol/L + N at 0.05 mol/L; 6: P at 0.05 mol/L + N at 0.5 mol/L; 7: P at 0.5 mol/L; 8: P at 0.5 mol/L + N at 0.05 mol/L; 9: P at 0.5 mol/L + N at 0.5 mol/L. The observed growth responses of *Epithemia, Rhopalodia,* and *Anabaena* were similar to growth predicted for species A. Growth of *Achnanthes, Gomphonema,* and *Cocconeis* resembled that for species B.

reed's (1978) study of periphyton in Carnation Creek, Vancouver Island, *A. minutissima* was a dominant species in stream troughs with no added nutrients or with added NO_3, but was overgrown by other taxa in troughs with added PO_4. Ecological preferences for particular nutrient concentrations are not well known for *Gomphonema tenellum* or *Cocconeis placentula.*

Algal response to N + P

The filamentous green alga *Stigeoclonium tenue,* the clear dominant in all combinations of N + P, has been ranked as the sixth most tolerant alga to organic enrichment (Palmer 1969). Organic enrichment usually provides large quantities of both N and P. Considerable habitat modification by *Stigeoclonium* complicates the interpretation of growth stimulation of the naviculoid diatoms. Overgrowth by *Stigeoclonium* may also have reduced the abundance of *Epithemia* and *Rhopalodia.*

Periphyton species as nutrient specialists

A plausible explanation of the observed growth responses is shown in Fig. 4, modified from Smith's (1982) description of N:P requirements of phytoplankton. The placement of the nine nutrient treatments in Fig. 4 is judged accurate only in their relative supply rates of N and P. Lines A and B denote the Zero Net Growth Isoclines (ZNGI) for two hypothetical periphyton species potentially competing for two essential resources (Tilman 1982). Optimal N:P requirements are shown as dotted lines for each. The amounts of available N and P should both decline over time as periphyton biomass and uptake increase. Further growth for a given species is curtailed when its ZNGI is reached, and may imply either N or P limitation, depending upon the portion of the ZNGI intercepted.

The increased growth of *Achnanthes, Gomphonema,* and *Cocconeis* on pots with added N suggests that their ZNGI's may be similar to that of species B. Although presumably N limited on the control pots, when N was supplied these taxa probably became P limited. Their increased growth is consistent with their being superior competitors for P.

Likewise, *Epithemia, Rhopalodia,* and *Anabaena,* which were P limited on the control pots, may have nutrient requirements more similar to species A. Their considerable growth on the pots with added P appears to be the result of N fixation under otherwise N-limiting conditions.

Stigeoclonium showed rapid growth on all pots with N + P, with N:P ratios ranging over approximately two orders of magnitude. Francke and Den Oude (1983) have suggested that the N and P requirements of *Stigeoclonium* spp. are high relative to most algae, and the dominance of *S. tenue* in this study may well reflect the advantages of a filamentous growth form highly adapted to obtaining both light and nutrients in nutrient-enriched waters.

The higher biotic density of periphyton communities relative to the phytoplankton may accentuate the growth-limiting potential of other resources such as light and available attachment sites for many species. Despite the addition of two principal nutrients deemed critical to the growth of periphyton in Douglas Lake, only 8 of 46 taxa actually showed significantly enhanced growth. Because the overall community response to nutrient addition may thus be dependent upon the growth of a small number of species, an understanding of such indicator species can be critical to interpretations of overall growth as a bioassay of nutrient limitation.

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